# KINETIC STUDIES IN MICELLAR SYSTEMS

## THESIS

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**BY**

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**UNDER THE SUPERVISION OF**

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**2014**

*Dedicated to*

*My Adorable Parents*

*My Family*

*&*

*My Teachers*

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# Certificate

This is to certify that the thesis entitled "**Kinetic Studies in Micellar Systems**" is the original work carried out by **Mr. Adel Ahmed Mohammed Saeed** under my supervision and is suitable for submission for the award of **Ph. D.** degree in **Chemistry**.

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This is to certify that **Mr. ADEL AHMED MOHAMMED SAEED,** Department of **Chemistry,** has satisfactorily completed the course work/ comprehensive examination and pre-submission seminar requirement which are part of his Ph.D. programme.

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## ***List of Publications***

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2. Micellar, Salts, and Organic Solvent Effects on the Rate of [Cu(II)-Gly-L-Ala]$^{+}$ Complex-Ninhydrin Reaction.
   Mohd. Akram, **Adel A. M. Saeed** and Kabir-ud-Din
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3. Catalytic Effect of Cationic Gemini Micelles on the Rate of Condensation between Glycyl-DL-Aspartic Acid and Ninhydrin in Absence and Presence of Organic Solvents.
   Mohd. Akram, **Adel A. M. Saeed** and Kabir-ud-Din
   *J. Solution Chem.*, **44**, 1529-1544 (2015).

4. Role of Alkanediyl-α,ω-bis(Dimethyltetradecylammonium Bromide) Gemini Surfactants in Dipeptide (Glycyl-L-Alanine)-Ninhydrin Reaction in Aqueous and Aqueous-Organic Solvents.
   Mohd. Akram, **Adel A. M. Saeed** and Kabir-ud-Din
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   Mohd. Akram and **Adel A. M. Saeed**
   International Conference on Chemistry Frontiers & Challenges, Department of Chemistry, Aligarh Muslim University, Aligarh, March 2-3, 2013, p. 201.

2. Effect of Gemini Surfactant, Alkanediyl-α, ω- bis- (Dimethyl-tetradecylammonium Bromide) on Ninhydrin/ Dipeptide Reaction.
   Mohd. Akram and **Adel A. M. Saeed**
   16th National Conference on Surfactants, Emulsions and Biocolloids, (NATCOSEB-XVI), CSIR-Central Leather Research Institute, Adyar, Chennai-20, November 28-30, 2013, p. 49.

3. Interaction of Dipeptide (Glycyl-DL-Aspartic Acid) with Ninhydrin in Absence and Presence of Cationic/ Dicationic Micelles: A Kinetic Study.
   **Adel A. M. Saeed** and Mohd. Akram
   National Symposium on Chemistry (NSC-2014), Department of Chemistry, Aligarh Muslim University, March 22, 2014, p.89.

4. Effect of Cationic Surfactant (TTAB) on Rate of Ninhydrin-Glycyl-DL-Aspartic Acid Reaction in Absence and Presence of Organic Solvents.
   Mohd. Akram, **Adel A. M. Saeed** and Kabir-ud-Din
   National Conference on Emerging Areas in Chemical Education & Research and National Convention on Chemistry Teachers (NCCT-2014), The IIS University, Jaipur, October 16-18, 2014, P-17F, p.78.

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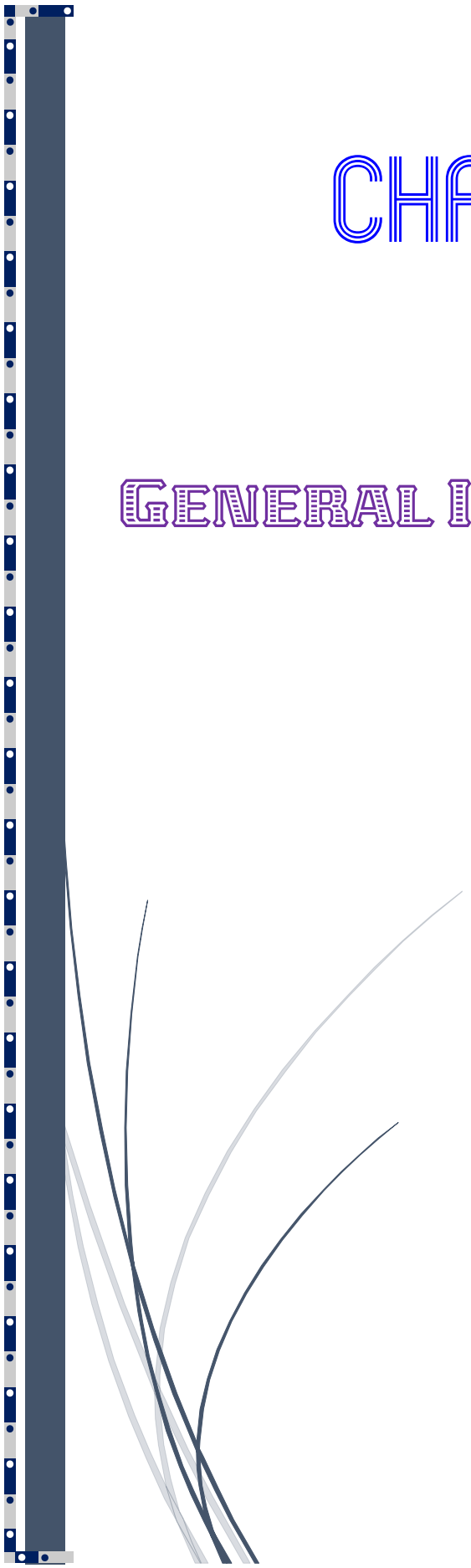
# General Introduction



# Chapter One

## 1.1 Introduction

The word *'kinetic'* comes from the Greek word for 'motion'. In chemistry, kinetics is the study of how fast reactions occur [1]. In 1864, Peter Waage and Cato Guldberg pioneered the development of chemical kinetics by formulating the law of mass action, which states that the speed of a chemical reaction is proportional to the quantity of the reacting substances [2].

In many chemical reactions where there are a number of possible products, the first one formed may be the one that is formed most quickly, not necessarily the one that is most stable; if you leave the reaction going, you should eventually form the product that involves the greatest change in bond energy - the thermodynamic product [1].

Catalysis is the phenomenon in which a relatively small amount of a foreign material, called a catalyst, increases the rate of a chemical reaction without itself being consumed. Although widely utilized now in many industrial processes, catalysis was not even recognized until the 19th century when Berzelius introduced the term in 1836 [3]. Catalyst research has been devoted to increase the catalyst activity and selectivity to improve process economics and reduce environmental impact through better feedstock utilization [4].

It is recognized that the rates of chemical reactions can be modified by self-organized assemblies such as micelles and the study of influence of micelles on reaction kinetics is known as "kinetics in micellar systems" or "micellar catalysis".

The development of our knowledge of solutions is on touch to some extent the development of chemistry itself. It is known that the water is the most abundant, the most important and best-known terrestrial fluid and is called the universal solvent because more substances dissolve in it than in any other solvent. This has to do with the polarity of each water molecule [5]. For some cases, if the solute has nonpolar part, selecting a suitable solvent should be considered according to the concept "*like dissolves like*".

*Micelles*, a Latin term meaning “small bit” which was first assigned by J. W. McBain [6]. Micelles have become a subject of great interest to the organic chemist and the biochemist—to the former because of their unusual catalysis of organic reactions [7] and to the latter because of their similarity to biological membranes and globular proteins [8].

Micelles, or surfactant organized assemblies in general, are of interest both from academic and applied points of view. A fundamental understanding of the physical chemistry of the surfactant organized assemblies, their unusual properties, and phase behavior is essential for the most industrial chemists.

The terms *amphiphile* and surfactant are often used interchangeably. The term surfactant (short for SURFace-ACTive-AgeNT) designates a substance that exhibits some superficial or interfacial activity according to its chemical structure which makes it particularly favorable to reside at interfaces.

The word *amphiphile* was coined by Paul Winsor 60 years ago [9]. It comes from two Greek roots: the prefix *amphi* which means "from both sides" and the root *philos* which means "affinity". An *amphiphilic* substance exhibits a double affinity, which can be defined from the physico-chemical point of view as a polar-apolar duality. A typical *amphiphilic* molecule contains two parts: on the one hand a polar part which contains heteroatoms such as O, S, P, or N, included in functional groups such as amine, amide, alcohol, acid, ether, ester, sulfate, sulfonate, phosphate, *etc*. On the other hand, apolar part which is in general a hydrocarbon chain (alkyl or alkyl derivatives). The polar portion exhibits a strong affinity for polar solvents, particularly water, and it is often called *hydrophilic* part or *hydrophile*. The apolar part is called *hydrophobe* or *lipophile*, from Greek roots *phobos* (fear) and *lipos* (grease) [10].

Whether a surfactant is a man-made or naturally occurring, its molecules are a frequent component of colloidal systems [11]. It is worth remarking that all *amphiphiles* do not display such activity; in effect, only the *amphiphiles* with more or less equilibrated *hydrophilic* and *lipophilic* tendencies are likely to migrate to the surface or interface. It

does not happen if the *amphiphilic* molecule is too *hydrophilic* or too *hydrophobic*, in which case it stays in one of the phases.

A micelle is an organized blob of surfactant molecules with all the *hydrophobic* tails pointing inwards to create a tiny *hydrophobic* phase. Dissolving surfactant molecules in solvent, continue up to a point as more surfactant is added, and then any additional surfactant will form micelles. Under the same conditions, a particular surfactant will always form micelles of the same size and containing almost the same number of surfactant molecules.

Proteins as macromolecules are an important target of reactive species and have attracted enormous scientific interest over the last century according to their characteristic chemistry importance in relation with origin of life on Earth [12]. Protein structure is useful in understanding biochemical functions such as enzyme catalysis [13] and it may be done by the help of the kinetic and mechanistic studies on protein units (amino acids/dipeptides) condensation. For metal ions, proteins are important binding sites and this complexation forms the prominent interactions in nature and biological systems.

The application of ninhydrin (1,2,3-indanetrione or, 1,2,3-triketohydrindene) for the detection/estimation of amine functionality in the fields of chemistry, forensic science and biochemistry [14,15] has a great ability in disclosing latent fingerprints [16-21]. On comparison with other fingerprint reagents such as fluorescamine and o-phthalaldehyde (OPA) [22,23] ninhydrin has (1) a long shelf life making it practical in routine analysis, (2) it is stable in aqueous solutions (there is no competition between product formation and hydrolytic deactivation of the reagent), and (3) the powdered form is not hygroscopic. Ninhydrin is thus an "ideal" reagent due to its high sensitivity and it provides excellent background contrast with high intensity when using it to detect fingerprints. The use depends on the formation of diketohydrindylidene-diketohydrindamine (DYDA) commonly called "*Ruhemann's purple (RP)"* [24-31]. Several investigations have been made to modify ninhydrin reactions' interest across a broad spectrum of disciplines. The method, though useful, still has much room for improvements. Therefore, continuous efforts are being made to improve the method such

as addition of surfactant micelles, hydrotropes, organic solvents, metal ions and the order of addition of reagents [32-50].

In this context, reactions of ninhydrin with dipeptide/metal ion-coordinated dipeptide were performed in micellar systems in absence and presence of organic solvents with a view to find some applications to improve contrast and visualization of ninhydrin developed fingerprints that may prove a step forward from the methods already used in current forensic, agricultural, food, histochemical, biomedical, clinical, microbiological, nutritional, plant, analytical and other fields studies [21,51-53].

Performing the reactions in organized (micellar) system can influence rates and pathways of all kinds of chemical reactions instead of pure bulk solvents [54]. These reactions often occur at the interface between the solvent, which is usually water or an aqueous-organic mixture, and the submicroscopic particles or aggregates. Motivation for studying reactions in micellar systems may be derived from three sources: first, to further understanding of those factors which influence the rates and course of organic reactions; second, and closely related to the first, to gain additional insight into the exceptional catalysis characteristics of enzyme reactions; third, to explore the utility of micellar systems for the purpose of organic synthesis.

## 1.2 Amphiphiles and Surfactants: Definition and Types

### 1.2.1 Definition

Because of its dual affinity, an *amphiphilic* molecule does not feel "at ease" in any solvent, be it polar or non-polar, since there is always one of the groups which "does not like" the solvent environment. This is why *amphiphilic* molecules exhibit a very strong tendency to migrate to interfaces or surfaces and to orientate so that the polar group lies in water and the apolar group is placed out of it, and eventually in oil.

*Amphiphiles* exhibit other properties than tension lowering and this is why they are often labeled according to their main use such as: soap, detergent, wetting agent,

dispersant, emulsifier, foaming agent, bactericide, corrosion inhibitor, antistatic agent, *etc*. [10,55].

Surfactants (the ubiquitous *amphiphiles*) are organic substances that contain polar or ionic head groups and apolar tails (Figure 1.1) and when dissolved in water and/or organic solvent at low concentration, have the ability to adsorb (or locate) at interfaces, thereby altering significantly the physical properties of those interfaces [56]. Because surfactants are adsorbed mainly on the surface of the solution, creating a thin monolayer, they are called surface-active substances. When dissolving them, after they reach a certain value of concentration, molecules or ions of surfactants begin to associate and to organize themselves into more complex units, also called micelles. Surfactants have become the subject of intense investigation by researchers in the field of chemical kinetics and biochemistry because of the unusual properties of the aggregated forms (*e.g.*, micelles) of these materials.

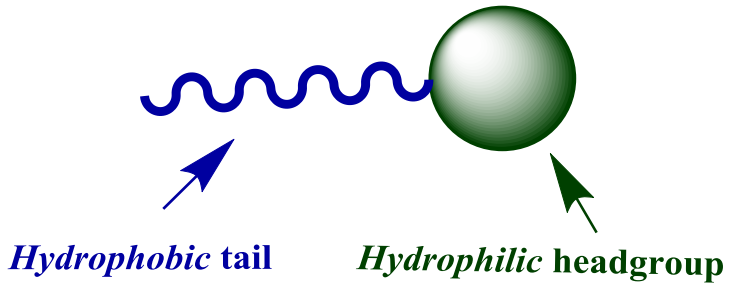



**Figure 1.1:** Schematic representation of a surfactant monomer.

A micelle is an aggregate of surfactant molecules (with a nano size (~3-50 nm) [57]) dispersed in a liquid colloid. Micelles are approximately spherical in shape. Other phases, including shapes such as ellipsoids, cylinders, and bilayers, are also possible. The shape and size of a micelle are a function of the molecular geometry of its surfactant molecules and solution conditions such as surfactant concentration, temperature, *p*H, and ionic strength. The process of forming micelles is known as micellization and forms part of the phase behavior of many lipids according to their *polymorphism* [58].

The majority of practical surfactant systems have water as their main liquid component. The next largest class of surfactant systems utilizes a water-immiscible

organic solvent as the dominant liquid. Additional surfactant applications are being developed for polar solvents other than water, such as, glycerol, ethylene glycol, formamide, and hydrazine [59]. Other significant applied research concerns the development of surfactants for fluids such as critical carbon dioxide.

### 1.2.2 Surfactant Types

Surfactants are often classified on the basis of an empirical scale called *hydrophilic–lipophilic* balance (HLB) number, which gives a simple index for the molecular balance of surfactant at an oil–water interface. A general classification of the surfactants may be made on the basis of the nature of *hydrophilic* (polar) group.

#### *1.2.2.1 Ionic Surfactants*

##### *(a) Cationic Surfactants*

Cationic surfactants have a high proportion of all surfactants. The surface active portion of the molecule bears a positive charge. The prime use of cationic surfactants is their tendency to adsorb on negatively charged surfaces, *e.g.*, anticorrosive agents for steel, flotation collectors for mineral ores, dispersants for inorganic pigments, antistatic agents, fabric softeners, hair conditioners, anticaking agent for fertilizers and as bactericides.

*Examples*:

Hexadecyltrimethylammonium chloride

$CH_3(CH_2)_{15}N^+(CH_3)_3\,Cl^-$

Dodecylpyridinium bomide

$CH_3(CH_2)_{11}C_6H_4N^+Br^-$

##### *(b) Anionic Surfactants*

The surface active portion of the molecule has a negative charge. Anionic surfactants are the second famous and most widely used class of surfactants in

industrial applications [60,61] coming after cationic surfactants. Due to their low cost of manufacture, they are used in practically every type of detergent.

*Examples:*

Sodium dodecyl sulfate

$CH_3(CH_2)_{11}OSO_3^-\ Na^+$

Sodium dodecylbenzene sulfonate

$CH_3(CH_2)_{11}C_6H_4SO_3^-\ Na^+$

***(c) Amphoteric Surfactants***

*Amphoteric* (zwitterionic) surfactants [62] have both positive and negative charges in the surface active portion, and can behave as either an anionic, nonionic, or cationic species, depending upon the *p*H of the solution, which gives it the properties of zwitterions and thus, lead to head group *hydrophilicity*, an intermediate between that of ionic and nonionic surfactants [63]. *Amphoteric* surfactants have excellent dermatological properties as they are less irritating to skin than many ionic surfactants [64] and have thus useful applications when combined with ionic and nonionic surfactants in cosmetics and pharmaceutical industries. In *amphoteric* surfactants, whereas the positive charge is almost invariably ammonium, the source of negative charge may vary, although carboxylate is by far the most common. Neither the acid nor the basic site is permanently charged, *i.e.*, the compound is only *amphoteric* over a certain *p*H range.

*Examples:*

Tetradecyl betaine

$C_{14}H_{29}N^+(CH_3)_2CH_2COO^-$

*N*-dodecyl-*N*,*N*-dimethyl-3-ammonio-1-propanesulfonate

$CH_3(CH_2)_{11}N^+(CH_3)_2CH_2CH_2CH_2SO_3^-$

***1.2.2.2 Nonionic Surfactants***

The surface-active portion bears no apparent ionic charge, but has a polar head group (containing hydroxyl groups or polyoxyethylene chains). Many nonionic

surfactants are structurally analogous to anionic and cationic surfactants, except that the head group is uncharged. An important group of nonionic surfactants includes those where the *hydrophilic* portion comprises a chain of ethoxy group and is known as ethoxylates [65,66].

*Examples:*

Polyethyleneglycol tert-octylphenyl ether

$t\text{-}C_8H_{17}\text{-}C_6H_4\text{-}(OCH_2CH_2)_nOH$

Polyethylene glycol (23) lauryl ether

$CH_3(CH_2)_{11}(OCH_2CH_2)_{23}OH$

### *1.2.2.3 Bola-amphiphile Surfactants*

*Bola-amphiphile* surfactants or (also known as bolaform or α–ω–type surfactants) are amphiphilic molecules which consist of two *hydrophilic* head groups, connected by a long, linear *hydrophobic* skeleton (*e.g.*, one, two, or three alkyl chains, a steroid, or a porphyrin) [67-69]. One example of a one alkyl chain is a polymethylene chain (Figure. 1.2).

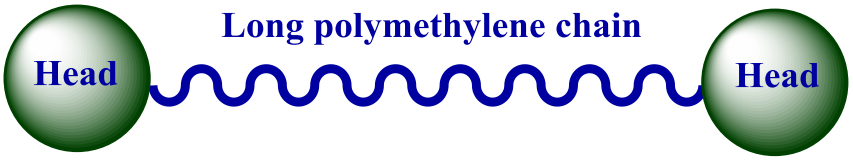



**Figure 1.2:** Schematic representation of a *bola-amphiphile* surfactant.

Their self-association ability is less, compared to conventional ionic surfactants. However, they show biological activity [70,71] such as working as perfect vehicles for DNA delivery to mitochondria [69]. Some special *bola-amphiphiles* are capable of giving rise to organized assemblies of peculiar structure [72].

*Examples:*

Hexadecanediyl-1,16-bis(trimethylammonium bromide)

$(CH_3)_3N^+(CH_2)_{16}\,N^+(CH_3)_3\;2Br^-$

1,1'-decane-1,10-diylbis(4-amino-2-methylquinolinium) dichloride

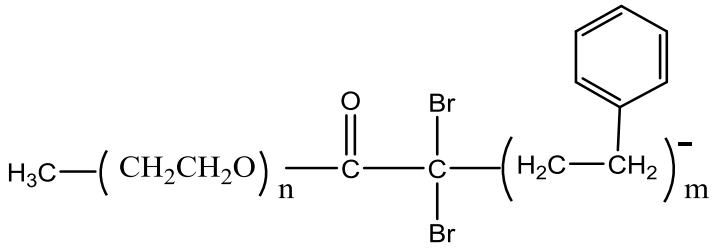
$C_{10}H_8NH_2N^+(CH_2)_{10}C_{10}H_8NH_2N^+ \ 2Cl^-$

### *1.2.2.4 Polymeric Surfactants*

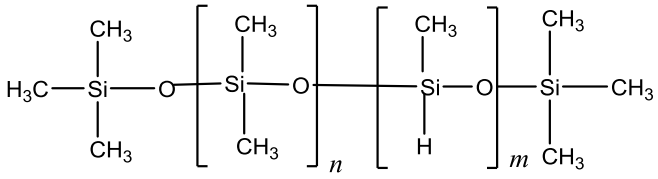
Association of one or several macromolecular structures exhibiting *hydrophilic* and *lipophilic* characters forms polymeric surfactants. Recently, there has been considerable interest in this surfactants category due to their wide application as stabilizers and rheology controlling for suspensions and emulsions (disperse systems). The most convenient polymeric surfactants are those of the block and graft copolymer type. [8,73]

*Examples:*

Polystyrene-block-poly(vinyl acetate):



Polydimethylsiloxane-block-polymethylhydrogensiloxane



### *1.2.2.5 Dimeric (Gemini) Surfactants*

The period between the late 1980s and early 1990s gave birth to one of the most exciting developments in the field of surfactant chemistry that is the emergence of the dimeric surfactants. However, the first known dicationic (dimeric) surfactant was marked

to Bunton et. al [74] in 1971 who studied catalysis of *nucleophilic* substitutions by "dicationic detergents". Menger and Littau [75] coined the term "*gemini*" (Latin word for 'twins' ) for describing dimeric surfactants (or bis-surfactants), that is, one surfactant molecule containing two *hydrophobic* groups and two *hydrophilic* groups, connected by a linkage (spacer) close to *hydrophilic* groups and this term still used by researchers [76-81].

A schematic representation of a *gemini* surfactant is shown in Figure 1.3.

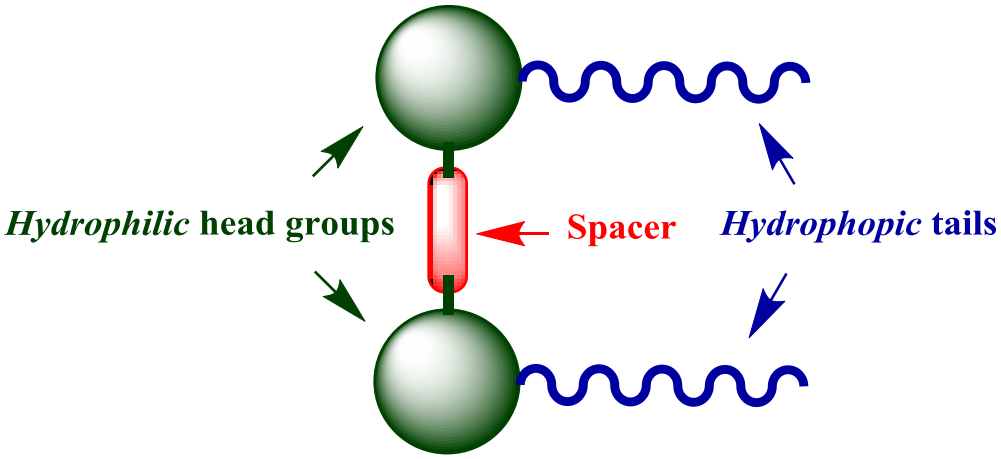



**Figure 1.3:** Schematic representation of a *gemini* surfactant.

Rosen opened the door of interest in *gemini* surfactant synthesis and use due to the paper [78] which pointed out that these surfactants could be more surface-active by orders of magnitude than comparable monomeric (conventional) surfactants. *Gemini* surfactants with a great variety of chemical structures have been obtained by acting on the nature of the head group and spacer group, as illustrated in Figure 1.3. As a result, numerous papers appeared in the literature describing the fundamental properties of *gemini* surfactants. Among these *gemini* surfactants, the cationic bis(alkyldimethylammonium)alkane dibromide type, with two tails and a spacer separating the two quaternary nitrogen atoms in the heads, designated as m-s-m, where m refers to symmetric side chains of carbon atoms and s refers to the spacer, has received more attention [76-80,82-85].

According to many achieved patents and published papers, the head group can be cationic [86-88], anionic [89-91], nonionic [92,93], or amphoteric [94] while the spacer group can be either hydrophilic or hydrophobic, rigid or flexible [95-97]. Other types of *geminis*: sugar-based *gemini* surfactants [98,99], asymmetric *gemini* surfactants with different head groups have been also synthesized [100-102].

Nowadays, some other types of surfactants have been developed, such as trimeric surfactants and eco-friendly biodegradable surfactants [103,104].

### 1.3 Micelle Formation and Critical Micelle Concentration (CMC)

Since the beginning of the study of surfactant solutions, it was observed that surfactants (*amphiphiles*) distort the structure of the solvent/water when dissolve in it, (because of unfavorable interactions), thereby, the free energy of the system increases. To minimize system instability, the system responds in such a way to minimize contact between the *lyophobic*/*hydrophobic* groups and solvent/water. As a result of this distortion, some of the surfactant molecules are expelled to the interface/surface of the system with their *hydrophobic* groups oriented predominantly away from the polar solvent (*e.g.*, water) so as to minimize the free energy of the solution. This results in a decrease in the surface tension of solvent. However, there is a particular concentration which leads to aggregate surfactant molecules. This concentration is narrow enough to be called critical, at which the surfactant molecules begin to organize by ordering themselves in structures called micelles.

The formation of colloidal-sized clusters of individual surfactant molecules in solution is now better known as micelle formation, or micellization which is an important phenomenon not only because a number of important interfacial phenomena, such as detergency and solubilization, depend on the existence of micelles in solution, but also because it affects other interfacial phenomena, such as surface or interfacial tension reduction, that do not directly involve micelles.

The physical properties of surface active agents differ from those of smaller or *nonamphipathic* molecules in one major aspect, namely the abrupt changes in their

properties above a critical concentration and the concentration at which this phenomenon occurs is called the critical micelle concentration (CMC).

In some cases, particularly where the hydrophobic group is long (*e.g.*, $>C_{16}$), the surfactant self-assembly leads to a range of different structures. It has been suggested [105] that this indicates change in the micellar structure (Figure 1.4).

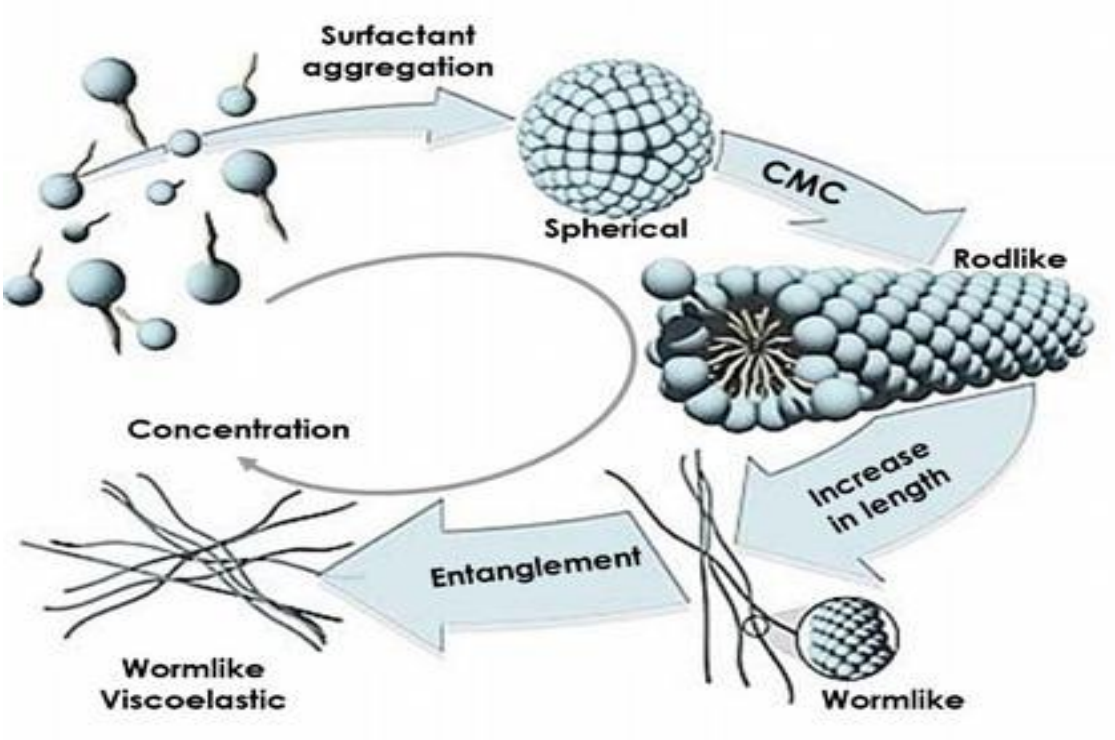



**Figure 1.4:** Micellar self-assembling structure dependency on surfactant concentration.

The increase in the concentration of a particular surfactant in an aqueous solvent reveals a sudden change in various aqueous surfactant solution properties that can be determined by several methods such as surface tension, UV-Vis/fluorescence spectra of solutes, equivalent conductivity, solubilization, turbidity, osmotic pressure, light scattering, self-diffusion, magnetic resonance, and reaction rate.

Figure 1.5, proposed by W.C. Preston [106] and developed by B. Lindman [107] illustrates with plots of several physical properties (osmotic pressure, turbidity,

solubilization, magnetic resonance, surface tension, equivalent conductivity and self-diffusion) as a function of concentration for an ionic surfactant.

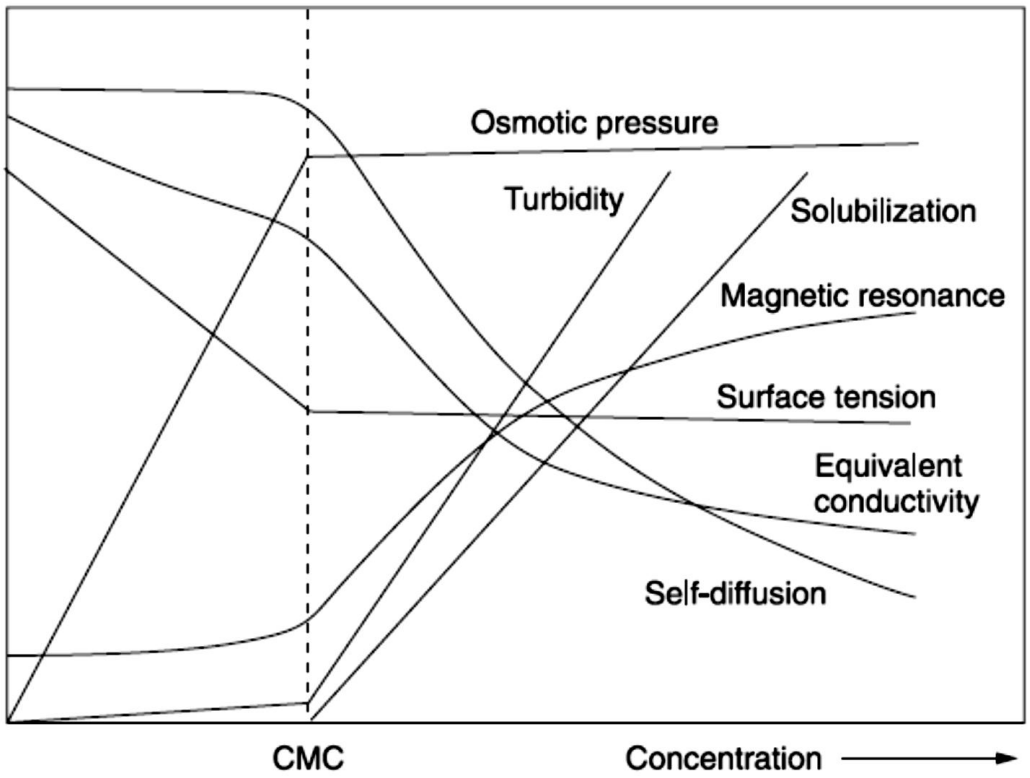



**Figure 1.5:** Several physical properties of surfactant solution abruptly change at CMC.

Broxton and co-workers [108] have advanced a method to determine CMC under the reaction kinetic conditions. In their method, the point of intersection of two linear plots of $k_{obs}$ *vs.* $[Surf.]_T$ drawn just below and above CMC gives the value of CMC. It must be mentioned that the CMC definition is only for normal micelles; for the case of reversed micelles, it is not necessary to have a CMC.

### 1.3.1 Direct (Normal), Reverse and Mixed Micelles

According to nature and direction of head group-tail parts, micelles can be classified into three main types: direct, reverse and mixed micelles (Figure 1.6).

#### *1.3.1.1 Direct Micelles*

Direct micelles are formed in water or in polar media. Their polar heads stretch out and the assembled *hydrophobic* tails form the low-polar 'nano-phase', which can solubilize low-polar molecules and the structure above the CMC can be roughly considered as spherical [109-113]. When the hydrocarbon portion of the *amphiphile* is a *hydrophobic* chain, the micelle will consist of a liquid like hydrocarbon core with radius of nearly equal to the fully extended hydrocarbon chain length (12-30 Å). The polar head groups with the surrounding water are arranged at the rough micellar surface [114]. The fluorescence and $^{1}$H-NMR measurements support the idea proposed by the Menger that water can penetrate inside the micelle up to a certain level [115,116]. Partial molar volume determinations indicate that the alkyl chains in the core are more expanded than those in the normal liquid state [117].

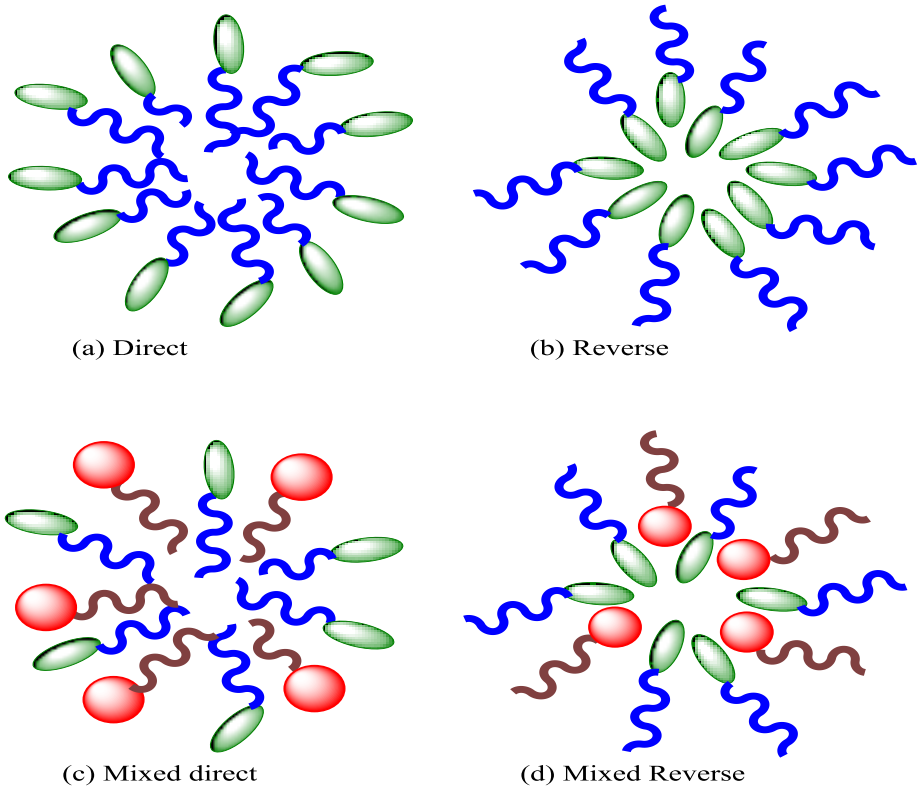



**Figure 1.6:** Schematic representation of types of micelles.

In ionic micelles, the surface properties are high [111,118] and a significant fraction of the counterions (60-90%) [119] are located in a compact region, known as Stern layer, which extends from the core to within a few angstroms of the shear surface of the micelle. The core and the Stern layer form the 'kinetic micelle'. Most of the remaining counterions are, however, located outside the shear surface in the region called 'Gouy–Chapman electrical double–layer' (Figure 1.7). According to Hartley model [109] the overall volume of a micelle is approximately twice that of Stern layer [120,121].

Counterions are bound primarily by the strong electric field created by the head groups but also by specific interactions that depend upon head group and counterion type [122]. A two–site model has been successfully applied to the distributions of counterions; *i.e.*, they are assumed to be either "bound" to the micellar *pseudophase* or "free" in the aqueous phase [107,123,124]. The head group and counterion conicentrations in the interfacial region of an ionic micelle are on the order of 3–5 mol $dm^{-3}$, which gives the micellar surface some of the properties of concentrated salt solutions [125]. Although the solution as a whole is electrically neutral, both the micellar and aqueous *pseudophases* carry a net charge because thermal forces distribute a fraction of the counterions radially into the aqueous phase.

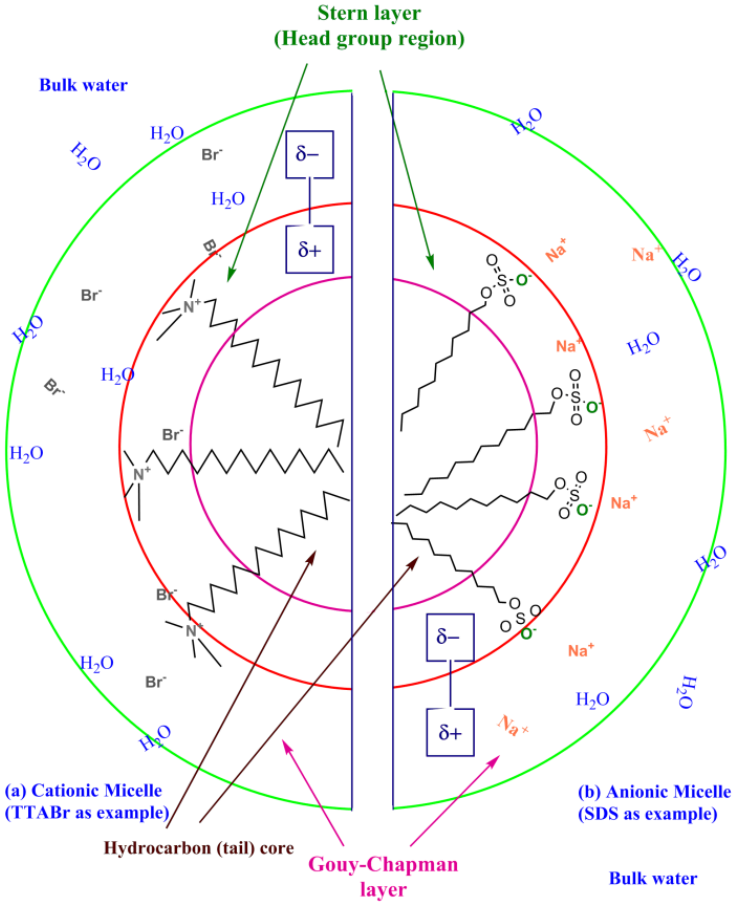



**Figure 1.7:** A two dimensional schematic representation of regions of spherical direct ionic micelle: (a) cationic micelle (*i.e.*, TTABr), (b) anionic micelle (*i.e.*, SDS).

### *1.3.1.2 Reverse Micelles*

On the other hand, if *amphiphiles* dissolved in non-polar solvents in presence of traces of water, it associates to form the so-called reverse, inverted or reverted micelles. The low-polar tails stretch out and polar heads assemble. They can solubilize water and other highly polar molecules.

The head groups of surfactant molecules are located inside to form a polar core while the hydrocarbon tails are directed towards the bulk solvent to form the outside shell of the micelle [126-132]. Dipole-dipole [133,134] interactions hold the *hydrophilic* head groups together in the core. Water in reverse micelles is expected to behave very differently from ordinary water because of extensive binding and orientation effects induced by polar heads forming the water core [135].

Continuously, the field of reverse micelles has witnessed a significant growth of interest, partly due to the finding that proteins, other biopolymers, and even bacterial cell can be solubilized in the reverse micellar system: in fact, this has permitted the extension of area of interest to new domains, *i.e.*, biocatalysis, chemical biotechnology and nanotechnology [136-140].

#### *1.3.1.3 Mixed Micelles*

Mixing of two or more surfactants in solution leads to the formation of mixed micelles. A mixed micelle is an aggregate of surfactant molecules composed of different types of surfactants present in solution. Mixed micellar system is used to improve the properties of the final product and to provide better performance characteristics in their applications than those consisting of only one type of surfactants [141-146].

Mixed micelles may also form when low molecular weight solutes are solubilized by micelles of *amphiphiles* containing a relatively larger non-polar side chain. The solubilized substances, also called as the penetrating additives [147], may be located in both the hydrocarbon core and in the *hydrophilic* mantle [148-150].

From the application point of view, mixed micelles are of fundamental, technological, pharmaceutical and biological considerations [151]. Due to numerous applications of such systems, a lot of attention has been devoted for the understanding of mixing behavior using various techniques such as conductivity, surface tension, viscosity, density, calorimetry, potentiometry, fluorimetry, NMR, scattering techniques, cryo-TEM and cryo-FESEM, *etc*. [152-167].

### 1.3.2 The Importance of CMC and Aggregation Number

CMC values are important indicators when considering which surfactant will provide optimal performance benefits. Surfactant solutions with concentrations above the CMC can dissolve considerably larger quantities of organic materials than can pure water or surfactant solutions at concentrations below the CMC [168]. With knowledge of the surfactant CMC and aggregation number ($N_{agg.}$), one can determine several important parameters including the concentration of micelles present in solution and the aggregate molecular weight of the micelle.

The CMC is also important in determining which method should be used to remove excess or unwanted surfactant. Surfactants may interfere with certain applications and must be removed when reconstituting into liposomes [169,170].

Surfactants with high CMCs are easily removed by dialysis; surfactant solutions can be diluted below their CMC so that micelles disintegrate into monomers which can easily pass through dialysis tubing over time [171].

Micelle aggregation number ($N_{agg}$) which is the number of monomers making up a micelle is a fundamental parameter concerning the micelle. It gives an idea about the size of the micelle and is vital in determining the stability and practical applications of the investigated systems [53,107].

Generally, in aqueous medium greater the dissimilarity between *amphiphile* and solvent, greater the aggregation number. Hence, aggregation number appears to increase with increase in *hydrophobic* character of the *amphiphile*. An increase in the temperature appears to cause a small decrease in the aggregation number in aqueous medium for ionics. For nonionic surfactants, it increases markedly [41,172,173].

### 1.3.3 Factors Affecting the CMC and $N_{agg}$ of Micelles

There are several physico-chemical factors that can affect the CMC of a given surfactant. Generally, the CMC decreases as the *hydrophobicity* of the surfactant

increases. Other properties that directly affect the CMC are the characteristics of the *hydrophobic* and *hydrophilic* groups and solution additives such as electrolytes.

Lowering the CMC usually increase the lifetimes of the micelles as well as the residence times of the surfactant molecules in the micelle [174]. Because of this dynamic character, the size and shape of micelles are subjected to appreciable structural fluctuations. Therefore, micellar aggregates are polydisperse, as is demonstrated by light scattering techniques [175,176]. Most surfactants used for biochemical applications have $N_{agg}$ typically fall between 50 and 100 [177-179] which depends on their hydrocarbon chains in a micelle and these hydrocarbon chains have high mobility as is indicated by NMR, in comparable with a relative liquid alkane [180].

An aggregation number is a description of the number of molecules present in a micelle once the CMC has been reached. Aggregation number is affected by different factors such as concentration of surfactant [143,181,182], temperature [107,183-185], concentration of added electrolyte [181,186-189], organic additives [190-192], *etc*. Various experimental techniques like dynamic light scattering (DLS), small-angle neutron scattering (SANS), steady-state fluorescence quenching (SSFQ), and time-resolved fluorescence quenching (TRFQ), *etc*. may be used for the determination of aggregation number [143,181,193-198].

The factors known to affect the *CMC* in aqueous micellar solution markedly are briefly discussed below.

#### *1.3.3.1 Hydrophilic/Hydrophobic Parts of the Surfactants*

The *hydrophilic* head group variations affect the surfactant CMC. In general, surfactants containing ionic head groups have a higher CMC than those containing nonionic head groups [53]. This is due to electronic repulsion between the head groups of neighboring surfactant monomers within the micelles. Surfactants containing zwitterionic head groups tend to have smaller CMCs than those containing ionic head groups.

If the surfactant has long *hydrophobic* tail (straight or branched chains with saturated or unsaturated bonds) that makes the surfactant more *hydrophobic*, CMC

decreases as the number of the carbon atoms in the *hydrophobic* group increases. In aqueous medium, ionic surfactants have much higher CMCs than non-ionic surfactants containing equivalent groups. Zwitterionic surfactants appear to have about the same CMCs as ionics with the same number of carbon atoms in *hydrophobic* group. The CMC increases as the head group is closer to the two branches of the chain partially shielding one another, interfacial energy effects are smallest. In aqueous medium, the CMCs of ionic surfactants decrease with decrease in the hydrated radius of the counterion.

### *1.3.3.2 Effect of Electrolytes Addition*

Influence of electrolytes on CMC as well as on aggregation behavior of charged surfactants in aqueous solutions are important to many applications in industry. When surfactant and electrolyte are mixed in solution, salting-out phenomenon often happens [85,199-201]. Salting-out is the result of preferential movement of water molecules, which immobilize and quench their role as solvents, from coordination shells of surfactant molecules to those of salts. The counterions decrease the CMC of ionic surfactants, for a particular cationic surfactant, as the counterion is changed according to the series $F^-$, $Cl^-$, $Br^-$, and for a particular anionic surfactant, as the counterion is changed according to the series $Li^+$, $Na^+$, $K^+$, $Cs^+$. Divalent inorganic counterions give a reduction of the CMC by roughly a factor of 4. Addition of high concentrations of salt to ionic surfactants decreases the CMC. Electrolyte has small effect on the CMC of non-ionic surfactants.

Counterions tend to reduce the CMC of surfactant solutions due to a reduction in the thickness of the ionic atmosphere surrounding the polar head groups and a consequent decreased repulsion between head groups of ionic micelles. Addition of electrolytes decreases the repulsion between similarly charged ionic head groups within a micelle and therefore, the surfactant monomers can pack tightly and the CMC is reduced [53]. These effects are manifest as a reduction in CMC and an increase in aggregation number, the effect being more pronounced for anionic and cationic than for zwitterionic surfactants, and more pronounced for zwitterionics than for nonionics. The effect of the concentration of electrolyte on the CMC of ionics is given by the following relation.

$$\log CMC = a \log c_1 + b \quad (1.1)$$

where a and b are constants for a particular ionic group and $c_1$ denotes the total counterion concentration in mole per $dm^3$ [202].

For nonionics and zwitterionics, Eq. (1.1) does not hold. Instead, the effect is given by equation (1.2) [164]

$$\log CMC = -k c_1 + \text{constant} \qquad (c_1 < 1) \quad (1.2)$$

where k is the constant for a particular surfactant, electrolyte and temperature and $c_1$ is concentration of electrolyte in mole per $dm^3$.

The size of counterion is also a determining factor for the CMC value. As the size of counterion increases, counterion binding also increases due to decrease in hydrated radius of ion, and hence decrease in CMC occurs [53].

There have been continuous attempts to examine the salts effect on micelle formation in the light of Hofmeister (*lyotropic*) series [203, 204]. The series plays a remarkable role in biological and physicochemical phenomena. However, depending on the system and type, there may be changes in order in the series.

Addition of inorganic salts leads to reduce electrostatic repulsion among the surfactant head groups that is a key factor to influence the morphology of aggregates in ionic surfactant solutions. For monomeric cationic surfactants, micelles shape may change from spherical to wormlike or rodlike with the addition of inorganic salts. [205, 206].

#### *1.3.3.3 Organic Additives*

The addition of organic molecules affects CMC in a variety of ways. Polar organic molecules with medium chain-length and strong polar organic molecules can decrease the CMC while polar organic molecules with low molecular weight can increase the CMC.

Generally, addition small amounts of the organic materials changes the CMC in aqueous media by two ways, *i.e.*, either by being incorporated into the micelle (type-1) or by modifying the solvent/solvent-surfactant interaction (type-2).

Type-1 is composed of molecules (like alcohols, with moderate to long hydrocarbon chains) that appear to be adsorbed in the outer region of the micelles, forming a palisade (*i.e.*, fence like) structure with *amphiphilic* molecules. This lowers the free energy of micellization to more negative values, so reduces the CMC [207]; such molecules can also influence the micelle shape. Water soluble compounds in type-1 may operate as members of type-1 while, at high bulk phase concentration, as members of type-2. Addition of alcohols produces both increase and decrease in CMC of surfactants [208-210]. Additives like sugar has been known to reduce the CMC of the system [211, 212]. Urea additive have been shown to increase the CMC of ionic [213, 214] and nonionic surfactants [215, 216]. For fluorocarbon surfactants, the CMC slightly decreases when urea is added [217].

Organic salts with an aromatic phenyl group, so-called hydrotropes, have also been studied in ionic surfactant systems [218-223]. In comparison with inorganic salts, most organic salts have additional *hydrophobic* interaction with ionic surfactants in aqueous solutions besides electrostatic interaction [224]. Benzene rings in organic salts may penetrate into micelles, inducing strong *hydrophobic* interaction, reducing electrostatic repulsion between the *hydrophilic* head groups, and finally leading to tight packing and possible reduced curvature of surfactant aggregates [225]. Therefore, wormlike micelles were often observed when organic salts were added to ionic surfactant solutions. [226-232].

#### *1.3.3.4 Organic Solvents*

Small amounts of organic solvent can have a significant influence on the CMC of ionic surfactants due to the tendency of the added organic solvent either to break or make the water structure through solvation of the *hydrophobic* tail of the surfactant by the hydrocarbon (*hydrophobic*) part of the organic solvent [233,234].

Knowledge of the effects of organic solvents on the CMC of surfactants is therefore of great importance for both the theoretical and practical purposes for example nonpolar medium offers environment similar to the surfactant tail so that the tendency of self-association is reduced and this environment is favored for inverted micelles. So, micellization can be understood in terms of *hydrophobic* effect, which is the main driving force behind the formation of micelles in solution [233,235]. In nonaqueous solvents, the term "*solvophobic* interaction" has been coined, in analogy with "*hydrophobic* interactions" which causes micellization in aqueous medium [236,237]. The micelles formed due to "*solvophobic* interactions" are similar in many respects to the micelles that are formed in aqueous medium, although in general, micelle formation is not as favored in nonaqueous solvents (of low dielectric constants) as in water for a given surfactant [236,238,239].

*Hydrophobicity* of the solvent media (like as the *hydrophobicity* of the surfactant molecule) is of importance in understanding the process of micelle formation [240-242]. A perusal of the literature reveals that formation of micelles has been observed in solvents having high degree of hydrogen bonding such as hydrazine, glycol, formamide, *N*,*N*-dimethyl acetamide, *etc*. [235,243-245]. In addition to the criterion of the solvent's ability to form hydrogen bond, changes in the polarity or *hydrophobicity* of the solvent media are also expected to play a critical role in determining the micellar behavior of ionic surfactants [244].

#### *1.3.3.5 Effect of pH*

In case of surfactant molecules having ionizable groups such as –COOH, –$(CH_3)_2N\rightarrow O$ and –$NH_2$, the degree of dissociation of the polar group will be dependent on *p*H [246]. In general, the CMC will be high at *p*H values where the group is charged (high *p*H for –COOH and low *p*H for – $(CH_3)_2N\rightarrow O$ and –$NH_2$) and low when uncharged. Ataci et al. [247] found that the CMC of cationic surfactant was *p*H-independent in alkaline (between 6 and 10), but it was *p*H-dependent in acidic (below 5). Some zwitterionic surfactants become cationic at low *p*H, a change that can be accompanied by a rapid rise in the CMC [248], or a more modest rise [249] depending on the structure and hence hydrophilicity of the zwitterionic form.

#### *1.3.3.6 Temperature*

An increase in temperature can have varying effect on the micellization of different surfactants. In the case of ionic surfactants, the influence of CMC is usually weak by temperature reflecting subtle changes in bonding, heat capacity and volume that accompany the transition. However, the CMC of non-ionic surfactants decrease with increase in temperature [250].

The CMC value at a particular temperature is affected by two different ways: (i) dehydration of *hydrophilic* group (ii) disruption of structured water around the *hydrophobic* group. Dehydration of *hydrophilic* part favors the micellization while disruption of structured water around the *hydrophobic* part disfavors the micellization. The relative magnitude of these two opposing effects, therefore, determines whether the CMC increases or decreases over a particular temperature range.

#### *1.3.3.7 Pressure*

Several reports have appeared on the effect of pressure on micelle formation for ionic and nonionic surfactants [251, 252]. With pressure the CMC of ionic surfactants increases upto 100MPa followed by a decrease above this pressure due to an increase in the dielectric constant of water, solidification of the micellar interior [253], making less electrical work necessary to bring a monomer into a micelle.

Just as in the case of temperature, data for the variation of CMC for surfactants with pressure also fall on a reduced curve, which passes through a maximum CMC at pressure *p*. For nonionic surfactants, the CMC values increase monotonously and then level off with increasing pressure.

### 1.3.4 Micelle Geometry and Micellar Dynamic Aggregates

As is well known, the shapes of the micelles produced in aqueous media are of importance in determining various properties of the surfactant solution, such as its viscosity, its capacity to solubilize water-insoluble materials, and its cloud point and the shape of micelles depends strongly upon the actual packing parameters in the micellar assembly [254,255]. Packing considerations constitute a factor which involves the nature

of the head group/tail of the surfactant. Ninham and coworkers [225,254] have devised a critical ratio ($R_p$) (called packing parameter) with associated limits for possible aggregation shapes given as

$$R_p = v_h/a_o l_c \quad (1.3)$$

where $v_h$ is the volume of the *amphiphile's* hydrocarbon tail, $a_o$ is the cross-sectional area per surfactant molecule, and $l_c$ is the length of the fully extended hydrocarbon tail. $a_o$ is determined experimentally by X-ray diffraction (XRD) of bilayer systems, while $v_h$ and $l_c$ can be calculated using Tanford equations [256]. The hydrocarbon chain of $n_c$ carbon atoms can be approximated by correlations of experimental data as:

$$v_h = 27.4 + 26.9\, n_c\ (Å^3) \quad (1.4)$$

$$l_c = 1.54 + 1.265\, n_c\ (Å) \quad (1.5)$$

As it shown in Table 1.1, spherical micelles are formed when $R_p$ is in between 0-1/3; wormlike micelles with cylindrical structures are formed when $R_p$ has a value in between 1/3 to 1/2; vesicles or bilayers are formed when $1/2 < R_p < 1$ and inverted micelles are formed when the volume of the hydrocarbon part is large relative to the head group area.

Micelles are extremely dynamic aggregates. Rates of uptake of monomers into micellar aggregates are close to diffusion controlled [257]. The residence times of the individual surfactant molecules in the aggregate are typically in the order of $10^{-5}$ - $10^{-6}$ seconds [258,259], whereas the lifetime of the micellar entity is about $10^{-1}$ - $10^{-3}$ seconds [260]. However, it is to be noted that the solution parameters such as concentration, *p*H, temperature and solvent polarity may heavily modify the specific structures formed.

### 1.3.5 Thermodynamics of Micellization

Thermodynamics is a science, and more importantly an engineering tool, that is necessary for describing the processes that involve changes in temperature, transformation of energy, and relationships between heat and work.

Since *amphiphilic* self-assembly involves structures of finite yet large size (micelles containing tens to hundreds of molecules), the prevalent model of micellization has been that of Israelachvili, Mitchell, and Ninham [183,225], in which the CMC and aggregate shape and size are derived from thermodynamic analysis and simple geometrical arguments related to molecular packing.

Thermodynamic parameters of micelle formation have been calculated in a number of ways. Equation (1.6) has been used in conductivity experiments for calculation of the standard free energy, $\Delta G^0_m$,

$$\Delta G^0_m = RT \ln X_{CMC} \tag{1.6}$$

$X_{CMC}$ is the CMC expressed as a mole fraction, therefore,

$$X_{CMC} = n_s/(n_s+n_{H_2O}) \tag{1.7}$$

Since the number of moles of free surfactant, $n_s$, is small compared to number of moles of water, $n_{H2O}$, therefore, Eq. (1.7) can be written as

$$X_{cmc} = n_s/n_{H_2O} \tag{1.8}$$

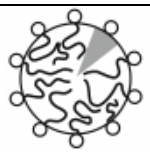
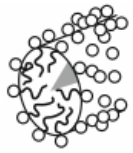
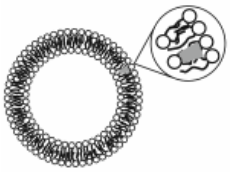
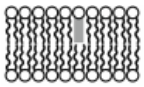
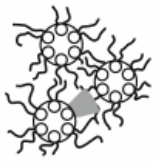
Substituting the value of Eq. (1.8) into the Eq. (1.6) and applying logarithm we get

$$\Delta G^0_m = -2.303RT\,(\log CMC - \log w) \tag{1.9}$$

The second terms of Eqs. (1.7) and (1.8) are also dropped from the right hand side. A relatively small error in the calculated thermodynamics quantities is introduced by this approximation. The $\Delta H^0_m$ of micelle formation can also be determined by calorimetry, and it is of interest to compare enthalpy changes determined by the two approaches. Finally, the entropy of micelle formation, $\Delta S^0_m$, is most often obtained from the equation

$$\Delta G^0_m = \Delta H^0_m - T\,\Delta S^0_m \tag{1.10}$$

**Table 1.1:** Illustration of packing parameters on micelles geometry structure.

| Surfactant | Critical packing parameter | Critical packing shape | Structures formed |
|---|---|---|---|
| Single- chained with large head group areas: SDS in low salt | < 1/3 |  Cone |  Spherical micelles |
| Single- chained with small head group areas :SDS and CTAB in high salt and nonionic | 1/3-1/2 |  Truncated cone (Wedge) |  Cylindrical rod-like micelles (globular micelles) |
| Double-chained with large head group areas: dihexadecyl phosphate | 1/2-1 |  Truncated cone |  Flexible bilayer micelles |
| Double-chained with small head group areas: anionic in high salt | ≈ 1 |  Cylinder |  Planer bilayer |
| Double-chained with small head group areas: nonionics | >1 |  Inverted truncated cone |  Inverted micelles |

**1.4 Kinetics in Micellar Systems**

Chemists have long recognized the important role the reaction media plays in controlling rates, products distribution and stereochemistry. Recently, much effort has been directed toward the use of micellar systems to modify reactivity, as compared to that in isotropic liquids. A major goal of such studies is to utilize the order of the medium so as to increase the rate and selectivity of the chemical process involved in much the same way that enzymes modify the reactivity of the substrates to which they are bound. Among the many ordered or constrained systems utilized to organize the reactants, the notable ones are micelles, microemulsions, liquid crystals, inclusion complexes, monolayers and solid phases such as adsorbed surface and crystals. Judicious selection of a given organized assembly for a given application requires a sufficient understanding and properties of the organized assembly themselves and those of the substrate interactions therein.

Charged colloidal assemblies such as micelles are believed to mimic the biological system [119]. It possesses structural similarities between globular proteins and spherical micelles and analogies between the catalytic effects of enzymes and functional micelles between catalysis and phase transfer catalysis and as a result numerous researchers [261-269] have directed their attention towards the reactions occurring in micellar systems. It is the micelle, rather than individual surfactant molecules, which are responsible for altering the rates of reactions in solutions of surfactants.

The kinetic data on the rates of micellar-mediated reactions, obtained until the mid-1960s, have been explained only qualitatively because of the lack of an acceptable kinetic micellar model based on logical and convincing mechanisms of micellar-mediated reactions.

The term “micellar catalysis” was first applied to the increases in rates of reactions produced by aqueous association colloids, in particular micelles [270]. Surfactant micelles can enhance the sensitivity and can bring about changes in chemical equilibria, reaction rates and mechanisms, $pK_a$, solubility, spectral distributions and

intensities and the stereoselectivity of some chemical processes. Surfactants increase the absorptivity of the analytes and some of them also facilitate solubilization of the analytical system [271,272].

Rates of chemical reactions ($R_\psi$) in micellar solutions are usually considered to be the sum of rates in the continuous aqueous phase ($R_w$) and the micellar *pseudophase* ($R_m$) [103,119].

$$R_\psi = R_w + R_m \qquad (1.11)$$

Micellar solutions are macroscopically homogeneous, but the total volumes of the uniformly distributed dynamic aggregates of surfactant monomers is assumed to act as a separate phase, the micellar *pseudophase*, of constant properties [106,273]. *Pseudophase* formation begins at the CMC, and all additional surfactant forms micelles with the monomer concentration remaining constant and equal to CMC.

To diagnose the reaction mechanism, kinetic method is the most important method/technique. By the late 1960s, the accumulated kinetic data on micellar-mediated reactions were enough to warrant a logical kinetic micellar model to explain these kinetic data quantitatively. The kinetic studies of reaction rates provide perhaps the most extensive fine details of changes at the molecular level of chemical reactions.

The micellar kinetic models developed so far for apparent quantitative explanations of the effects of micelles on reaction rates. The father of micellar kinetics (Bunton) has observed [274]: "The development of a quantitative understanding of chemical reactivity in solution has depended on the willingness chemists to use models that are no more than crude approximation. For this reason, it is useful to accept the *pseudophase* model, despite its limitation, until it either fails to fit the data, or is replaced by a better model".

The *pseudophase* model (PP) considers micellar solutions as two-phase systems, composed of a bulk phase (usually water) and a microphase (*pseudophase*). It treats the reaction kinetics in these two phases as if they were two separate homogeneous solutions with particular concentrations of dissolved species.

A micelle-bound substrate will experience a reaction environment different from bulk water, leading to a kinetic medium effect. Hence, micelles are able to catalyse or inhibit organic reactions. Research on micellar catalysis has focused on the kinetics of the organic reactions involved.

An aqueous solution of surfactant at $[Surf]_T$ less and greater than CMC remains transparent to UV-Visible radiation and, consequently, it is defined as a single homogeneous phase. Thus, by a simple definition of a real phase, micelles cannot be considered to constitute a real phase and, for this technical reason, micelles are said to represent a *pseudophase* (PP). The word *pseudophase* of micelle is probably the most appropriate term, because various kinds of experimental data show that micelles are surfactant molecular aggregates with aggregation numbers varying from <100 to >100 depending on the nature and concentration of micelle forming surfactant and additives as well as temperature of aqueous surfactant solutions. Menger and Portnoy proposed [262] the concept of micellar phase, which does not seem to fit well within the domain of a formal definition of the real phase. A number of influential researchers in this field suggested the concept of the PP rather than real phase of micelles. The PP model of micelles, considers the following assumptions [275]:

I. Substrate does not give complexation with monomers.

II. Bulk aqueous and micelles solvents are regarded as separate reaction regions.

III. There is a 1:1 stoichiometry associated between substrate and micelles.

IV. Micellization does not perturb by substrate.

V. At the CMC, micellization starts to occur.

VI. The relationship $[S_n] = \{[Surf]_T - CMC\}/n$ is valid where $[S_n]$ is the concentration of micellized surfactant.

VII. Micelles shape and size does not perturb micellar effects on reaction rates and equilibria.

VIII. There is no cross-interaction between equilibrium constants of micellar incorporation of different solubilizates.

IX. The equilibrium constant $K_M$ for the formation of micelles is independent of equilibrium constants $K_A$ for micellar solubilization of different solubilizates, and rate constants $k_M$ for micellar-mediated reactions (Scheme1.1).

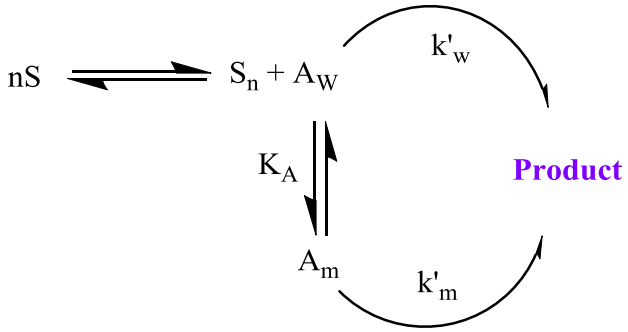
$$\{n\text{-}N/N_A\}\ \text{monomers} \underset{}{\overset{K_M}{\rightleftharpoons}} (N/rN_A)\ \text{micelles}$$

**Scheme1.1:** Micellization equilibrium course

where n represents total number of surfactant molecules, N is the total number of surfactant molecules used up in the formation of number of micelles (N/r), r the mean aggregation number of a micelle, and $N_A$ is Avogadro's number.

X. For a bimolecular reaction, the reaction between a substrate ($A_m$) in the micellar *pseudophase* and the other substrate ($A_w$) in the aqueous *pseudophase* does not occur.

Micelle-catalyzed reactions can be treated in a manner analogous to that used for enzymatic catalysis:




**Scheme1.2:** A *pseudophase* model for a unimolecular reaction

where $S_n$ is the micellized surfactant, A is the substrate, and $k'_w$ and $k'_m$ are the first-order rate constants for product formation in the bulk solvent and in the micellar phase, respectively.

The rate equation for the Scheme 1.2 is given by

$$\frac{-d([A_w] + [A_m])}{dt} = \frac{-d[A]_t}{dt} = \frac{d[P]}{dt} \qquad (1.12)$$

and

$$\frac{d[P]}{dt} = k'_w[A_w] + k'_m[A_m] \qquad (1.13)$$

where $[A]_t$ is the stoichiometric concentration of the substrate at time t. (Here, and elsewhere, the quantities in square brackets denote molarity in terms of total solution volume, which is approximately that of the aqueous *pseudophase*). The observed rate constant for the product formation, $k_\psi$, is given by:

$$k_\psi = \frac{-d[A]_t}{dt} \Big/ [A]_t = k'_w F_w + k'_m F_m \qquad (1.14)$$

where $F_w$ and $F_m$ are the fractions of the uncomplexed and complexed substrate. Often, for a *pseudo*-first-order process $[S_n] >> [A_m]$ and $F_m$ is constant. The equilibrium constant, $K_A$, can be expressed in terms of concentrations and also in terms of the fractions of the complexed and uncomplexed substrate:

$$K_A = \frac{[A_m]}{([A]_t - [A_m])\,[S_n]} = \frac{F_m}{[S_n]\,(1 - F_m)} \qquad (1.15)$$

Combination of equations (1.14) and (1.15) and rearrangement leads to:

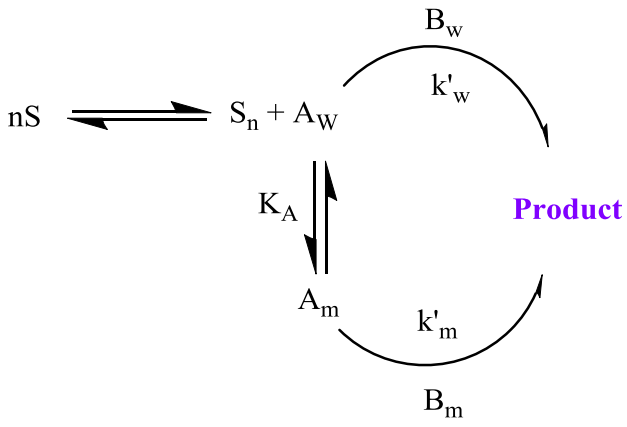
$$k_{\psi} = \frac{k'_{w} + k'_{m} K_A[S_n]}{1 + K_A[S_n]} \qquad (1.16)$$

Equation (1.16) is similar in form to the Michaelis-Menten equation of enzyme kinetics [276] and successfully fits the sigmoidal rate–surfactant profiles of micellar–catalyzed unimolecular reactions; *i.e.*, $k_{\psi}$ increases initially and then plateaus once all the substrate is bound. Rearrangement of equation (1.16) to the linear double reciprocal form of equation (1.17), similar to the Lineweaver-Burk equation [277], allows both $K_A$ and $k'_m$ to be estimated from the kinetic data. Rate enhancements of 3-700 fold are observed for a number of spontaneous hydrolyses and decarboxylations [278, 279]. Values of $K_A$ cannot be measured independently for these substrates because they decompose spontaneously, but the kinetically determined values are reasonable.

$$\frac{1}{(k'_{w} - k_{\psi})} = \frac{1}{(k'_{w} - k'_{m})} + \frac{1}{(k'_{w} - k'_{m}) K_A[S_n]} \qquad (1.17)$$

The simple distribution model applied to unimolecular reactions fails for higher order reactions. Bimolecular reactions, for example, show the same increase in observed rate above the CMC, but with increasing surfactant concentration the rate passes through a maximum and then gradually decreases instead of remaining constant [280]. The results for the addition of $CN^-$ to *N*-alkyl-3-carbamoylpyridinium ions are typical [281]. This consistent pattern, except for certain predictable limiting cases [282], was surprising at first because experimental conditions were selected to mimic those of enzyme catalyzed reactions [265,266]. The concentration of the second reactant was either buffered, if $H^+$ or $OH^-$, or in large excess over the substrate, with salt added to control ionic strength. The observed rate was expected to plateau once all the substrate was bound. However, unlike enzyme kinetics experiments, the surfactant concentration in micellar catalyzed reactions is usually in large excess over both reactants. This difference is crucial because, unlike enzymes, increasing the micelle concentration can significantly alter the concentrations of both reactants in both *pseudophases*.

The maxima in rate–surfactant profiles are produced by two opposing effects. Binding of reactants begins at the CMC, and transfers them into the small volume of the micellar *pseudophase*. If the binding with substrate and *nucleophile* (it is necessary to consider the transfer of the second reactant, *e.g.*, a *nucleophile*, B, between the two *pseudophases*) are large, the reactants' concentration within the micellar *pseudophase* in moles per $dm^3$ of micellar volume can be 100-1000 times greater than their stoichiometric concentrations. The concentration effect is opposed by continuous dilution of the reactants within the micellar *pseudophase* with increasing surfactant concentration. Thus, the shape of rate–surfactant profiles is primarily a phase transfer phenomena, but the extent of the change depends on the size of the binding and the difference in rate constants for reaction in the micellar and aqueous *pseudophases*.




**Scheme 1.3:** A *pseudophase* model for a bimolecular reaction

Scheme 1.3 shows reaction between the substrate, A, and *nucleophile*, B (or any second reactant). The second reactant is generally in large excess over the substrate establishing *pseudo*-first-order conditions, so that:

$$k'_w = k_w [B_w] \qquad (1.18)$$

and

$$k'_m = k_m M_B^S \qquad (1.19)$$

where $k_w$ and $k_m$ are second-order rate constants for reaction in aqueous and micellar *pseudophases*, respectively, and the mole ratio $M_B^S = [B_m]/S_n$. Substitution of equations (1.18) and (1.19) into equation (1.16) gives:

$$k_\psi = \frac{k_w[B_w] + k_m K_A M_B^S [S_n]}{1 + K_A[S_n]} \qquad (1.20)$$

$$= \frac{k_w[B_w] + k_m K_A [B_m]}{1 + K_A[S_n]} \qquad (1.21)$$

Equations (1.20) or (1.21), essentially identical but written in different ways, can be applied to bimolecular micelle-assisted reactions provided that the distribution of both reactants can be determined.

Estimation of the extent of micellar binding can be done if the organic ion is very *hydrophobic*, because then it is completely micellar bound under essentially all conditions [283]. Perhaps for this reason, there are many examples of good fits between experimental rate constant–surfactant profiles and those calculated using equations (1.20), (1.21) or equivalent expressions.

The final form of the kinetic equation (1.20) will depend upon the properties of the second reactant: whether it is a neutral molecule, a hydrophilic or hydrophobic coion, a counterion to the micelle, or in complex systems, an anion of a weak organic acid XH.

## 1.5 Statement of the Problem

Micellar catalysis has received considerable attention in view of analogies to enzyme catalyzed reactions. A considerable amount of research work has been directed towards determining the physicochemical properties of micelles. The interest in elucidating the physicochemical properties of micelles can be understood by mainly three reasons. First, one can consistently and easily prepare aqueous micellar solutions which have aggregates of colloidal dimensions with characteristic shape, size and, more

importantly, have *hydrophobic* core and polar surfaces. Therefore, these systems have been employed as models for enzyme action in investigations. Second, the similarities between surfactant aggregates and biological lipid membranes have not gone unnoticed. Thus, in many studies micelle-like aggregates have served as rudimentary model systems for biological lipid membrane systems. Also, it has been found that micelles can act as unique reaction media and can affect rate of reactions due to several factors; by differential distribution of the substrates inside and outside the micelles and by perturbing the thermodynamic parameters of the reaction [264,284-286].

In last two decades, extensive attentions in research have been paid to the solution behavior of *geminis* [74-78,287]. In comparison to conventional single-tail/single head surfactants, *gemini* surfactants have many unique properties [288,289], such as significant low CMC (one to two orders of magnitude lower), higher efficiency in decreasing the surface tension of water, unfamiliar aggregation morphologies, much higher surface activity, better wetting, solubilizing, emulsifying, foaming, solid dispersion enhancing, and biological importance.

No doubt, a number of improvements have been introduced to increase stability of the so-called *ninhydrin reaction* [11,290], the problem related with the kinetics and mechanism under various conditions remains poorly explored. Many investigators modified the ninhydrin reagent by impairing it with metal ions and reported different colored products. It is also known that enzyme and metal ions show marginal improvement for older fingerprints [16,17]. Thus, it follows that a study of the condensation reactions of the metal ion-dipeptide complexes with ninhydrin in the presence of micelles may be a better model than studies in water from which to draw conclusions concerning the mechanism of the development of latent fingerprints by ninhydrin.

The effect of solvents on chemical reactions was firstly reported in 1862 [291-293] while the first theory to explain solvent effect on reaction rates was proposed by Hughes and Ingold in 1935 [294]. It has been suggested that any change in solvent from a

polar solvent to a nonpolar solvent leads to increased or decreased reaction rates depending on the type of reactions [295].

The present study was, therefore, targeted at exploring the influence of micelles upon the reactions of dipeptide/metal ion-coordinated dipeptide complexes with ninhydrin and to check whether micelles change the aqueous reaction mechanism. In micellar systems, organic solvents can affect the reaction rate constant $k_\psi$ which was further investigated in this work.

### 1.6 Lay-out of the Thesis

The work described in the thesis deals with systematic kinetic studies of the reactions of ninhydrin with [Gly-L-Ala], [Gly-DL-Asp], $[Hg(II)\text{-Gly-L-Ala}]^+$, $[Cu(II)\text{-Gly-Dl-Asp}]^+$, $[Cu(II)\text{-Gly-L-Ala}]^+$, and $[Hg(II)\text{-Gly-DL-Asp}]^+$. The whole thesis consists of the following chapters:

Chapter I: **General Introduction** includes starting attempts and up-to-date literature survey related to the topic.

Chapter II **Experimental** includes methodologies which are utilized, the list of chemicals used, their formulae, make and % purity and related Figures and Tables.

Chapter III: **Kinetics of the Dipeptide–Ninhydrin Reactions in Aqueous and Micellar Systems and Effect of Organic Solvents** describes the study of Gly-L-Ala–ninhydrin and Gly-DL-Asp–ninhydrin reactions in absence and presence of TTABr/14-s-14 surfactants in aqueous and aqueous-organic solvent systems.

Chapter IV: **Kinetics of the Metal Ion-Coordinated Dipeptide–Ninhydrin Reactions in Aqueous and Micellar Systems and Effect of Organic Solvents** devotes to study [Hg(II)-Gly-L-Ala]$^{+}$–ninhydrin, [Hg(II)-Gly-DL-Asp]$^{+}$–ninhydrin, [Cu(II)-Gly-L-Ala]$^{+}$–ninhydrin and [Cu(II)-Gly-DL-Asp]$^{+}$–ninhydrin reactions in absence and presence of TTABr/14-s-14 surfactants in aqueous and aqueous-organic solvent systems.

## 1.7 References

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# CHAPTER-II

# EXPERIMENTAL

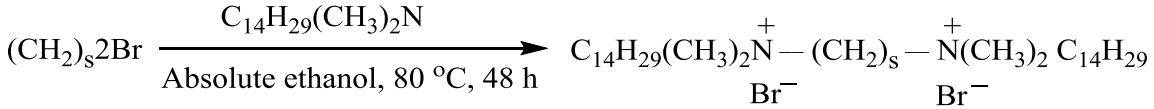
## 2.1 Materials

The surfactants (both conventional and *geminis* (14-s-14, s = 4, 5, 6)), reagents used for synthesis, reactants, salts, organic solvents and all other chemicals used throughout the present study are mentioned in the Table 2.1, which also includes their abbreviation, formula, make and purity. The *gemini* surfactants were synthesized in the laboratory using the procedure given below.

### 2.1.1 Synthesis of Gemini Surfactants

There are two main factors, which are important in their preparation: one is synthesis and the other is purification. Simple cationic *geminis* of tetradecyl series with methylene spacers were prepared as shown in protocol (Scheme 2.1). This method is attractive and is preferable only for $s \geq 3$ [1]. To synthesize a required *gemini*, a 2.1:1 equivalent mixture of corresponding *N*,*N*-dimethyltetradecylamine with α,ω-dibromoalkane (s = 4, 5, 6) in absolute ethanol was refluxed at 80 $^{o}$C for 48 h to ensure as much as possible a complete biquaternization. The progress of the reaction was monitored using TLC technique. At the end of the reaction, the solvent was removed under vacuum and the solid thus obtained was washed/recrystallized more than three times with hexane/ethyl acetate to obtain the *geminis* in pure form [1,2]. The overall yield of the surfactants was ~ 80%. Purity of yielded *geminis* was ascertained on the basis of $^{1}$H NMR and C, H, N data.

Spectral data for the *gemini* surfactants is given in Table 2.2 and Figures 2.1-2.3.




**Scheme 2.1**: Protocol for the synthesis of 14-s-14 surfactants (s = 4, 5, 6)

**Table 2.1:** Names and structural formulas of the chemicals used.

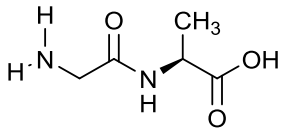
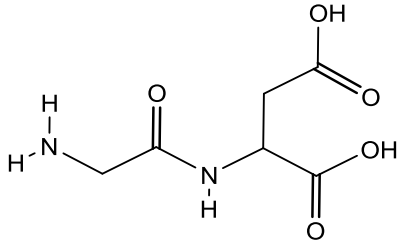
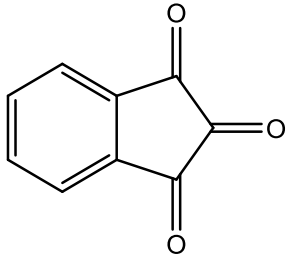
| Name | Abbreviation | Formula | Make | % Purity |
|---|---|---|---|---|
| ***Reagents used for synthesis*** | | | | |
| *N*,*N*-Dimethyltetradecylamine | —— | $C_{16}H_{35}N$ | Fluka (USA) | ≥95.0 |
| 1, 6-Dibromohexane | —— | $C_6H_{12}Br_2$ | Fluka (France) | ≥97.0 |
| 1, 5-Dibromopentane | —— | $C_5H_{10}Br_2$ | Fluka (India) | ≥98.0 |
| 1, 4-Dibromobutane | —— | $C_4H_8Br_2$ | Aldrich (China) | >99.0 |
| Ethanol (absolute) | EtOH | $C_2H_5OH$ | Merck (Germany) | 99.8 |
| Ethyl acetate | EtOAc | $C_4H_8O_2$ | Merck (India) | 99.0 |
| Hexane (for HPLC and spectroscopy) | —— | $C_6H_{14}$ | Merck (India) | 95.0 |

*contd…*

| | | | | |
|---|---|---|---|---|
| ***Conventional surfactants*** | | | | |
| Tetradecyltrimethylammonium bromide | TTABr | $C_{17}H_{38}N\ Br$ | Sigma (India) | ≥99 |
| ***Gemini surfactants*** | | | | |
| Tetramethylene-1,4-bis (dimethyltetradecylammonium bromide) | 14-4-14 | $C_{36}H_{78}N_2Br_2$ | Self synthesized | —— |
| Pentamethylene-1,5-bis (dimethyltetradecylammonium bromide) | 14-5-14 | $C_{37}H_{80}N_2Br_2$ | Self synthesized | —— |
| Hexamethylene-1,6-bis (dimethyltetradecylammonium bromide) | 14-6-14 | $C_{38}H_{82}N_2Br_2$ | Self synthesized | —— |

*contd…*

***Reactants***

| | | | | |
|---|---|---|---|---|
| Glycyl-L-Alanine | Gly-L-Ala |  | Aldrich (Switzerland) | ≥99 |
| Glycyl-DL-Aspartic acid | Gly-DL-Asp |  | Sigma (Switzerland) | ≥99 |
| Ninhydrin | Nin |  | Merck (India) | 99 |

*contd…*

***Organic solvents***

| | | | | |
|---|---|---|---|---|
| Acetonitrile (pure) | AN | $C_2H_3N$ | Merck (India) | ≥99.0 |
| Dimethyl sulfoxide (for synthesis) | DMSO | $C_2H_6OS$ | Merck (India) | ≥99.0 |
| 1,4-Dioxane (extra pure) | DO | $C_4H_8O_2$ | Merck (India) | ≥99.0 |

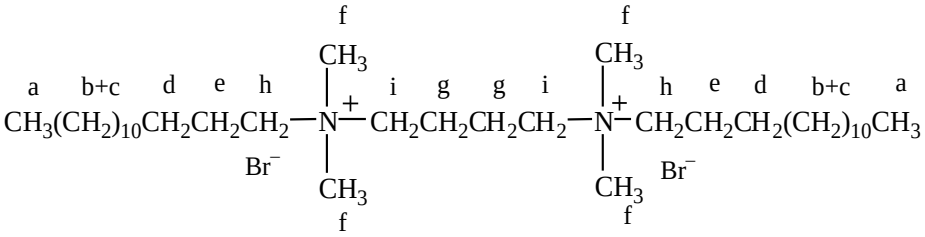
***Salts***

| | | | | |
|---|---|---|---|---|
| Sodium acetate anhydrous (pure) | NaAc | $CH_3COONa$ | Merck (India) | ≥99.0 |
| Mercuric nitrate (extra pure) (Mercury(II) nitrate) | —— | $Hg(NO_3)_2•H_2O$ | s.d.fine (India) | ≥ 58.0 (as Hg) |
| Copper(II) sulfate pentahydrate (pure) | —— | $CuSO_5•5H_2O$ | Merck (India) | ≥99.0 |
| Potassium permanganate (purified) | —— | $KMnO_4$ | E. Merck (India) | 98.5 |
| Potassium dichromate (pure) | —— | $K_2Cr_2O_7$ | Himedia (India) | >99.5 |

*contd…*

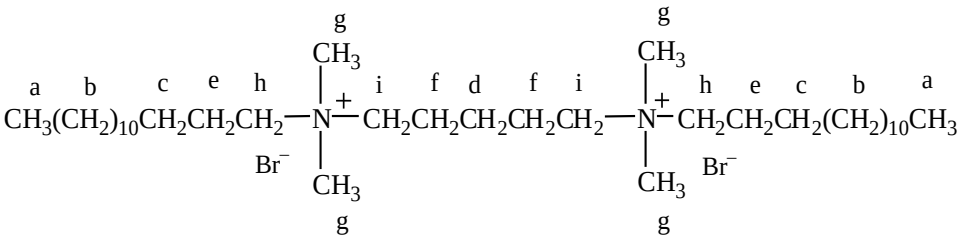
| | | | | |
|---|---|---|---|---|
| Ammonium iron(III) sulfate | —— | $NH_4Fe(SO_4)_2 \cdot 12H_2O$ | Sigma (Germany) | 99 |
| Ammonium thiocyanate | —— | $NH_4SCN$ | Merck (Germany) | 98 |
| ***Acids*** | | | | |
| Acetic acid glacial (for synthesis) | HA | $CH_3COOH$ | Merck (India) | 99-100 |
| Sulfuric acid | —— | $H_2SO_4$ | Rankem (India) | 97.0 |
| Nitric acid | —— | $HNO_3$ | Rankem (India) | >98.0 |
| ***Bases*** | | | | |
| Sodium hydroxide (pellets) | —— | NaOH | Fisher (India) | >97.0 |
| Calcium hydroxide | —— | $Ca(OH)_2$ | Sara bhai (India) | >95.0 |

**Table 2.2:** Spectral data of the synthesized gemini surfactants.

| Compound | Structure | $^1$H NMR δ (ppm) (Solvent $CDCl_3$) | | No. of protons |
|---|---|---|---|---|
| | | Assignment | δ (ppm) | |
| 14-4-14 |  | a | 0.864-0.878 | 6 |
| | | b+c | 1.255-1.351 | 40 |
| | | d | 1.752 | 4 |
| | | | 2.068 | 4 |
| | | f | 3.311 | 12 |
| | | g | 3.419-3.461 | 4 |
| | | h | 3.613 | 4 |
| | | i | 3.789 | 4 |

*contd…*

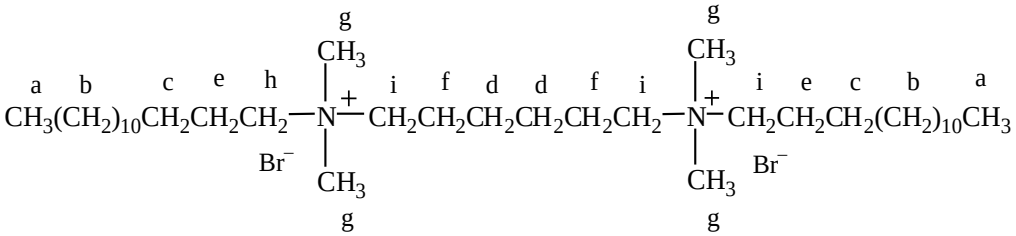
14-5-14




| | | |
|---|---|---|
| a | 0.863-0.897 | 6 |
| b | 1.355-2.556 | 40 |
| c | 1.582-1.615 | 4 |
| d | 1.733 | 2 |
| e | 2.037-2.074 | 4 |
| f | 2.953 | 4 |
| g | 3.387 | 12 |
| h | 3.512-3.554 | 4 |
| i | 3.813-3.853 | 4 |

*contd…*

14-6-14




| | | |
|---|---|---|
| a | 0.863-0.897 | 6 |
| b+c | 1.254-1.353 | 44 |
| d | 1.557 | 4 |
| e | 1.724 | 4 |
| f | 1.973 | 4 |
| g | 2.844 | 12 |
| h | 3.396 | 4 |
| i | 3.509-3.711 | 4 |

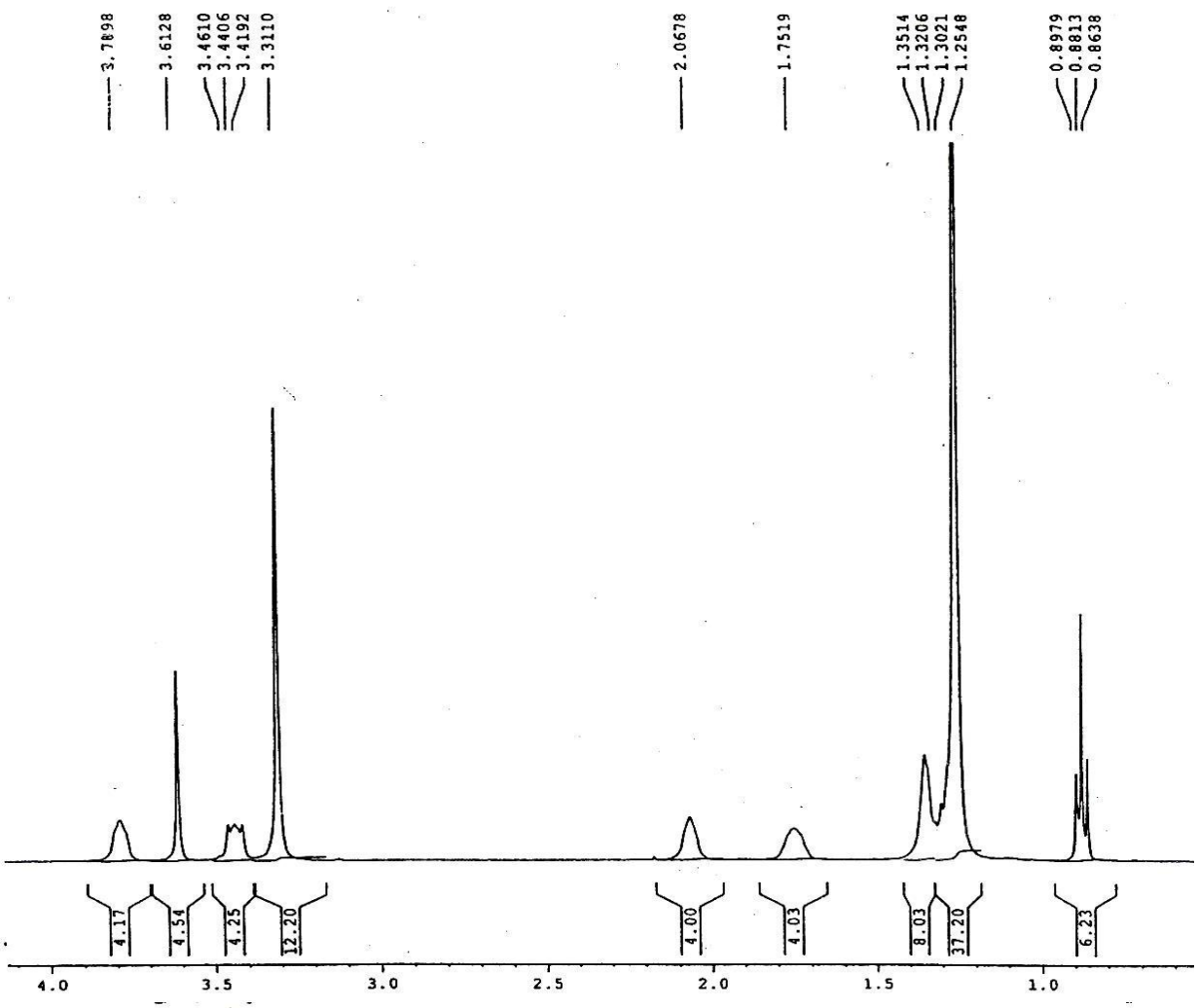



**Figure 2.1:** $^{1}H$ NMR spectrum of 14-4-14 in $CDCl_3$.

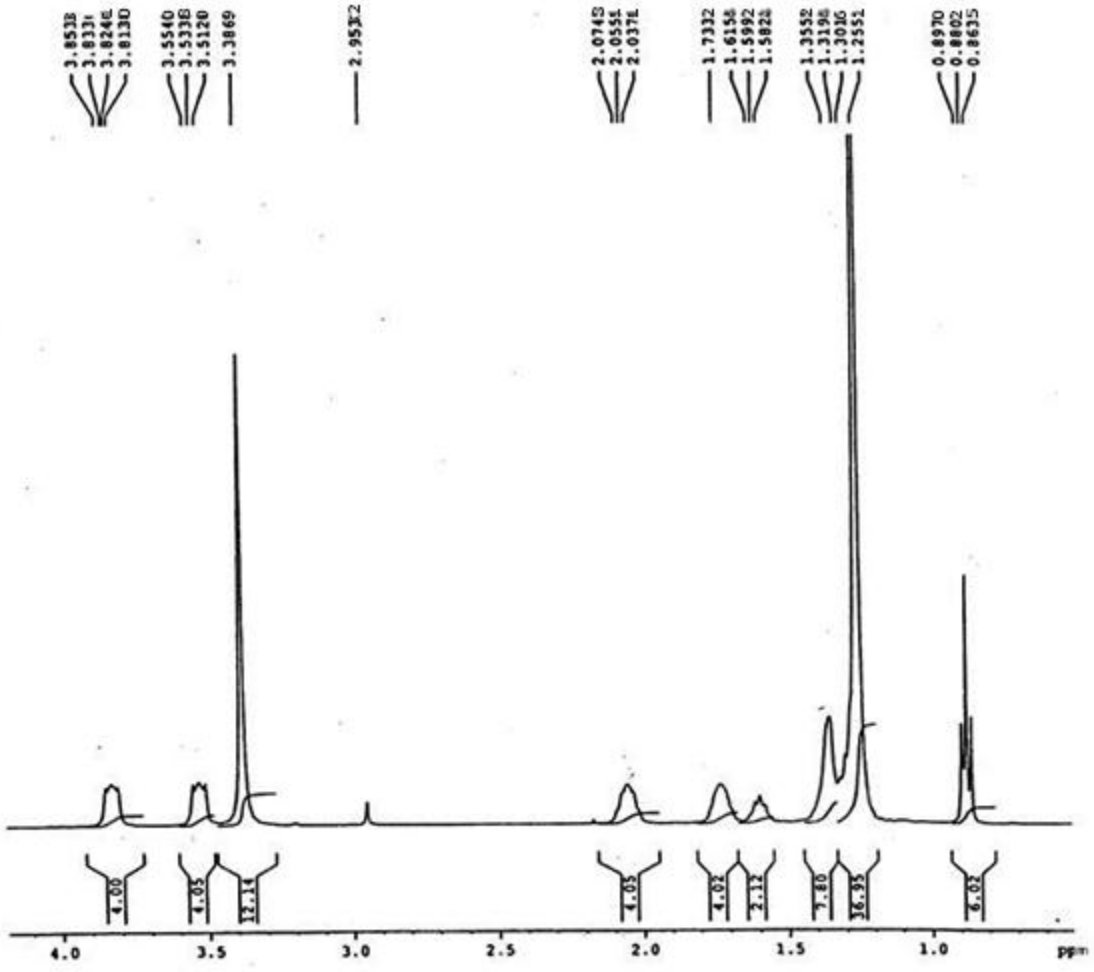



**Figure 2.2:** $^{1}H$ NMR spectrum of 14-5-14 in $CDCl_3$.

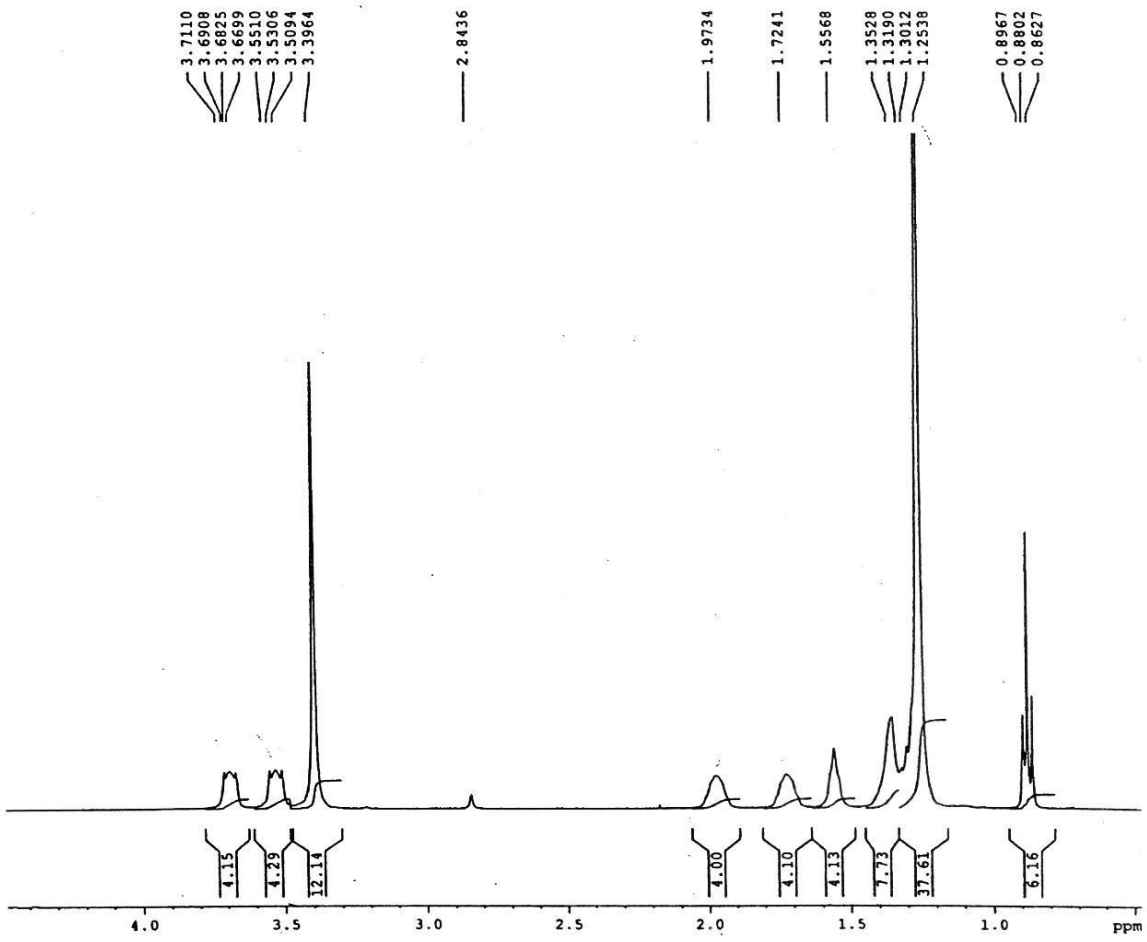



**Figure 2.3:** $^{1}H$ NMR spectrum of 14-6-14 in $CDCl_3$.

## 2.2 Preparation of Solutions

All stock solutions were freshly prepared (just before using) to avoid aging in demineralized doubly distilled water. The specific conductivity (κ) of distilled water was in between (0.9–2.2) x $10^{-6}$ $\Omega^{-1}$ $cm^{-1}$. All glasswares were properly cleaned with chromic acid (freshly prepared by mixing a desired amount of concentrated sulfuric acid with potassium dichromate), then with water, and finally by rinsing with doubly distilled water and/or acetone.

### 2.2.1 Acetate Buffer Solutions

The controlled acetic acid–sodium acetate buffer (*p*H 5.0) was prepared by mixing acetic acid (0.2 mol $dm^{-3}$) and sodium acetate (0.2 mol $dm^{-3}$) in the ratio 3:7 [3].

For studying *p*H effect, the examined range solutions (*p*H = 4.0 - 6.5) were prepared as follows [3,4]:

*p*H = 4.0 : prepared by mixing 80 $cm^3$ acetic acid (0.2 mol $dm^{-3}$) with 20 $cm^3$ sodium acetate (0.2 mol $dm^{-3}$) and checked by *p*H meter.

*p*H = 4.5 : prepared by mixing 60 $cm^3$ acetic acid (0.2 mol $dm^{-3}$) with 40 $cm^3$ sodium acetate (0.2 mol $dm^{-3}$) and checked by *p*H meter.

*p*H = 5.5 : prepared by mixing 14 $cm^3$ acetic acid (0.2 mol $dm^{-3}$) with 86 $cm^3$ sodium acetate (0.2 mol $dm^{-3}$) and checked by *p*H meter.

*p*H = 6.0 : prepared by mixing 5 $cm^3$ acetic acid (0.2 mol $dm^{-3}$) with 95 $cm^3$ sodium acetate (0.2 mol $dm^{-3}$) and checked by *p*H meter.

*p*H = 6.5 : prepared by adding a desired volume of sodium acetate (0.2 mol $dm^{-3}$), to a freshly prepared *p*H =6.0 solution and checked by *p*H meter.

### 2.2.2 Surfactant Solutions

Surfactant solutions were prepared by dissolving appropriate amount of TTABr or *geminis* in the desired buffer solution.

### 2.2.3 Dipeptide Solutions

Stock solutions of dipeptides were always prepared in the buffer solution.

### 2.2.4 Ninhydrin Solution

Stock solution of ninhydrin was prepared in the buffer solution and was stored in a dark bottle.

### 2.2.5 Organic Solvent Solutions

In all cases, mixing of pre-calculated volumes of the organic solvents was done with appropriate volumes of buffer solution at controlled temperature to prepare different volume percentages of the aqueous-organic solvent mixtures.

### 2.2.6 Preparation and Standardizing of Mercuric Nitrate: 0.01M [0.3426 g $Hg(NO_3)_2$ per 100 $cm^3$]

To prepare 0.01M of mercury(II) nitrate, about 0.35g of $Hg(NO_3)_2 \cdot H_2O$ was dissolved in a mixture of 0.5 $cm^3$ concentrated nitric acid and 50 $cm^3$ water, and then diluted with water to 100 $cm^3$. Standardizing the solution was done by transferring an accurately measured volume of 20 $cm^3$ of the solution into Erlenmeyer flask, then adding 0.5 $cm^3$ of nitric acid and 1 $cm^3$ ammonium iron(III) sulfate. The titration of Hg(II) solution was started by adding 0.02N ammonium thiocyanate till the first appearance of a permanent brown color, then the calculation was done to find the exact molarity [5].

### 2.2.7 Preparation of Copper Nitrate: 0.01M [0.2497g $CuSO_4$ per 100 $cm^3$]

To prepare 0.01M of copper (II) sulphate, an appropriate amount of $CuSO_5 \cdot 5H_2O$ was dissolved in a buffer solution and kept as a stock solution.

## 2.3 Instrumentation and Techniques

### 2.3.1 $^1$H NMR Measurements

$^1$H NMR spectra of the synthesized geminis were recorded on 300 MHz by Bruker Avance II 300 NMR spectrometer (Central Drug Research Institute, Lucknow in $CDCl_3$ solvent with $^1$H chemical shifts relative to internal standard tetramethylsilane (TMS).

The stock solutions of geminis were prepared in $CDCl_3$. For characterization studies, about 0.6 $cm^{-3}$ of each solution was taken in 5 mm NMR tube and chemical shifts were recorded on the δ (ppm) scale (reproducibility within 0.01 ppm).

### 2.3.2 *p*H-Measurements

The *p*H measurements of the solutions were made using a digital Systronics *p*H meter model MK-VI (India) in conjugation with a combined electrode (glass-saturated calomel electrode). The electrode was stored in *p*H 7.0 buffer and was washed in double-distilled water before use; it was then rinsed with *p*H 7.0 buffer and the *p*H-meter was standardized using WTW buffer solution (*p*H 4.0 (Germany)). Whenever the solution was changed, the electrode was rinsed with double-distilled water and the surplus water removed and the *p*H-meter was restandardized using the *p*H 4.0 buffer solution. All *p*H measurements were made at least in triplicate and they agreed within ± 0.02.

### 2.3.3 Spectra of the Reaction Product

The amino acid/peptides–ninhydrin reaction always yields purple colored product (DYDA) in absence and presence of surfactants with two absorption maxima (~400 nm and 570 nm)[6-15]. On the other hand, the color of the final product in presence of a metal ion depends upon the order of mixing of the metal ion [16]. In our investigations we first made metal ion-coordinated dipeptide then the reaction was started by adding ninhydrin and other reagent (when required). The UV-Vis spectra of the product, recorded in the absence and presence of conventional and related *gemini* surfactant micelles (TTABr/ 14-s-14 (s = 4, 5, 6)) and in the absence and presence of organic solvents were done using Shimadzu single beam spectrophotometer (model UV mini 1240, Kyoto, Japan). The absorption spectra of mixtures containing the reactants in different solvents and *gemini* surfactants exhibited negligible shift in the absorption maxima as that of a solution of *Ruhemann's purple* in aqueous medium (Figures 2.4-2.13). The results also indicate that the dipeptide–ninhydrin/metal ion-coordinated dipeptide–ninhydrin reactions are catalyzed by TTABr/14-s-14 micelles and further catalytic effect was observed in presence of organic solvents.

For metal ion-coordinated dipeptides–ninhydrin [Figures 2.14-2.33], the Hg(II)-dipeptide and Cu(II)-dipeptide complexes were prepared as follows. Solutions of the reactants (1:1 molar) were taken in a three-necked vessel filled with an appropriate volume of buffer solution (*p*H=5.0) or buffer solution plus organic solvent in absence and presence of TTABr/14-s-14 micelles and heated in a controlled manner. The wavelength maximum ($\lambda_{max}$) depend upon the final yellow product of the reaction.

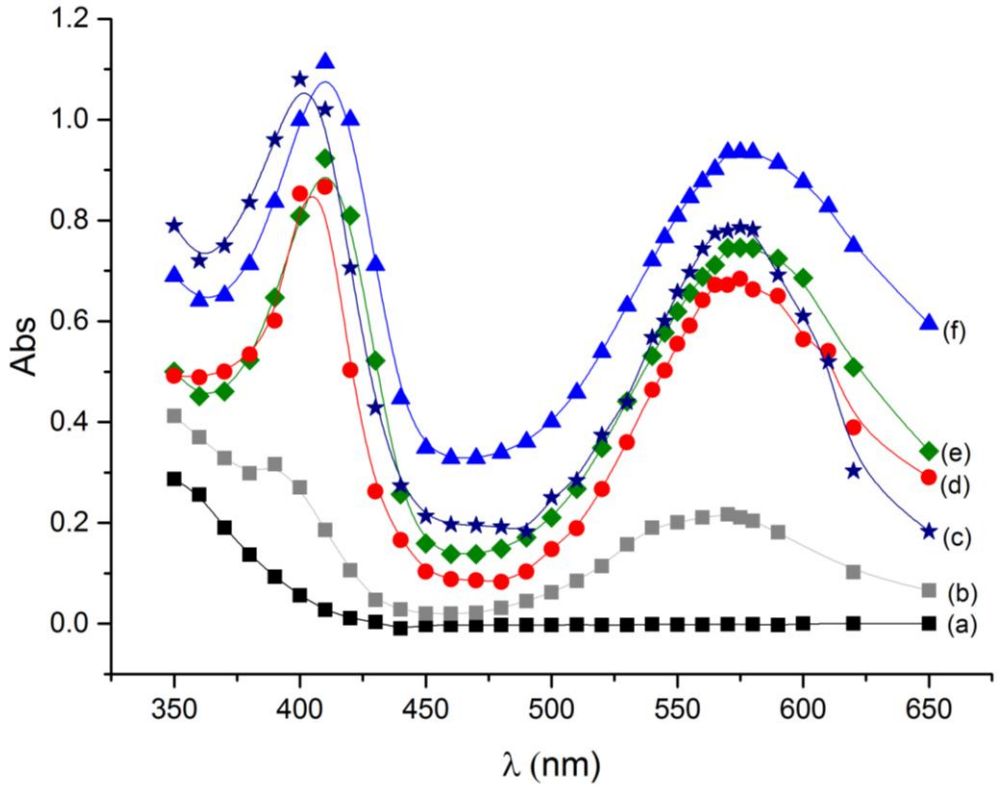



**Figure 2.4:** Absorption spectra of the reaction product of Gly-L-Ala with ninhydrin in (a, b) the absence and (c-f) presence of surfactants after completion of the reaction: (a) represents absorbance when the reaction was tried in the absence of surfactant showing the absence of any reaction under the conditions at zero time; (b) aqueous medium after completion of the reaction; (c) in presence of TTABr; (d) 14-6-14; (e) 14-5-14, and (f) 14-4-14. *Reaction conditions:* [Gly-L-Ala] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

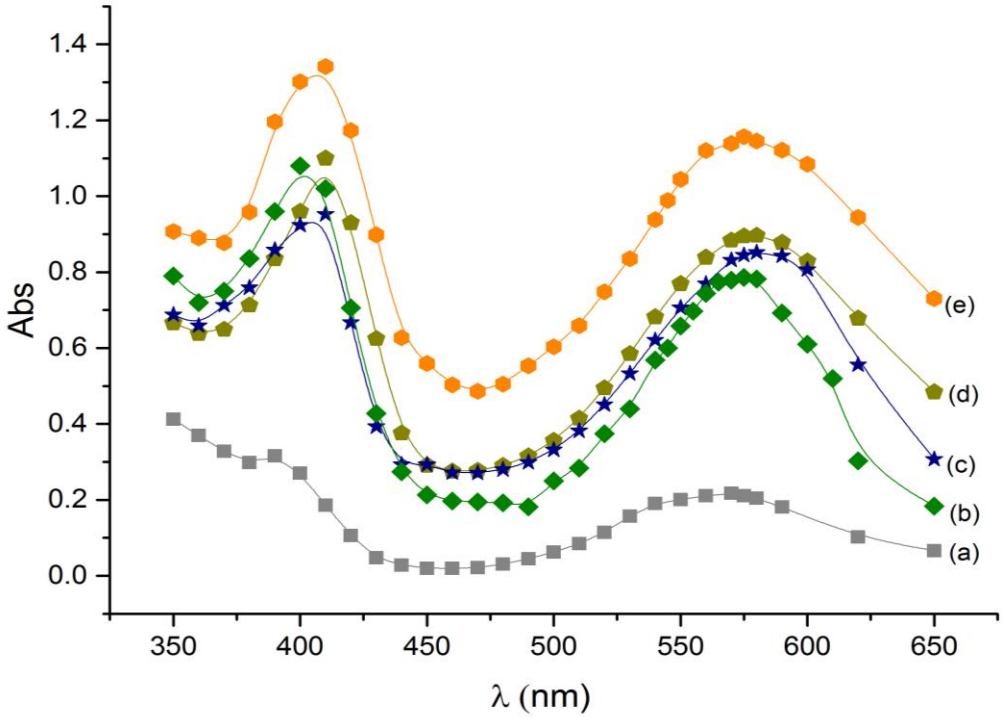



**Figure 2.5:** Absorption spectra of the reaction product of Gly-L-Ala with ninhydrin in (a) the absence and (b-e) presence of TTABr: (b) represents absorbance when the reaction was tried in the presence of TTABr without organic solvents additives; (c) in presence of 20.0% DMSO after completion of the reaction; (d) 20.0% DO; (e) 20.0% AN. *Reaction conditions:* [Gly-L-Ala] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

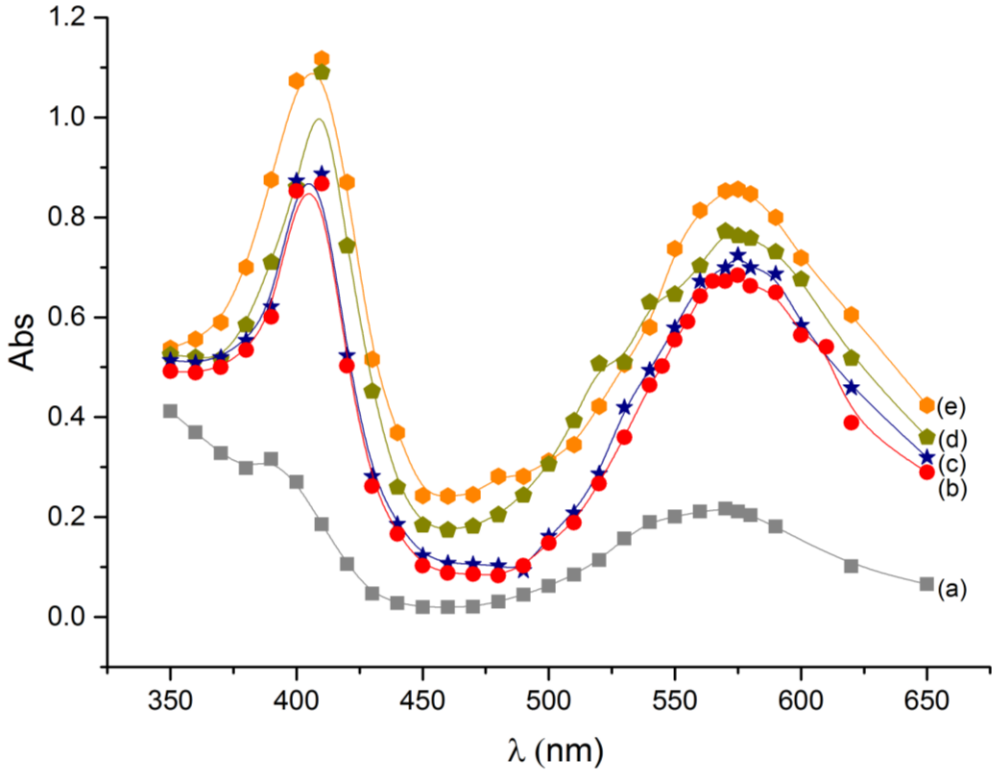



**Figure 2.6:** Absorption spectra of the reaction product of Gly-L-Ala with ninhydrin in (a) the absence and (b-e) presence of 14-6-14: (b) represents absorbance when the reaction was tried in the presence of 14-6-14 without organic solvents additives; (c) in the presence of 14-6-14 and 20.0% DMSO after completion of the reaction; (d) 20.0% DO; (e) 20.0% AN. *Reaction conditions:* [Gly-L-Ala] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [14-6-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

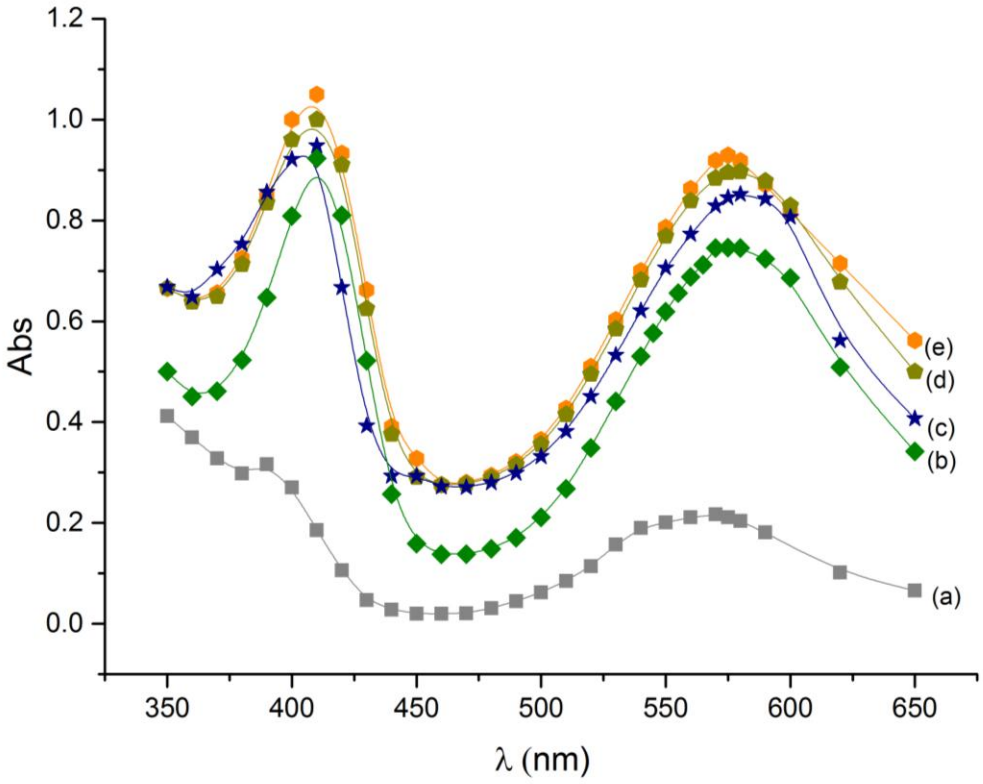



**Figure 2.7:** Absorption spectra of the reaction product of Gly-L-Ala with ninhydrin in (a) the absence and (b-e) presence of 14-5-14: (b) represents absorbance when the reaction was tried in the presence of 14-5-14 without organic solvents additives; (c) in presence of 14-5-14 and 20.0% DMSO after completion of the reaction; (d) 20.0% DO; (e) 20.0% AN. *Reaction conditions:* [Gly-L-Ala] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [14-5-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

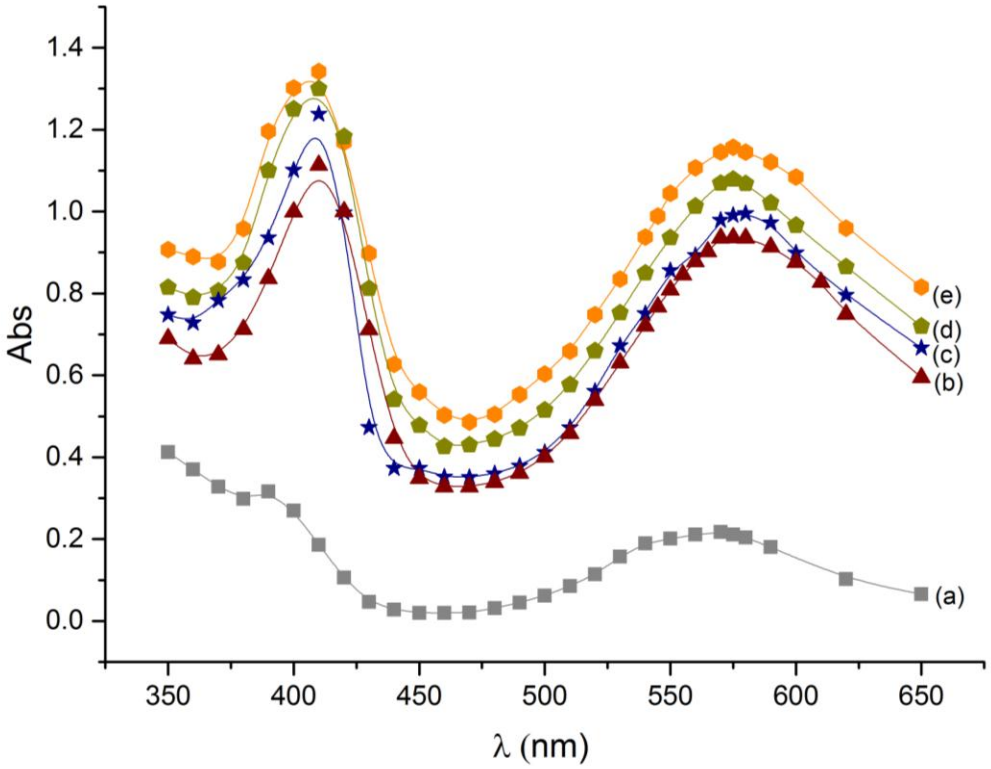



**Figure 2.8:** Absorption spectra of the reaction product of Gly-L-Ala with ninhydrin in (a) the absence and (b-e) presence of 14-4-14: (b) represents absorbance when the reaction was tried in the presence of 14-4-14 without organic solvents additives; (c) in presence of 14-4-14 and 20.0% DMSO after completion of the reaction; (d) 20.0% DO; (e) 20.0% AN. *Reaction conditions*: [Gly-L-Ala] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [14-4-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

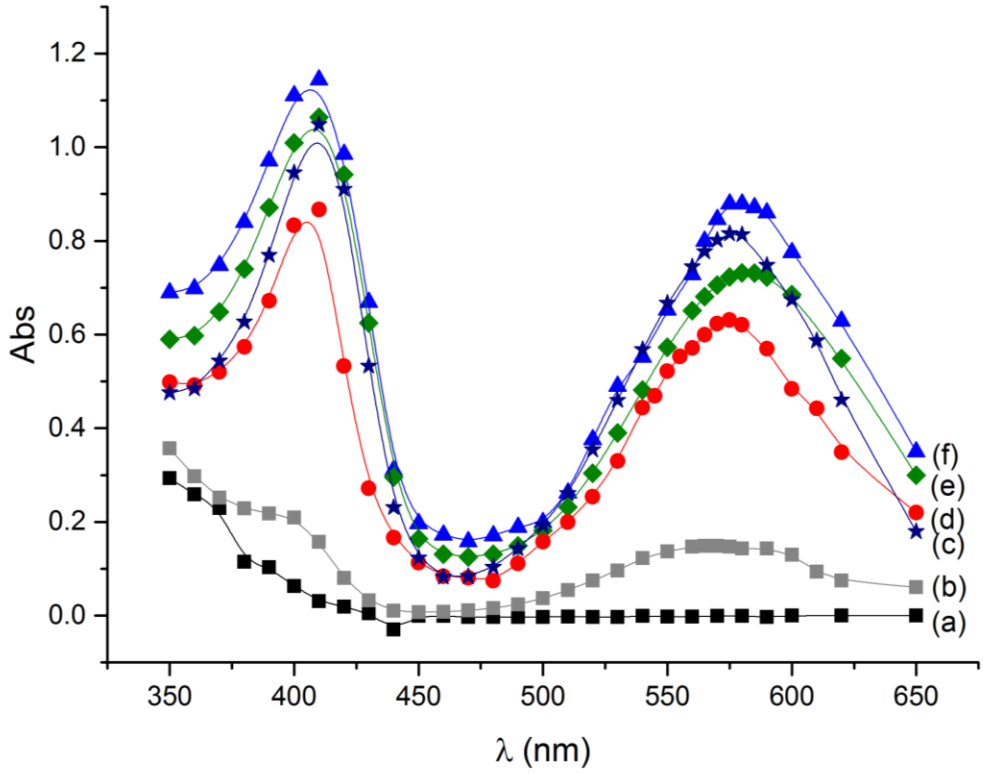



**Figure 2.9:** Absorption spectra of the reaction product of Gly-DL-Asp with ninhydrin in (a, b) the absence and (c-f) presence of surfactants after completion of the reaction: (a) represents absorbance when the reaction was tried in the absence of surfactant showing the absence of any reaction under the conditions at zero time; (b) aqueous medium after completion of the reaction; (c) in presence of TTABr; (d) 14-6-14; (e) 14-5-14, and (f) 14-4-14. *Reaction conditions*: [Gly-DL-Asp] = 3.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

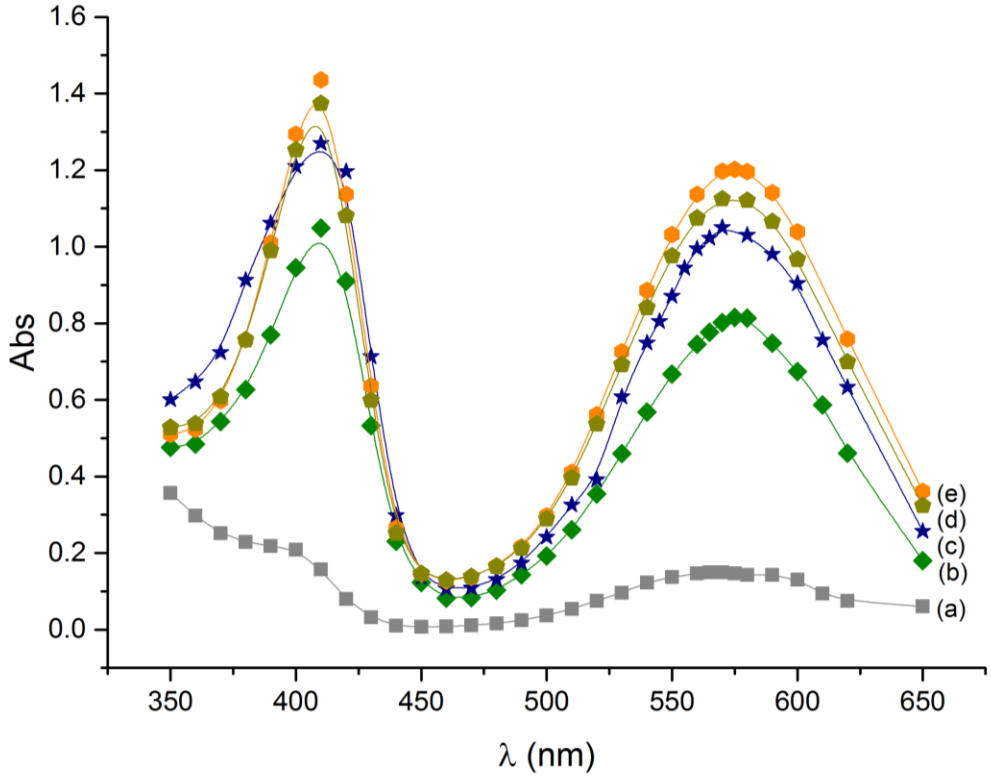



**Figure 2.10:** Absorption spectra of the reaction product of Gly-DL-Asp with ninhydrin in (a) the absence and (b-e) presence of TTABr: (b) represents absorbance when the reaction was tried in the presence of TTABr without organic solvents additives; (c) in presence of TTABr and 20.0% DMSO after completion of the reaction; (d) 20.0% DO; (e) 20.0% AN. *Reaction conditions:* [Gly-DL-Asp] = 3.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

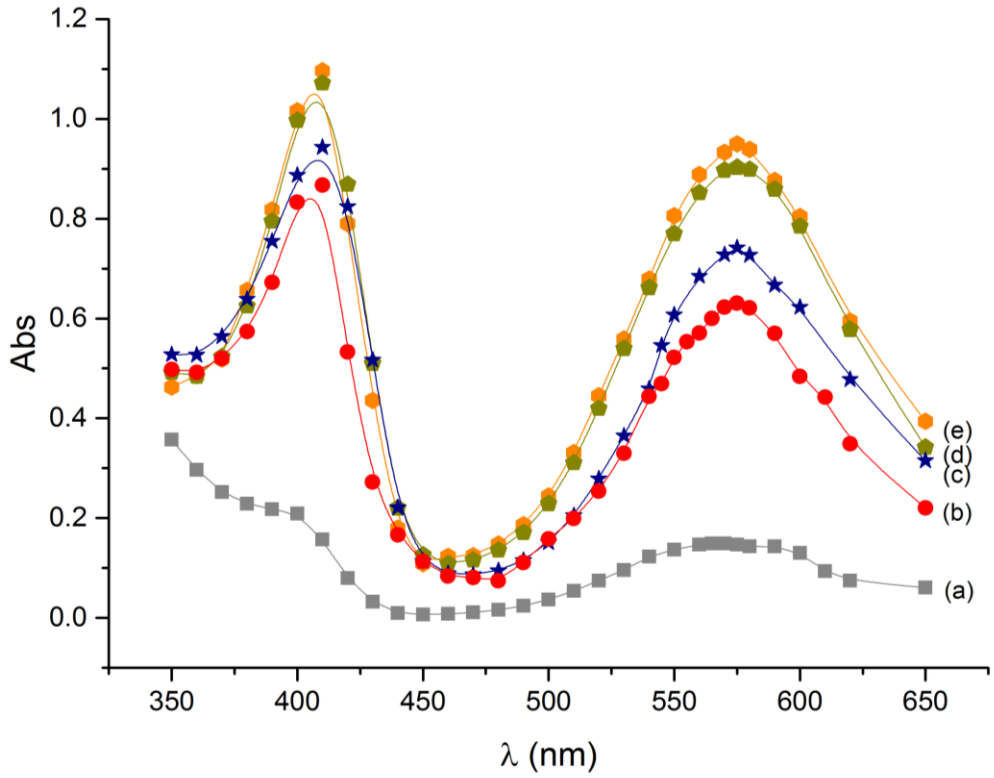



**Figure 2.11:** Absorption spectra of the reaction product of Gly-DL-Asp with ninhydrin in (a) the absence and (b-e) presence of 14-6-14: (b) represents absorbance when the reaction was tried in the presence of 14-6-14 without organic solvents additives; (c) in presence of 14-6-14 and 20.0% DMSO after completion of the reaction; (d) 20.0% DO; (e) 20.0% AN. *Reaction conditions:* [Gly-DL-Asp] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [14-6-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

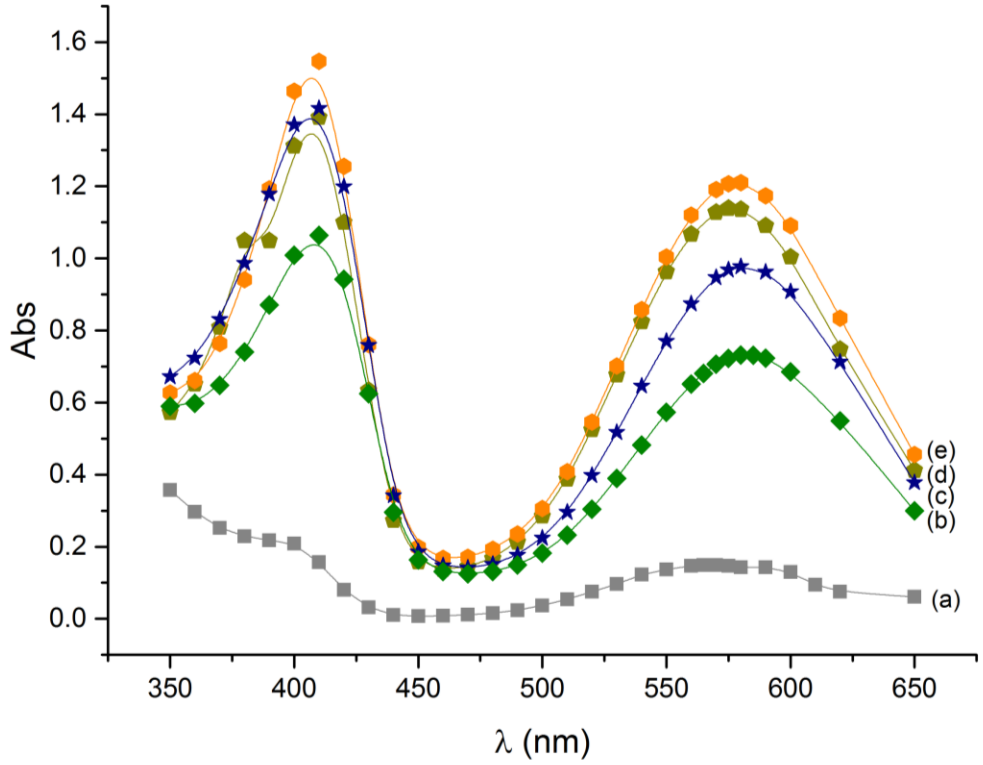



**Figure 2.12:** Absorption spectra of the reaction product of Gly-DL-Asp with ninhydrin in (a) the absence and (b-e) presence of 14-5-14: (b) represents absorbance when the reaction was tried in the presence of 14-5-14 without organic solvents additives; (c) in presence of 14-5-14 and 20.0% DMSO after completion of the reaction; (d) 20.0% DO; (e) 20.0% AN. *Reaction conditions:* [Gly-DL-Asp] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [14-5-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

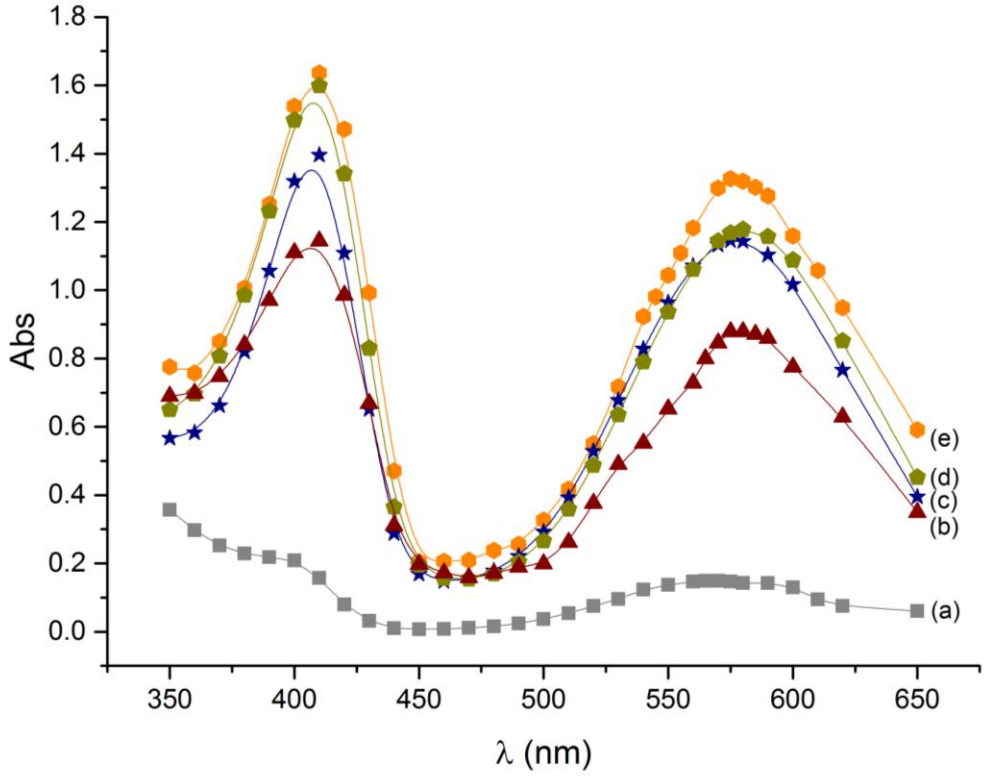



**Figure 2.13:** Absorption spectra of the reaction product of Gly-DL-Asp with ninhydrin in (a) the absence and (b-e) presence of 14-4-14: (b) represents absorbance when the reaction was tried in the presence of 14-4-14 without organic solvents additives; (c) in presence of 14-4-14 and 20.0% DMSO after completion of the reaction; (d) 20.0% DO; (e) 20.0% AN. *Reaction conditions:* [Gly-DL-Asp] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [14-4-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

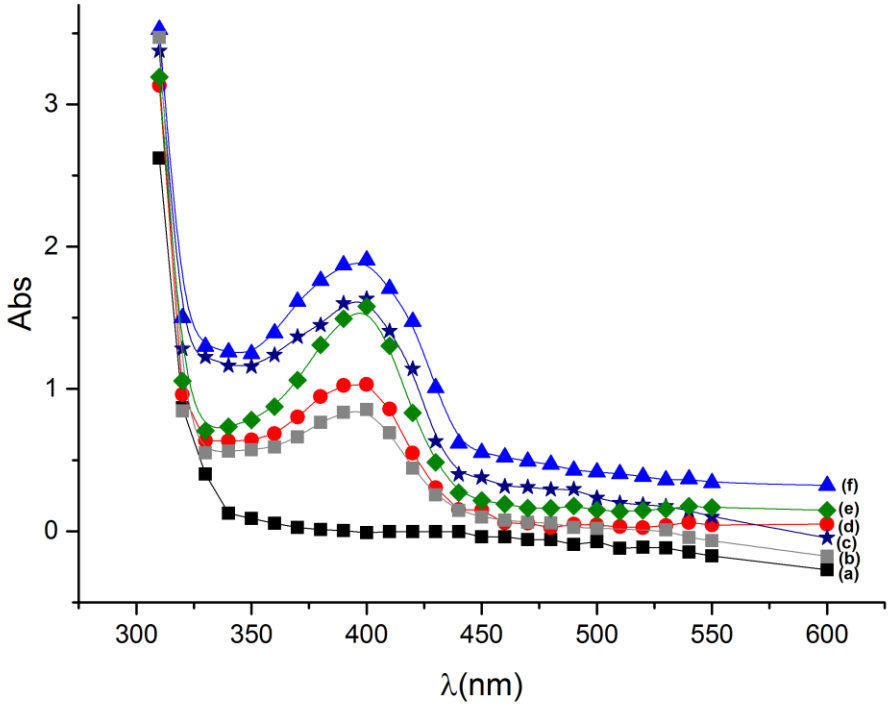



**Figure 2.14** Absorption spectra of the reaction product of Hg(II)-Gly-L-Ala complex with ninhydrin in (a, b) the absence and (c-f) presence of surfactants after completion of the reaction: (a) represents absorbance when the reaction was tried in the absence of surfactant showing the absence of any reaction under the conditions at zero time; (b) aqueous medium after completion of the reaction; (c) in presence of TTABr; (d) 14-6-14 ; (e) 14-5-14, and (f) 14-4-14. *Reaction conditions:* $[Hg(II)\text{-}Gly\text{-}L\text{-}Ala]^{+}$ = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

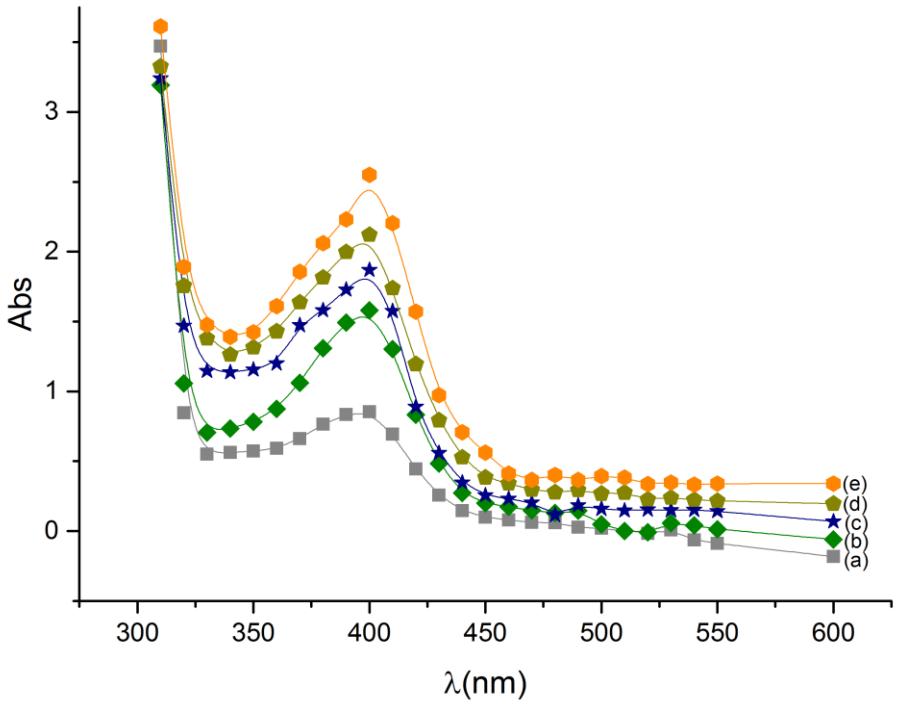



**Figure 2.15:** Absorption spectra of the reaction product of Hg(II)-Gly-L-Ala complex with ninhydrin in (a) the absence and (b-e) presence of TTABr: (b) represents absorbance when the reaction was tried in the presence of TTABr without organic solvents additives; (c) in presence of TTABr and 10.0% DMSO after completion of the reaction; (d) 10.0% DO; (e) 10.0% AN. *Reaction conditions:* $[Hg(II)\text{-}Gly\text{-}L\text{-}Ala]^{+}$ = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

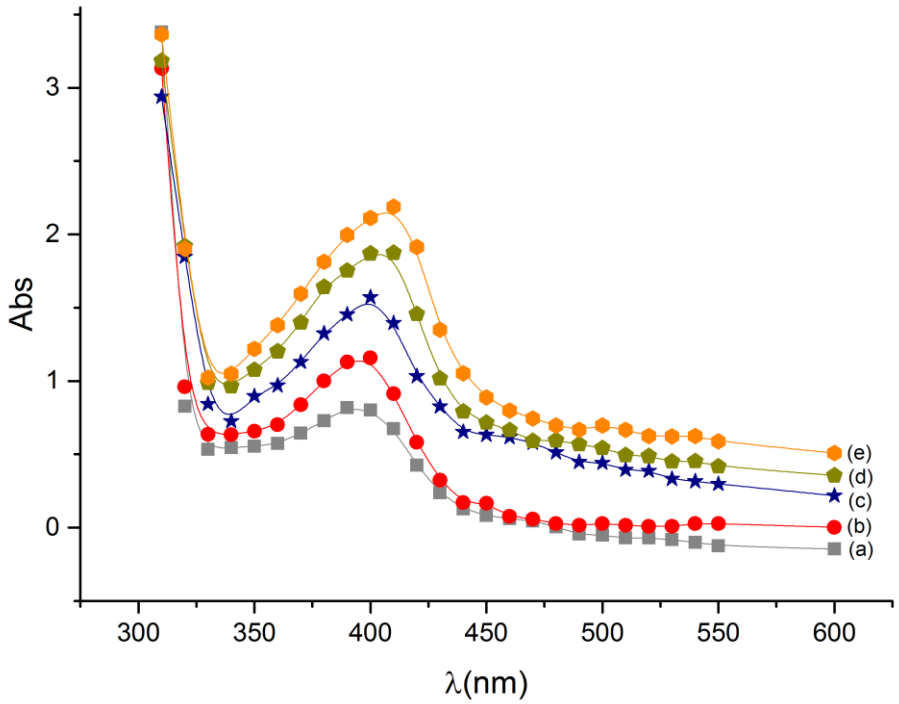



**Figure 2.16:** Absorption spectra of the reaction product of Hg(II)-Gly-L-Ala complex with ninhydrin in (a) the absence and (b-e) presence of 14-6-14: (b) represents absorbance when the reaction was tried in the presence of 14-6-14 without organic solvents additives; (c) in presence of 14-6-14 and 10.0% DMSO after completion of the reaction; (d) 10.0% DO; (e) 10.0% AN. *Reaction conditions*: $[Hg(II)\text{-Gly-L-Ala}]^{+}$ = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [14-6-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

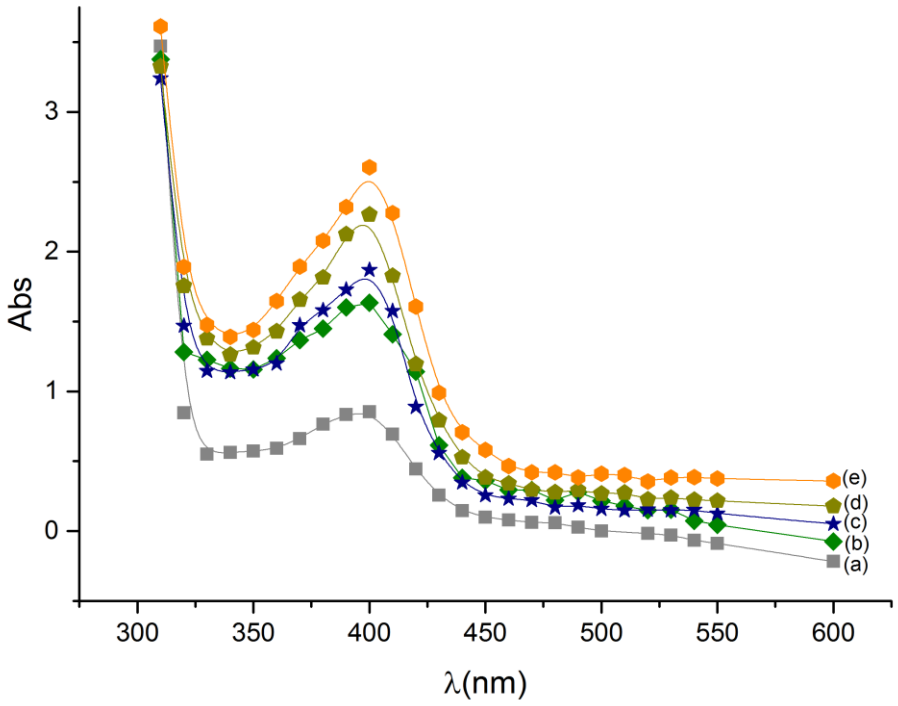



**Figure 2.17:** Absorption spectra of the reaction product of Hg(II)-Gly-L-Ala complex with ninhydrin in (a) the absence and (b-e) presence of 14-5-14: (b) represents absorbance when the reaction was tried in the presence of 14-5-14 without organic solvents additives; (c) in presence of 14-5-14 and 10.0% DMSO after completion of the reaction; (d) 10.0% DO; (e) 10.0% AN. *Reaction conditions:* $[Hg(II)\text{-Gly-L-Ala}]^{+}$ = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [14-5-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

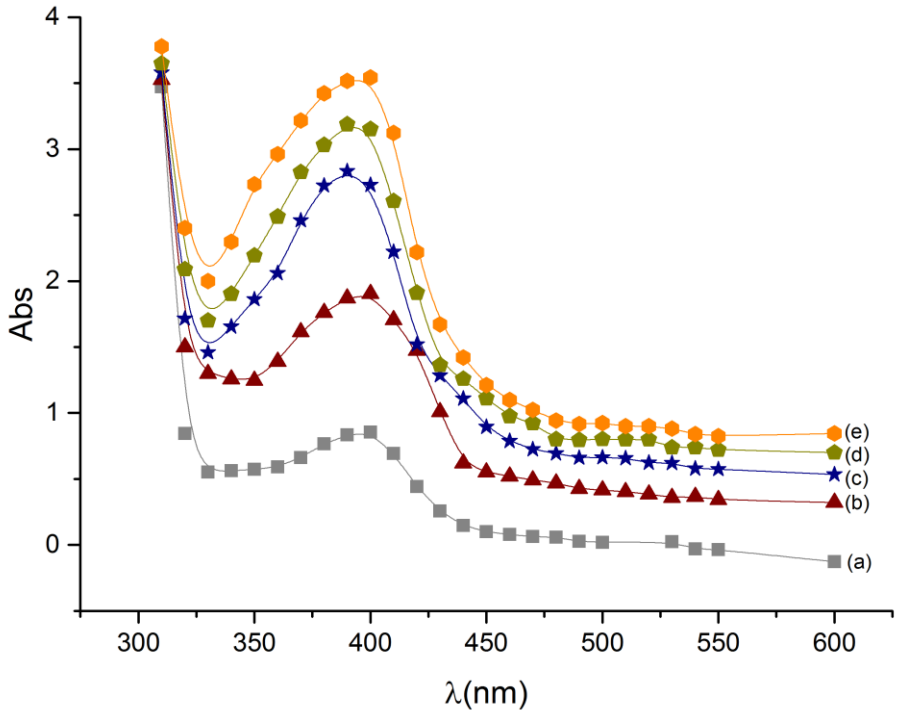



**Figure 2.18:** Absorption spectra of the reaction product of Hg(II)-Gly-L-Ala complex with ninhydrin in (a) the absence and (b-e) presence of 14-4-14: (b) represents absorbance when the reaction was tried in the presence of 14-4-14 without organic solvents additives; (c) in presence of 14-4-14 and 10.0% DMSO after completion of the reaction; (d) 10.0% DO; (e) 10.0% AN. *Reaction conditions*: $[Hg(II)\text{-Gly-L-Ala}]^{+}$ = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [14-4-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

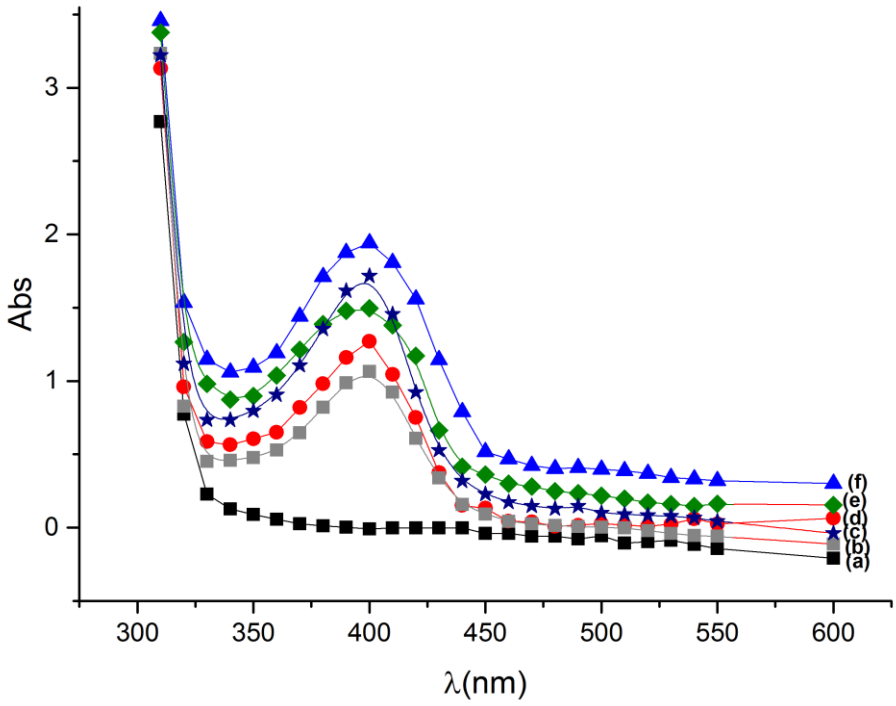



**Figure 2.19:** Absorption spectra of the reaction product of Hg(II)-Gly-DL-Asp complex with ninhydrin in (a, b) the absence and (c-f) presence of surfactants after completion of the reaction: (a) represents absorbance when the reaction was tried in the absence of surfactant showing the absence of any reaction under the conditions at zero time; (b) aqueous medium after completion of the reaction; (c) in presence of TTABr; (d) 14-6-14 ; (e) 14-5-14, and (f) 14-4-14. *Reaction conditions:* $[Hg(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x$10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

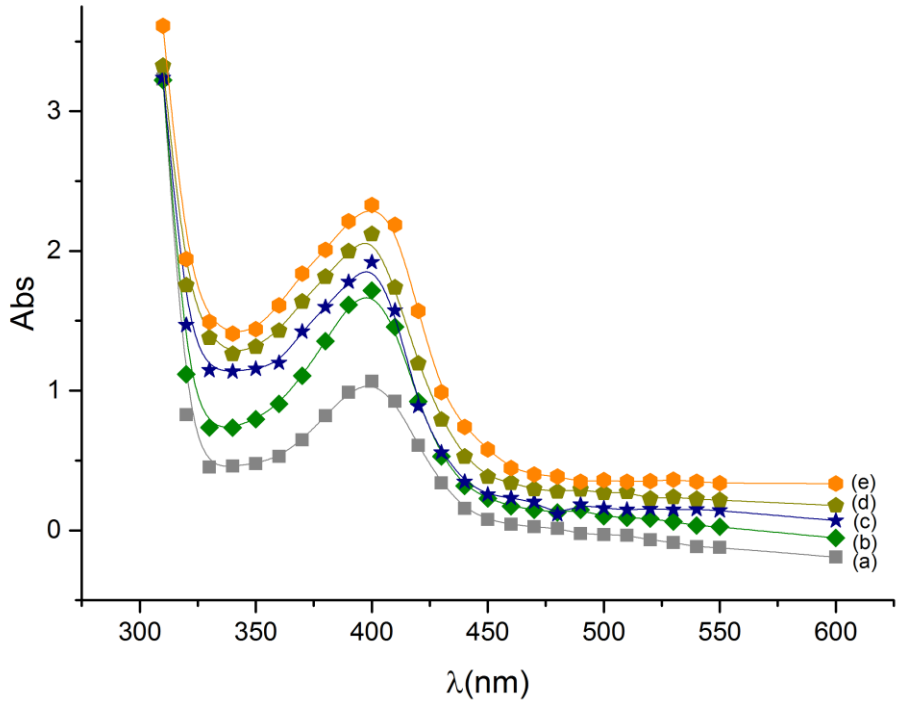



**Figure 2.20:** Absorption spectra of the reaction product of Hg(II)-Gly-DL-Asp complex with ninhydrin in (a) the absence and (b-e) presence of TTABr: (b) represents absorbance when the reaction was tried in the presence of TTABr without organic solvents additives; (c) in presence of TTABr and 10.0% DMSO after completion of the reaction; (d) 10.0% DO; (e) 10.0% AN. *Reaction conditions*: $[Hg(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

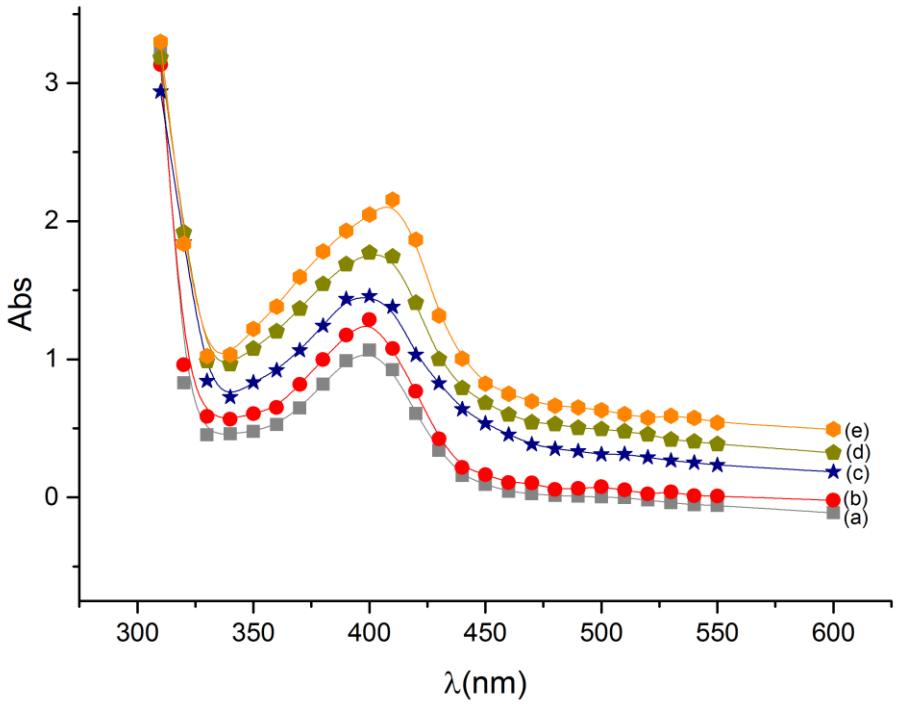



**Figure 2.21:** Absorption spectra of the reaction product of Hg(II)-Gly-DL-Asp complex with ninhydrin in (a) the absence and (b-e) presence of 14-6-14: (b) represents absorbance when the reaction was tried in the presence of 14-6-14 without organic solvents additives; (c) in presence of 14-6-14 and 10.0% DMSO after completion of the reaction; (d) 10.0% DO; (e) 10.0% AN. *Reaction conditions:* $[Hg(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [14-6-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

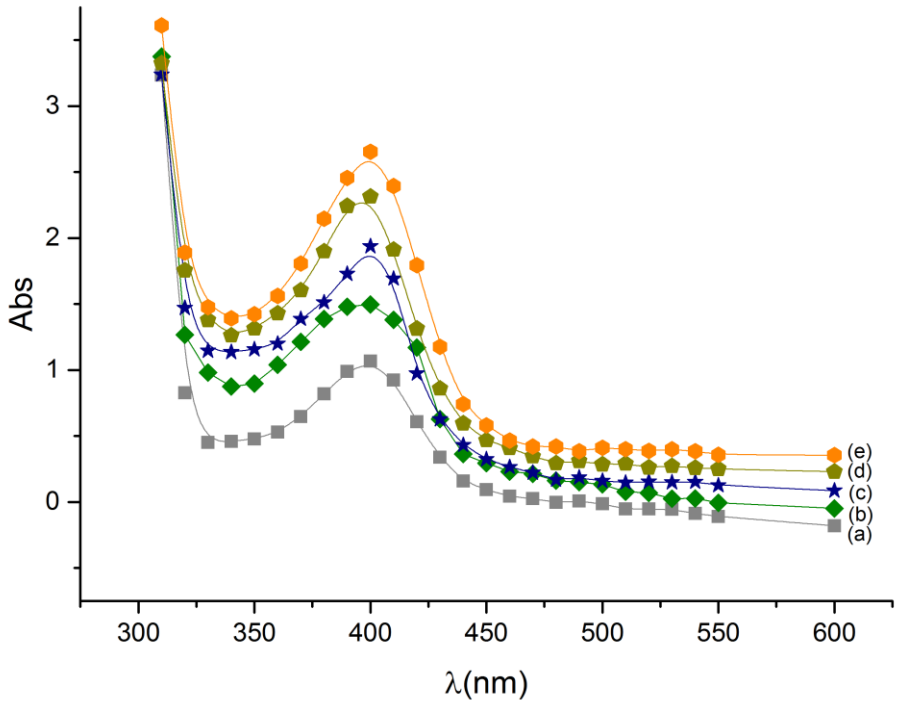



**Figure 2.22:** Absorption spectra of the reaction product of Hg(II)-Gly-DL-Asp complex with ninhydrin in (a) the absence and (b-e) presence of 14-5-14: (b) represents absorbance when the reaction was tried in the presence of 14-5-14 without organic solvents additives; (c) in presence of 14-5-14 and 10.0% DMSO after completion of the reaction; (d) 10.0% DO; (e) 10.0% AN. *Reaction conditions:* $[Hg(II)\text{-Gly-DL-Asp}]^{+}$ = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [14-5-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

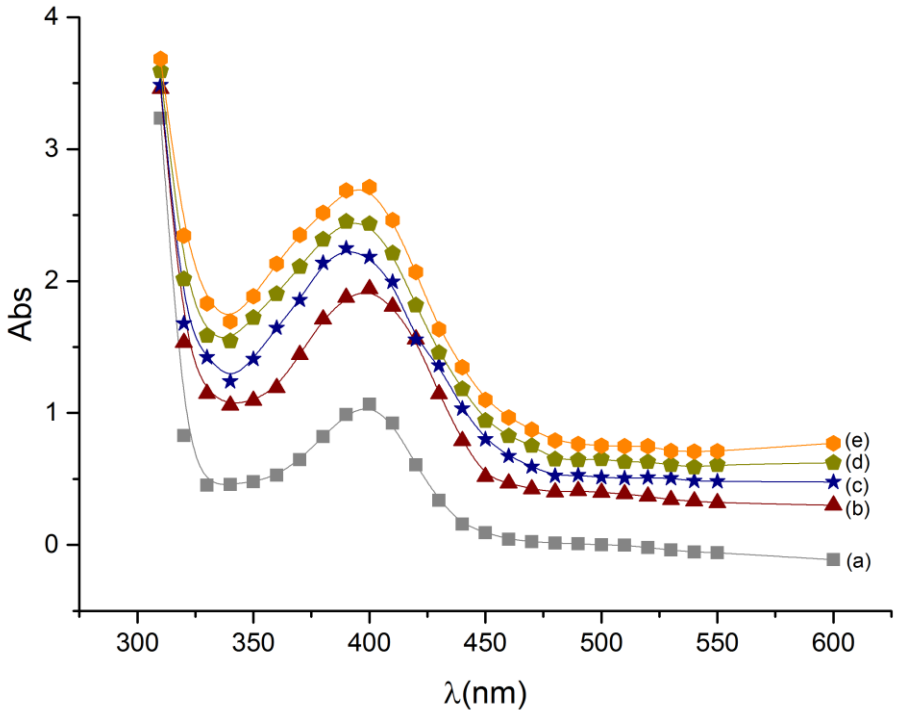



**Figure 2.23:** Absorption spectra of the reaction product of Hg(II)-Gly-DL-Asp complex with ninhydrin in (a) the absence and (b-e) presence of 14-4-14: (b) represents absorbance when the reaction was tried in the presence of 14-4-14 without organic solvents additives; (c) in presence of 14-4-14 and 10.0% DMSO after completion the reaction; (d) 10.0% DO; (e) 10.0% AN. *Reaction conditions:* $[Hg(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [14-4-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

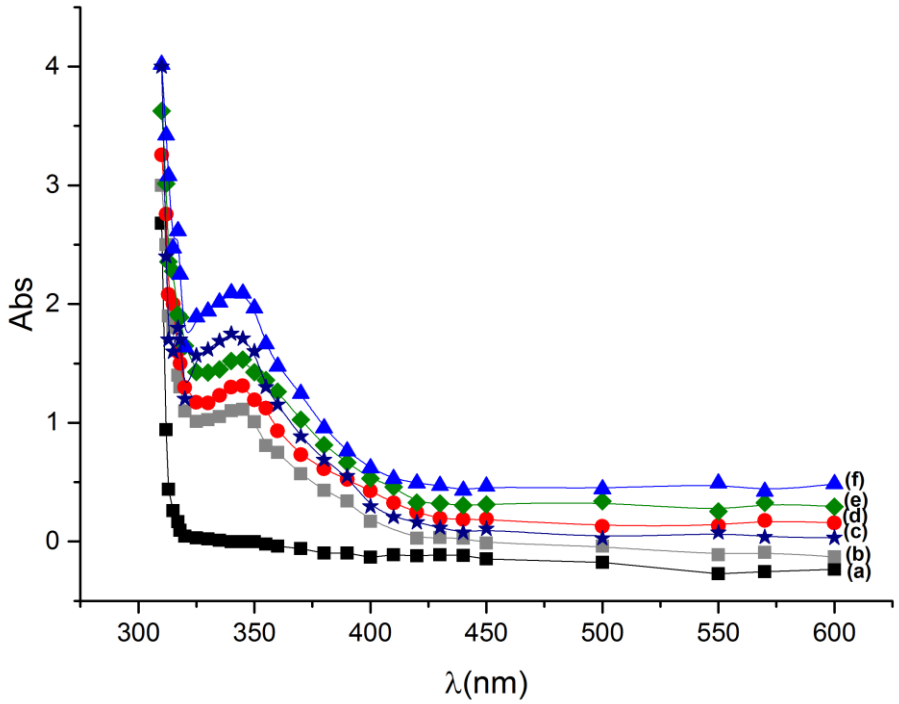



**Figure 2.24:** Absorption spectra of the reaction product of Cu(II)-Gly-L-Ala complex with ninhydrin in (a, b) the absence and (c-f) presence of surfactants after completion of the reaction: (a) represents absorbance when the reaction was tried in the absence of surfactant showing the absence of any reaction under the conditions at zero time; (b) aqueous medium after completion of the reaction; (c) in presence of TTABr; (d) 14-6-14 ; (e) 14-5-14, and (f) 14-4-14. *Reaction conditions:* $[Cu(II)\text{-Gly-L-Ala}]^{+}$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

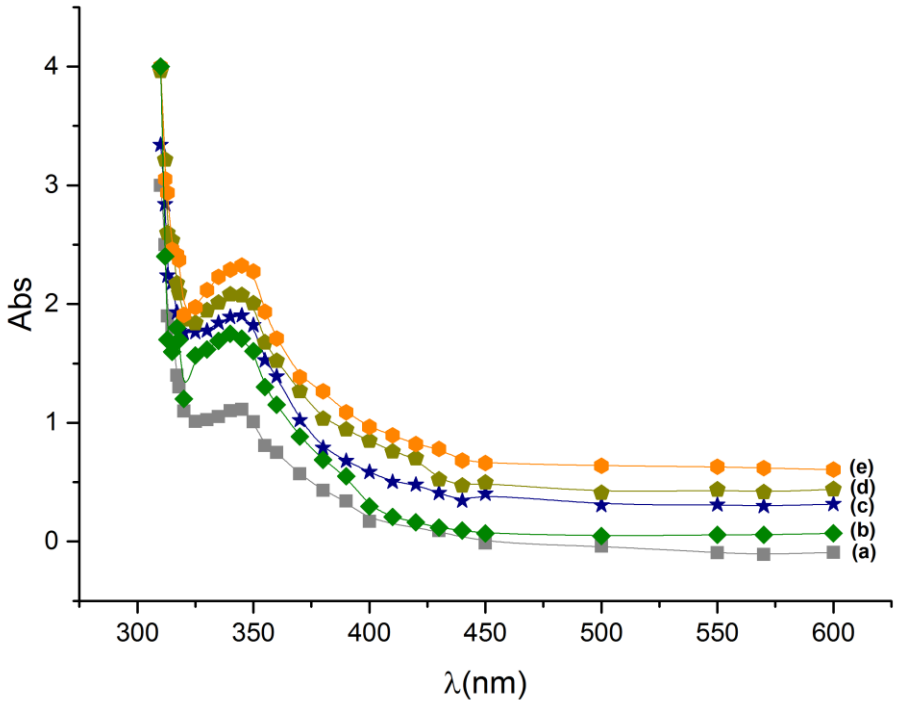



**Figure 2.25:** Absorption spectra of the reaction product of Cu(II)-Gly-L-Ala complex with ninhydrin in (a) the absence and (b-e) presence of TTABr: (b) represents absorbance when the reaction was tried in the presence of TTABr without organic solvents additives; (c) in presence of TTABr and 10.0% DMSO after completion of the reaction; (d) 10.0% DO; (e) 10.0% AN. *Reaction conditions:* $[Cu(II)\text{-Gly-L-Ala}]^{+}$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

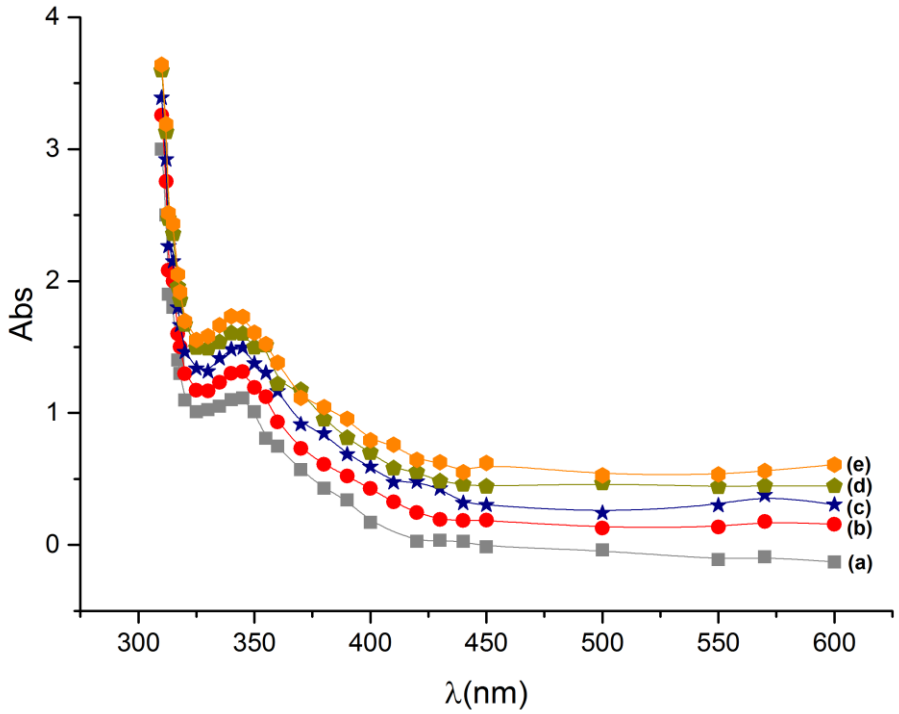



**Figure 2.26:** Absorption spectra of the reaction product of Cu(II)-Gly-L-Ala complex with ninhydrin in (a) the absence and (b-e) presence of 14-6-14: (b) represents absorbance when the reaction was tried in the presence of 14-6-14 without organic solvents additives; (c) in presence of 14-6-14 and 10.0% DMSO after completion of the reaction; (d) 10.0% DO; (e) 10.0% AN. *Reaction conditions:* $[Cu(II)\text{-Gly-L-Ala}]^{+}$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, [14-6-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

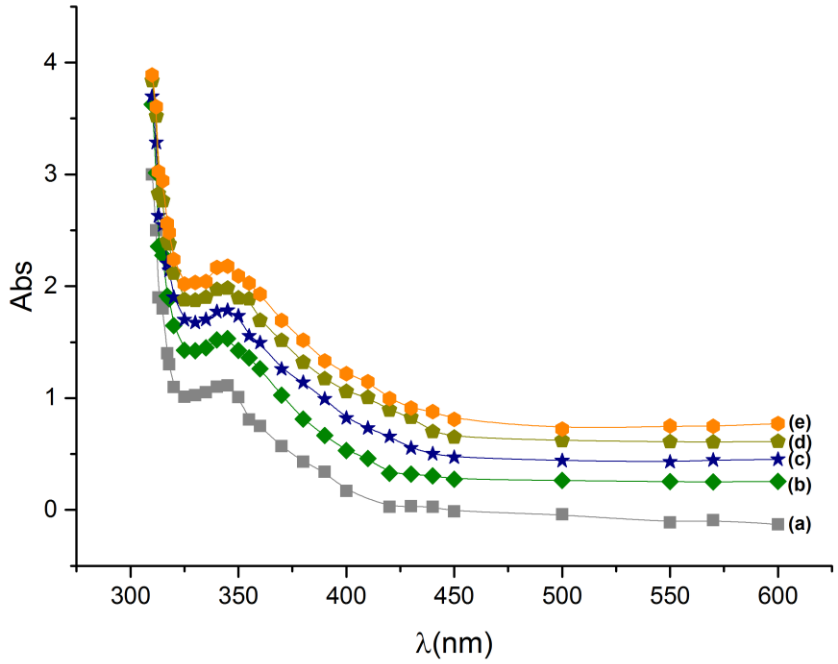



**Figure 2.27:** Absorption spectra of the reaction product of Cu(II)-Gly-L-Ala complex with ninhydrin in (a) the absence and (b-e) presence of 14-5-14: (b) represents absorbance when the reaction was tried in the presence of 14-5-14 without organic solvents additives; (c) in presence of 14-5-14 and 10.0% DMSO after completion of the reaction; (d) 10.0% DO; (e) 10.0% AN. *Reaction conditions*: $[Cu(II)\text{-Gly-L-Ala}]^{+}$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, [14-5-14] = 50 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

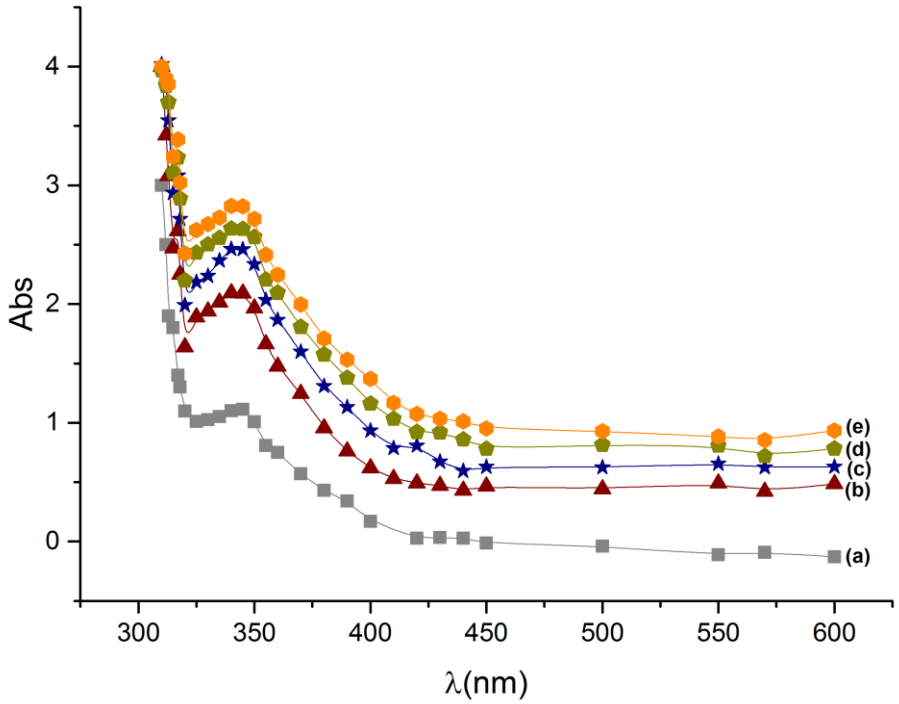



**Figure 2.28:** Absorption spectra of the reaction product of Cu(II)-Gly-L-Ala complex with ninhydrin in (a) the absence and (b-e) presence of 14-4-14: (b) represents absorbance when the reaction was tried in the presence of 14-4-14 without organic solvents additives; (c) in presence of 14-4-14 and 10.0% DMSO after completion of the reaction; (d) 10.0% DO; (e) 10.0% AN. *Reaction conditions:* $[Cu(II)\text{-Gly-L-Ala}]^{+}$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, [14-4-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

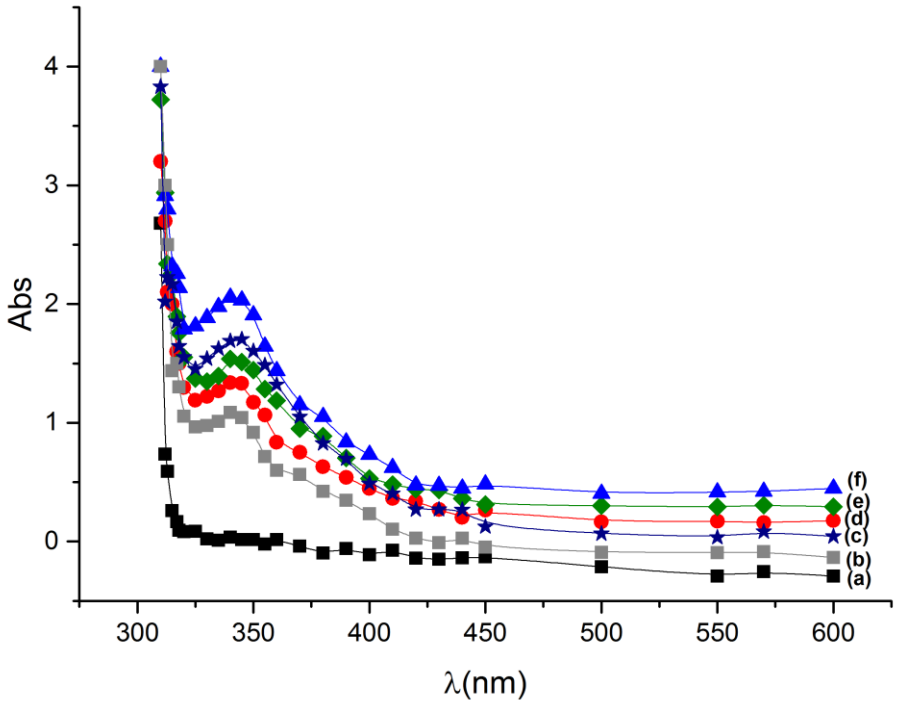



**Figure 2.29:** Absorption spectra of the reaction product of Cu(II)-Gly-DL-Asp complex with ninhydrin in (a, b) the absence and (c-f) presence of surfactants after completion of the reaction: (a) represents absorbance when the reaction was tried in the absence of surfactant showing the absence of any reaction under the conditions at zero time; (b) aqueous medium after completion of the reaction; (c) in presence of TTABr; (d) 14-6-14; (e) 14-5-14, and (f) 14-4-14. *Reaction conditions:* $[Cu(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+} = 4.0 \times 10^{-4}$ mol $dm^{-3}$, [ninhydrin] = $10.0 \times 10^{-3}$ mol $dm^{-3}$, [TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$, [14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

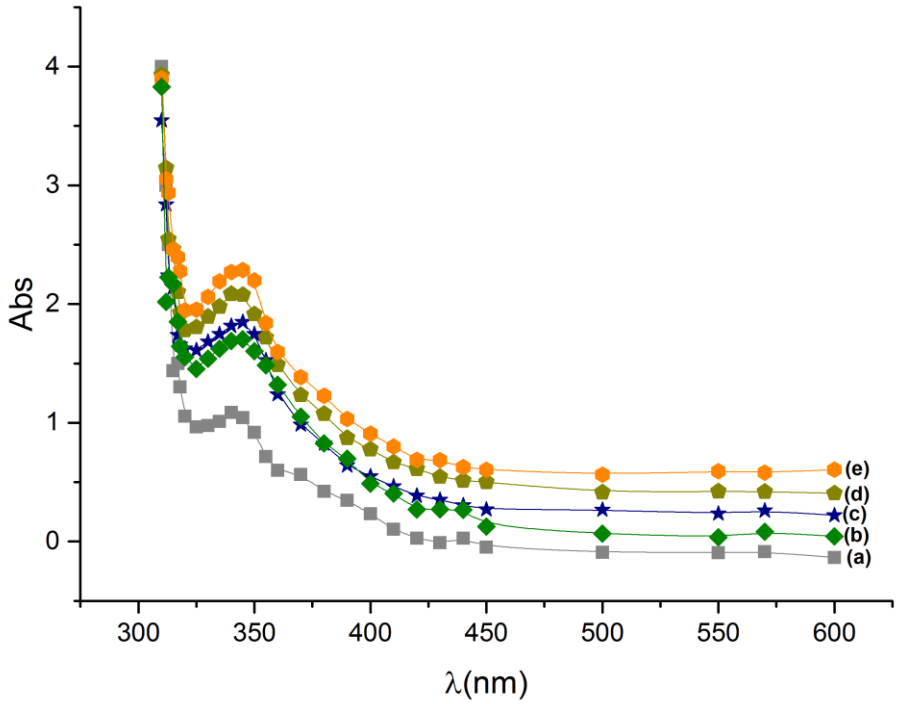



**Figure 2.30:** Absorption spectra of the reaction product of Cu(II)-Gly-DL-Asp complex with ninhydrin in (a) the absence and (b-e) presence of TTABr: (b) represents absorbance when the reaction was tried in the presence of TTABr without organic solvents additives; (c) in presence of TTABr and 10.0% DMSO after completion of the reaction; (d) 10.0% DO; (e) 10.0% AN. *Reaction conditions:* $[Cu(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

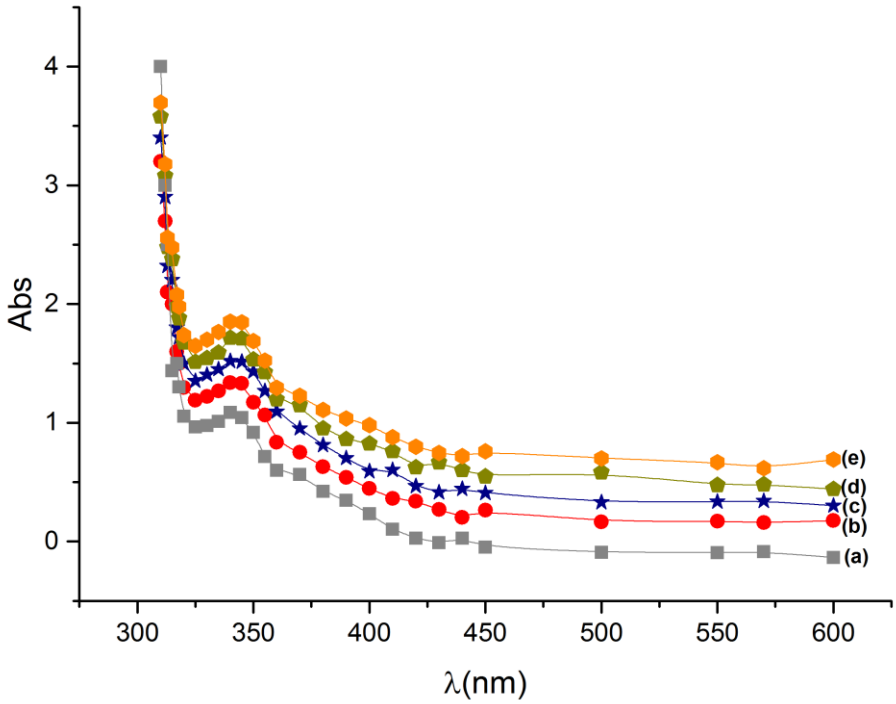



**Figure 2.31:** Absorption spectra of the reaction product of Cu(II)-Gly-DL-Asp complex with ninhydrin in (a) the absence and (b-e) presence of 14-6-14: (b) represents absorbance when the reaction was tried in the presence of 14-6-14 without organic solvents additives; (c) in presence of 14-6-14 and 10.0% DMSO after completion of the reaction; (d) 10.0% DO; (e) 10.0% AN. *Reaction conditions*: $[Cu(II)\text{-Gly-DL-Asp}]^{+}$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, [14-6-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

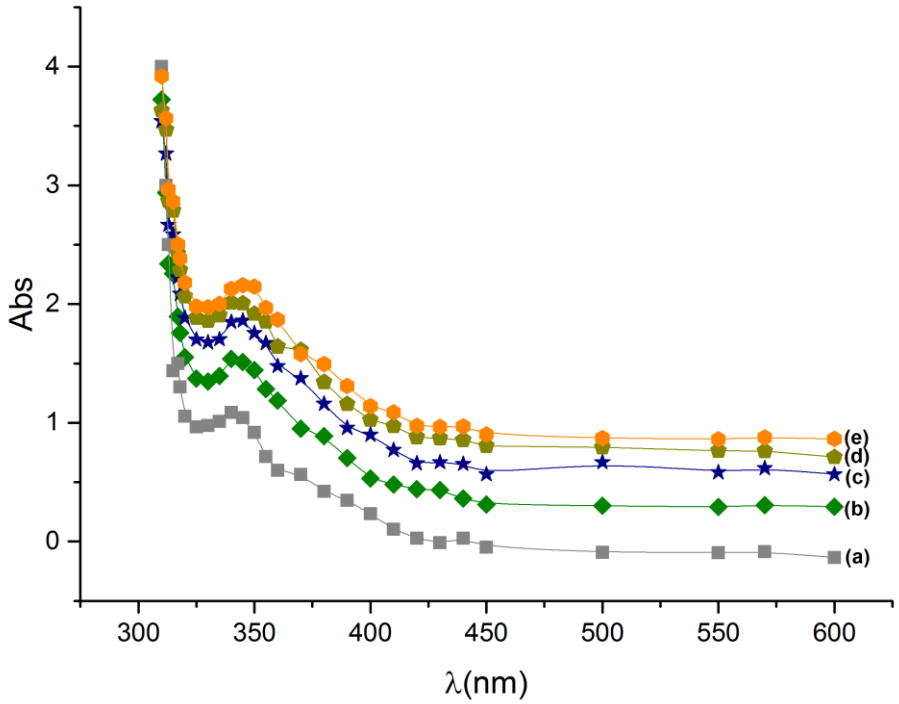



**Figure 2.32:** Absorption spectra of the reaction product of Cu(II)-Gly-DL-Asp complex with ninhydrin in (a) the absence and (b-e) presence of 14-5-14: (b) represents absorbance when the reaction was tried in the presence of 14-5-14 without organic solvents additives; (c) in presence of 14-5-14 and 10.0% DMSO after completion of the reaction; (d) 10.0% DO; (e) 10.0% AN. *Reaction conditions*: $[Cu(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, [14-5-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

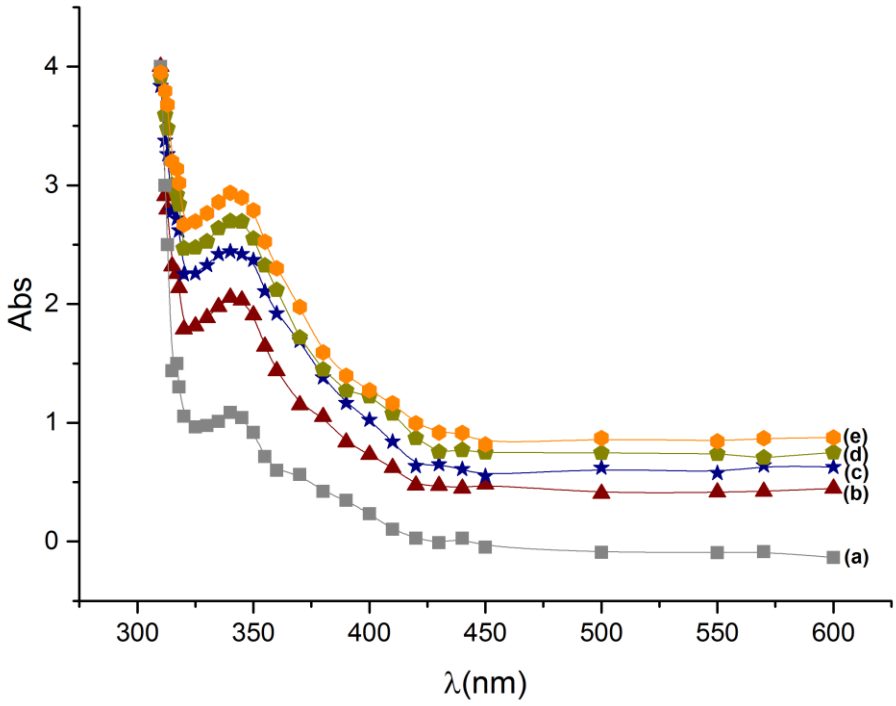



**Figure 2.33:** Absorption spectra of the reaction product of Cu(II)-Gly-DL-Asp complex with ninhydrin in the absence (a) and (b-e) presence of 14-4-14: (b) represents absorbance when the reaction was tried in the presence of 14-4-14 without organic solvents additives; (c) in presence of 14-4-14 and 10.0% DMSO after completion of the reaction; (d) 10.0% DO; (e) 10.0% AN. *Reaction conditions:* $[Cu(II)\text{-Gly-DL-Asp}]^{+}$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, [14-4-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$, temperature = 70 $^{o}$C, *p*H= 5.0.

Lines are drawn as a guide to the eye.

### 2.3.4 Stoichiometric Measurements of the Products Using Job's Method of Continuous Variations

A simple sensitive spectrophotometric method (Job's method of continuous variation) [17] was used to find out the stoichiometry of the reaction products. This method was employed in absence and presence of micelles by taking nine calibrated test-tubes and having 1,2,3,...,9 $cm^3$ of metal ion-coordinated dipeptide solutions in order. Ninhydrin solution of the same molarity was added to the respective test tubes to make the volume 10 $cm^3$. These mixtures were kept in thermostated oil bath at 95 °C for 2 h and then cooled to room temperature. Any loss in the volume was compensated by the addition of the buffer solution, after that their absorbances were recorded at appropriate selected wavelengths of maximum absorption. Absorbances of corresponding concentrations of the metal-dipeptide complex and ninhydrin solutions were also recorded. The difference in absorbance, ΔAbs, where ΔAbs = [absorbance of the product – (absorbance of metal-dipeptide complex + absorbance of ninhydrin)], was obtained for all the sets, which were then plotted against the mole fraction of ninhydrin. Similar steps were repeated in presence of TTABr/14-s-14 micelles or in presence of 10.0% organic solvents. Representative plots are shown in Figures 2.34–2.41 and a summary of the results is presented in Tables 2.3 and 2.4.

The results indicate that the yellow colored reaction products are the same in aqueous, aqueous-organic solvents and micellar media.

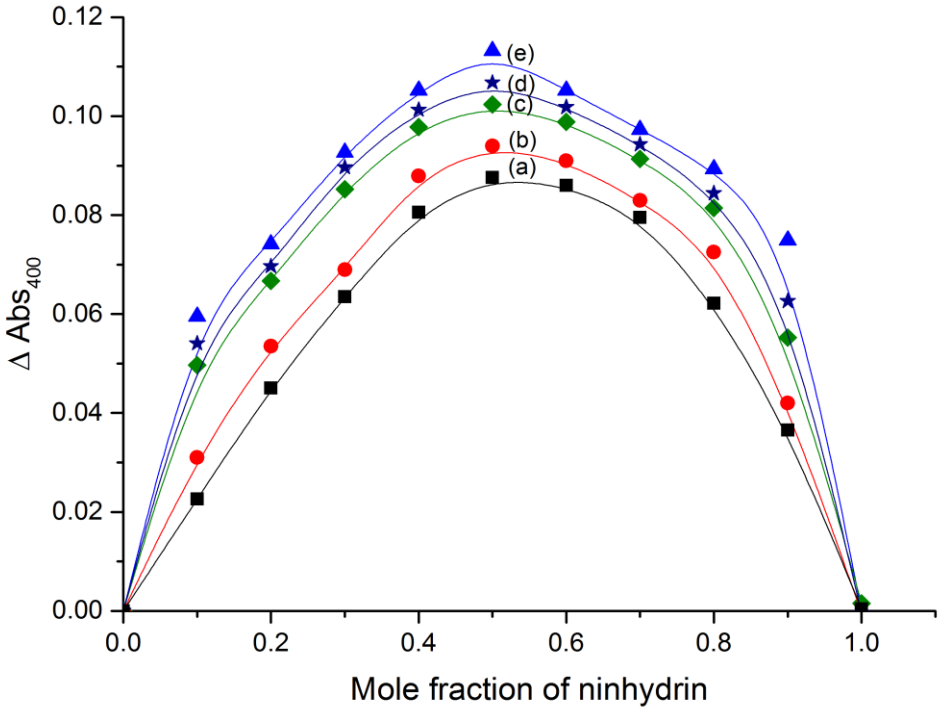



**Figure 2.34:** Plots of $\Delta Abs_{400}$ *vs.* mole fraction of ninhydrin for determination of composition of the product formed by the interaction of Hg(II)-Gly-L-Ala complex with ninhydrin: (a) in aqueous; (b-e) in presence of: (b)14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions:* [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, *p*H=5.0.

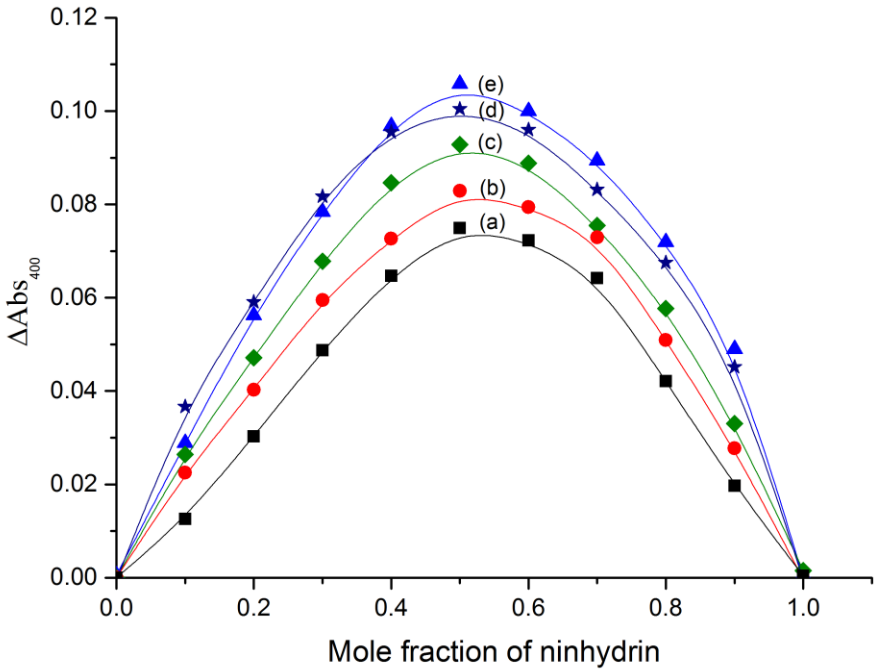



**Figure 2.35:** Plots of $\Delta Abs_{400}$ *vs.* mole fraction of ninhydrin for determination of composition of the product formed by the interaction of Hg(II)-Gly-DL-Asp complex with ninhydrin: (a) in aqueous; (b-e) in presence of: (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions*: [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ (s= 4, 5, 6), [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, *p*H=5.0.

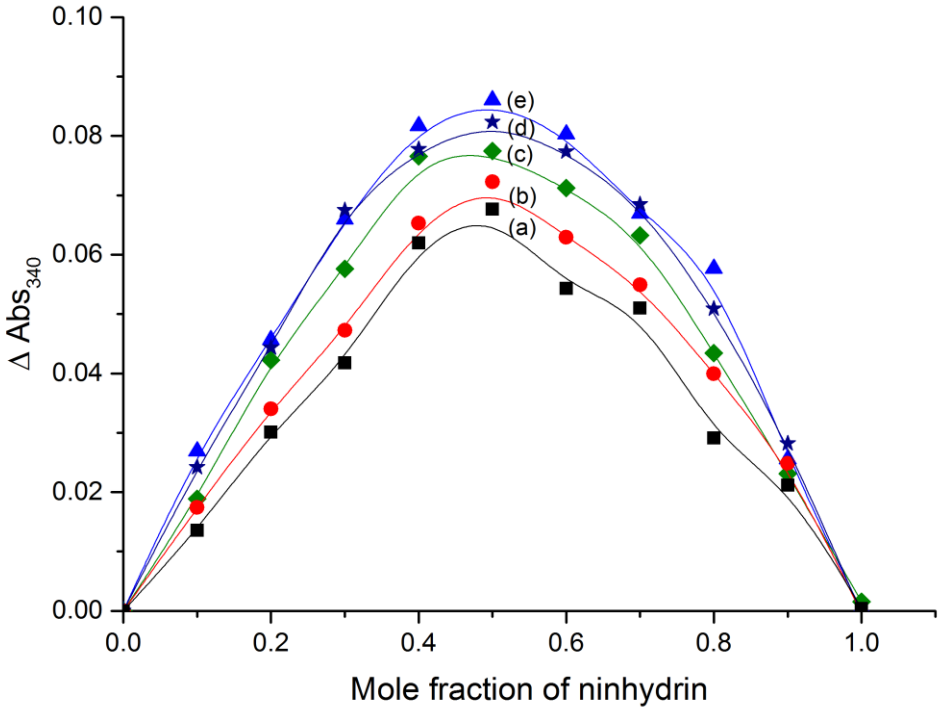



**Figure 2.36:** Plots of $\Delta Abs_{340}$ *vs.* mole fraction of ninhydrin for determination of composition of the product formed by the interaction of Cu(II)-Gly-L-Ala complex with ninhydrin: (a) in aqueous; (b-e) in presence of: (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions:* [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, *p*H=5.0.

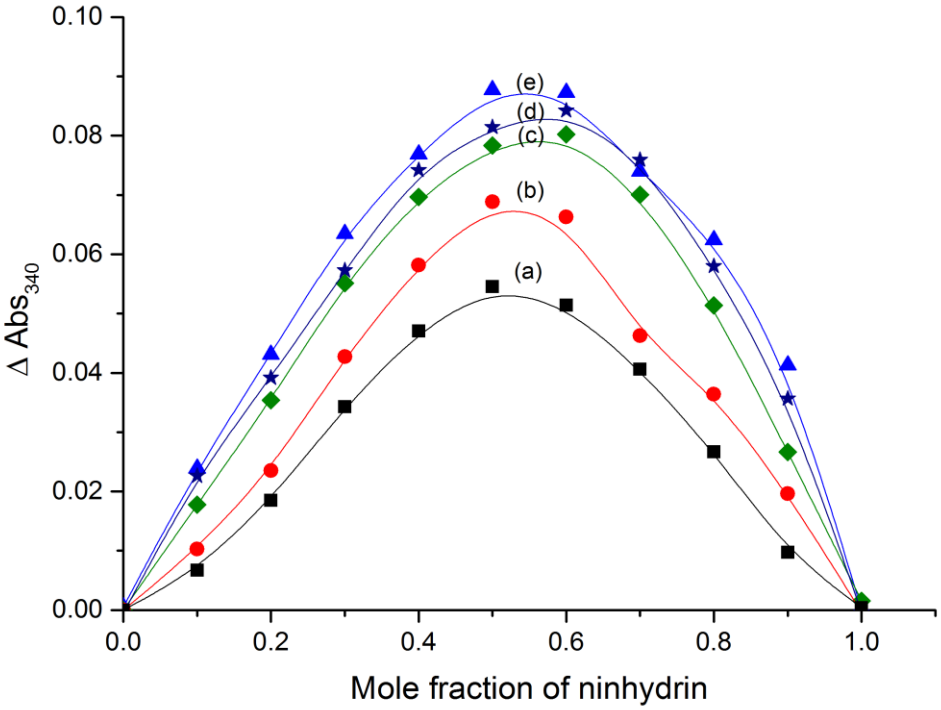



**Figure 2.37:** Plots of $\Delta Abs_{340}$ *vs.* mole fraction of ninhydrin for determination of composition of the product formed by the interaction of Cu(II)-Gly-DL-Asp complex with ninhydrin: (a) in aqueous; (b-e) in presence of: (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions*: [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ (s= 4, 5, 6), [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, *p*H=5.0.

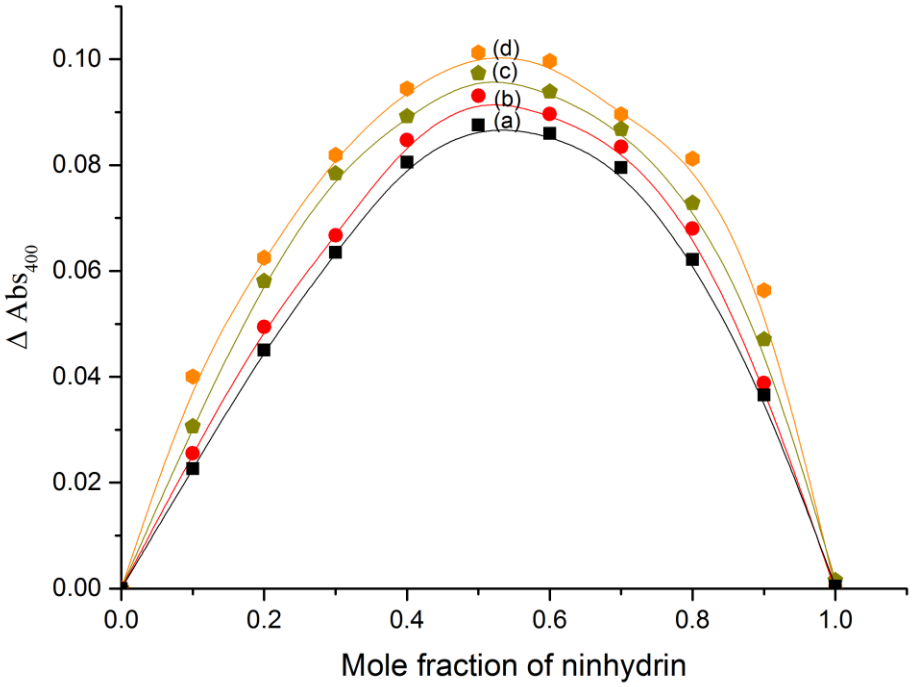



**Figure 2.38:** Plots of $\Delta Abs_{400}$ *vs.* mole fraction of ninhydrin for determination of composition of the product formed by the interaction of Hg(II)-Gly-L-Ala complex with ninhydrin: (a) in aqueous; (b-d) in presence of organic solvents: (b) DMSO; (c) DO; (d) AN; *Reaction conditions:* 10.0% organic solvent (v/v), *p*H=5.0.

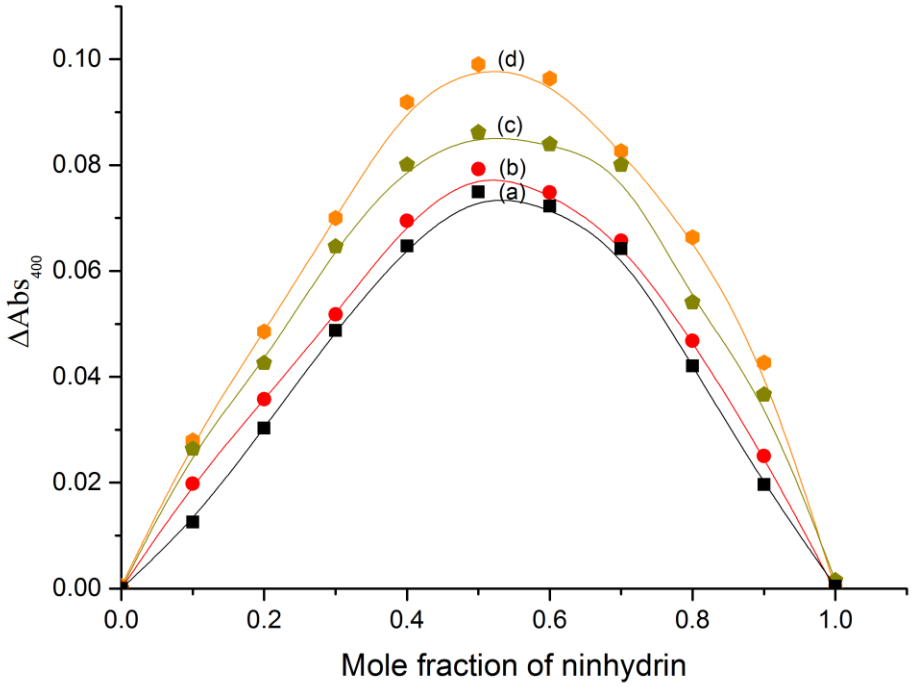



**Figure 2.39:** Plots of $\Delta Abs_{400}$ *vs.* mole fraction of ninhydrin for determination of composition of the product formed by the interaction of Hg(II)-Gly-DL-Asp complex with ninhydrin: (a) in aqueous; (b-d) in presence of organic solvents: (b) DMSO; (c) DO; (d) AN; *Reaction conditions:* 10.0% organic solvent (v/v), *p*H=5.0.

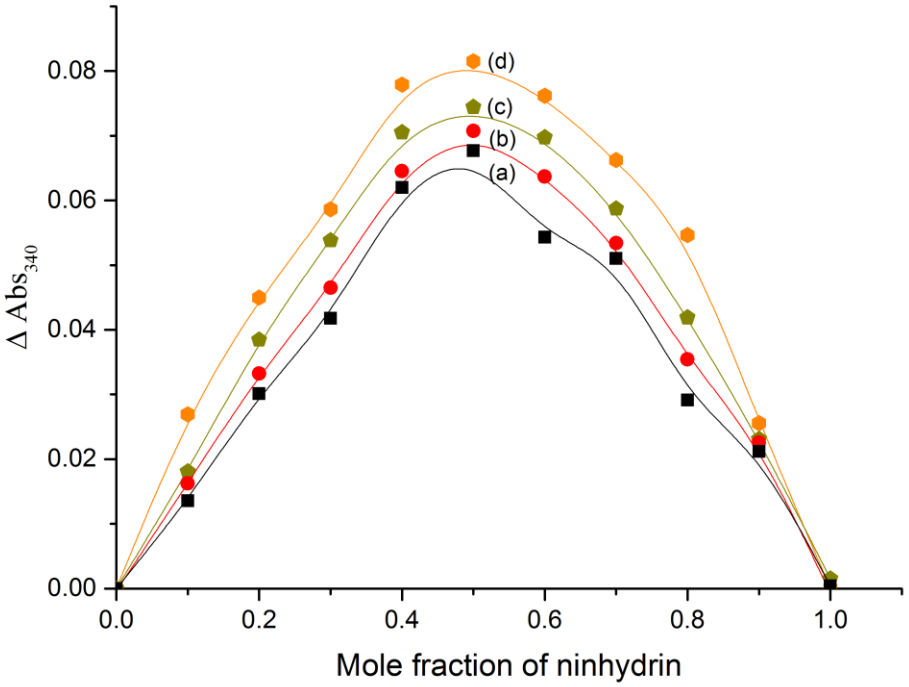



**Figure 2.40:** Plots of $\Delta Abs_{340}$ *vs.* mole fraction of ninhydrin for determination of composition of the product formed by the interaction of Cu(II)-Gly-L-Ala complex with ninhydrin: (a) in aqueous; (b-d) in presence of organic solvents: (b) DMSO; (c) DO; (d) AN; *Reaction conditions:* 10.0% organic solvent (v/v), *p*H=5.0.

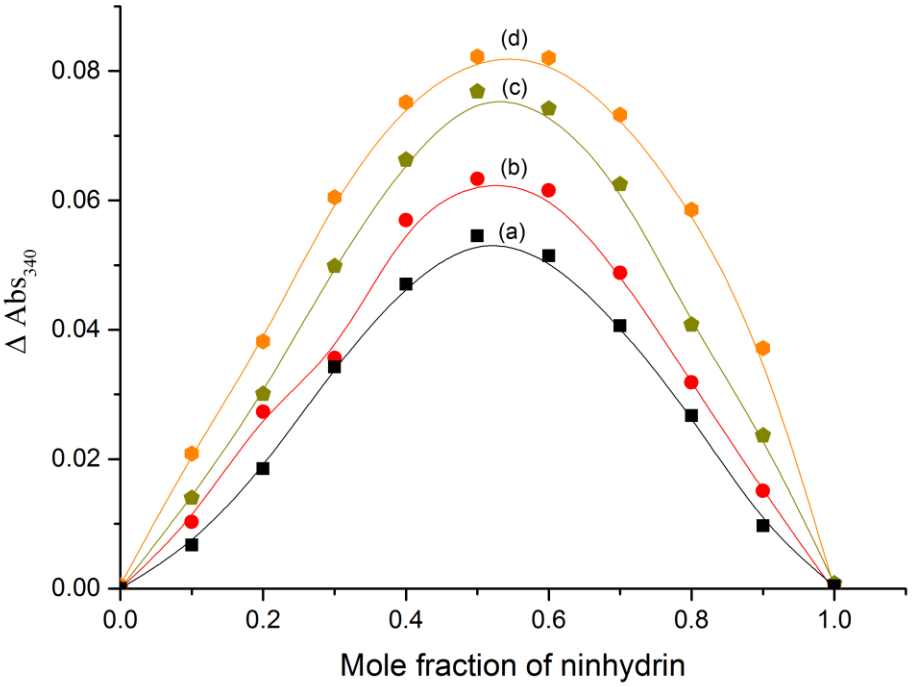



**Figure 2.41:** Plots of $\Delta Abs_{340}$ *vs.* mole fraction of ninhydrin for determination of composition of the product formed by the interaction of Cu(II)-Gly-DL-Asp complex with ninhydrin: (a) in aqueous; (b-d) in presence of organic solvents: (b) DMSO; (c) DO; (d) AN; *Reaction conditions:* 10.0% organic solvent (v/v), *p*H=5.0.

**Table 2.3:** Summary of the results of Job's method in aqueous and micellar media.

| Complex | Composition | | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-14 | 14-5-14 | 14-4-14 |
| [Hg(II)-Gly-L-Ala]$^{+}$–ninhydrin | 1:1 | 1:1 | 1:1 | 1:1 | 1:1 |
| [Hg(II)-Gly-DL-Asp]$^{+}$–ninhydrin | 1:1 | 1:1 | 1:1 | 1:1 | 1:1 |
| [Cu(II)-Gly-L-Ala]$^{+}$–ninhydrin | 1:1 | 1:1 | 1:1 | 1:1 | 1:1 |
| [Cu(II)-Gly-DL-Asp]$^{+}$–ninhydrin | 1:1 | 1:1 | 1:1 | 1:1 | 1:1 |

**Table 2.4:** Summary of the results of Job's method in aqueous and aqueous-organic solvent mixed systems.

| Complex | Composition | | | |
|---|---|---|---|---|
| | Aqueous | 10% DMSO | 10% DO | 10% AN |
| [Hg(II)-Gly-L-Ala]$^{+}$–ninhydrin | 1:1 | 1:1 | 1:1 | 1:1 |
| [Hg(II)-Gly-DL-Asp]$^{+}$–ninhydrin | 1:1 | 1:1 | 1:1 | 1:1 |
| [Cu(II)-Gly-L-Ala]$^{+}$–ninhydrin | 1:1 | 1:1 | 1:1 | 1:1 |
| [Cu(II)-Gly-DL-Asp]$^{+}$–ninhydrin | 1:1 | 1:1 | 1:1 | 1:1 |

### 2.3.5 Kinetic Measurements

In each kinetic run, the solution of dipeptide along with other reagents (when required) was prepared *in situ* by taking required volumes of it in a three-necked reaction vessel having provision for $N_2$-gas inlet/outlet and equipped with a double-surface water condenser to prevent evaporation. The reaction vessel was then immersed in a thermostated oil bath at the desired temperature within ± 0.1 °C. The reaction was initiated by adding the requisite volumes of thermally equilibrated ninhydrin solution. The zero time was recorded when half of the ninhydrin solution had been added. A slow stream of pure $N_2$-gas (free from $O_2$ and $CO_2$) was bubbled through the reaction mixture for stirring as well as to maintain an inert atmosphere. The progress of the reaction was followed spectrophotometrically by pipetting out aliquots at various time intervals and measuring the absorbance of yielded product at the selected wavelength ($\lambda_{max}$). *Pseudo*-first-order conditions were maintained by keeping the [ninhydrin] in excess (≥10 times). Values of *pseudo*-first-order rate constants ($k_{obs}$ in aqueous and $k_{\psi}$ in micellar media) were obtained up to completion of 80% of the reaction from plots of log $(Abs_{\infty} - Abs_t)/(Abs_o - Abs_t)$ *vs.* time (t) by a least-squares regression analysis of the data with the help of computer-based program. The values of absorbance at infinite time ($Abs_{\infty}$) for each system were obtained in the following manner. At the end of each kinetic run, 10 $cm^3$ of the solution mixture (after taking into a standard volumetric flask) was boiled for 2 min. It was then cooled to room temperature and, after adding buffer solution to compensate any volume loss, the complete absorbance spectrum was then recorded. The rate constants obtained from replicate kinetics runs agreed within ± 4%.

The dependence of *pseudo*-first-order rate constants was obtained as a function of [dipeptide], [ninhydrin], [surfactant], % organic solvents (v/v), *p*H, and temperature and the results are given in Chapters III and IV.

### 2.3.6 Conductance Measurements

To find out the critical micelle concentrations (CMC) of the surfactant solutions, conductivity measurements were employed at the desired temperature using a Systronics conductivity meter model 306 (India) equipped with a calibrated dip cell (cell constant 1.0 $cm^{-1}$). The conductivity measurements were carried out by adding progressively small concentrated surfactant stock solution into the thermostated solvent of known conductivity. The temperature of the system was kept at the desired point (± 0.1 $^{o}$C) by circulating water through jacketed container holding the solution under study. The conductivity was noted after each addition after ensuring complete mixing. The specific conductivity (κ, $\Omega^{-1}$ $cm^{-1}$) was calculated by applying solvent corrections. The CMC values of the surfactant solutions in absence and presence of reactants were obtained from the intersection of the two straight lines drawn before and after the break in the $\kappa$ vs. surfactant concentration plot [18]. The measurements were made at 30 $^{o}$C and 70 $^{o}$C under different conditions, *i.e.*, solvent being water, water + ninhydrin, water + dipeptide, water + metal ion, water + metal ion-dipeptide complex, and water + metal ion-dipeptide complex + ninhydrin (Tables 2.5-2.8). For the binary mixture of water-organic solvent systems, the values of CMC are recorded in Tables 2.9-2.12.

**Table 2.5:** Critical micelle concentration values of TTABr in the absence and presence of reactants at 30 $^{o}$C and 70 $^{o}$C.

| Solution | $10^3$ CMC (mol dm$^{-3}$) | |
|---|---|---|
| | 30 $^{o}$C | 70 $^{o}$C |
| Water | 3.900 | 5.110 |
| Gly-L-Ala | 3.800 | 4.302 |
| Gly-DL-Asp | 3.491 | 4.022 |
| Ninhydrin | 4.322 | 5.404 |
| Gly-L-Ala + ninhydrin | 4.251 | 5.528 |
| Gly-DL-Asp + ninhydrin | 3.953 | 4.741 |
| Hg (II) | 3.958 | 5.501 |
| Cu (II) | 3.262 | 4.082 |
| [Hg (II)-Gly-L-Ala]$^{+}$ | 3.914 | 5.173 |
| [Cu (II)-Gly-L-Ala]$^{+}$ | 2.768 | 4.211 |
| [Hg (II)-Gly-DL-Asp]$^{+}$ | 3.842 | 4.880 |
| [Cu (II)-Gly-L-Asp]$^{+}$ | 2.982 | 4.580 |
| [Hg (II)-Gly-L-Ala]$^{+}$ + ninhydrin | 4.187 | 5.420 |
| [Cu (II)-Gly-L-Ala]$^{+}$+ ninhydrin | 3.960 | 5.243 |
| [Hg (II)-Gly-DL-Asp]$^{+}$ + ninhydrin | 4.561 | 6.052 |
| [Cu (II)-Gly-DL-Asp]$^{+}$+ ninhydrin | 4.452 | 5.811 |

**Table 2.6:** Critical micelle concentration values of 14-6-14 in the absence and presence of reactants at 30 $^{o}$C and 70 $^{o}$C.

| Solution | $10^3$ CMC (mol dm$^{-3}$) | |
|---|---|---|
| | 30 $^{o}$C | 70 $^{o}$C |
| Water | 0.162 | 0.296 |
| Gly-L-Ala | 0.156 | 0.279 |
| Gly-DL-Asp | 0.150 | 0.276 |
| Ninhydrin | 0.170 | 0.322 |
| Gly-L-Ala + ninhydrin | 0.178 | 0.330 |
| Gly-DL-Asp + ninhydrin | 0.169 | 0.293 |
| Hg (II) | 0.163 | 0.301 |
| Cu (II) | 0.160 | 0.290 |
| [Hg (II)-Gly-L-Ala]$^{+}$ | 0.164 | 0.315 |
| [Cu (II)-Gly-L-Ala]$^{+}$ | 0.157 | 0.301 |
| [Hg (II)-Gly-DL-Asp]$^{+}$ | 0.156 | 0.293 |
| [Cu (II)-Gly-L-Asp]$^{+}$ | 0.145 | 0.290 |
| [Hg (II)-Gly-L-Ala]$^{+}$ + ninhydrin | 0.166 | 0.323 |
| [Cu (II)-Gly-L-Ala]$^{+}$+ ninhydrin | 0.163 | 0.310 |
| [Hg (II)-Gly-DL-Asp]$^{+}$ + ninhydrin | 0.179 | 0.360 |
| [Cu (II)-Gly-DL-Asp]$^{+}$+ ninhydrin | 0.177 | 0.348 |

**Table 2.7**: Critical micelle concentration values of 14-5-14 in the absence and presence of reactants at 30 $^{o}$C and 70 $^{o}$C.

| Solution | $10^3$ CMC (mol dm$^{-3}$) | |
|---|---|---|
| | 30 $^{o}$C | 70 $^{o}$C |
| Water | 0.145 | 0.287 |
| Gly-L-Ala | 0.140 | 0.269 |
| Gly-DL-Asp | 0.136 | 0.258 |
| Ninhydrin | 0.153 | 0.318 |
| Gly-L-Ala + ninhydrin | 0.156 | 0.314 |
| Gly-DL-Asp + ninhydrin | 0.148 | 0.278 |
| Hg (II) | 0.147 | 0.293 |
| Cu (II) | 0.146 | 0.280 |
| [Hg (II)-Gly-L-Ala]$^{+}$ | 0.146 | 0.290 |
| [Cu (II)-Gly-L-Ala]$^{+}$ | 0.141 | 0.280 |
| [Hg (II)-Gly-DL-Asp]$^{+}$ | 0.143 | 0.283 |
| [Cu (II)-Gly-L-Asp]$^{+}$ | 0.135 | 0.271 |
| [Hg (II)-Gly-L-Ala]$^{+}$ + ninhydrin | 0.152 | 0.311 |
| [Cu (II)-Gly-L-Ala]$^{+}$+ ninhydrin | 0.150 | 0.290 |
| [Hg (II)-Gly-DL-Asp]$^{+}$ + ninhydrin | 0.161 | 0.328 |
| [Cu (II)-Gly-DL-Asp]$^{+}$+ ninhydrin | 0.158 | 0.320 |

**Table 2.8:** Critical micelle concentration values of 14-4-14 in the absence and presence of reactants at 30 $^{o}$C and 70 $^{o}$C.

| Solution | $10^3$ CMC (mol dm$^{-3}$) | |
|---|---|---|
| | 30 $^{o}$C | 70 $^{o}$C |
| Water | 0.137 | 0.273 |
| Gly-L-Ala | 0.130 | 0.251 |
| Gly-DL-Asp | 0.112 | 0.217 |
| Ninhydrin | 0.150 | 0.301 |
| Gly-L-Ala + ninhydrin | 0.146 | 0.298 |
| Gly-DL-Asp + ninhydrin | 0.130 | 0.266 |
| Hg (II) | 0.140 | 0.300 |
| Cu (II) | 0.125 | 0.217 |
| [Hg (II)-Gly-L-Ala]$^{+}$ | 0.139 | 0.275 |
| [Cu (II)-Gly-L-Ala]$^{+}$ | 0.128 | 0.240 |
| [Hg (II)-Gly-DL-Asp]$^{+}$ | 0.136 | 0.268 |
| [Cu (II)-Gly-L-Asp]$^{+}$ | 0.110 | 0.237 |
| [Hg (II)-Gly-L-Ala]$^{+}$ + ninhydrin | 0.146 | 0.304 |
| [Cu (II)-Gly-L-Ala]$^{+}$+ ninhydrin | 0.140 | 0.298 |
| [Hg (II)-Gly-DL-Asp]$^{+}$ + ninhydrin | 0.156 | 0.317 |
| [Cu (II)-Gly-DL-Asp]$^{+}$+ ninhydrin | 0.152 | 0.307 |

**Table 2.9:** Critical micelle cocentration values of TTABr in the absence and presence of reactants at 30 $^{o}$C and 70 $^{o}$C with composition of organic solvents (% v/v).

| Solution | $10^3$ CMC (mol dm$^{-3}$) | | | | | |
|---|---|---|---|---|---|---|
| | In DMSO-$H_2O$ mixed system | | In DO-$H_2O$ mixed system | | In AN-$H_2O$ mixed system | |
| | 30 $^{o}$C | 70 $^{o}$C | 30 $^{o}$C | 70 $^{o}$C | 30 $^{o}$C | 70 $^{o}$C |
| Water+ org. solvent [(a)] | 4.500 | 8.121 | 5.648 | 11.892 | 6.108 | 12.561 |
| Water+ org. solvent [(b)] | 7.161 | 13.532 | 8.108 | 16.629 | 10.881 | 20.652 |
| Gly-L-Ala [(a)] | 4.311 | 7.900 | 5.509 | 11.737 | 6.042 | 12.302 |
| Gly-L-Ala [(b)] | 6.950 | 13.211 | 8.081 | 16.478 | 10.671 | 20.309 |
| Gly-DL-Asp [(a)] | 4.201 | 7.783 | 5.412 | 11.551 | 5.902 | 12.002 |
| Gly-DL-Asp [(b)] | 6.800 | 13.004 | 7.930 | 16.412 | 10.411 | 20.321 |
| Ninhydrin [(a)] | 4.692 | 8.382 | 5.852 | 12.201 | 6.352 | 12.903 |
| Ninhydrin [(b)] | 7.312 | 13.771 | 8.253 | 16.913 | 11.223 | 21.234 |
| Gly-L-Ala + ninhydrin [(a)] | 4.601 | 8.253 | 5.734 | 12.212 | 6.250 | 12.702 |
| Gly-L-Ala + ninhydrin [(b)] | 7.221 | 13.689 | 8.201 | 16.821 | 11.103 | 20.901 |
| Gly-DL-Asp + ninhydrin [(a)] | 4.573 | 8.201 | 5.687 | 12.104 | 6.202 | 12.614 |
| Gly-DL-Asp + ninhydrin [(b)] | 7.181 | 13.609 | 8.146 | 16.708 | 10.931 | 20.800 |

*contd…*

| | | | | | | |
|---|---|---|---|---|---|---|
| Hg (II) [(a)] | 4.601 | 8.208 | 5.920 | 12.101 | 6.302 | 12.712 |
| Cu (II) [(a)] | 4.303 | 7.900 | 5.702 | 11.123 | 5.975 | 12.401 |
| [Hg (II)-Gly-L-Ala]$^{+}$ [(a)] | 4.574 | 8.202 | 5.701 | 12.212 | 6.202 | 12.754 |
| [Cu (II)-Gly-L-Ala]$^{+}$ [(a)] | 4.032 | 7.801 | 5.468 | 11.724 | 6.034 | 12.473 |
| [Hg (II)-Gly-DL-Asp]$^{+}$ [(a)] | 4.581 | 8.303 | 5.601 | 11.800 | 6.082 | 12.402 |
| [Cu (II)-Gly-L-Asp]$^{+}$ [(a)] | 3.950 | 7.702 | 5.547 | 11.707 | 5.903 | 12.011 |
| [Hg (II)-Gly-L-Ala]$^{+}$ + ninhydrin [(a)] | 4.624 | 8.301 | 5.778 | 12.139 | 6.266 | 12.812 |
| [Cu (II)-Gly-L-Ala]$^{+}$+ ninhydrin [(a)] | 4.550 | 8.169 | 5.701 | 12.028 | 6.165 | 12.624 |
| [Hg (II)-Gly-DL-Asp]$^{+}$ + ninhydrin [(a)] | 4.722 | 8.408 | 6.026 | 12.300 | 6.390 | 12.932 |
| [Cu (II)-Gly-DL-Asp]$^{+}$+ ninhydrin [(a)] | 4.628 | 8.322 | 5.902 | 12.222 | 6.212 | 12.840 |

(a) 10.0% organic solvent (v/v)

(b) 20.0% organic solvent (v/v)

**Table 2.10:** Critical micelle cocentration values of 14-6-14 in the absence and presence of reactants at 30 $^{o}$C and 70 $^{o}$C with composition of organic solvents (% v/v).

| Solution | $10^3$ CMC (mol dm$^{-3}$) | | | | | |
|---|---|---|---|---|---|---|
| | In DMSO-$H_2O$ mixed system | | In DO-$H_2O$ mixed systeem | | In AN-$H_2O$ mixed system | |
| | 30 $^{o}$C | 70 $^{o}$C | 30 $^{o}$C | 70 $^{o}$C | 30 $^{o}$C | 70 $^{o}$C |
| Water+ org. solvent [(a)] | 0.401 | 0.750 | 0.485 | 1.028 | 0.503 | 1.128 |
| Water+ org. solvent [(b)] | 0.608 | 1.130 | 0.874 | 1.744 | 1.231 | 2.398 |
| Gly-L-Ala [(a)] | 0.393 | 0.729 | 0.477 | 1.017 | 0.540 | 1.121 |
| Gly-L-Ala [(b)] | 0.599 | 0.997 | 0.867 | 1.735 | 1.228 | 2.302 |
| Gly-DL-Asp [(a)] | 0.389 | 0.721 | 0.470 | 1.012 | 0.533 | 1.116 |
| Gly-DL-Asp [(b)] | 0.588 | 0.989 | 0.865 | 1.729 | 1.422 | 2.250 |
| Ninhydrin [(a)] | 0.413 | 0.773 | 0.501 | 1.249 | 0.564 | 1.358 |
| Ninhydrin [(b)] | 0.619 | 1.340 | 0.890 | 1.760 | 1.636 | 2.610 |
| Gly-L-Ala + ninhydrin [(a)] | 0.410 | 0.771 | 0.498 | 1.201 | 0.569 | 1.381 |
| Gly-L-Ala + ninhydrin [(b)] | 0.608 | 1.330 | 0.879 | 1.750 | 1.655 | 2.602 |
| Gly-DL-Asp + ninhydrin [(a)] | 0.407 | 0.751 | 0.497 | 1.043 | 0.547 | 1.345 |
| Gly-DL-Asp + ninhydrin [(b)] | 0.602 | 1.321 | 0.872 | 1.744 | 1.620 | 2.580 |

*contd…*

| | | | | | | |
|---|---|---|---|---|---|---|
| Hg (II) [(a)] | 0.405 | 0.757 | 0.491 | 1.066 | 0.514 | 1.142 |
| Cu (II) [(a)] | 0.400 | 0.747 | 0.490 | 1.020 | 0.509 | 1.130 |
| [Hg (II)-Gly-L-Ala]$^{+}$ [(a)] | 0.406 | 0.759 | 0.496 | 1.073 | 0.520 | 1.153 |
| [Cu (II)-Gly-L-Ala]$^{+}$ [(a)] | 0.401 | 0.752 | 0.487 | 1.025 | 0.502 | 1.124 |
| [Hg (II)-Gly-DL-Asp]$^{+}$ [(a)] | 0.402 | 0.748 | 0.483 | 1.022 | 0.498 | 1.120 |
| [Cu (II)-Gly-L-Asp]$^{+}$ [(a)] | 0.398 | 0.745 | 0.481 | 1.018 | 0.495 | 1.101 |
| [Hg (II)-Gly-L-Ala]$^{+}$ + ninhydrin [(a)] | 0.410 | 0.768 | 0.498 | 1.245 | 0.561 | 1.353 |
| [Cu (II)-Gly-L-Ala]$^{+}$+ ninhydrin [(a)] | 0.403 | 0.754 | 0.491 | 1.133 | 0.540 | 1.220 |
| [Hg (II)-Gly-DL-Asp]$^{+}$+ ninhydrin [(a)] | 0.419 | 0.780 | 0.511 | 1.254 | 0.570 | 1.364 |
| [Cu (II)-Gly-DL-Asp]$^{+}$+ ninhydrin [(a)] | 0.410 | 0.771 | 0.501 | 1.249 | 0.562 | 1.356 |

(a) 10.0% organic solvent (v/v)

(b) 20.0% organic solvent (v/v)

**Table 2.11:** Critical micelle cocentration values of 14-5-14 in the absence and presence of reactants at 30 $^{o}$C and 70 $^{o}$C with composition of organic solvents (% v/v).

| Solution | $10^3$ CMC (mol dm$^{-3}$) | | | | | |
|---|---|---|---|---|---|---|
| | In DMSO-$H_2O$ mixed system | | In DO-$H_2O$ mixed system | | In AN-$H_2O$ mixed system | |
| | 30 $^{o}$C | 70 $^{o}$C | 30 $^{o}$C | 70 $^{o}$C | 30 $^{o}$C | 70 $^{o}$C |
| Water+ org. solvent [(a)] | 0.383 | 0.735 | 0.391 | 0.932 | 0.402 | 0.967 |
| Water+ org. solvent [(b)] | 0.542 | 1.063 | 0.764 | 1.637 | 1.193 | 1.752 |
| Gly-L-Ala [(a)] | 0.375 | 0.715 | 0.387 | 0.936 | 0.398 | 0.964 |
| Gly-L-Ala [(b)] | 0.533 | 0.932 | 0.755 | 1.623 | 1.193 | 1.660 |
| Gly-DL-Asp [(a)] | 0.371 | 0.709 | 0.383 | 0.922 | 0.394 | 0.957 |
| Gly-DL-Asp [(b)] | 0.522 | 0.922 | 0.744 | 1.617 | 1.186 | 1.607 |
| Ninhydrin [(a)] | 0.393 | 0.758 | 0.425 | 0.969 | 0.461 | 1.194 |
| Ninhydrin [(b)] | 0.554 | 1.276 | 0.776 | 1.649 | 1.599 | 1.965 |
| Gly-L-Ala + ninhydrin [(a)] | 0.391 | 0.752 | 0.417 | 0.964 | 0.467 | 1.223 |
| Gly-L-Ala + ninhydrin [(b)] | 0.548 | 1.272 | 0.771 | 1.643 | 1.614 | 1.974 |
| Gly-DL-Asp + ninhydrin [(a)] | 0.385 | 0.738 | 0.411 | 0.951 | 0.448 | 1.187 |
| Gly-DL-Asp + ninhydrin [(b)] | 0.543 | 1.111 | 0.769 | 1.672 | 1.585 | 1.936 |

*contd…*

| | | | | | | |
|---|---|---|---|---|---|---|
| Hg (II) [a] | 0.387 | 0.744 | 0.401 | 0.951 | 0.411 | 0.974 |
| Cu (II) [a] | 0.382 | 0.734 | 0.395 | 0.942 | 0.406 | 0.970 |
| [Hg (II)-Gly-L-Ala]$^{+}$ [a] | 0.388 | 0.746 | 0.398 | 0.943 | 0.409 | 0.974 |
| [Cu (II)-Gly-L-Ala]$^{+}$ [a] | 0.383 | 0.739 | 0.391 | 0.933 | 0.403 | 0.968 |
| [Hg (II)-Gly-DL-Asp]$^{+}$ [a] | 0.382 | 0.735 | 0.396 | 0.939 | 0.408 | 0.972 |
| [Cu (II)-Gly-L-Asp]$^{+}$ [a] | 0.380 | 0.732 | 0.390 | 0.929 | 0.401 | 0.967 |
| [Hg (II)-Gly-L-Ala]$^{+}$ + ninhydrin [a] | 0.392 | 0.755 | 0.423 | 0.966 | 0.459 | 1.190 |
| [Cu (II)-Gly-L-Ala]$^{+}$+ ninhydrin [a] | 0.385 | 0.741 | 0.417 | 0.950 | 0.450 | 1.187 |
| [Hg (II)-Gly-DL-Asp]$^{+}$ + ninhydrin [a] | 0.401 | 0.767 | 0.429 | 0.972 | 0.471 | 1.201 |
| [Cu (II)-Gly-DL-Asp]$^{+}$+ ninhydrin [a] | 0.392 | 0.758 | 0.425 | 0.964 | 0.465 | 1.169 |

(a) 10.0% organic solvent (v/v)

(b) 20.0% organic solvent (v/v)

**Table 2.12:** Critical micelle cocentration values of 14-4-14 in the absence and presence of reactants at 30 $^{o}$C and 70 $^{o}$C with composition of organic solvents (% v/v).

| Solution | $10^3$ CMC (mol dm$^{-3}$) | | | | | |
|---|---|---|---|---|---|---|
| | In DMSO-$H_2O$ mixed system | | In DO-$H_2O$ mixed system | | In AN-$H_2O$ mixed system | |
| | 30 $^{o}$C | 70 $^{o}$C | 30 $^{o}$C | 70 $^{o}$C | 30 $^{o}$C | 70 $^{o}$C |
| Water+ org. solvent (a) | 0.322 | 0.687 | 0.336 | 0.701 | 0.355 | 0.755 |
| Water+ org. solvent [(b)] | 0.528 | 0.981 | 0.684 | 1.028 | 0.983 | 1.164 |
| Gly-L-Ala [(a)] | 0.314 | 0.669 | 0.331 | 0.708 | 0.349 | 0.751 |
| Gly-L-Ala [(b)] | 0.510 | 0.929 | 0.679 | 1.019 | 0.984 | 1.140 |
| Gly-DL-Asp [(a)] | 0.311 | 0.653 | 0.327 | 0.692 | 0.347 | 0.743 |
| Gly-DL-Asp [(b)] | 0.497 | 0.917 | 0.662 | 1.011 | 0.977 | 1.121 |
| Ninhydrin [(a)] | 0.341 | 0.699 | 0.353 | 0.737 | 0.391 | 0.811 |
| Ninhydrin [(b)] | 0.543 | 1.195 | 0.696 | 1.055 | 1.322 | 1.405 |
| Gly-L-Ala + ninhydrin [(a)] | 0.336 | 0.711 | 0.347 | 0.734 | 0.422 | 0.847 |
| Gly-L-Ala + ninhydrin [(b)] | 0.538 | 1.192 | 0.693 | 1.033 | 1.340 | 1.411 |
| Gly-DL-Asp + ninhydrin [(a)] | 0.326 | 0.691 | 0.342 | 0.721 | 0.376 | 0.803 |
| Gly-DL-Asp + ninhydrin [(b)] | 0.531 | 1.071 | 0.685 | 1.134 | 1.223 | 1.392 |

*contd…*

| | | | | | | |
|---|---|---|---|---|---|---|
| Hg (II) [(a)] | 0.325 | 0.692 | 0.342 | 0.719 | 0.373 | 0.765 |
| Cu (II) [(a)] | 0.323 | 0.685 | 0.338 | 0.713 | 0.359 | 0.761 |
| [Hg (II)-Gly-L-Ala]$^{+}$ [(a)] | 0.329 | 0.694 | 0.342 | 0.712 | 0.364 | 0.764 |
| [Cu (II)-Gly-L-Ala]$^{+}$ [(a)] | 0.323 | 0.687 | 0.336 | 0.704 | 0.357 | 0.759 |
| [Hg (II)-Gly-DL-Asp]$^{+}$ [(a)] | 0.321 | 0.684 | 0.338 | 0.708 | 0.362 | 0.761 |
| [Cu (II)-Gly-L-Asp]$^{+}$ [(a)] | 0.319 | 0.681 | 0.335 | 0.698 | 0.353 | 0.756 |
| [Hg (II)-Gly-L-Ala]$^{+}$ + ninhydrin [(a)] | 0.342 | 0.714 | 0.353 | 0.733 | 0.404 | 0.806 |
| [Cu (II)-Gly-L-Ala]$^{+}$+ ninhydrin [(a)] | 0.326 | 0.690 | 0.351 | 0.717 | 0.401 | 0.801 |
| [Hg (II)-Gly-DL-Asp]$^{+}$ + ninhydrin [(a)] | 0.351 | 0.729 | 0.359 | 0.736 | 0.421 | 0.853 |
| [Cu (II)-Gly-DL-Asp]$^{+}$+ ninhydrin [(a)] | 0.339 | 0.722 | 0.355 | 0.728 | 0.409 | 0.844 |

(a) 10.0% organic solvent (v/v)

(b) 20.0% organic solvent (v/v)

## 2.4 References

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# CHAPTER-III

# Kinetics of Dipeptide–Ninhydrin Reactions in Aqueous and Micellar Systems and Effect of organic Solvents

## 3.1 Introduction

Ninhydrin reacts with several amino acids/dipeptides (except proline and hydroxyproline as their amino group is involved in the ring formation) with different rate but all give the same purple-colored product (*i.e.*, *Ruhemann*'s *purple*)[1-3]. In our case, spectra of yielded product of dipeptides (*i.e.* Gly-L-Ala and Gly-DL-Asp) with ninhydrin reaction in desired *p*H solutions (5.0 or any, as required) have been taken in aqueous and in TTABr/14-s-14 micellar systems. Increasing absorbance has been noticed parallel with increasing the concentration of investigated surfactants. The absorption spectra of mixtures containing the reactants in presence of surfactants exhibited no shift in the absorption maxima (570 nm) as that of a solution of *Ruhemann*'s *purple* in aqueous system. This implies that the reaction between dipeptides and ninhydrin gives the same product in both systems. Thus, maximum absorbance ($\lambda_{max}$ =570 nm) is used for qualitative and quantitative studies.

Surfactant micelles (formed by self-aggregation of surfactant monomers under appropriate solution conditions) are in dynamic equilibrium with soluble monomeric species. It is known that micellar solutions affect the rates of chemical reactions and the position of chemical equilibria [4-10]. In many cases, all kinds of chemical reaction rates and pathways can be altered by carrying out the reactions in micellar systems instead of pure bulk solvents. Kinetic studies have earlier been carried out to explore the usefulness of micellar systems for organic synthesis, to explain the factors that influence the course of reactions and rates, and to gain insight into the exceptional catalytic characteristics of enzymatic reactions [11]. Enhancing the reactions by micelles can be achieved in which interactions between the micelles and the reacting species affect the kinetics; the micelles are reagents; and the micelles carry catalytically active substituents [12]. In non-aqueous micellar systems, selection of the solvent is a vital factor for controlling the reaction rate by increasing the solubility of insoluble/poorly soluble substrates in water [13].

Here, we have studied kinetics and mechanism of dipeptide-ninhydrin reactions systematically in aqueous and TTABr/14-s-14 micellar systems. The effect of organic

solvents on the reaction rate constants ($k_\psi$) in micellar systems was also seen. The results and discussions are given in the next pages.

## 3.2 Results

### 3.2.1 Influence of [Dipeptide] on the Reaction Rate

In order to verify the reaction mechanism in micellar vis-a-vis aqueous medium, several kinetics runs were carried out at fixed [surfactants] and various [dipeptide] under *pseudo*-first-order conditions of [ninhydrin] >> [dipeptide] in the range of ($1.0 \times 10^{-4}$ to $4.0 \times 10^{-4}$ mol $dm^{-3}$ of [dipeptide] at constant [ninhydrin] ($6.0 \times 10^{-3}$ mol $dm^{-3}$), temperature (70 °C) and *p*H (5.0). The rate constant values are recorded in Tables 3.1 and 3.2. As the values of rate constants ($k_{obs}$ and $k_\psi$) were found to be independent of the initial concentration of dipeptide, the order of reaction with respect to [dipeptide] is unity in both the systems (aqueous as well as TTABr/14-s-14 micellar systems).

### 3.2.2 Influence of *p*H on the Reaction Rate

Variation of *p*H was studied (Tables 3.3 and 3.4, Figures 3.1 and 3.2) to examine the medium effect on the rate of dipeptide–ninhydrin reaction in absence and presence of surfactants (TTABr/14-s-14). It is known [14] that the rate at which Schiff base (see later) is formed is generally high near a *p*H value of five, and drops at higher and lower *p*H's. But for Gly-DL-Asp–ninhydrin reaction, as it is depicted graphically in Figure 3.2 at high *p*H, a slightly increase in the rate values is due to side chain effect of dipeptide which contains carboxylic acid group ($H^+$ donor) leading to protonation of the OH in the intermediate to allow for removal as $H_2O$. At low *p*H, most of the amine reactant will be tied up as its ammonium conjugate acid and will become non-nucleophilic [15]. Therefore, the detailed kinetics runs were performed at *p*H 5.0 keeping other experimental variables constants.

### 3.2.3 Influence of [Ninhydrin] on the Reaction Rate

The dependence of the rate constant on [ninhydrin] was determined at different [ninhydrin] (6.0 – 40.0) x $10^{-3}$ mol $dm^{-3}$ keeping concentrations of other reaction ingredients constant at *p*H 5.0 and 70 °C. The rate constant values obtained in the two systems are summarized in Tables 3.5 and 3.6. The plots of rate constants *vs.* [ninhydrin] (Figures 3.3 and 3.4) are non-linear passing through the origin that indicates the order to be fractional with respect to [ninhydrin] in both aqueous and micellar systems.

### 3.2.4 Influence of Temperature on the Reaction Rate

For the determination of activation parameters, several series of kinetic runs were carried out at different temperatures (60-80 °C), with fixed reactants concentration both in the absence and presence of TTABr/14-s-14 micelles. The observed data were found to fit Arrhenius and Eyring equations

$$k = A \exp(-E_a/(RT)) \quad (3.1)$$

and

$$k = (k_B T/h) \exp(\Delta S^{\neq}/R) \exp(-\Delta H^{\neq}/RT) \quad (3.2)$$

where k, A, R, $k_B$, h, $E_a$, $\Delta S^{\neq}$ and $\Delta H^{\neq}$ are respectively, rate constant (in aqueous ($k_{obs}$) and in micellar systems ($k_{\psi}$)), frequency factor, gas constant, Boltzmann constant, Planck's constant, activation energy, activation entropy and activation enthalpy. The activation energy ($E_a$) was calculated from the slope of the plot of ln k (y-axis) *vs.* 1000/T (x-axis). The activation enthalpy ($\Delta H^{\neq}$) and activation entropy ($\Delta S^{\neq}$) were calculated using linear least squares regression technique (Tables 3.7 and 3.8).

**Table 3.1:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on [Gly-L-Ala] for the reaction of Gly-L-Ala with ninhydrin.

*Reaction conditions*:

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| $10^4$ [Gly-L-Ala] (mol $dm^{-3}$) | $10^5$ $k_{obs}$ ($s^{-1}$) | $10^5$ $k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 1.0 | 13.0 | 60.9 | 32.1 | 41.1 | 60.9 |
| 1.5 | 13.5 | 63.2 | 30.1 | 42.3 | 61.6 |
| 2.0 | 14.1 | 63.9 | 31.5 | 43.0 | 62.2 |
| 2.5 | 14.3 | 61.0 | 32.5 | 42.9 | 63.9 |
| 3.0 | 14.6 | 64.8 | 31.8 | 43.5 | 63.0 |

**Table 3.2:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on [Gly-DL-Asp] for the reaction of Gly-DL-Asp with ninhydrin.

*Reaction conditions*:

| | | |
|---|---|---|
| [TTABr] | = | $20.0 \times 10^{-3}$ mol $dm^{-3}$ |
| [14-s-14] | = | $50.0 \times 10^{-5}$ mol $dm^{-3}$ |
| [ninhydrin] | = | $6.0 \times 10^{-3}$ mol $dm^{-3}$ |
| *p*H | = | 5.0 |
| Temperature | = | 70 °C |

| $10^4$ [Gly-DL-Asp] (mol $dm^{-3}$) | $10^5 k_{obs}$ ($s^{-1}$) | $10^5 k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 2.0 | 4.9 | 19.7 | 9.8 | 15.9 | 28.7 |
| 2.5 | 5.1 | 20.2 | 10.0 | 16.0 | 28.5 |
| 3.0 | 5.3 | 20.1 | 10.1 | 16.3 | 28.6 |
| 3.5 | 5.2 | 20.5 | 10.3 | 16.2 | 28.8 |
| 4.0 | 5.2 | 21.1 | 10.4 | 16.3 | 28.7 |

**Table 3.3:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on *p*H for the reaction of Gly-L-Ala with ninhydrin.

*Reaction conditions*:

[Gly-L-Ala] = $2.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

Temperature = 70 °C

| *p*H | $10^5 k_{obs}$ ($s^{-1}$) | $10^5 k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 4.0 | 1.1 | 11.3 | 5.7 | 6.7 | 8.9 |
| 4.5 | 2.3 | 45.6 | 8.1 | 12.6 | 20.4 |
| 5.0 | 14.1 | 63.9 | 31.5 | 43.0 | 62.2 |
| 5.5 | 10.0 | 55.5 | 32.8 | 44.9 | 62.5 |
| 6.0 | 6.5 | 58.4 | 34.3 | 46.3 | 61.9 |
| 6.5 | 8.4 | 52.8 | 30.8 | 40.4 | 59.4 |

**Table 3.4:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on *p*H for the reaction of Gly-DL-Asp with ninhydrin.

*Reaction conditions*:

[Gly-DL-Asp] = $3.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

Temperature = 70 °C

| *p*H | $10^5 k_{obs}$ ($s^{-1}$) | $10^5 k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 4.0 | 0.8 | 4.9 | 4.5 | 5.9 | 6.3 |
| 4.5 | 4.8 | 13.0 | 7.8 | 12.4 | 14.1 |
| 5.0 | 5.3 | 20.1 | 10.1 | 16.3 | 20.7 |
| 5.5 | 7.1 | 20.5 | 10.7 | 16.9 | 21.5 |
| 6.0 | 6.2 | 20.9 | 10.9 | 17.2 | 21.7 |
| 6.5 | 7.2 | 21.7 | 11.4 | 17.7 | 22.0 |

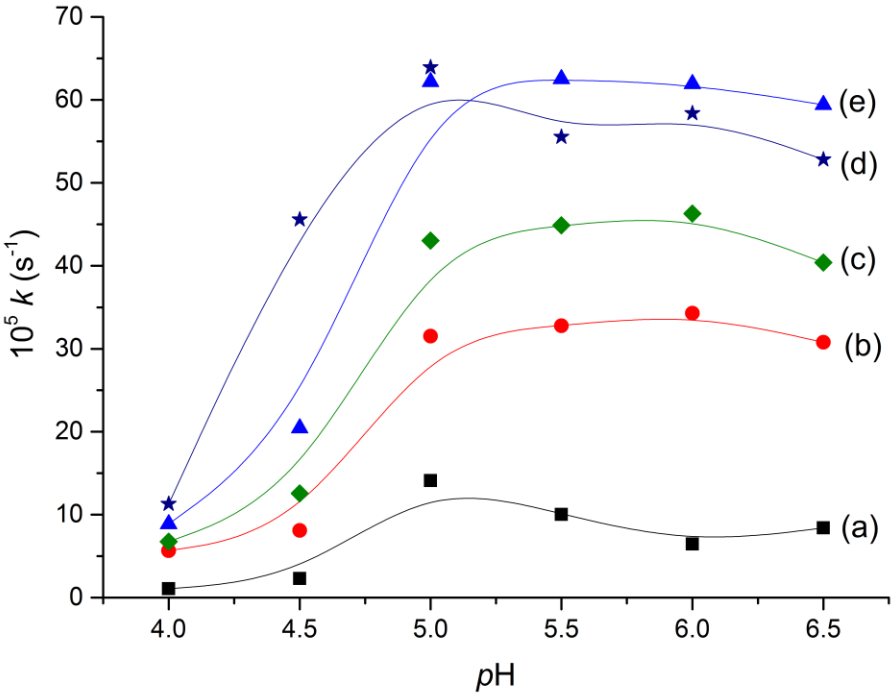



**Figure 3.1:** Influence of *p*H on the reaction rate of Gly-L-Ala with ninhydrin in (a) aqueous and (b-e) presence of surfactants: (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions*: [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [Gly–L-Ala] = 2.0 x $10^{-4}$ mol $dm^{-3}$, temperature = 70 °C.

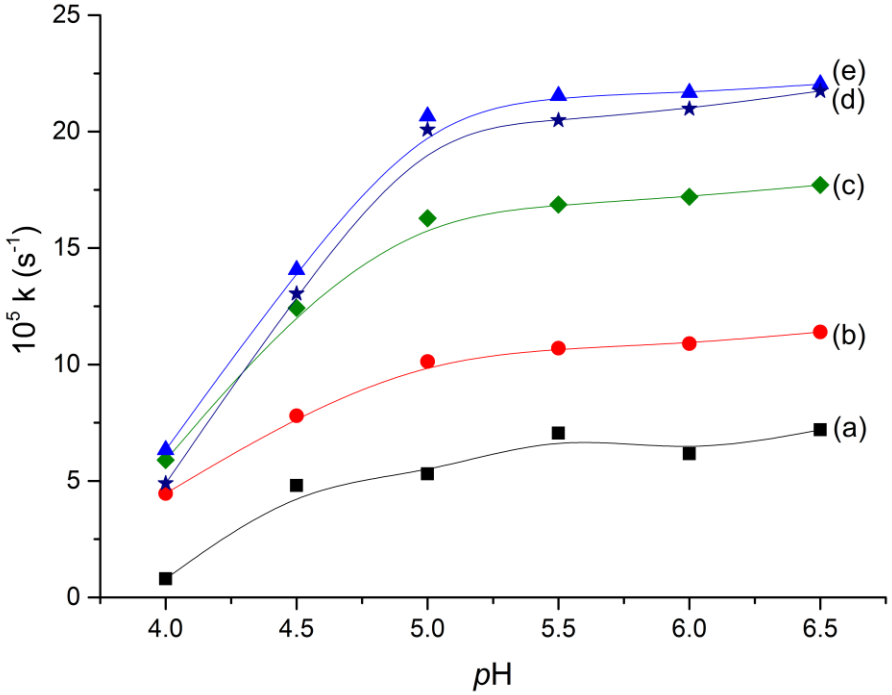



**Figure 3.2:** Influence of *p*H on the reaction rate of Gly-DL-Asp with ninhydrin in (a) aqueous and (b-e) presence of surfactants: (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions*: [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [Gly–DL-Asp] = 3.0 x $10^{-4}$ mol $dm^{-3}$, temperature = 70 °C.

**Table 3.5:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on [ninhydrin] for the reaction of Gly-L-Ala with ninhydrin.

*Reaction conditions*:

| | | |
|---|---|---|
| [Gly-L-Ala] | = | $2.0 \times 10^{-4}$ mol $dm^{-3}$ |
| [TTABr] | = | $20.0 \times 10^{-3}$ mol $dm^{-3}$ |
| [14-s-14] | = | $50.0 \times 10^{-5}$ mol $dm^{-3}$ |
| *p*H | = | 5.0 |
| Temperature | = | 70 °C |

| $10^3$ [ninhydrin] (mol $dm^{-3}$) | $10^5$ $k_{obs}$ ($s^{-1}$) | $10^5$ $k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 6.0 | 14.1(14.0) | 63.9 | 31.5 | 43.0 | 62.2 |
| 10.0 | 16.5(15.5) | 96.8 | 63.8 | 89.9 | 110.1 |
| 15.0 | 31.5(30.2) | 110.0 | 94.1 | 116.9 | 144.8 |
| 20.0 | 47.8(47.2) | 124.0 | 112.0 | 138.0 | 165.9 |
| 25.0 | 45.5(48.3) | 122.0 | 122.9 | 146.0 | 180.3 |
| 30.0 | 52.7(53.5) | 126.0 | 134.0 | 170.0 | 197.1 |
| 35.0 | 53.5(54.6) | 123.0 | 139.8 | 184.0 | 209.0 |
| 40.0 | 51.2(54.9) | 115.0 | 130.0 | 176.0 | 199.8 |

**Table 3.6:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on [ninhydrin] for the reaction of Gly-DL-Asp with ninhydrin.

*Reaction conditions*:

| | | |
|---|---|---|
| [Gly-DL-Asp] | = | $3.0 \times 10^{-4}$ mol dm$^{-3}$ |
| [TTABr] | = | $20.0 \times 10^{-3}$ mol dm$^{-3}$ |
| [14-s-14] | = | $50.0 \times 10^{-5}$ mol dm$^{-3}$ |
| *p*H | = | 5.0 |
| Temperature | = | 70 °C |

| $10^3$ [ninhydrin] (mol dm$^{-3}$) | $10^5$ $k_{obs}$ ($s^{-1}$) | $10^5$ $k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 6.0 | 5.3(5.4) | 20.1 | 10.1 | 16.3 | 28.6 |
| 10.0 | 12.1(12.0) | 29.7 | 12.2 | 17.7 | 30.1 |
| 15.0 | 16.2(15.9) | 49.8 | 20.2 | 24.4 | 51.4 |
| 20.0 | 22.7(22.5) | 60.3 | 32.5 | 40.1 | 64.4 |
| 25.0 | 38.6(38.3) | 82.5 | 45.9 | 51.1 | 79.2 |
| 30.0 | 48.3(49.0) | 89.9 | 53.7 | 60.4 | 88.7 |
| 35.0 | 50.9(51.6) | 96.4 | 57.1 | 65.5 | 95.5 |
| 40.0 | 54.4(53.7) | 101 | 62.4 | 73.7 | 105.0 |

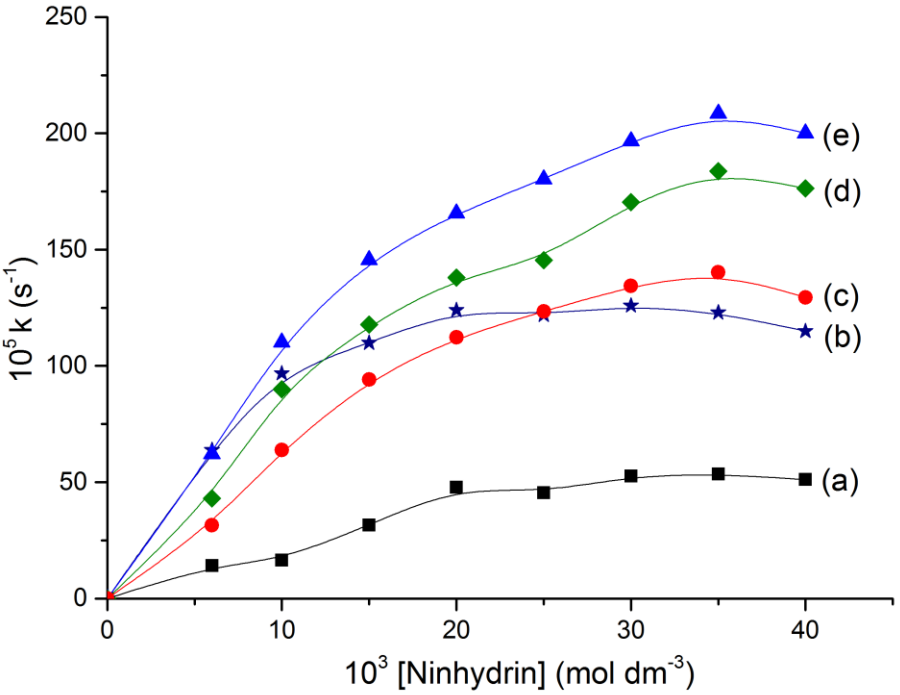



**Figure 3.3:** Influence of [ninhydrin] on the reaction rate of Gly-L-Ala with ninhydrin in (a) aqueous and (b-e) presence of surfactants: (b) TTABr; (c)14-6-14; (d)14-5-14; (e) 14-4-14. *Reaction conditions*: [Gly-L-Ala] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), temperature = 70 °C, *p*H = 5.0.

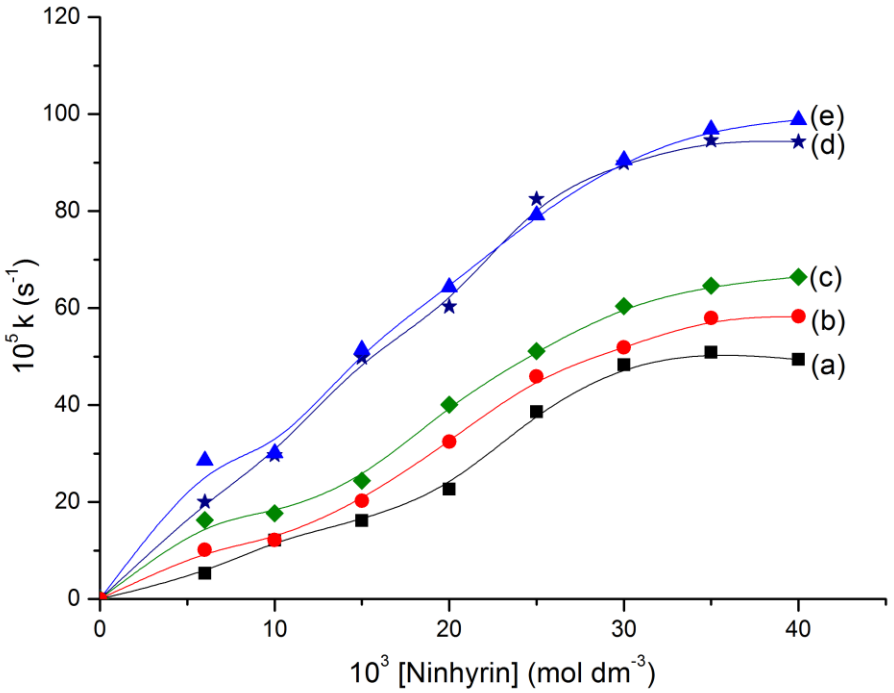



**Figure 3.4:** Influence of [ninhydrin] on the reaction rate of Gly-DL-Asp with ninhydrin in (a) aqueous and (b-e) presence of surfactants: (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions*: [Gly-DL-Asp] = 3.0 x $10^{-4}$ mol $dm^{-3}$, [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), temperature = 70 °C, *p*H = 5.0.

**Table 3.7:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on temperature and related thermodynamic parameters for the reaction of Gly-L-Ala with ninhydrin.

*Reaction conditions*:

[Gly-L-Ala] = $2.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

*p*H = 5.0

| Temperature (°C) | $10^5 k_{obs}$ ($s^{-1}$) | $10^5 k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 60 | 3.2 | 18.9 | 11.6 | 13.5 | 17.9 |
| 65 | 8.2 | 33.2 | 20.6 | 28.4 | 34.2 |
| 70 | 14.1 | 63.9 | 31.5 | 43.0 | 62.2 |
| 75 | 20.8 | 100.9 | 73.2 | 80.2 | 105.3 |
| 80 | 47.9 | 127.0 | 89.9 | 100.1 | 109.1 |
| Parameters | | | | | |
| $E_a$ (kJ $mol^{-1}$) | 127±0.8 | 98.1±0.7 | 108±0.8 | 102±0.7 | 96.7±0.7 |
| $\Delta H^{\neq}$ (kJ $mol^{-1}$) | 124±0.7 | 95.3±0.7 | 105±0.7 | 99.2±0.6 | 93.9±0.6 |
| $-\Delta S^{\neq}$ ($JK^{-1}mol^{-1}$) | 259±2 | 265 ±3 | 262 ±3 | 263 ±3 | 264 ±2 |

**Table 3.8:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on temperature and related thermodynamic parameters for the reaction of Gly-DL-Asp with ninhydrin.

*Reaction conditions*:

[Gly-DL-Asp] = $3.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

*p*H = 5.0

| Temperature (°C) | $10^5 k_{obs}$ ($s^{-1}$) | $10^5 k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 60 | 1.3 | 4.9 | 5.4 | 6.6 | 7.8 |
| 65 | 3.3 | 11.5 | 7.4 | 10.9 | 16.7 |
| 70 | 5.3 | 20.1 | 10.1 | 16.3 | 28.6 |
| 75 | 12.0 | 34.4 | 22.4 | 28.1 | 41.1 |
| 80 | 14.3 | 38.6 | 31.6 | 39.2 | 46.3 |
| Parameters | | | | | |
| $E_a$ (kJ $mol^{-1}$) | 120±0.7 | 104±0.7 | 92.0±0.8 | 89.6±0.8 | 89.4±0.7 |
| $\Delta H^{\neq}$ (kJ $mol^{-1}$) | 117±0.6 | 102± 0.6 | 89.2± 0.7 | 86.7±0.7 | 86.5±0.6 |
| $-\Delta S^{\neq}$ ($JK^{-1}mol^{-1}$) | 256±2 | 260±4 | 258±3 | 259±3 | 260±3 |

### 3.2.5 Influence of [Surfactant] on the Reaction Rate

To explore the cationic/dicationic surfactants' concentration effect on the reaction rate, [TTABr]/[14-s-14] were varied at constant [ninhydrin], [dipeptide] and *p*H 5.0 at 70 °C. An enhancement in the reaction rate was observed for the reaction in presence of conventional TTABr micelles. The *pseudo*-first-order rate constants ($k_\psi$, $s^{-1}$) increase with increasing TTABr concentration, up to an optimum value, and then, any further increase in TTABr concentration (> 20.0 $x10^{-3}$ mol $dm^{-3}$) leads to decrease in the reaction rate.

On the other hand, with geminis (dicationic) surfactants, the rate constant follows three zones: first zone (I), adding [14-s-14] below CMC the geminis accelerate the reaction as reflected by $k_\psi$ values. After that, at zone (II), the reaction rate becomes almost constant up to definite concentration then increases again at zone (III).

The results for [surfactant] effect on the reaction rate are tabulated in Tables 3.9-3.12 and are depicted in Figures 3.5-3.8.

### 3.2.6 Influence of Organic Solvents on the Reaction Rate

The influence of presence of organic solvents, *viz.*, acetonitrile (AN), 1,4-dioxane (DO) and dimethyl sulfoxide (DMSO) on the rate of product formation was also examined at fixed [dipeptide], [ninhydrin], [TTABr], [14-s-14], *p*H (=5.0) and temperature (70 $^oC$) (Tables 3.13 and 3.14 and Figures 3.9 – 3.15).

**Table 3.9:** Dependence of *pseudo*-first-order rate constants ($k_\psi$) on [TTABr] for the reaction of Gly-L-Ala with ninhydrin.

*Reaction conditions*:

[Gly-L-Ala] = $2.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| $10^3$[TTABr] (mol $dm^{-3}$) | $10^5\ k_\psi$ ($s^{-1}$) | $10^5\ k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ |
|---|---|---|---|
| 0 | 14.1 | -- | -- |
| 5.0 | 26.5 | 23.4 | +0.11 |
| 7.0 | 33.3 | 26.9 | +0.19 |
| 10.0 | 40.5 | 43.9 | - 0.08 |
| 12.0 | 43.0 | 50.2 | - 0.17 |
| 15.0 | 47.6 | 56.1 | - 0.16 |
| 20.0 | 63.9 | 61.1 | +0.04 |
| 30.0 | 59.4 | 62.8 | - 0.05 |
| 40.0 | 48.6 | 63.2 | - 0.30 |
| 50.0 | 45.3 | 64.3 | - 0.42 |
| 60.0 | 36.6 | 64.4 | - 0.76 |
| 70.0 | 30.4 | 65.7 | - 1.16 |
| 90.0 | 20.9 | 66.3 | - 2.17 |

**Table 3.10:** Dependence of *pseudo*-first-order rate constants ($k_\psi$) on [TTABr] for the reaction of Gly-DL-Asp with ninhydrin.

*Reaction conditions*:

[Gly-DL-Asp] = $3.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| $10^3$[TTABr] (mol $dm^{-3}$) | $10^5\ k_\psi$ ($s^{-1}$) | $10^5\ k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ |
|---|---|---|---|
| 0 | 5.3 | -- | -- |
| 5.0 | 6.9 | 6.2 | +0.10 |
| 7.0 | 8.2 | 8.1 | +0.01 |
| 10.0 | 10.5 | 9.6 | +0.08 |
| 15.0 | 13.3 | 15.9 | - 0.19 |
| 20.0 | 20.1 | 19.0 | +0.05 |
| 30.0 | 19.8 | 22.2 | - 0.12 |
| 40.0 | 20.5 | 22.0 | - 0.07 |
| 50.0 | 16.5 | 17.6 | - 0.07 |
| 60.0 | 12.4 | 13.9 | - 0.13 |
| 70.0 | 9.2 | 10.0 | - 0.09 |
| 80.0 | 8.5 | 9.3 | - 0.09 |
| 90.0 | 7.2 | 8.7 | - 0.21 |

**Table 3.11:** Dependence of *pseudo*-first-order rate constants ($k_\psi$) on [14-s-14] for the reaction of Gly-L-Ala with ninhydrin.

*Reaction conditions*:

[Gly-L-Ala] = $2.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| $10^5$ [14-s-14] (mol $dm^{-3}$) | 14-6-14 | | | 14-5-14 | | | 14-4-14 | | |
|---|---|---|---|---|---|---|---|---|---|
| | $10^5 k_\psi$ ($s^{-1}$) | $10^5 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ | $10^5 k_\psi$ ($s^{-1}$) | $10^5 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ | $10^5 k_\psi$ ($s^{-1}$) | $10^5 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ |
| 0 | 14.1 | -- | -- | 14.1 | -- | -- | 14.1 | -- | -- |
| 10.0 | 14.8 | -- | -- | 15.2 | -- | -- | 16.4 | -- | -- |
| 15.0 | 16.1 | 14.2 | +0.12 | 22.8 | 19.9 | +0.13 | 26.5 | 24.4 | +0.08 |
| 20.0 | 19.8 | 18.3 | +0.08 | 30.3 | 28.8 | +0.05 | 32.8 | 30.1 | +0.08 |
| 30.0 | 24.3 | 25.1 | - 0.03 | 34.0 | 33.7 | +0.01 | 48.9 | 48.1 | +0.02 |
| 50.0 | 31.5 | 29.9 | +0.05 | 43.0 | 42.8 | 0.00 | 62.2 | 62.0 | 0.00 |
| 70.0 | 36.2 | 36.8 | - 0.02 | 51.5 | 51.8 | - 0.01 | 74.1 | 74.4 | 0.00 |

*contd…*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 100.0 | 43.0 | 44.2 | - 0.03 | 60.3 | 61.1 | - 0.01 | 80.0 | 81.1 | - 0.01 |
| 150.0 | 44.6 | 45.3 | +0.02 | 61.2 | 61.8 | - 0.01 | 83.5 | 84.7 | - 0.01 |
| 200.0 | 44.8 | 46.4 | - 0.04 | 63.8 | 64.3 | - 0.01 | 81.6 | 85.1 | - 0.04 |
| 300.0 | 43.6 | 46.9 | - 0.08 | 62.4 | 63.4 | - 0.02 | 84.3 | 86.3 | - 0.02 |
| 500.0 | 43.7 | 48.1 | - 0.10 | 60.5 | 62.1 | - 0.03 | 82.5 | 87.4 | - 0.05 |
| 700.0 | 54.5 | 57.3 | - 0.05 | 71.3 | 73.4 | - 0.03 | 102.0 | 108.2 | - 0.06 |
| 1000.0 | 55.8 | -- | -- | 75.5 | -- | -- | 115.9 | -- | -- |
| 1500.0 | 60.6 | -- | -- | 84.3 | -- | -- | 121.1 | -- | -- |
| 2000.0 | 61.2 | -- | -- | 86.4 | -- | -- | 125.2 | -- | -- |
| 2500.0 | 62.7 | -- | -- | 88.4 | -- | -- | 130.0 | -- | -- |
| 3000.0 | 63.4 | -- | -- | 90.5 | -- | -- | 133.8 | -- | -- |

**Table 3.12:** Dependence of *pseudo*-first-order rate constants ($k_\psi$) on [14-s-14] for the reaction of Gly-DL-Asp with ninhydrin.

*Reaction conditions*:

[Gly-DL-Asp] = $3.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| $10^5$ [14-s-14] (mol $dm^{-3}$) | 14-6-14 | | | 14-5-14 | | | 14-4-14 | | |
|---|---|---|---|---|---|---|---|---|---|
| | $10^5 k_\psi$ ($s^{-1}$) | $10^5 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ | $10^5 k_\psi$ ($s^{-1}$) | $10^5 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ | $10^5 k_\psi$ ($s^{-1}$) | $10^5 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ |
| 0 | 5.3 | -- | -- | 5.3 | -- | -- | 5.3 | -- | -- |
| 10.0 | 6.0 | -- | -- | 7.2 | -- | -- | 7.8 | -- | -- |
| 15.0 | 6.7 | 5.9 | +0.12 | 8.4 | 8.0 | +0.05 | 9.6 | 8.5 | +0.11 |
| 20.0 | 7.4 | 7.0 | +0.05 | 10.6 | 9.6 | +0.09 | 13.8 | 11.3 | +0.18 |
| 30.0 | 9.0 | 8.7 | +0.04 | 14.6 | 14.1 | +0.03 | 20.1 | 20.0 | 0.00 |
| 50.0 | 10.1 | 10.0 | +0.01 | 16.3 | 16.5 | - 0.01 | 28.6 | 28.4 | +0.01 |
| 70.0 | 14.0 | 14.2 | - 0.01 | 23.4 | 24.0 | - 0.02 | 33.8 | 34.1 | - 0.01 |
| 100.0 | 18.0 | 18.5 | - 0.03 | 23.9 | 24.5 | - 0.02 | 34.0 | 35.1 | - 0.03 |

*contd…*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 150.0 | 18.5 | 19.2 | - 0.04 | 24.2 | 25.3 | - 0.04 | 36.4 | 37.4 | - 0.03 |
| 200.0 | 19.2 | 19.8 | - 0.03 | 24.6 | 26.7 | - 0.08 | 37.6 | 37.5 | 0.0 |
| 300.0 | 21.2 | 22.0 | - 0.04 | 24.8 | 27.3 | - 0.10 | 40.0 | 41.4 | -0.04 |
| 500.0 | 23.0 | 23.7 | - 0.03 | 25.2 | 28.1 | - 0.11 | 41.2 | 42.2 | -0.02 |
| 700.0 | 27.6 | 28.6 | - 0.04 | 30.2 | 31.3 | - 0.04 | 50.3 | 51.4 | 0.0 |
| 1000.0 | 31.2 | -- | -- | 34.6 | -- | -- | 55.1 | -- | -- |
| 1500.0 | 36.2 | -- | -- | 39.9 | -- | -- | 60.6 | -- | -- |
| 2000.0 | 37.2 | -- | -- | 41.1 | -- | -- | 67.4 | -- | -- |
| 2500.0 | 38.8 | -- | -- | 43.6 | -- | -- | 72.3 | -- | -- |
| 3000.0 | 39.5 | -- | -- | 45.2 | -- | -- | 74.3 | -- | -- |

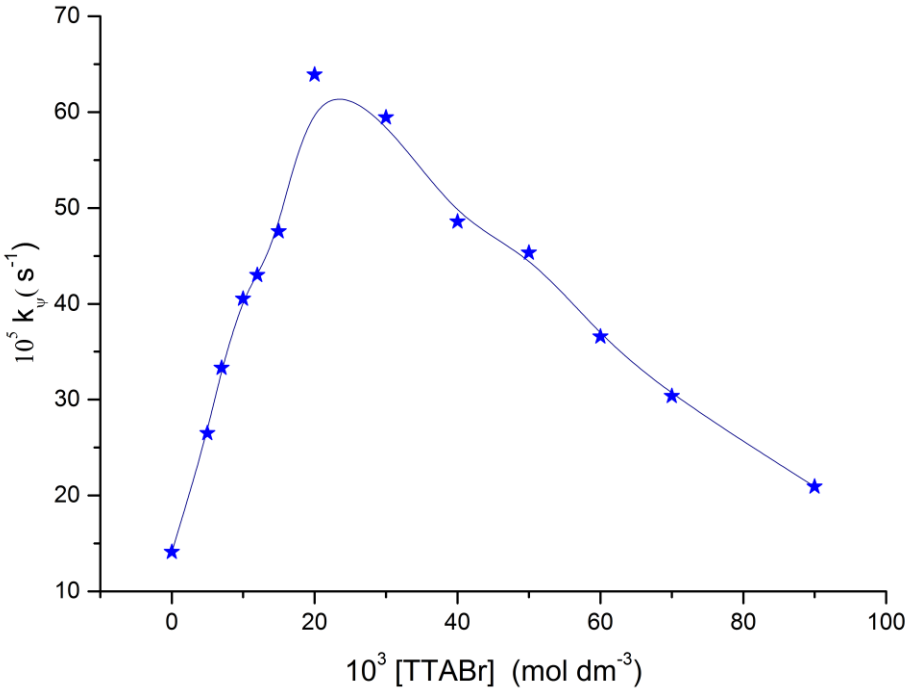



**Figure 3.5:** Influence of [TTABr] on the reaction rate of Gly-L-Ala with ninhydrin. *Reaction conditions*: [Gly-L-Ala] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

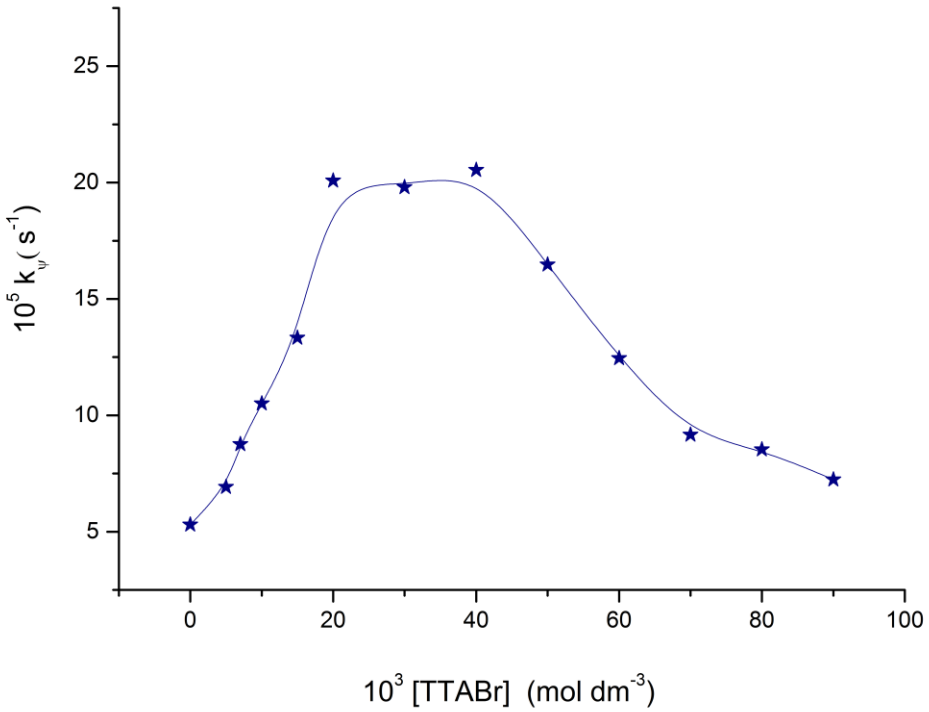



**Figure 3.6:** Influence of [TTABr] on the reaction rate of Gly-DL-Asp with ninhydrin. *Reaction conditions*: [Gly-DL-Asp] = 3.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

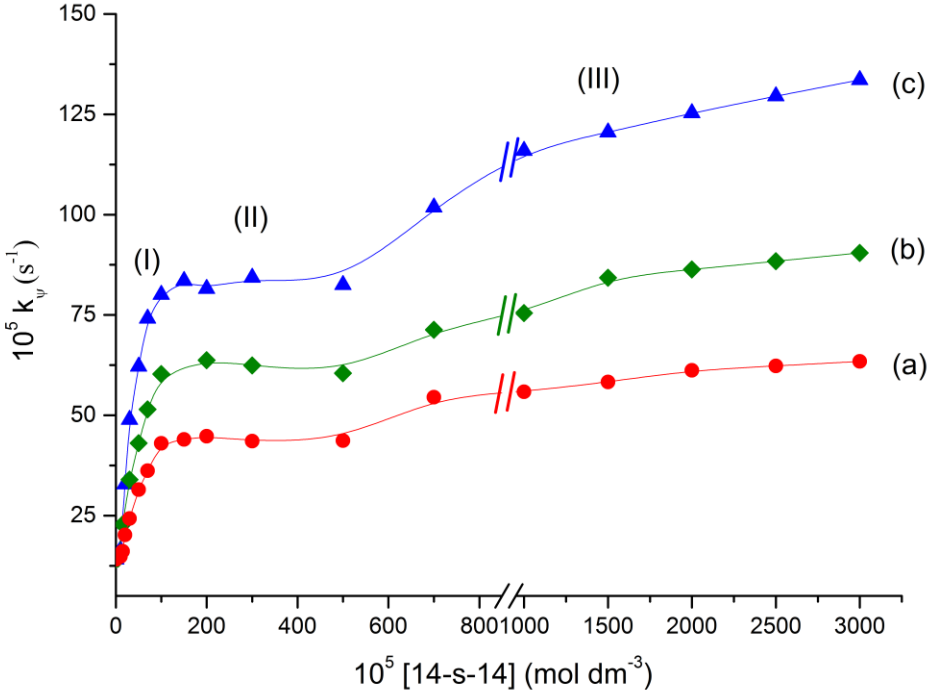



**Figure 3.7:** Influence of [geminis] on the reaction rate of Gly-L-Ala with ninhydrin. *Reaction conditions*: [Gly-L-Ala] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C. (a) 14-6-14; (b) 14-5-14; (c) 14-4-14.

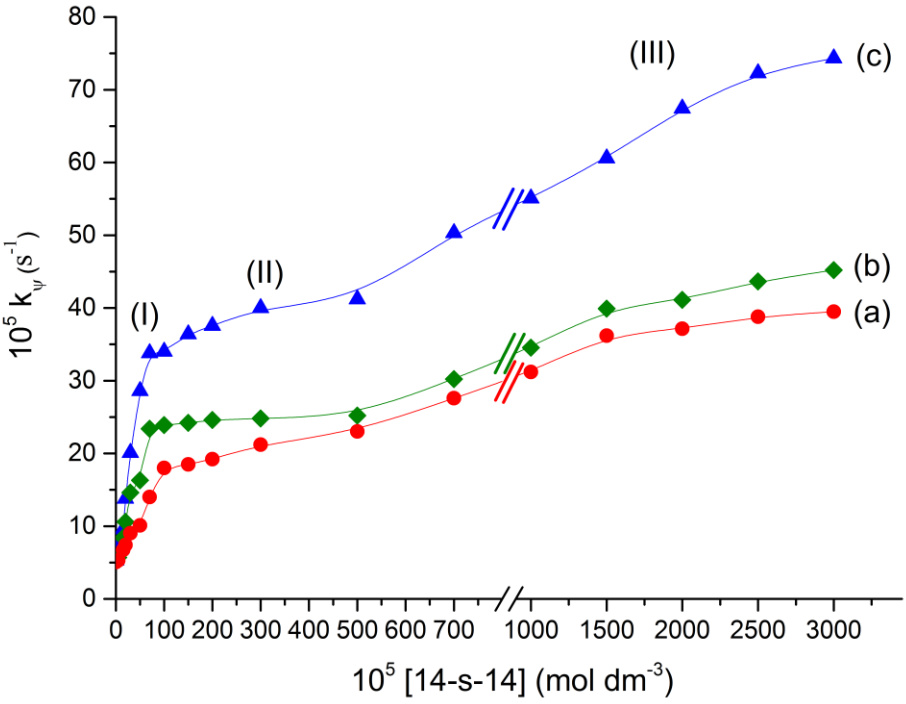



**Figure 3.8:** Influence of [geminis] on the reaction rate of Gly-DL-Asp with ninhydrin. *Reaction conditions*: [Gly-DL-Asp] = 3.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C. (a) 14-6-14; (b) 14-5-14; (c) 14-4-14.

**Table 3.13** Rate constants (k) for the reaction of Gly-L-Ala with ninhydrin in the absence and presence of surfactants in aqueous-organic medium.

*Reaction conditions*

[Gly-L-Ala] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x$10^{-3}$ mol $dm^{-3}$, [14-s-14] = 50.0 x$10^{-5}$ mol $dm^{-3}$, at *p*H = 5.0 and temperature = 70 $^{o}$C.

| % Solvent (v/v) | ($10^5$) k ($s^{-1}$) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DMSO | | | | | DO | | | | | AN | | | | |
| | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 |
| 0.0 | 14.1 | 63.9 | 31.5 | 43.0 | 62.2 | 14.1 | 63.9 | 31.5 | 43.0 | 62.2 | 14.1 | 63.9 | 31.5 | 43.0 | 62.2 |
| 10.0 | 13.8 | 62.7 | 32.2 | 42.4 | 62.4 | 16.1 | 64.7 | 34.7 | 44.1 | 63.6 | 21.2 | 68.9 | 42.3 | 50.6 | 64.5 |
| 15.0 | 14.3 | 70.8 | 33.2 | 44.0 | 63.3 | 31.6 | 68.8 | 43.3 | 56.5 | 70.5 | 25.8 | 73.4 | 51.5 | 57.6 | 69.6 |
| 20.0 | 16.5 | 80.0 | 37.6 | 46.8 | 67.6 | 46.3 | 79.8 | 58.7 | 65.1 | 77.7 | 35.1 | 89.6 | 63.7 | 73.6 | 86.4 |
| 25.0 | 18.6 | 86.8 | 34.3 | 45.3 | 65.5 | 50.3 | 82.2 | 60.2 | 67.8 | 81.3 | 42.3 | 94.4 | 69.4 | 81.2 | 91.4 |
| 30.0 | 17.6 | 85.3 | 30.5 | 41.3 | 67.5 | 59.8 | 86.4 | 63.5 | 70.3 | 85.7 | 55.8 | 104.0 | 78.7 | 93.2 | 99.5 |
| 40.0 | 18.7 | 76.8 | 24.4 | 36.4 | 60.1 | 63.6 | 93.5 | 69.1 | 75.5 | 89.1 | 60.1 | 121.1 | 97.8 | 114.0 | 116.7 |
| 45.0 | 15.7 | 68.4 | 20.0 | 33.4 | 54.5 | 70.2 | 96.3 | 76.9 | 78.4 | 91.2 | 63.6 | 134.9 | 102.0 | 124.1 | 129.6 |
| 50.0 | 14.5 | 55.4 | 17.4 | 30.6 | 45.3 | 78.8 | 103.2 | 80.2 | 83.5 | 94.5 | 69.9 | 143.2 | 127.1 | 131.2 | 139.1 |

**Table 3.14:** Rate constants (k) for the reaction of Gly-DL-Asp with ninhydrin in the absence and presence of surfactants in aqueous-organic medium.

*Reaction conditions*

[Gly-DL-Asp] = 3.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x$10^{-3}$ mol $dm^{-3}$, [14-s-14] = 50.0 x$10^{-5}$ mol $dm^{-3}$, at *p*H = 5.0 and temperature = 70 $^{o}$C.

| % Solvent (v/v) | ($10^5$) k ($s^{-1}$) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DMSO | | | | | DO | | | | | AN | | | | |
| | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 |
| 0.0 | 5.3 | 20.1 | 10.1 | 16.3 | 28.6 | 5.3 | 20.1 | 10.1 | 16.3 | 28.6 | 5.3 | 20.1 | 10.1 | 16.3 | 28.6 |
| 10.0 | 8.9 | 17.5 | 12.2 | 17.6 | 31.4 | 14.3 | 22.2 | 14.3 | 18.7 | 30.2 | 15.0 | 25.5 | 14.3 | 26.7 | 33.3 |
| 15.0 | 9.0 | 21.5 | 15.4 | 20.2 | 35.5 | 18.9 | 25.6 | 20.3 | 21.2 | 33.4 | 17.0 | 30.8 | 20.2 | 32.5 | 38.5 |
| 20.0 | 9.1 | 26.5 | 19.9 | 24.6 | 38.9 | 19.5 | 30.9 | 26.6 | 29.1 | 36.1 | 21.9 | 35.7 | 24.7 | 38.6 | 41.2 |
| 25.0 | 13.5 | 34.6 | 25.5 | 33.3 | 46.6 | 20.2 | 37.6 | 30.2 | 34.4 | 40.5 | 24.4 | 41.4 | 30.2 | 45.5 | 43.3 |
| 30.0 | 18.7 | 38.3 | 31.1 | 37.9 | 50.0 | 22.2 | 43.3 | 33.6 | 40.8 | 51.2 | 30.0 | 45.1 | 34.5 | 50.4 | 56.6 |
| 40.0 | 26.7 | 43.3 | 37.9 | 40.5 | 57.2 | 28.8 | 55.7 | 45.2 | 58.0 | 60.2 | 35.8 | 60.9 | 46.7 | 63.3 | 67.6 |
| 45.0 | 30.3 | 55.6 | 45.9 | 52.2 | 67.3 | 31.2 | 70.1 | 59.6 | 63.4 | 77.7 | 44.3 | 78.9 | 58.8 | 80.3 | 87.7 |
| 50.0 | 32.4 | 76.8 | 67.7 | 72.5 | 85.5 | 39.6 | 80.7 | 70.7 | 76.7 | 91.3 | 54.2 | 90.1 | 79.6 | 91.6 | 103.1 |

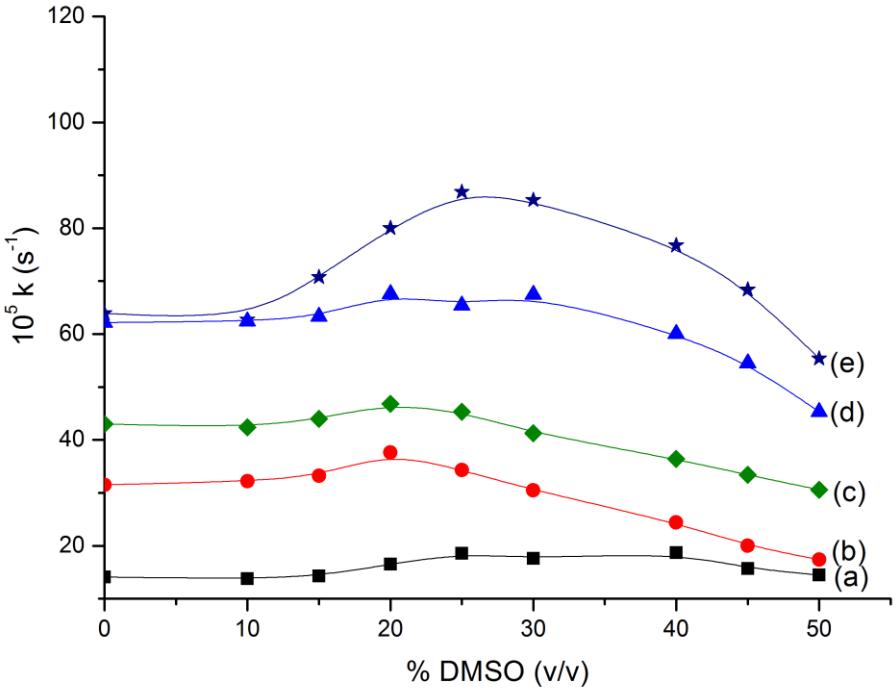



**Figure 3.9:** Influence of composition of DMSO on the reaction rate of Gly-L-Ala with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) 14-5-14; (d) 14-4-14 and (e) TTABr. *Reaction conditions*: [Gly-L-Ala] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] =20.0 x$10^{-3}$ mol $dm^{-3}$, [14-s-14] =50.0 x$10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

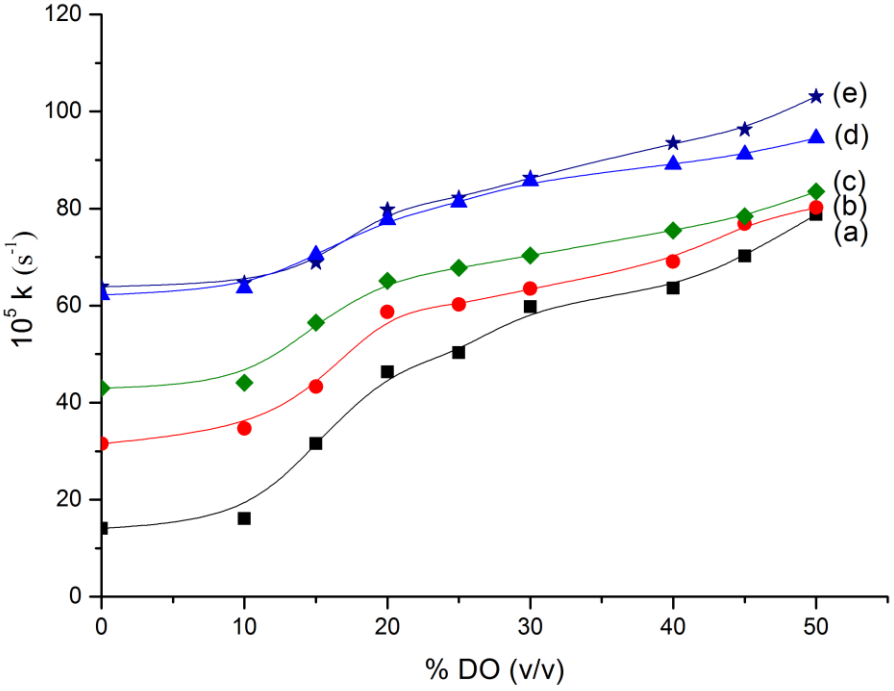



**Figure 3.10:** Influence of composition of DO on the reaction rate of Gly-L-Ala with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) 14-5-14; (d) 14-4-14 and (e) TTABr. *Reaction conditions*: [Gly-L-Ala] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] =20.0 x$10^{-3}$ mol $dm^{-3}$, [14-s-14] =50.0 x$10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

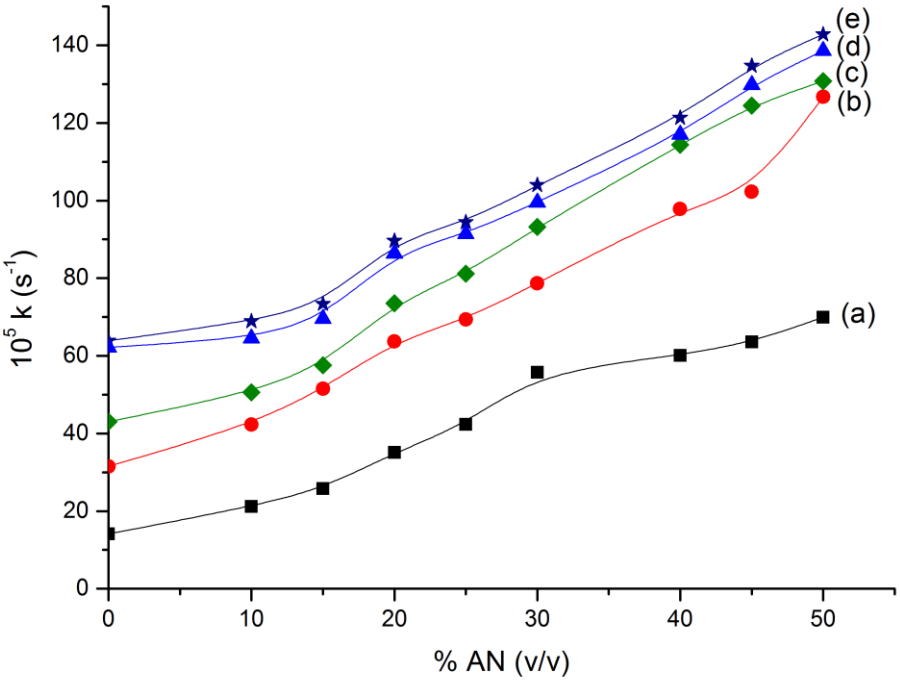



**Figure 3.11:** Influence of composition of AN on the reaction rate of Gly-L-Ala with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) 14-5-14; (d) 14-4-14 and (e) TTABr. *Reaction conditions*: [Gly-L-Ala] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] =20.0 x$10^{-3}$ mol $dm^{-3}$, [14-s-14] =50.0 x$10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

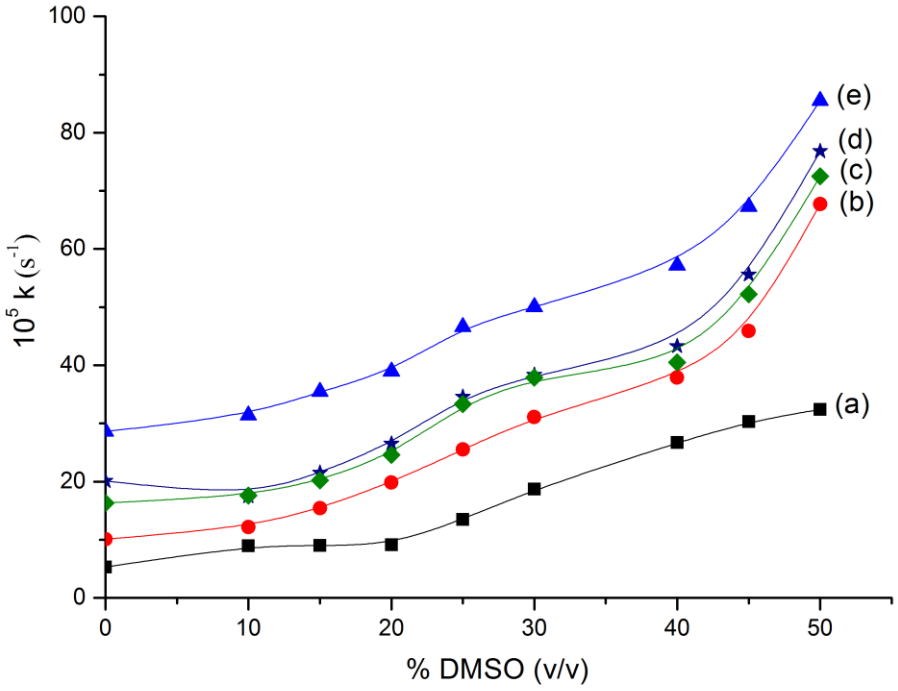



**Figure 3.12:** Influence of composition of DMSO on the reaction rate of Gly-DL-Asp with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) 14-5-14; (d) TTABr and (e) 14-4-14. *Reaction conditions*: [Gly-L-Asp] = 3.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] =20.0 x$10^{-3}$ mol $dm^{-3}$, [14-s-14] =50.0 x$10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

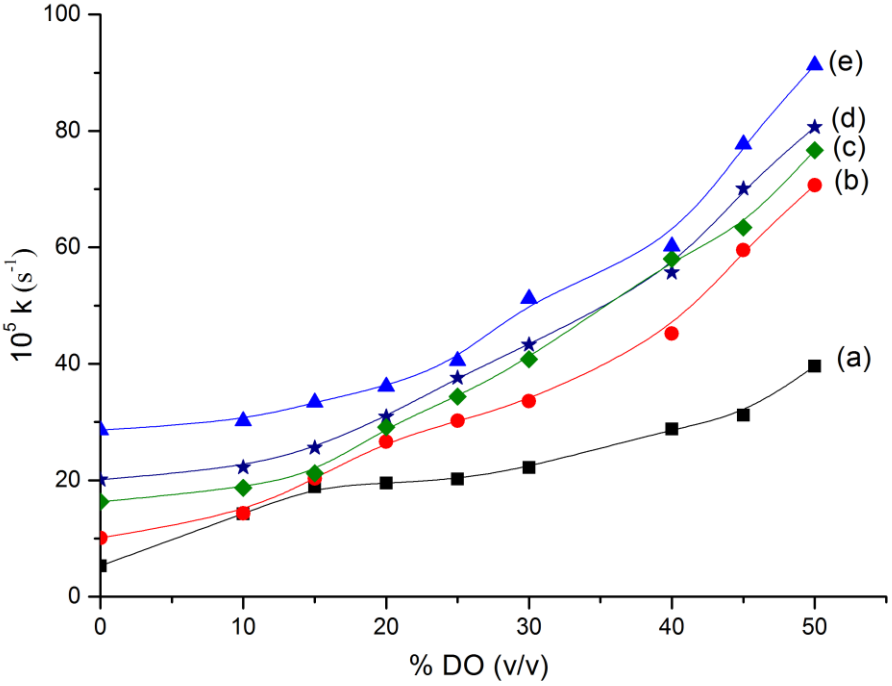



**Figure 3.13:** Influence of composition of DO on the reaction rate of Gly-DL-Asp with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) 14-5-14; (d) TTABr and (e) 14-4-14. *Reaction conditions*: [Gly-DL-Asp] = 3.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] =20.0 x $10^{-3}$ mol $dm^{-3}$, [14-s-14] =50.0 x $10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

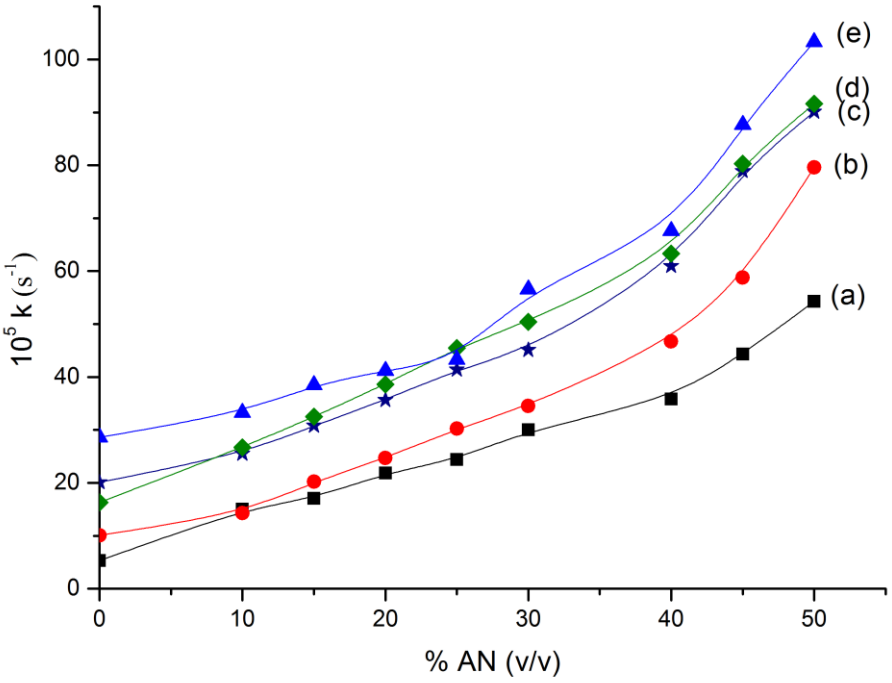



**Figure 3.14:** Influence of composition of AN on the reaction rate of Gly-DL-Asp with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) TTABr; (d) 14-5-14; (e) 14-4-14. *Reaction conditions*: [Gly-DL-Asp] = 3.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] =20.0 x $10^{-3}$ mol $dm^{-3}$, [14-s-14] =50.0 x $10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

## 3.3 Discussion

### 3.3.1 Reactions in Absence of Surfactants

As per the common mechanism of dipeptide-ninhydrin reactions in aqueous medium, Scheme 3.1, it is known [16, 17] that every elementary reaction of amino acids/dipeptides and ninhydrin depends upon the hydrogen ion concentration because the reaction proceeds through the formation of an intermediate which has Schiff base linkage (>C=N-) [3,18]. Ninhydrin with the anhydride form ($Nin_b$, 1,2,3-indanetrione) condenses with dipeptide (Pep) and the reaction proceeds through the formation of A which is in equilibrium with B (Schiff base), which undergoes hydrolysis and decarboxylation to yield an intermediate (2-amino-indanedione, C), which is very reactive. 2-Amino-indanedione is highly sensitive to oxygen molecules and a yellowish colored product is formed (instead of *Ruhemann's purple*) in the presence of atmospheric oxygen. The hydrolysis step could not be rate controlling either, as rate should be governed by steric factors alone [19]. The interaction of C with another ninhydrin molecule also involves an addition-elimination type reaction to give condensation product (DYDA) (route (1)). Hydrolysis of C yields ammonia gas and hydrindantin at *p*H < 5.0 (route (2)); however, at *p*H more than 5.0, route (1) predominates and the color formation is the basis of the analytical methodology [1]. The formation of hydrindantin, if formed, reduces the yield of DYDA.

It was found that the order of the reaction with respect to [Pep] is unity. The rate law is, therefore,

$$d[P]/dt = k_{obs}\,[Pep] \qquad (3.3)$$

The proposed mechanism (Scheme 3.1) shows condensation of amino group to carbonyl group (route 1) and leads to

$$d[P]/dt = kK[Nin][Pep]/(1+K[Nin]) \qquad (3.4)$$

which, when compared with Eq. (3.3), gives

$$k_{obs} = kK[Nin]/(1+K[Nin]) \quad (3.5)$$

where [Nin] = total concentration of ninhydrin.

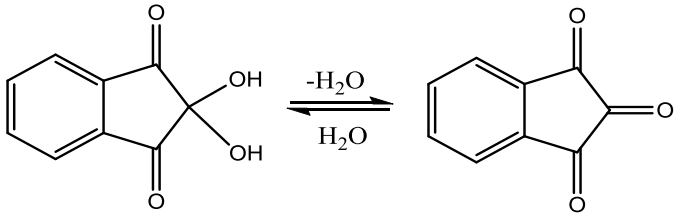
Equation (3.5) can be rearranged as:

$$1/ k_{obs} = 1/k + 1/(kK[Nin]) \quad (3.6)$$

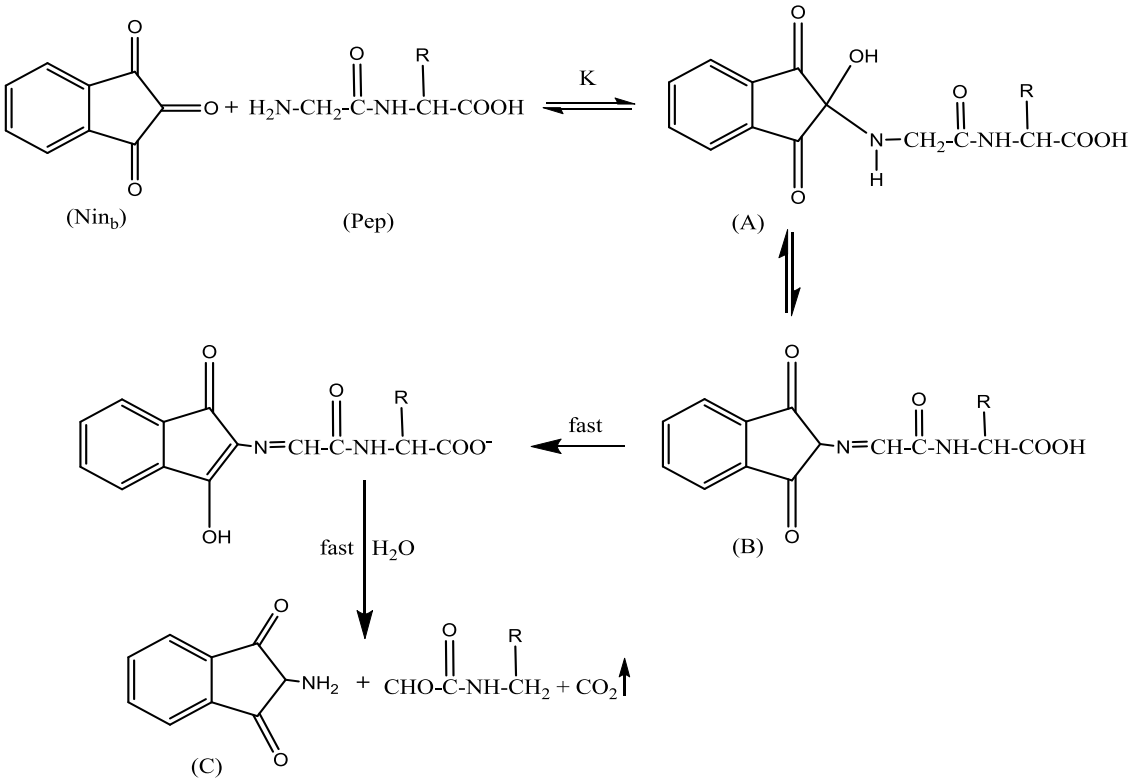
which envisages linearity between $1/k_{obs}$ and 1/[Nin]. The values of *k* and K (the rate and equilibrium constants) were evaluated from the intercept and slope in aqueous medium. The calculated values of rate constants (obtained by substituting *k* and K in Eq. (3.6)) are in close agreement with $k_{obs}$ (given in parenthesis, Tables 3.5 and 3.6) which confirms the proposed mechanism; as also supporting the validity of the rate law and Eq. (3.6).

(a) $H_2N\text{-}CH_2\text{-}C(=O)\text{-}NH\text{-}CH(R)\text{-}COOH \rightleftharpoons H_3N^+\text{-}CH_2\text{-}C(=O)\text{-}NH\text{-}CH(R)\text{-}COO^-$

(Pep) (Pep$_1$)

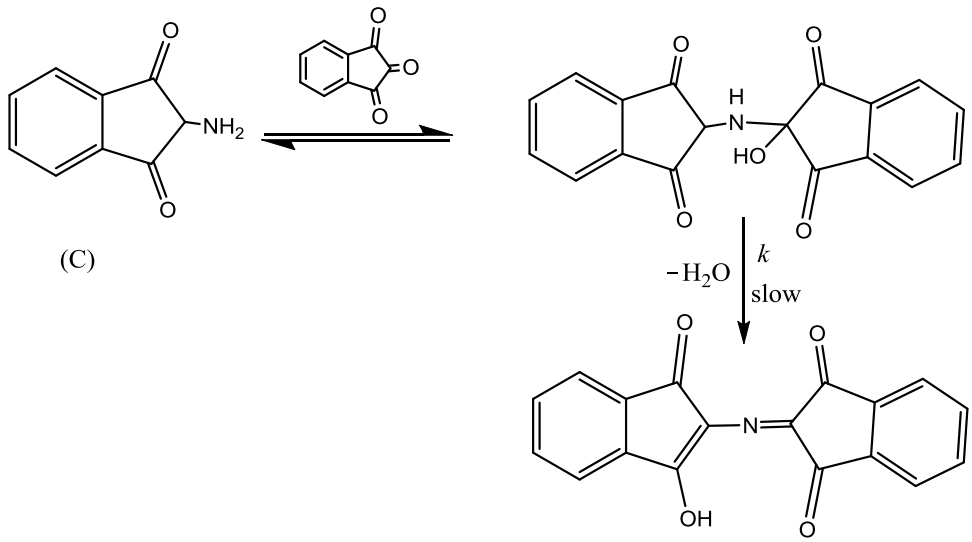
(b) 


(Nin$_a$) (Nin$_b$)

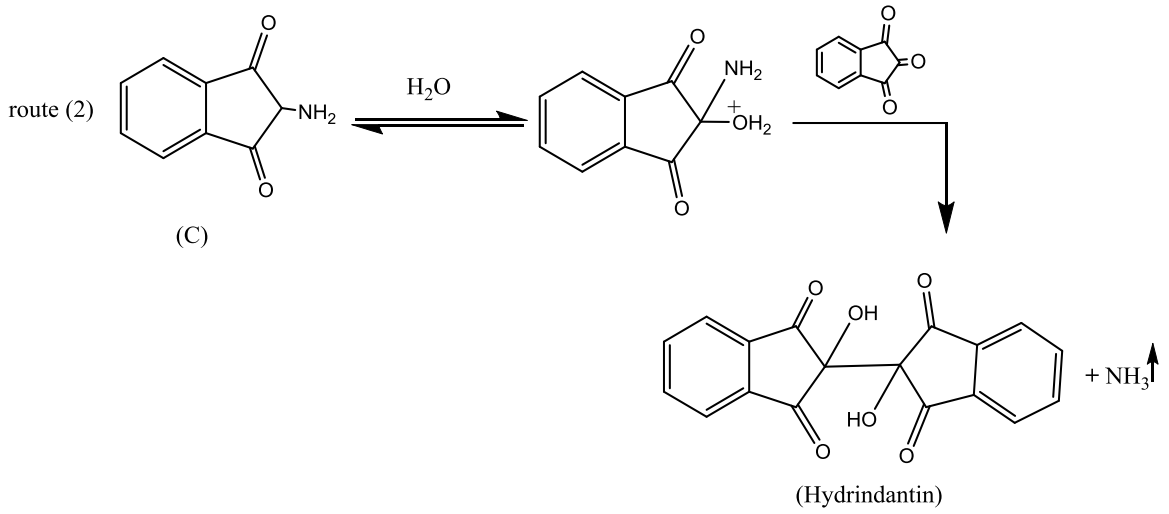



($Nin_b$) (Pep) (A) (B) (C)

route (1)




(C) (Ruhemann's purple (DYDA))




**Scheme 3.1**: Mechanism of dipeptide-ninhydrin reaction. R: $–CH_3$ for Gly-L-Ala, $–CH_2COOH$ for Gly-DL-Asp.

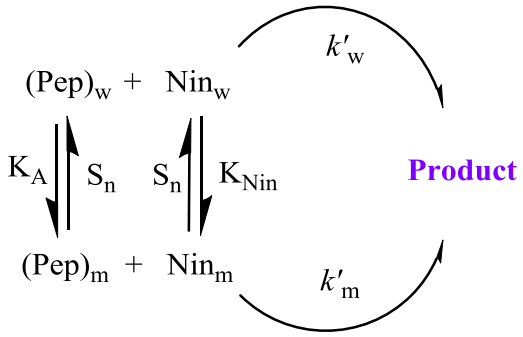
### 3.3.2 Reactions in Presence of TTABr/14-s-14 Surfactants

In comparison with aqueous medium, the experiments were performed in the presence of TTABr/14-s-14 micelles which indicate that absorbance of DYDA increases but the wavelength of maximum absorbance ($\lambda_{max}$) remains the same. Also, no shift in $\lambda_{max}$ was noted when using aqueous-organic solvent medium (Figures 2.4-2.13). This confirms that the product (DYDA) of the reaction remains the same in aqueous-micellar and aqueous-micellar-organic solvent systems. Also, the absorbance and the intensity of the purple-colored product is higher in presence of TTABr/14-s-14 micelles than in aqueous and becomes the highest in mixed aqueous-organic solvents. These results are in conformity that there is a strong association between DYDA and TTABr/14-s-14 micelles which becomes stronger when adding polar organic solvents due to effect on the characteristics of the bulk water. Another possibility is that side reactions are blocked in the presence of TTABr/14-s-14 micelles which, in turn, suppress the loss of amino nitrogen (Scheme 3.1).

As mentioned before, several sets with varying [TTABr]/[14-s-14] were performed at constant [dipeptide], [ninhydrin] and *p*H 5.0 at 70 $^oC$. The rate constant ($k_\psi$)

is affected by [TTABr]/[14-s-14] changes and increases *ca.* seven-fold for Gly-L-Ala-ninhydrin reaction and *ca.* fourteen-fold for Gly-DL-Asp-ninhydrin reaction.

Just like aqueous system, the same first- and fractional-order in [dipeptide] and [ninhydrin], respectively, are being followed in micellar and aqueous-micellar-organic systems. In TTABr/14-s-14 micelles, the reaction between ninhydrin and dipeptide mainly involves three steps: (1) the substrate-micelle binding, (2) chemical transformation at the micellar surface, and (3) releasing DYDA, and the catalytic effects of [TTABr]/[geminis] can be explained by the *pseudophase* kinetic model [5,20,21] which indicates the total volume of micelles as a separate phase regularly distributed in the aqueous phase. The reaction scheme for dipeptide-ninhydrin interaction in the presence of micelles may be given as Scheme 3.2.




**Scheme 3.2**: The *pseudophase* kinetic model for the reaction of dipeptide with ninhydrin in aqueous and in micellar system.

where $K_A$ is the binding constant of dipeptide (Pep) to the surfactant micelles, and $S_n$ represents the micellized surfactant (= $[Surfactant]_T$ – CMC), Nin is a symbol for ninhydrin in aqueous ($Nin_w$) and in micellar ($Nin_m$) system and, $K_{Nin}$ the binding constant of the ninhydrin to the TTABr/gemini micelle. $k'_w$ and $k'_m$ are the *pseudo*-first-order rate constants for condensation of ninhydrin in aqueous and micellar *pseudo*-phase, respectively.

Scheme 3.2 in conjunction with the observed rate being unity in [dipeptide], leads to Eq. (3.7)

$$K_A = [(Pep)_m] / \{[(Pep)_w] [S_n]\} \quad (3.7)$$

$k'_w$ and $k'_m$ are given by Eqs. (3.8 and 3.9)

$$k'_w = k_w[Nin_w] \quad (3.8)$$

$$k'_m = k_m [Nin_m] /[S_n] = k_m M_N^S \quad (3.9)$$

where $k_w$ is a second-order rate constant in aqueous medium and $k_m$ is a second-order rate constant in micellar medium and $M_N^S$ is written in terms of the mole ratio of ninhydrin bound to the micellar head groups:

$$M_N^S = [Nin_m]/[S_n] \quad (3.10)$$

Values of $M_N^S$ were estimated in the following manner. Upon solving $K_{Nin} = [Nin_m]/[Nin_w]([S_n]-[Nin_m])$ and mass balance $[Nin] = [Nin_w] + [Nin_m]$, quadratic Eq. (3.11) resulted which was solved for $[Nin_m]$ with the help of a computer program with $K_{Nin}$ as an adjustable parameter. $M_N^S$ was then calculated with the help of Eq. (3.10)

$$K_{Nin}[Nin_m]^2-(1+K_{Nin}[S_n]+K_{Nin}[Nin])[Nin_m]+K_{Nin}[S_n][Nin] = 0 \quad (3.11)$$

From Scheme 3.2, rate Eq. (3.12) is derived.

$$k_\psi = \{k_w[Nin] + (K_A k_m - k_w) M_N^S [S_n] \} /1 + K_A [S_n] \quad (3.12)$$

The best values of $k_m$ and $K_A$ were calculated using a computer based program with the help of the non-linear least-square analysis. For $K_{Nin}$, the best value was considered to be one for which the value of $\Sigma d_i^2$ ($d_i = k_{\psi obsi} - k_{\psi cali}$) turned out to be minimum. The CMC values under kinetic conditions were required for the calculation, which were determined conductimetrically. Such calculations were carried out given in Tables 3.15 and 3.16.

To verify the Scheme 3.1 mechanism, influence of variable on the rate constants was seen in the presence of constant [TTABr] or [14-s-14]. It was observed that the

reaction follows the same first- and fractional-order kinetics with respect to concentrations of dipeptide and ninhydrin. This indicates that the reaction mechanism remains the same in presence of micelles as that in aqueous medium with all possible intermediary situations. In the micellar medium the reaction of both $(Pep)_w$ and $(Pep)_m$ with $Nin_w$ and $Nin_m$ takes place. The rate constant ($k_\psi$) increased with increase in [TTABr], reached a maximum value, and, then with further increase in [TTABr], a decreasing effect was observed. The enhancement of rate in presence of cationic micelles could then be attributed to stabilization of intermediate (*i.e.*, Schiff base (B)) on the positively charged micellar surface, thereby increasing the concentration of the intermediate in the Stern layer. Therefore, both the reactants get effectively incorporated/associated into the aqueous surface of the micelles (*i.e.*, the Stern layer considered to be the usual site of ionic micelle-mediated organic reactions). Thus, the overall increase of reaction rate is due to concentrating both the reactants in the micellar zone. The $k_\psi$– [TTABr] profile shapes (Figures 3.5 and 3.6) are perfectly general being a common characteristic of bimolecular reactions catalyzed by micelles [5,20.22-25].

A much debatable question in the micelle assisted reactions is that of the locale of the reaction [26,27]. Most of the ionic mediated reactions are believed to occur either inside the Stern layer or at the interface between micellar surface and bulk water solvent [11,22] (reports revealing the occurrence of reactions at the junctural region of Stern and Gouy-Chapman layers [18,19] and cross micelles are scanty [20-22]). The main factor involved in the kinetic micellar effects on bimolecular reactions is the increased concentration of both the reactants, *i.e.*, ninhydrin and dipeptide into a small volume (through electrostatic and *hydrophobic* interactions). Beside this, micelles also exert a medium effect influencing reactivity. The location of reactants in the micellar structure and the degree of penetration of water into micellar structure has a major influence on reactivity. The fact is that the micellar *pseudophase* is regarded as a microenvironment having varying degrees of polarity, water activity, and hydrophobicity increasing with distance from the interfacial region to its core [28]. It is therefore not possible to precisely locate the site of reaction but, at least, the localization of the reactants can be considered. Based on purely electrostatic considerations, ninhydrin (due to presence of electron cloud [3]) will be located predominantly in the Stern layer and a lesser extent in the counter ion

diffuse layer surrounding the cationic micelles, whereas *hydrophobic* interactions can bring about the incorporation of the dipeptide into micelles. The micelles thus help in bringing the reactants together which may now orient in a manner suitable for the condensation.

With TTABr, optimum $k_\psi$ was found around [TTABr] ≈ 20.0 x$10^{-3}$ mol $dm^{-3}$ while further increase in TTABr concentration led to decrease in rate constant values. The explanation of this decreasing behavior can be as follows. At [TTABr] > 20.0 x$10^{-3}$ mol $dm^{-3}$, practically all the substrate has been incorporated into the micellar phase. When bulk of the substrate is incorporated into micelles, addition of more TTABr generates more cationic micelles, which simply take up the ninhydrin molecules into the Stern layer, and thereby deactivate them; because a ninhydrin molecule in one micelle should not react with the other in another [29]. This effect is the one responsible for the decrease in $k_\psi$ observed at high surfactant concentrations. Another reason for decreasing $k_\Psi$ could be a result of counterion ($Br^-$) inhibition.

In contrast, with gemini surfactants (Figures 3.7 and 3.8), The $k_\psi$–[14-s-14] profile can be described as follows. At the beginning, adding gemini surfactant increases the rate constants (zone I), then remains constant up to certain concentration (zone II). At the end, the rate constant increases again (zone III). The character for zones I and II and behavior of $k_\psi$ are akin to monomeric surfactant micelles with much better catalyzing effect for geminis than their single chain analogues [5,23,30-33].

In zone I, at concentrations lower than CMC, $k_\psi$ increases abnormally. The noticeable catalytic effect may be due to presence of premicelles [34]. In the second part (zone II), the $k_\psi$ values remain almost unchanged up to ~ 700 x$10^{-5}$ mol $dm^{-3}$ of gemini surfactants. Within the range of concentrations in zones I and II, the 14-s-14 surfactants show a much better catalyzing power than TTABr. This could be due to the presence of spacer in the geminis which decreases the water content in the aggregates providing the reaction environments less polar and thus causing enhancement in the rate [10]. Menger et al. [20] have already concluded that due to the proximity of positive charges in gemini surfactants, anion binding at the surfaces is increased at the expense of binding of $H_2O$

molecules. After leveling off (zone II), further increase at higher [14-s-14] (zone III, 800-3000 x $10^{-5}$ mol $dm^{-3}$), the values of $k_\psi$ increase slowly due to probable association with a change of micellar structure.

The influence of temperature on the TTABr/14-s-14-catalyzed reactions of dipeptides with ninhydrin in presence of constant concentration of TTABr/geminis were studied and the activation parameters such as activation energy ($E_a$), enthalpy of activation ($\Delta H^{\neq}$) and entropy of activation ($\Delta S^{\neq}$) are given in Tables 3.7 and 3.8. The values of $E_a$ clearly suggest that TTABr acts as a catalyst and provides a new reaction path with lower activation energy. The variation of the activation parameters in TTABr micelles compared in water is as expected, because one might expect stabilization of transition state due to presence of micelles that facilitate the occurrence of the reaction.

The activation energies obtained in the case of the gemini surfactants are according to their efficacy of catalyzing the reaction, *i.e.*, 14-4-14>14-5-14>14-6-14. Gemini surfactants lower the activation parameters ($E_a$ and $\Delta H^{\neq}$) than that in aqueous. This decrease in parameters occurs not only through the stabilization of transition state but also through adsorption of substrate on micellar surface.

The decrease in $\Delta S^{\neq}$ indicates that the formation of a well-structured transition state in which the reactive groups are closely associated with less degree of freedom. The $\Delta H^{\neq}$ and $\Delta S^{\neq}$ values are associated to overall rate of reaction. In a complex reaction each elementary step has its own value of enthalpy and entropy. The observed rate constants are representative of total rate and are complex function of true rate, binding and ionization constant. Therefore, for a complex reaction path, a meaningful mechanistic explanation is not possible on the basis of $\Delta H^{\neq}$ and $\Delta S^{\neq}$. The fitting of observed $k_\psi$ at different temperatures to the equation was examined and it was found that Eyring equation is applicable to the micellar systems and the sensitivity of micelle structure to temperature is kinetically insignificant.

### *3.3.2.1 Influence of s-Value of Gemini Surfactants*

The effect of s-values (spacer chain length variation) on the reaction rate of the formation of DYDA product under experimental reaction condition is illustrated in Figures 3.15 and 3.16, which represents maximum $k_\psi$ at s = 4. No significant change in $k_\psi$ has been found when using the single chain TTABr surfactant at the same condition ([TTABr] = 50.0 x $10^{-5}$ mol $dm^{-3}$). Spacer length (s) in 14-s-14 series is the key for enhancing the reaction rate by providing different less polar microenvironments when decreasing the amount of water in aggregates [10].

The $k_\psi$ – values for the 14-s-14 series at the range concentration (0 – 3000 x $10^{-5}$ mol $dm^{-3}$) follow the order 14-4-14 > 14-5-14 > 14-6-14 and have the same characteristics. It is known that type and length of the spacer moiety dictates the conformation of the gemini molecule [35,36]. The micellar growth is greater when the s-value is shorter in the order 4 > 5 > 6 which is most likely due to the increasing geometrical constraint in the formation of aggregation with decreasing length of spacer unit. SANS and microviscosity data support the argument that, within the gemini surfactants, micellar morphology tends to be less ellipsoidal with increasing s-value [37]. It is well known that, to minimize its contact with water, a spacer longer than the "equilibrium" distance between two $-^{+}NMe_2$ head groups (the "equilibrium" distance happens at s = 4 in 14-s-14 surfactants) tends to loop towards the micellar interior [38]. Increased looping of the spacer (s > 4) will make the Stern layer more wet that will cause decrease in the rate constant value. Therefore, the results are in agreement with the earlier findings that, on increasing in the water content, the reaction environment leads to an inhibiting effect [39-45].

Thus, because of the spacer greatly influencing the surfactant morphology that provides different reaction environment, the $k_\psi$ values obtained in our studies are reliable with the expectation being maximum with geminis in the order 14-4-14>14-5-14>14-6-14.

### *3.3.2.2 Influence of Organic Solvents*

Organic solvents can direct the completion of reactions slowly or quickly according to the solvation property, and in oppose with *hydrophobic* effect. Changing the solvent composition provides an opportunity to study the role of the so-called *solvophobic* effect on the reaction rate [46] so that the ability of the organic solvent to solvate anions and cations must be considered. The effect of DMSO, DO and AN on the rate of dipeptide–ninhydrin reaction in absence and presence of micelles is reported in Tables 3.13 and 3.14 and in Figures 3.9-3.14. We see that the addition of water-soluble organic solvents markedly increase the rate as well as intensity of the color (Figures 2.4-2.13) as DYDA product is preferably soluble in organic solvents [1,47–50]. On the other hand, the combined presence of organic solvent and micelles in micellar system shows a synergistic effect, which may lead to direct the mechanism (Scheme 3.1) to route 1 and decrease or block a side reaction of hydrolysis in route 2 as well. In given kinetic sets, increasing of solvent volume leads to decrease the bulk of water which results in a decrease of the rate of hydrolysis.

For Gly-L-Ala–ninhydrin reaction (Table 3.13, Figures 3.9- 3.11), it has been found that the enhancing of the reaction rate (except for DMSO at >20.0% v/v, which shows inhibition effect) follows the pattern: AN (dipolar aprotic)> DO (nonpolar aprotic) > DMSO (dipolar aprotic). The term "solvent polarity" is not precisely defined so that the polarity of the medium alone cannot be a primary guide for this pattern. The presence of all the studied organic solvents delay the micellization processes (*i.e.*, increase the CMC values as shown in Tables 2.8- 2.11) and can be explained on the basis of subtle balance of *hydrophobic* interactions of the long chain hydrocarbon tails, repulsive interactions between the ionic head groups, and any modifications to the above interactions by the presence of organic solvents. Thus, in all the cases of the studied water–organic solvent mixed systems, transfer of hydrocarbon tails of gemini surfactants into the micellar core and that of the methylene groups in the spacer and methyl groups in the head part to the micellar surface/interior part of the micelles becomes progressively less favorable with the increase of the organic solvent in the mixture.

AN and DMSO can form hydrogen bonds with water molecules which lead to increase the CMC and postpone the micellization of surfactants. The dipolar *aprotic* solvent AN can disrupt the micelle formation through better solvation of the surfactant monomers than pure water [51,52]. AN where presence of nitrogen atom may somehow block a side reaction more effectively and increase the solubility of the reactants more effectively than DO and DMSO due to the decrease of micelle number density which leads to the formation of H–bonds between AN molecules and bulk water. The non-polar aprotic cyclic ether DO, which can exist in two isomeric (either boat or chair) forms [53], may give larger hydrophobic surface area and this would be solvating more surfactant monomers than the pure water. As a consequence, *solvophobicity* of the TTABr/14-s-14 surfactants are decreased, formation of micelles becomes less favorable, and a higher surfactant concentration is required to start aggregation. DMSO, in comparison with AN and DO, inhibits the reaction rate (especially at higher concentration) due to its strong interaction with water and increasing of the structuring of the DMSO–$H_2O$ system, known to form stoichiometric hydrates with water in the ratio 1DMSO:2$H_2O$ [44,54].

In case of Gly-DL-Asp–ninhydrin reaction, concentration variation (v/v %) of binary solvent systems: DMSO–$H_2O$, DO–$H_2O$ and AN–$H_2O$ was also used to check the catalytic effect on this reaction in absence and presence of TTABr/14-s-14 micelles (Table 3.14, Figures 3.12- 3.14). It has been found that all considered solvents accelerate Gly-DL-Asp–ninhydrin reaction in absence and presence of surfactants with the highest enhancement in the presence of TTABr/14-s-14 (14-4-14>14-5-14>14-6-14) micelles while the lowest enhancement in the absence of surfactants.

In comparison with Gly-L-Ala–ninhydrin reaction, DMSO doesn't show any decrease in $k_\psi$ at high solvent concentration with Gly-DL-Asp. The main reason for this behavior may be the protonated hydrogen of the side chain (–$CH_2$COOH) in Gly-DL-Asp that reduces the hydrogen bonding between H of water and O of DMSO molecules.

**Table 3.15:** Rate and binding constants for the reaction of Gly-L-Ala with ninhydrin.

*Reaction conditions*:

| | | |
|---|---|---|
| [Gly-L-Ala] | = | $2.0 \times 10^{-4}$ mol $dm^{-3}$ |
| [ninhydrin] | = | $6.0 \times 10^{-3}$ mol $dm^{-3}$ |
| [TTABr] | = | $20.0 \times 10^{-3}$ mol $dm^{-3}$ |
| [14-s-14] | = | $50.0 \times 10^{-5}$ mol $dm^{-3}$ |
| *p*H | = | 5.0 |
| Temperature | = | 70 °C |

| Constants | Surfactant | | | |
|---|---|---|---|---|
| | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| $10^3 k_m$ ($s^{-1}$) | 0.7 | 4.0 | 13.7 | 10.8 |
| $10^3 k_w$ ($mol^{-1} dm^3 s^{-1}$) | 23.5 | 23.5 | 23.5 | 23.5 |
| $K_A$ ($mol^{-1} dm^3$) | 270.0 | 209.0 | 200.0 | 197.0 |
| $K_{Nin}$ ($mol^{-1} dm^3$) | 45.5 | 48.3 | 52.6 | 51.6 |

**Table 3.16:** Rate and binding constants for the reaction of Gly-DL-Asp with ninhydrin.

*Reaction conditions*:

[Gly-DL-Asp] = $3.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| Constants | Surfactant | | | |
|---|---|---|---|---|
| | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| $10^3 k_m$ ($s^{-1}$) | 1.6 | 3.5 | 8.5 | 13.6 |
| $10^3 k_w$ ($mol^{-1} dm^3 s^{-1}$) | 8.8 | 8.8 | 8.8 | 8.8 |
| $K_A$ ($mol^{-1} dm^3$) | 106.0 | 166.0 | 160.0 | 150.0 |
| $K_{Nin}$ ($mol^{-1} dm^3$) | 77.9 | 69.7 | 71.6 | 75.6 |

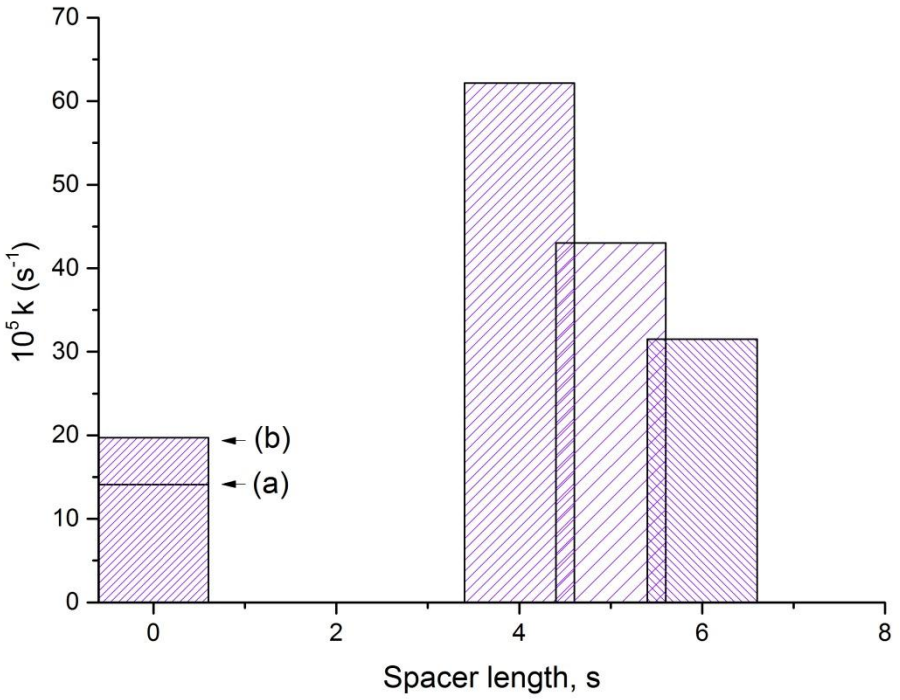



**Figure 3.15:** Spacer length (s = 4, 5, 6) influence on the reaction rate of Gly-L-Ala (2.0 x$10^{-4}$ mol $dm^{-3}$) with ninhydrin (6.0 x$10^{-3}$ mol $dm^{-3}$), (a) in aqueous, and (b) in [TTABr] = 50.0 x $10^{-5}$ mol $dm^{-3}$ at *p*H= 5.0 and temperature = 70 $^{o}$C. Others are for [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ with the respective s values.

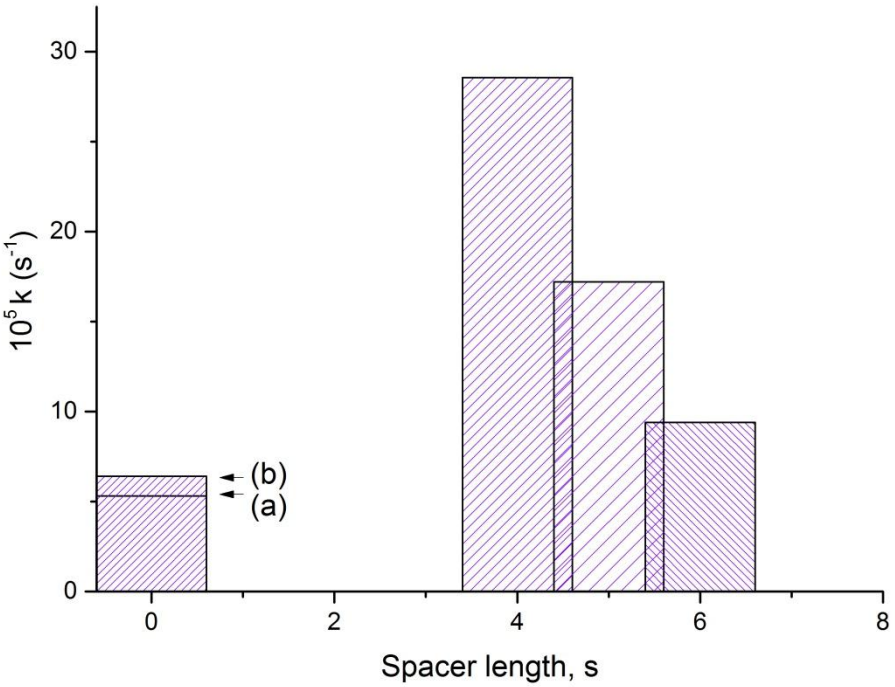



**Figure 3.16:** Spacer length (s = 4, 5, 6) influence on the reaction rate of Gly-DL-Asp (3.0 x $10^{-4}$ mol $dm^{-3}$) with ninhydrin (6.0 x$10^{-3}$ mol $dm^{-3}$), (a) in aqueous, and (b) in [TTABr] = 50.0 x $10^{-5}$ mol $dm^{-3}$ at *p*H= 5.0 and temperature = 70 $^{o}$C. Others are for [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ with the respective s values.

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# CHAPTER-IV

# Kinetics of Metal ion-Coordinated Dipeptide –Ninhydrin Reactions in Aqueous and Micellar Systems and Effect of organic Solvents

## 4.1 Introduction

The study of coordination compounds has been focused on the behavior of the metal ions rather than the attached ligands. It is evident that metals play essential roles in biological systems both structurally and functionally and the coordination of metal is a base stone in molecular identification [1] and conjugation [2-6] between biomedicine and chemistry. Both the three-dimensional structures [7,8] and enzymatic functions [9-11] of many biological systems depend on complexation of metals.

Metal ions form complexes with aldehydes, amines and Schiff bases and stabilize or labilize the double bond of Schiff bases thermodynamically [12]. The mechanism of this intermediate formation is of importance in biochemistry [13]. Although ninhydrin as a fingerprint material is largely used daily in thousands of chemistry, forensic and biochemistry laboratories throughout the world [14-16], the technique is still far from satisfaction. The color-forming ninhydrin-amino acids/dipeptide reaction have characteristics of common addition-elimination type reactions. As the purple-colored dye faints at room temperature, many attempts were carried out to stabilize it. Metal ion complex formations are the prominent interactions in nature. The effects of metal ions on this reaction were also studied with the viewpoint of promoting the nucleophilic attack. The condensation of ninhydrin with the dipeptide (coordinated with metal ion) acts as a potential tridentate metal binding ONO donor ligand producing stable five membered metal chelate. As a result, interaction of metal ion-dipeptide complexes with ninhydrin was also studied, and the color yield was indeed affected [17,18].

The use of micelles, as a microenvironment for reactions to take place, has been investigated by many workers [19-26]. In kinetic studies the most important advantage, as compared to water media, is that micellar systems can enhance/inhibit the reactions more effectively according to their properties such as solubility, binding and structure. Ionic micelles affect rates of unimolecular and bimolecular water-catalyzed reactions because the reaction region at the micelle-water interface is less polar than water [27]. The so-called “Hartley’s Rules” or charge-charge interactions between micelles and ions

in solution played a major role in subsequent development of treatments of micellar effects on reactions.

Even though some qualitative information is available on the role of organic solvents [15, 28-30], kinetic evidence to distinguish their role is limited especially with metal ion coordinated-dipeptide complex–ninhydrin reactions. For this reason, and in the hope that introduction of organic solvents may cause the use of low concentration of reactants, a systematic kinetic study of metal ion coordinated-dipeptide–ninhydrin reactions has been made in the presence of cationic/dicationic TTABr/14-s-14 micelles in the absence and presence of different fixed compositions of solvents (dimethyl sulfoxide (DMSO), 1,4-dioxane (DO) and acetonitrile (AN)).

The present investigations on the kinetics and mechanism of metal ion–dipeptide (Met(II)-Pep) complexes (dipeptide = Gly-L-Ala and Gly-DL-Asp) with ninhydrin were carried out to explore other aspects of the effect of presence of TTABr/14-s-14 micelles in aqueous and aqueous-organic solvents upon Hg(II)- and Cu(II)-dipeptide complex-ninhydrin reactions, to assist in binding the substrate to the micelle, to improve the catalytic efficiency of dipeptide towards condensation and to check whether TTABr/14-s-14 micelles change the aqueous reaction mechanism.

The results are described in the following pages.

## 4.2 Results

The UV-visible spectra of metal coordinated dipeptide $[Met(II)\text{-}Pep]^{+}$–ninhydrin complexes were recorded using UV-mini 1240 SHIMADZU spectrophotometer (Figures 2.14 – 2.33). In comparison with aqueous medium, the absorbance is higher in presence of TTABr/14-s-14 which becomes even higher when organic solvents are present. Further, no shift in the absorbance maxima for $[Hg(II)\text{-}Pep]^{+}$–ninhydrin complex ($\lambda_{max}$= 400 nm) or $[Cu(II)\text{-}Pep]^{+}$–ninhydrin complex ($\lambda_{max}$= 340 nm) was observed: this confirms that the same products are formed in both the systems (*i.e.*, aqueous and TTABr/14-s-14 micellar systems).

### 4.2.1 Influence of [Met(II)-Pep]$^+$ Complex on the Reaction Rate

To find out the order of reaction with respect to metal-dipeptide complex concentration, the rate constants were determined at different initial concentrations of [Met(II)-Pep]$^+$ complex ranging from 1.0 x $10^{-4}$ to 5.0 x $10^{-4}$ mol $dm^{-3}$. The concentration of ninhydrin was kept constant at fixed temperature and *p*H. The values of *pseudo*-first order rate constants are given in Tables 4.1-4.4. Similar studies were performed in TTABr/14-s-14 surfactant micelles. As the values of rate constants ($k_{obs}$ or $k_{\psi}$) were found to be independent of the initial concentration of Met(II)-Pep complex, the order with respect to [Met(II)-Pep]$^+$ complex concentration is unity in both the media. Hence, the rate law is:

$$\text{rate} = k\,[\text{Met(II)-Pep}]^+ \qquad (4.1)$$

where [Met(II)-Pep]$^+$ is the total concentration of metal ion coordinated-dipeptide complex.

### 4.2.2 Influence of *p*H on the Reaction Rate

The influence of *p*H on the rate constants of [Met(II)–Pep]$^+$ with ninhydrin reaction was studied in the *p*H range 4.0–6.0 (Tables 4.5– 4.8) at 70 $^oC$. At *p*H 5.0, Gly-L-Ala is neutral while Gly-DL-Asp is negatively charged. Irrespective of the charge, it is the unprotonated $–NH_2$ (terminal) which should be available for the condensation. Hence, in this case also the mechanism of reaction would remain the same [31]. As is evident (Figures 4.1– 4.4), the optimum rate constant is at *p*H 5.0. Consequently, all the subsequent kinetic runs were made at *p*H 5.0.

### 4.2.3 Influence of [Ninhydrin] on the Reaction Rate

The rate constants were determined by carrying out the kinetic experiments with different concentrations of ninhydrin keeping the [Met(II)-Pep]$^+$, temperature and *p*H constant in both the media (Tables 4.9 – 4.12). The plots of rate constant values (k, $s^{-1}$) *vs.* [ninhydrin] are curved passing through the origin (Figures 4.5– 4.8). This verifies that the order is fractional with respect to [ninhydrin] in aqueous and micellar media.

### 4.2.4 Influence of Temperature on the Reaction Rate

The influence of temperature on the rate of metal-coordinated dipeptide-ninhydrin reactions was studied at five different temperatures with five degree interval (range 60–80 $^{o}$C) in absence and presence of TTABr/14-s-14 surfactants. The *pseudo*-first order constants increased with rise in temperature from 60 to 80 $^{o}$C in both the media. The *pseudo*-first order rate constants (k, $s^{-1}$), as summarized in Tables 4.13– 4.16, were used to calculate $E_a$, $\Delta S^{\neq}$ and $\Delta H^{\neq}$ from the Eyring equation by plotting of log k *vs.* 1/T (which was found to be linear with negative slope in both the media) and the values of these activation parameters are recorded in Tables 4.13– 4.16.

### 4.2.5 Influence of [Surfactant] on the Reaction Rate

The effect of cationic surfactants on the reaction were studied under varying concentrations of TTABr/geminis at constant $[Met(II)–Pep]^+$, [ninhydrin], temperature (70 $^{o}$C) and *p*H (5.0). The study showed that the reaction follows first- and fractional-order kinetics with respect to concentrations of metal-dipeptide complex and ninhydrin, respectively. Thus, the order is the same with respect to $[Met(II)–Pep]^+$ and [ninhydrin] as that in aqueous medium. In the conventional surfactant (TTABr), as noted earlier, the values of rate constant for spontaneous reactions generally increase monotonically with increasing surfactant concentration and after the substrates completely bind the micelles, $k_\psi$ values decrease. The plot of $k_\psi$ *vs.* [TTABr] has perfectly common characteristic of bimolecular reactions catalyzed by micelles [32-38]. However, with gemini surfactants, the $k_\psi$–[14-s-14] profiles follow a special phenomenon: at low [14-s-14] (below CMC) the $k_\psi$ values increase (zone I). After that there is no critical change in $k_\psi$ up to certain concentration (zone II) (the characteristics of zone I and zone II are just like the conventional counterpart TTABr [32,37]), and then $k_\psi$ values increase again (zone III).

The results for [surfactant] effect on the reaction rate are collected in Tables 4.17–4.24 and plotted in Figures 4.9– 4.16.

### 4.2.6 Influence of Organic Solvents on the Reaction Rate

To find out the effect of solvents on [Met(II)-Pep]$^{+}$–ninhydrin reactions, a number of solvents; acetonitrile (AN), 1,4-dioxane (DO) and dimethyl sulfoxide (DMSO) have been used for the purpose. It has been observed that addition of small quantities of the water-soluble organic solvents significantly increases the rate as well as the intensity of the color in micellar systems (Tables 4.25– 4.28, Figures 4.17– 4.28).

**Table 4.1:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on $[Hg(II)\text{-}Gly\text{-}L\text{-}Ala]^{+}$ for the reaction of Hg(II)-Gly-L-Ala complex with ninhydrin.

*Reaction conditions*:

| | | |
|---|---|---|
| [TTABr] | = | $20.0 \times 10^{-3}$ mol $dm^{-3}$ |
| [14-s-14] | = | $50.0 \times 10^{-5}$ mol $dm^{-3}$ |
| [ninhydrin] | = | $6.0 \times 10^{-3}$ mol $dm^{-3}$ |
| *p*H | = | 5.0 |
| Temperature | = | 70 °C |

| $10^4$ $[Hg(II)\text{-}Gly\text{-}L\text{-}Ala]^{+}$ (mol $dm^{-3}$) | $10^4$ $k_{obs}$ ($s^{-1}$) | $10^4$ $k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 1.0 | 36.6 | 46.4 | 41.3 | 44.8 | 49.5 |
| 2.0 | 37.5 | 47.3 | 42.3 | 45.6 | 50.4 |
| 2.5 | 36.8 | 46.2 | 42.1 | 46.3 | 51.3 |
| 3.0 | 37.8 | 47.5 | 43.4 | 46.0 | 50.7 |
| 3.5 | 36.8 | 46.9 | 42.4 | 46.2 | 51.1 |

**Table 4.2:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on $[Hg(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ for the reaction of Hg(II)-Gly-DL-Asp complex with ninhydrin.

*Reaction conditions*:

| | | |
|---|---|---|
| [TTABr] | = | $20.0 \times 10^{-3}$ mol $dm^{-3}$ |
| [14-s-14] | = | $50.0 \times 10^{-5}$ mol $dm^{-3}$ |
| [ninhydrin] | = | $6.0 \times 10^{-3}$ mol $dm^{-3}$ |
| *p*H | = | 5.0 |
| Temperature | = | 70 °C |

| $10^4$ $[Hg(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ (mol $dm^{-3}$) | $10^4$ $k_{obs}$ ($s^{-1}$) | $10^4$ $k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 1.0 | 34.6 | 48.9 | 40.0 | 42.9 | 47.6 |
| 2.0 | 34.9 | 50.9 | 40.5 | 43.4 | 48.3 |
| 2.5 | 35.8 | 51.5 | 41.2 | 44.0 | 49.0 |
| 3.0 | 34.6 | 50.7 | 42.0 | 43.5 | 48.6 |
| 3.5 | 35.0 | 49.8 | 41.1 | 44.1 | 49.1 |

**Table 4.3:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on $[Cu(II)\text{-}Gly\text{-}L\text{-}Ala]^{+}$ for the reaction of Cu(II)-Gly-L-Ala complex with ninhydrin.

*Reaction conditions*:

| | | |
|---|---|---|
| [TTABr] | = | $20.0 \times 10^{-3}$ mol $dm^{-3}$ |
| [14-s-14] | = | $50.0 \times 10^{-5}$ mol $dm^{-3}$ |
| [ninhydrin] | = | $10.0 \times 10^{-3}$ mol $dm^{-3}$ |
| *p*H | = | 5.0 |
| Temperature | = | 70 °C |

| $10^4$ $[Cu(II)\text{-}Gly\text{-}L\text{-}Ala]^{+}$ (mol $dm^{-3}$) | $10^4$ $k_{obs}$ ($s^{-1}$) | $10^4$ $k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 2.0 | 19.6 | 29.8 | 21.8 | 30.1 | 34.1 |
| 3.0 | 19.7 | 30.3 | 22.2 | 31.2 | 34.9 |
| 4.0 | 19.9 | 30.2 | 22.4 | 30.9 | 34.7 |
| 4.5 | 20.0 | 30.2 | 23.0 | 31.0 | 35.1 |
| 5.0 | 19.9 | 30.3 | 22.6 | 30.9 | 34.9 |

**Table 4.4:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on $[Cu(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ for the reaction of Cu(II)-Gly-DL-Asp complex with ninhydrin.

*Reaction conditions*:

| | | |
|---|---|---|
| [TTABr] | = | $20.0 \times 10^{-3}$ mol $dm^{-3}$ |
| [14-s-14] | = | $50.0 \times 10^{-5}$ mol $dm^{-3}$ |
| [ninhydrin] | = | $10.0 \times 10^{-3}$ mol $dm^{-3}$ |
| *p*H | = | 5.0 |
| Temperature | = | 70 °C |

| $10^4$ $[Cu(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ (mol $dm^{-3}$) | $10^4$ $k_{obs}$ ($s^{-1}$) | $10^4$ $k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 2.0 | 18.5 | 28.8 | 20.9 | 24.4 | 30.1 |
| 3.0 | 18.1 | 29.6 | 21.4 | 24.6 | 30.4 |
| 4.0 | 18.3 | 29.1 | 21.6 | 24.8 | 30.2 |
| 4.5 | 18.8 | 29.4 | 21.8 | 25.0 | 30.5 |
| 5.0 | 18.0 | 28.9 | 21.7 | 24.9 | 30.2 |

**Table 4.5:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on *p*H for the reaction of Hg(II)-Gly-L-Ala complex with ninhydrin.

*Reaction conditions*:

$[Hg(II)\text{-}Gly\text{-}L\text{-}Ala]^{+}$ = $2.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

Temperature = 70 °C

| *p*H | $10^4 k_{obs}$ ($s^{-1}$) | $10^4 k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 4.0 | 20.0 | 30.1 | 22.3 | 27.7 | 29.3 |
| 4.5 | 27.0 | 33.4 | 28.4 | 30.2 | 32.5 |
| 5.0 | 37.5 | 47.3 | 42.3 | 45.6 | 50.4 |
| 5.5 | 38.3 | 47.6 | 41.9 | 45.6 | 50.9 |
| 6.0 | 36.3 | 46.9 | 40.3 | 44.7 | 48.7 |

**Table 4.6:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on *p*H for the reaction of Hg(II)-Gly-DL-Asp complex with ninhydrin.

*Reaction conditions*:

$[Hg(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = $2.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

Temperature = 70 °C

| *p*H | $10^4 k_{obs}$ ($s^{-1}$) | $10^4 k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 4.0 | 18.3 | 23.6 | 20.3 | 22.3 | 25.1 |
| 4.5 | 30.5 | 37.9 | 32.6 | 35.7 | 37.2 |
| 5.0 | 34.9 | 50.9 | 40.5 | 43.4 | 48.3 |
| 5.5 | 39.6 | 55.1 | 44.3 | 49.3 | 53.6 |
| 6.0 | 39.8 | 57.4 | 45.3 | 50.3 | 57.8 |

**Table 4.7:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on *p*H for the reaction of Cu(II)-Gly-L-Ala complex with ninhydrin.

*Reaction conditions*:

$[Cu(II)\text{-}Gly\text{-}L\text{-}Ala]^{+}$ = $4.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $10.0 \times 10^{-3}$ mol $dm^{-3}$

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

Temperature = 70 °C

| *p*H | $10^4 k_{obs}$ ($s^{-1}$) | $10^4 k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 4.0 | 10.0 | 18.1 | 13.3 | 16.8 | 18.9 |
| 4.5 | 12.4 | 20.8 | 16.3 | 19.6 | 22.4 |
| 5.0 | 19.9 | 30.2 | 22.4 | 30.9 | 34.7 |
| 5.5 | 20.7 | 32.8 | 23.4 | 32.1 | 35.3 |
| 6.0 | 20.0 | 31.4 | 22.2 | 30.8 | 34.7 |

**Table 4.8:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on *p*H for the reaction of Cu(II)-Gly-DL-Asp complex with ninhydrin.

*Reaction conditions*:

$[Cu(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = $4.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $10.0 \times 10^{-3}$ mol $dm^{-3}$

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

Temperature = 70 °C

| *p*H | $10^4 k_{obs}$ ($s^{-1}$) | $10^4 k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 4.0 | 10.9 | 17.6 | 13.2 | 15.7 | 17.4 |
| 4.5 | 13.7 | 20.6 | 15.9 | 18.4 | 20.6 |
| 5.0 | 18.3 | 29.1 | 21.6 | 24.8 | 30.2 |
| 5.5 | 19.5 | 29.8 | 21.7 | 25.6 | 30.6 |
| 6.0 | 19.6 | 30.1 | 22.8 | 25.9 | 31.1 |

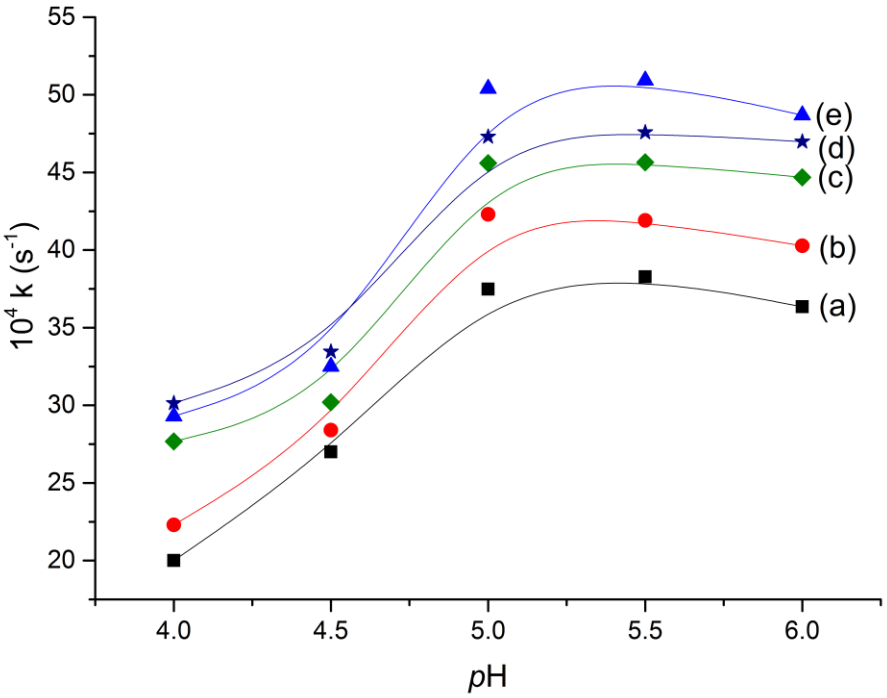



**Figure 4.1:** Influence of *p*H on the reaction rate of Hg(II)-Gly-L-Ala complex with ninhydrin in (a) aqueous and (b-e) presence of surfactants: (b) 14-6-14; (c)14-5-14; (d)TTABr: (e) 14-4-14. *Reaction conditions*: $[Hg(II)\text{-}Gly\text{-}L\text{-}Ala]^+ = 2.0 \times 10^{-4}$ mol $dm^{-3}$, [ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$, [TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$, [14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), temperature = 70 °C.

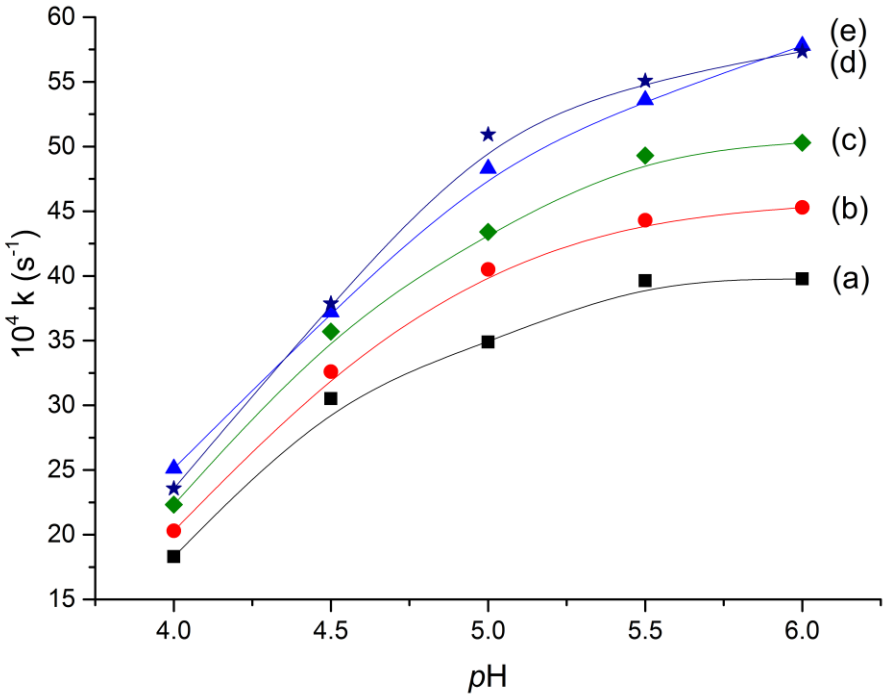



**Figure 4.2:** Influence of *p*H on the reaction rate of Hg(II)-Gly-DL-Asp complex with ninhydrin in (a) aqueous and (b-e) presence of surfactants: (b) 14-6-14; (c) 14-5-14; (d) TTABr and (e) 14-4-14. *Reaction conditions*: $[Hg(II)\text{-}Gly\text{–}DL\text{-}Asp]^{+}$ = 2.0 x$10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), temperature = 70 °C.

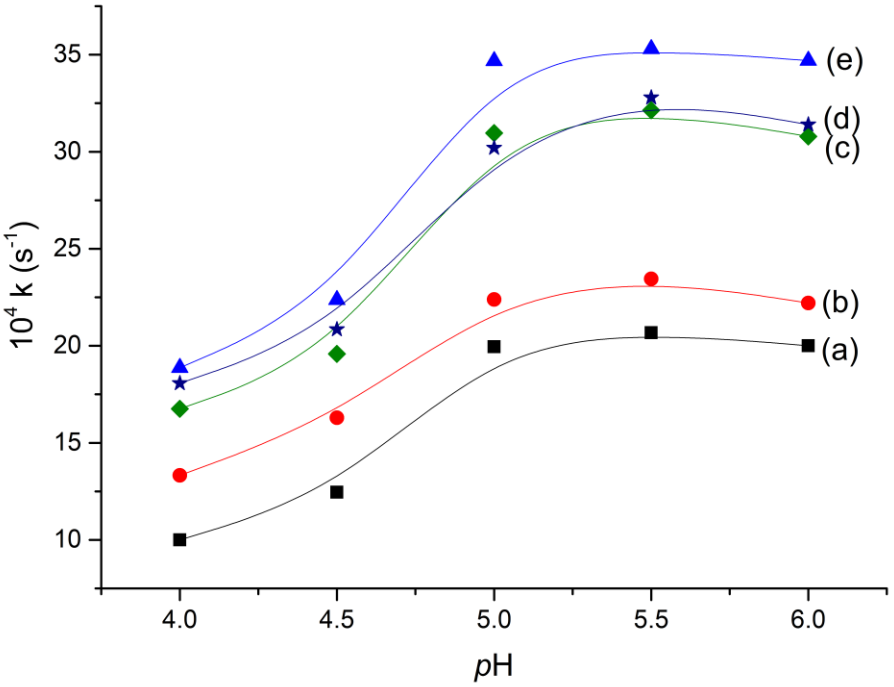



**Figure 4.3:** Influence of *p*H on the reaction rate of Cu(II)-Gly-L-Ala complex with ninhydrin in (a) aqueous and (b-e) presence of surfactants: (b) 14-6-14; (c) 14-5-14; (d) TTABr and (e) 14-4-14. *Reaction conditions*: $[Cu(II)\text{-}Gly\text{–}L\text{-}Ala]^+$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), temperature = 70 °C.

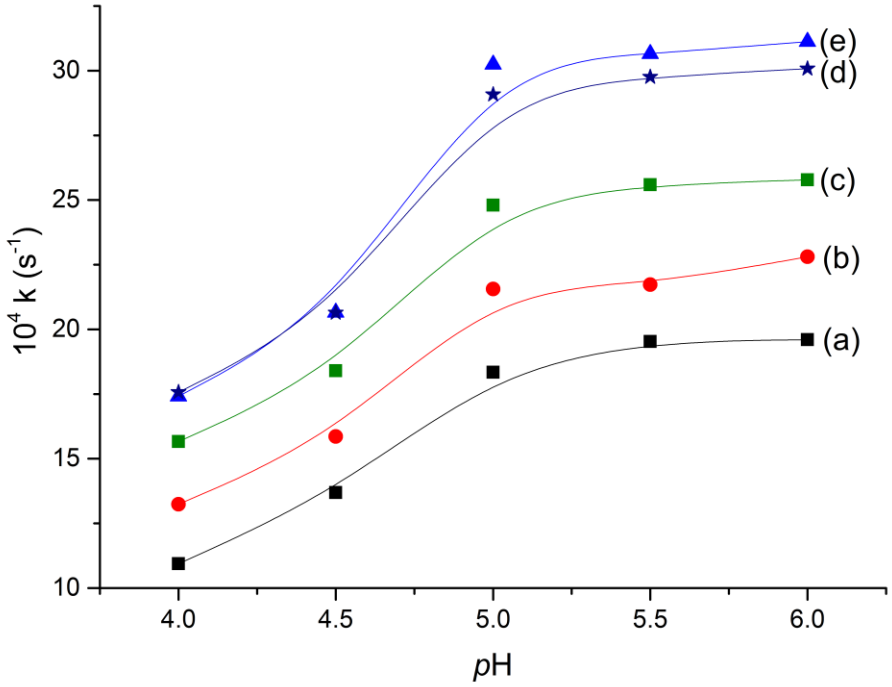



**Figure 4.4:** Influence of *p*H on the reaction rate of Cu(II)-Gly-DL-Asp complex with ninhydrin in (a) aqueous and (b-e) presence of surfactants: (b) 14-6-14; (c) 14-5-14; (d) TTABr and (e)14-4-14. *Reaction conditions*: $[Cu(II)\text{-}Gly\text{–}DL\text{-}Asp]^{+}$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x $10^{-3}$ mol $dm^{-3}$, [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), temperature = 70 °C.

**Table 4.9:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on [ninhydrin] for the reaction of Hg(II)-Gly-L-Ala complex with ninhydrin.

*Reaction conditions*:

$[Hg(II)\text{-}Gly\text{-}L\text{-}Ala]^{+}$ = $2.0 \times 10^{-4}$ mol $dm^{-3}$

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| $10^3$ [ninhydrin] (mol $dm^{-3}$) | $10^4$ $k_{obs}$ ($s^{-1}$) | $10^4$ $k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 6.0 | 37.5(38.7) | 47.3 | 42.3 | 45.6 | 50.4 |
| 10.0 | 50.8(51.2) | 58.7 | 53.9 | 56.8 | 60.5 |
| 15.0 | 55.8(53.4) | 61.1 | 58.2 | 60.8 | 62.9 |
| 20.0 | 62.3(56.7) | 69.4 | 65.1 | 67.7 | 69.7 |
| 25.0 | 65.1(59.1) | 70.2 | 67.8 | 69.7 | 72.4 |
| 30.0 | 65.1(61.0) | 73.3 | 68.3 | 70.9 | 75.6 |
| 35.0 | 68.4(62.4) | 79.6 | 70.4 | 70.9 | 76.2 |
| 40.0 | 65.6(63.3) | 78.2 | 68.9 | 72.8 | 81.2 |

**Table 4.10:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on [ninhydrin] for the reaction of Hg(II)-Gly-DL-Asp complex with ninhydrin.

*Reaction conditions*:

$[Hg(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = $2.0 \times 10^{-4}$ mol $dm^{-3}$

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| $10^3$ [ninhydrin] (mol $dm^{-3}$) | $10^4$ $k_{obs}$ ($s^{-1}$) | $10^4$ $k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 6.0 | 34.9(36.4) | 50.9 | 40.5 | 43.4 | 48.3 |
| 10.0 | 44.6(45.3) | 56.6 | 49.9 | 53.3 | 55.4 |
| 15.0 | 52.8(50.4) | 61.6 | 57.5 | 59.7 | 61.9 |
| 20.0 | 60.1(52.8) | 65.8 | 62.7 | 64.9 | 67.6 |
| 25.0 | 62.2(55.5) | 69.0 | 66.4 | 69.0 | 70.4 |
| 30.0 | 64.7(58.9) | 70.1 | 66.7 | 68.9 | 72.7 |
| 35.0 | 66.8(60.1) | 73.7 | 68.1 | 70.2 | 73.9 |
| 40.0 | 67.2(63.6) | 75.0 | 69.8 | 72.2 | 78.2 |

**Table 4.11:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on [ninhydrin] for the reaction of Cu(II)-Gly-L-Ala complex with ninhydrin.

*Reaction conditions*:

$[Cu(II)\text{-}Gly\text{-}L\text{-}Ala]^{+}$ = $4.0 \times 10^{-4}$ mol $dm^{-3}$

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| $10^3$ [ninhydrin] (mol $dm^{-3}$) | $10^4$ $k_{obs}$ ($s^{-1}$) | $10^4$ $k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 6.0 | 11.3(13.2) | 20.1 | 13.2 | 18.5 | 22.1 |
| 10.0 | 19.9(20.5) | 30.2 | 22.4 | 30.9 | 34.7 |
| 15.0 | 23.2(22.4) | 35.6 | 25.5 | 31.9 | 37.6 |
| 20.0 | 26.3(24.1) | 44.6 | 28.8 | 40.2 | 47.2 |
| 25.0 | 34.8(31.9) | 52.1 | 38.4 | 47.4 | 55.2 |
| 30.0 | 43.6(35.5) | 63.5 | 48.3 | 57.3 | 61.9 |
| 35.0 | 43.5(37.8) | 62.4 | 53.6 | 57.4 | 64.9 |
| 40.0 | 46.4(41.2) | 65.3 | 51.3 | 60.8 | 68.5 |

**Table 4.12:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on [ninhydrin] for the reaction of Cu(II)-Gly-DL-Asp complex with ninhydrin.

*Reaction conditions*:

$[Cu(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = $4.0 \times 10^{-4}$ mol $dm^{-3}$

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| $10^3$ [ninhydrin] (mol $dm^{-3}$) | $10^4 k_{obs}$ ($s^{-1}$) | $10^4 k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 6.0 | 12.4(13.5) | 17.3 | 13.0 | 15.4 | 17.4 |
| 10.0 | 18.3(19.4) | 29.1 | 21.6 | 24.8 | 30.2 |
| 15.0 | 24.5(21.1) | 33.3 | 28.1 | 30.2 | 34.8 |
| 20.0 | 27.1(25.3) | 40.9 | 33.6 | 35.8 | 38.3 |
| 25.0 | 30.5(29.0) | 43.9 | 38.9 | 41.4 | 48.4 |
| 30.0 | 38.3(35.6) | 48.7 | 41.9 | 47.7 | 51.9 |
| 35.0 | 39.6(37.8) | 56.5 | 47.2 | 48.9 | 53.5 |
| 40.0 | 42.5(40.1) | 57.0 | 48.4 | 54.8 | 59.1 |

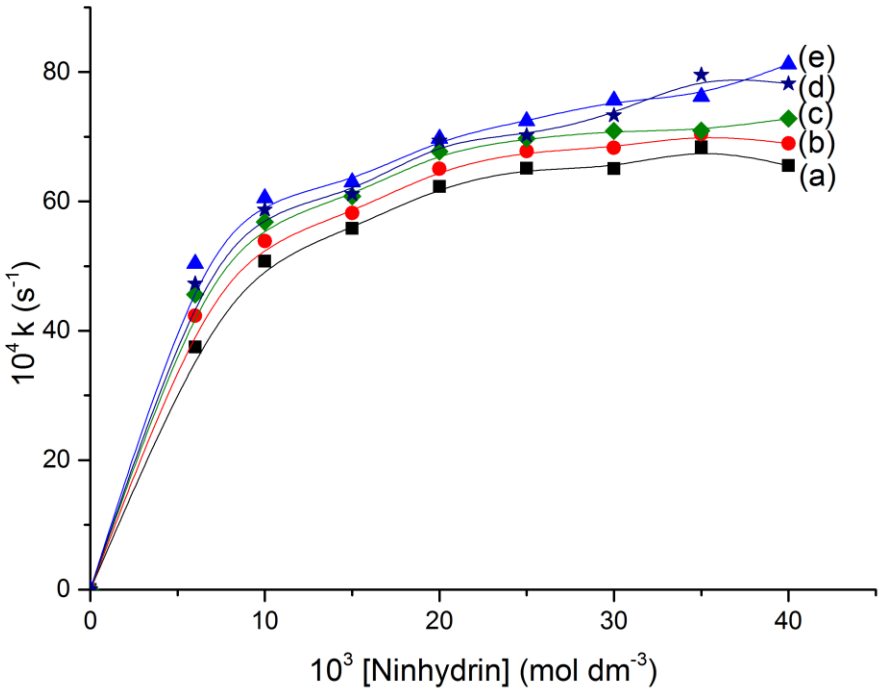



**Figure 4.5:** Influence of [ninhydrin] on the reaction rate of Hg(II)-Gly-L-Ala complex with ninhydrin in (a) aqueous and (b-e) presence of surfactants: (b) 14-6-14; (c) 14-5-14; (d) TTABr and (e) 14-4-14. *Reaction conditions*: $[Hg(II)\text{-Gly-L-Ala}]^+ = 2.0 \times 10^{-4}$ mol $dm^{-3}$, [TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$, [14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), temperature = 70 °C, *p*H = 5.0.

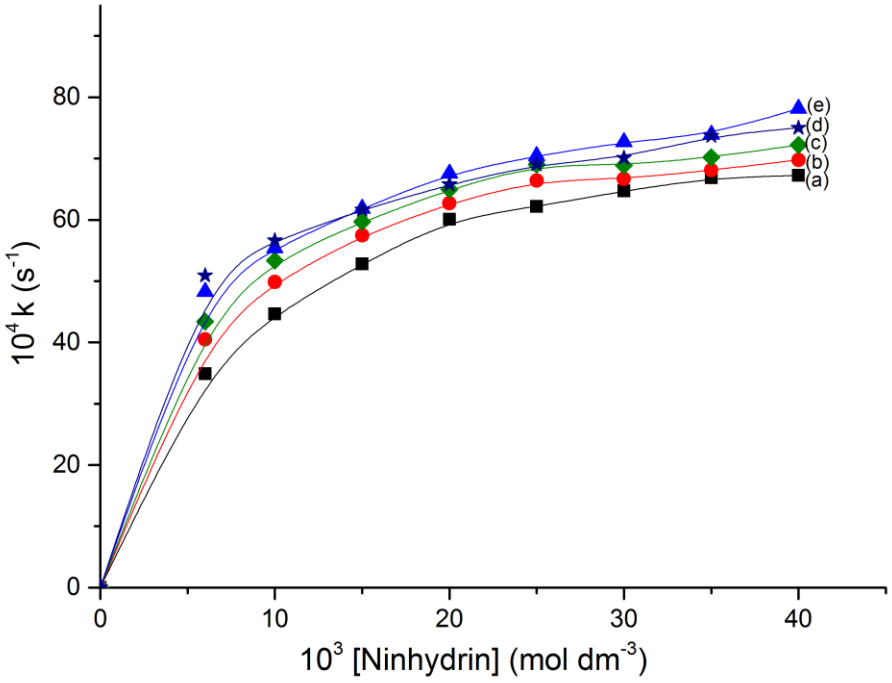



**Figure 4.6:** Influence of [ninhydrin] on the reaction rate of Hg(II)-Gly-DL-Asp complex with ninhydrin in (a) aqueous and (b-e) presence of surfactants: (b)14-6-14; (c) 14-5-14; (d) TTABr and (e)14-4-14. *Reaction conditions*: $[Hg(II)\text{-Gly-DL-Asp}]^+ = 2.0 \times 10^{-4}$ mol $dm^{-3}$, [TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$, [14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), temperature = 70 °C, *p*H = 5.0.

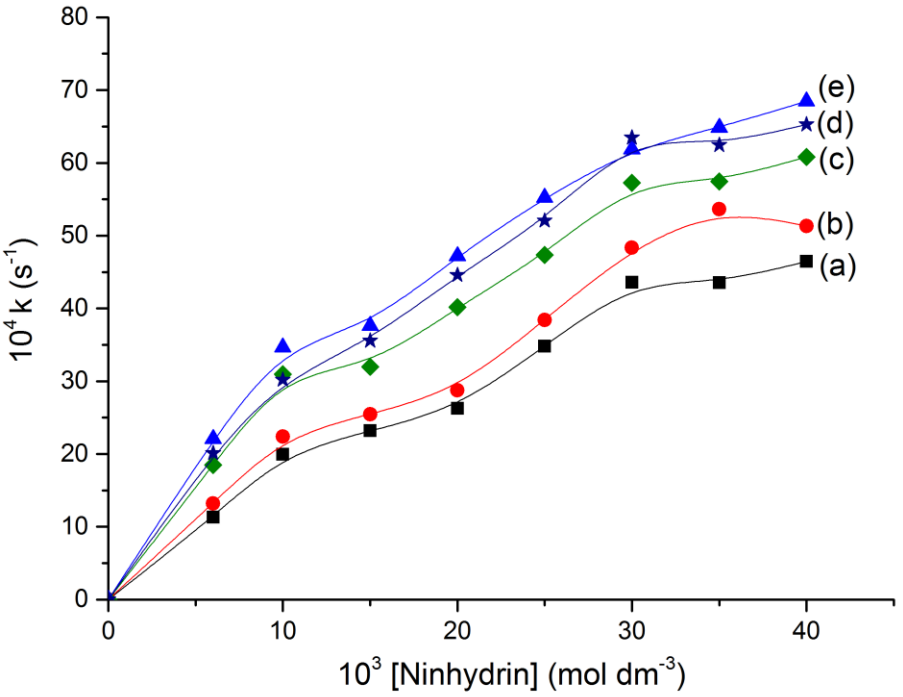



**Figure 4.7:** Influence of [ninhydrin] on the reaction rate of Cu(II)-Gly-L-Ala complex with ninhydrin in (a) aqueous and (b-e) presence of surfactants: (b) 14-6-14; (c) 14-5-14; (d) TTABr and (e) 14-4-14. *Reaction conditions*: $[Cu(II)\text{-Gly-L-Ala}]^+ = 4.0 \times 10^{-4}$ mol $dm^{-3}$, [TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$, [14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), temperature = 70 °C, *p*H = 5.0.

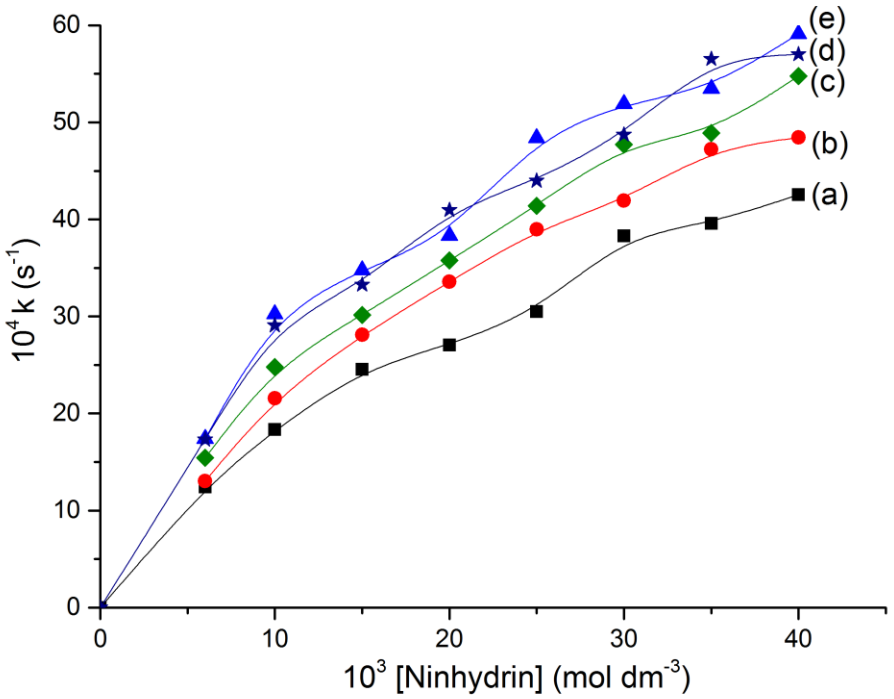



**Figure 4.8:** Influence of [ninhydrin] on the reaction rate of Cu(II)-Gly-DL-Asp complex with ninhydrin in (a) aqueous and (b-e) presence of surfactants: (b) 14-6-14; (c) 14-5-14, (d) TTABr and (e) 14-4-14. *Reaction conditions*: $[Cu(II)\text{-Gly-DL-Asp}]^+ = 4.0 \times 10^{-4}$ mol $dm^{-3}$, [TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$, [14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$ (s = 4, 5, 6), temperature = 70 °C, *p*H = 5.0.

**Table 4.13:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on temperature and related thermodynamic parameters for the reaction of Hg(II)-Gly-L-Ala complex with ninhydrin.

*Reaction conditions*:

$[Hg(II)\text{-}Gly\text{-}L\text{-}Ala]^{+}$ = $2.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

*p*H = 5.0

| Temperature (°C) | $10^4 k_{obs}$ ($s^{-1}$) | $10^4 k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 60 | 20.6 | 27.6 | 21.2 | 24.4 | 30.0 |
| 65 | 25.8 | 34.3 | 27.2 | 28.4 | 39.0 |
| 70 | 37.5 | 47.3 | 42.3 | 45.6 | 50.4 |
| 75 | 48.6 | 58.9 | 50.2 | 60.0 | 67.0 |
| 80 | 66.5 | 78.4 | 71.0 | 84.0 | 88.0 |
| Parameters | | | | | |
| $E_a$ (kJ $mol^{-1}$) | 74.3±0.4 | 53.7±0.3 | 65.3±0.4 | 61.6±0.4 | 54.8±0.3 |
| $\Delta H^{\neq}$ (kJ $mol^{-1}$) | 71.4±0.4 | 50.9±0.3 | 62.4±0.3 | 58.7±0.3 | 51.9±0.3 |
| $-\Delta S^{\neq}$ ($JK^{-1}mol^{-1}$) | 278±3 | 283±3 | 279±2 | 281±3 | 283±4 |

**Table 4.14:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on temperature and related thermodynamic parameters for the reaction of Hg(II)-Gly-DL-Asp complex with ninhydrin.

*Reaction conditions*:

$[Hg(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = $3.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

*p*H = 5.0

| Temperature (°C) | $10^4 k_{obs}$ ($s^{-1}$) | $10^4 k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 60 | 12.1 | 28.5 | 18.5 | 20.2 | 29.6 |
| 65 | 17.5 | 37.3 | 27.4 | 27.5 | 38.8 |
| 70 | 34.9 | 50.9 | 40.5 | 43.4 | 48.3 |
| 75 | 44.1 | 68.8 | 57.3 | 60.9 | 69.1 |
| 80 | 59.9 | 81.2 | 78.2 | 79.7 | 83.2 |
| Parameters | | | | | |
| $E_a$ (kJ $mol^{-1}$) | 83.3±0.5 | 55.2±0.4 | 72.8±0.5 | 68.2±0.4 | 53.9±0.4 |
| $\Delta H^{\neq}$ (kJ $mol^{-1}$) | 80.5±0.4 | 52.4±0.3 | 69.9±0.4 | 65.3±0.3 | 51.0±0.4 |
| $-\Delta S^{\neq}$ ($JK^{-1}mol^{-1}$) | 277±3 | 283±3 | 278±3 | 282±4 | 283±3 |

**Table 4.15:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on temperature and related thermodynamic parameters for the reaction of Cu(II)-Gly-L-Ala complex with ninhydrin.

*Reaction conditions*:

$[Cu(II)\text{-}Gly\text{-}L\text{-}Ala]^{+}$ = $4.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $10.0 \times 10^{-3}$ mol $dm^{-3}$

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

*p*H = 5.0

| Temperature (°C) | $10^4 k_{obs}$ ($s^{-1}$) | $10^4 k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 60 | 9.29 | 14.0 | 12.2 | 14.0 | 16.0 |
| 65 | 14.9 | 21.4 | 18.3 | 19.7 | 22.3 |
| 70 | 19.9 | 30.2 | 22.4 | 30.9 | 34.7 |
| 75 | 31.8 | 39.7 | 34.1 | 37.4 | 46.0 |
| 80 | 40.6 | 48.8 | 43.8 | 46.5 | 49.9 |
| Parameters | | | | | |
| $E_a$ (kJ $mol^{-1}$) | 74.3±0.5 | 63.1±0.4 | 64.0±0.4 | 61.9±0.4 | 61.1±0.4 |
| $\Delta H^{\neq}$ (kJ $mol^{-1}$) | 71.5±0.5 | 60.2±0.3 | 61.2±0.4 | 59.1±0.3 | 58.3±0.3 |
| $-\Delta S^{\neq}$ ($JK^{-1}mol^{-1}$) | 287±5 | 289±4 | 288±5 | 288±4 | 289±4 |

**Table 4.16:** Dependence of *pseudo*-first-order rate constants ($k_{obs}/k_{\psi}$) on temperature and related thermodynamic parameters for the reaction of Cu(II)-Gly-DL-Asp complex with ninhydrin.

*Reaction conditions*:

$[Cu(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = $4.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $10.0 \times 10^{-3}$ mol $dm^{-3}$

[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$

[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$

*p*H = 5.0

| Temperature (°C) | $10^4 k_{obs}$ ($s^{-1}$) | $10^4 k_{\psi}$ ($s^{-1}$) | | | |
|---|---|---|---|---|---|
| | Aqueous | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| 60 | 9.18 | 19.4 | 12.6 | 16.2 | 20.1 |
| 65 | 11.0 | 23.2 | 16.2 | 19.8 | 22.9 |
| 70 | 18.3 | 29.1 | 21.6 | 24.8 | 30.2 |
| 75 | 21.9 | 35.5 | 25.6 | 30.2 | 36.7 |
| 80 | 29.2 | 48.8 | 36.6 | 44.8 | 47.3 |
| Parameters | | | | | |
| $E_a$ (kJ $mol^{-1}$) | 61.1±0.3 | 46.7±0.2 | 52.9±0.3 | 50.2±0.3 | 45.1±0.2 |
| $\Delta H^{\neq}$ (kJ $mol^{-1}$) | 58.2±0.3 | 43.7±0.2 | 50.1±0.3 | 47.4±0.3 | 42.3±0.2 |
| $-\Delta S^{\neq}$ ($JK^{-1}mol^{-1}$) | 286±4 | 289±3 | 287±4 | 287±34 | 289±4 |

**Table 4.17:** Dependence of *pseudo*-first-order rate constants ($k_\psi$) on [TTABr] for the reaction of Hg(II)-Gly-L-Ala complex with ninhydrin.

*Reaction conditions*:

$[Hg(II)\text{-}Gly\text{-}L\text{-}Ala]^+$ = $2.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| $10^3$[TTABr] (mol $dm^{-3}$) | $10^4\ k_\psi$ ($s^{-1}$) | $10^4\ k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ |
|---|---|---|---|
| 0 | 37.5 | - | - |
| 3.0 | 38.1 | 36.2 | +0.05 |
| 5.0 | 41.4 | 38.6 | +0.07 |
| 7.0 | 42.8 | 40.1 | +0.06 |
| 10.0 | 42.8 | 42.1 | +0.02 |
| 15.0 | 43.9 | 43.0 | +0.02 |
| 20.0 | 47.3 | 46.2 | +0.02 |
| 25.0 | 49.9 | 48.7 | +0.02 |
| 30.0 | 51.6 | 53.8 | - 0.04 |
| 40.0 | 52.8 | 57.4 | - 0.09 |
| 50.0 | 47.3 | 61.5 | - 0.30 |
| 70.0 | 46.5 | 64.4 | - 0.38 |
| 80.0 | 44.1 | 67.2 | - 0.52 |
| 90.0 | 41.5 | 68.9 | -0.66 |

**Table 4.18:** Dependence of *pseudo*-first-order rate constants ($k_\psi$) on [TTABr] for the reaction of Hg(II)-Gly-DL-Asp complex with ninhydrin.

*Reaction conditions*:

$[Hg(II)\text{-}Gly\text{-}L\text{-}Asp]^+$ = $2.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| $10^3$[TTABr] (mol $dm^{-3}$) | $10^4\ k_\psi$ ($s^{-1}$) | $10^4\ k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ |
|---|---|---|---|
| 0 | 34.9 | -- | -- |
| 3.0 | 35.2 | 33.5 | +0.05 |
| 5.0 | 35.4 | 34.0 | +0.04 |
| 7.0 | 36.8 | 35.9 | +0.02 |
| 10.0 | 44.5 | 44.6 | 0.00 |
| 15.0 | 45.3 | 45.1 | 0.00 |
| 20.0 | 50.2 | 50.4 | 0.00 |
| 25.0 | 50.1 | 50.6 | - 0.01 |
| 30.0 | 51.6 | 52.3 | - 0.01 |
| 40.0 | 52.0 | 52.5 | - 0.01 |
| 50.0 | 49.7 | 53.7 | - 0.08 |
| 70.0 | 46.7 | 57.1 | - 0.22 |
| 80.0 | 45.1 | 60.2 | - 0.33 |
| 90.0 | 44.3 | 61.4 | - 0.39 |

**Table 4.19:** Dependence of *pseudo*-first-order rate constants ($k_\psi$) on [TTABr] for the reaction of Cu(II)-Gly-L-Ala complex with ninhydrin.

*Reaction conditions*:

$[Cu(II)\text{-}Gly\text{-}L\text{-}Ala]^+$ = $4.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $10.0 \times 10^{-3}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| $10^3$[TTABr] (mol $dm^{-3}$) | $10^4\ k_\psi$ ($s^{-1}$) | $10^4\ k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ |
|---|---|---|---|
| 0 | 19.9 | -- | -- |
| 3.0 | 21.2 | 20.0 | +0.06 |
| 5.0 | 22.8 | 21.3 | +0.06 |
| 7.0 | 24.9 | 23.9 | +0.04 |
| 10.0 | 27.6 | 27.5 | 0.00 |
| 15.0 | 28.2 | 28.0 | +0.01 |
| 20.0 | 30.2 | 30.4 | - 0.01 |
| 25.0 | 33.3 | 31.1 | +0.07 |
| 30.0 | 34.7 | 33.9 | +0.02 |
| 40.0 | 33.9 | 34.4 | - 0.01 |
| 50.0 | 24.7 | 37.8 | - 0.50 |
| 60.0 | 19.0 | 39.1 | - 1.06 |
| 70.0 | 16.2 | 40.5 | - 1.50 |
| 80.0 | 14.2 | 43.3 | - 2.05 |
| 90.0 | 14.7 | 44.5 | - 2.03 |

**Table 4.20:** Dependence of *pseudo*-first-order rate constants ($k_\psi$) on [TTABr] for the reaction of Cu(II)-Gly-DL-Asp complex with ninhydrin.

*Reaction conditions*:

$[Cu(II)\text{-}Gly\text{-}L\text{-}Asp]^+$ = $4.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $10.0 \times 10^{-3}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| $10^3$[TTABr] (mol $dm^{-3}$) | $10^4\ k_\psi$ ($s^{-1}$) | $10^4\ k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ |
|---|---|---|---|
| 0 | 18.3 | -- | -- |
| 3.0 | 18.9 | 16.5 | +0.13 |
| 5.0 | 19.8 | 17.9 | +0.09 |
| 7.0 | 20.9 | 18.8 | +0.10 |
| 10.0 | 23.9 | 20.1 | +0.16 |
| 15.0 | 27.3 | 24.5 | +0.10 |
| 20.0 | 29.1 | 28.8 | +0.01 |
| 25.0 | 33.3 | 32.7 | +0.02 |
| 30.0 | 29.3 | 33.1 | - 0.13 |
| 40.0 | 22.8 | 34.0 | - 0.49 |
| 50.0 | 11.1 | 35.3 | - 2.18 |
| 60.0 | 10.4 | 38.1 | - 2.66 |
| 70.0 | 5.06 | -- | -- |
| 80.0 | 5.9 | -- | -- |
| 90.0 | 2.9 | -- | -- |

**Table 4.21:** Dependence of *pseudo*-first-order rate constants ($k_\psi$) on [14-s-14] for the reaction of Hg(II)-Gly-L-Ala complex with ninhydrin.

*Reaction conditions*:

$[Hg(II)\text{-}Gly\text{-}L\text{-}Ala]^+$ = $2.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| $10^5$ [14-s-14] (mol $dm^{-3}$) | 14-6-14 | | | 14-5-14 | | | 14-4-14 | | |
|---|---|---|---|---|---|---|---|---|---|
| | $10^4 k_\psi$ ($s^{-1}$) | $10^4 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ | $10^4 k_\psi$ ($s^{-1}$) | $10^4 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ | $10^4 k_\psi$ ($s^{-1}$) | $10^4 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ |
| 0 | 37.5 | -- | -- | 37.5 | -- | -- | 37.5 | -- | -- |
| 5.0 | 37.6 | -- | -- | 38.0 | -- | -- | 38.1 | -- | -- |
| 15.0 | 40.1 | 35.5 | +0.11 | 42.3 | 46.7 | - 0.10 | 44.4 | 41.2 | +0.07 |
| 20.0 | 39.3 | 37.8 | +0.04 | 43.1 | 42.0 | +0.02 | 45.2 | 44.1 | +0.02 |
| 30.0 | 41.2 | 35.7 | +0.13 | 44.3 | 41.5 | +0.06 | 47.7 | 43.3 | +0.09 |
| 50.0 | 42.3 | 41.1 | +0.03 | 45.6 | 45.9 | - 0.01 | 50.4 | 51.2 | - 0.01 |

*contd…*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 70.0 | 42.5 | 42.9 | - 0.01 | 46.3 | 47.9 | - 0.03 | 51.2 | 52.4 | - 0.02 |
| 100.0 | 42.6 | 43.4 | - 0.02 | 47.3 | 48.4 | - 0.02 | 52.3 | 54.1 | - 0.03 |
| 150.0 | 43.5 | 43.9 | - 0.01 | 46.5 | 50.0 | - 0.07 | 53.4 | 58.5 | - 0.09 |
| 200.0 | 46.7 | 47.5 | - 0.02 | 49.0 | 52.2 | - 0.06 | 60.2 | 63.4 | - 0.05 |
| 300.0 | 46.8 | 48.3 | - 0.03 | 48.8 | 54.6 | - 0.12 | 61.2 | 66.7 | - 0.09 |
| 500.0 | 47.0 | 51.1 | - 0.09 | 47.9 | 60.3 | - 0.26 | 61.0 | 70.2 | - 0.15 |
| 700.0 | 53.0 | 58.1 | - 0.09 | 55.8 | 63.1 | - 0.13 | 65.2 | 73.4 | - 0.12 |
| 1000.0 | 53.5 | -- | -- | 60.2 | -- | -- | 75.4 | -- | -- |
| 1500.0 | 58.5 | -- | -- | 66.9 | -- | -- | 78.3 | -- | -- |
| 2000.0 | 60.1 | -- | -- | 68.8 | -- | -- | 80.1 | -- | -- |
| 2500.0 | 63.2 | -- | -- | 72.3 | -- | -- | 82.1 | -- | -- |
| 3000.0 | 65.8 | -- | -- | 78.0 | -- | -- | 84.4 | -- | -- |

**Table 4.22:** Dependence of *pseudo*-first-order rate constants ($k_\psi$) on [14-s-14] for the reaction of Hg(II)-Gly-DL-Asp complex with ninhydrin.

*Reaction conditions*:

$[Hg(II)\text{-}Gly\text{-}DL\text{-}Asp]^+$ = $2.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| $10^5$ [14-s-14] (mol $dm^{-3}$) | 14-6-14 | | | 14-5-14 | | | 14-4-14 | | |
|---|---|---|---|---|---|---|---|---|---|
| | $10^4 k_\psi$ ($s^{-1}$) | $10^4 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ | $10^4 k_\psi$ ($s^{-1}$) | $10^4 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ | $10^4 k_\psi$ ($s^{-1}$) | $10^4 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ |
| 0 | 34.9 | -- | -- | 34.9 | -- | -- | 34.9 | -- | -- |
| 5.0 | 35.3 | -- | -- | 35.7 | -- | -- | 36.3 | -- | -- |
| 15.0 | 37.2 | 36.5 | +0.02 | 38.1 | 37.2 | +0.02 | 39.8 | 38.1 | +0.04 |
| 30.0 | 38.4 | 38.1 | +0.01 | 41.5 | 41.2 | +0.01 | 44.5 | 44.0 | +0.01 |
| 50.0 | 40.5 | 40.7 | 0.00 | 43.4 | 43.6 | 0.00 | 48.3 | 48.5 | 0.00 |

*contd…*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 70.0 | 41.7 | 41.8 | - 0.00 | 44.6 | 45.1 | - 0.01 | 49.1 | 49.3 | 0.00 |
| 100.0 | 42.4 | 43.1 | - 0.02 | 45.2 | 46.2 | - 0.02 | 50.4 | 50.2 | 0.00 |
| 150.0 | 44.9 | 45.2 | - 0.01 | 46.1 | 46.0 | 0.00 | 51.4 | 52.1 | 0.01 |
| 200.0 | 45.6 | 46.4 | - 0.02 | 47.3 | 48.8 | - 0.03 | 52.4 | 53.4 | - 0.02 |
| 300.0 | 47.2 | 48.5 | - 0.03 | 48.1 | 49.7 | - 0.03 | 53.4 | 53.9 | - 0.01 |
| 500.0 | 48.8 | 49.4 | - 0.01 | 50.9 | 51.5 | - 0.01 | 54.9 | 56.1 | - 0.02 |
| 700.0 | 52.8 | 53.7 | - 0.02 | 52.6 | 54.1 | - 0.03 | 57.2 | 58.2 | - 0.02 |
| 1000.0 | 60.3 | -- | -- | 58.9 | -- | -- | 62.5 | -- | -- |
| 1500.0 | 62.1 | -- | -- | 60.5 | -- | -- | 70.3 | -- | -- |
| 2000.0 | 64.8 | -- | -- | 69.8 | -- | -- | 74.1 | -- | -- |
| 2500.0 | 66.5 | -- | -- | 71.4 | -- | -- | 79.2 | -- | -- |
| 3000.0 | 67.7 | -- | -- | 73.5 | -- | -- | 81.7 | -- | -- |

**Table 4.23:** Dependence of *pseudo*-first-order rate constants ($k_\psi$) on [14-s-14] for the reaction of Cu(II)-Gly-L-Ala complex with ninhydrin.

*Reaction conditions*:

$[Cu(II)\text{-}Gly\text{-}L\text{-}Ala]^+$ = $4.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $10.0 \times 10^{-3}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| $10^5$ [14-s-14] (mol $dm^{-3}$) | 14-6-14 | | | 14-5-14 | | | 14-4-14 | | |
|---|---|---|---|---|---|---|---|---|---|
| | $10^4 k_\psi$ ($s^{-1}$) | $10^4 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ | $10^4 k_\psi$ ($s^{-1}$) | $10^4 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ | $10^4 k_\psi$ ($s^{-1}$) | $10^4 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ |
| 0 | 19.9 | -- | -- | 19.9 | -- | -- | 19.9 | -- | -- |
| 5.0 | 13.9 | -- | -- | 20.0 | -- | -- | 24.1 | -- | -- |
| 10.0 | 16.1 | 15.7 | +0.02 | 20.1 | 19.1 | +0.05 | 27.3 | 25.1 | +0.08 |
| 15.0 | 17.1 | 16.5 | +0.04 | 21.2 | 20.7 | +0.02 | 29.9 | 28.3 | +0.05 |
| 20.0 | 18.4 | 18.1 | +0.02 | 22.9 | 22.5 | +0.02 | 31.6 | 31.1 | +0.02 |
| 30.0 | 19.9 | 19.5 | +0.02 | 26.8 | 26.3 | +0.02 | 32.4 | 32.2 | +0.01 |
| 50.0 | 22.4 | 22.5 | 0.00 | 30.9 | 30.8 | 0.00 | 34.7 | 34.8 | 0.00 |

*contd…*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 70.0 | 24.4 | 24.7 | - 0.01 | 34.3 | 34.1 | 0.01 | 42.1 | 42.0 | 0.00 |
| 100.0 | 27.6 | 28.5 | - 0.03 | 35.6 | 36.2 | - 0.02 | 42.1 | 42.5 | - 0.01 |
| 150.0 | 32.8 | 32.1 | +0.02 | 36.8 | 37.4 | - 0.02 | 45.3 | 46.1 | - 0.02 |
| 200.0 | 31.5 | 32.5 | - 0.03 | 36.1 | 38.1 | - 0.06 | 47.8 | 48.3 | - 0.01 |
| 300.0 | 32.7 | 33.3 | - 0.02 | 36.7 | 38.5 | - 0.05 | 50.1 | 51.5 | - 0.03 |
| 500.0 | 34.4 | 35.3 | - 0.03 | 38.7 | 39.8 | - 0.03 | 50.6 | 52.3 | - 0.03 |
| 700.0 | 37.9 | 39.1 | - 0.03 | 39.5 | 40.4 | - 0.02 | 52.5 | 54.1 | - 0.03 |
| 1000.0 | 39.3 | -- | -- | 46.2 | -- | -- | 59.2 | -- | -- |
| 1500.0 | 43.8 | -- | -- | 52.9 | -- | -- | 62.9 | -- | -- |
| 2000.0 | 52.0 | -- | -- | 53.2 | -- | -- | 70.4 | -- | -- |
| 2500.0 | 54.2 | -- | -- | 59.2 | -- | -- | 73.5 | -- | -- |
| 3000.0 | 57.4 | -- | -- | 63.5 | -- | -- | 77.0 | -- | -- |

**Table 4.24:** Dependence of *pseudo*-first-order rate constants ($k_\psi$) on [14-s-14] for the reaction of Cu(II)-Gly-DL-Asp complex with ninhydrin.

*Reaction conditions*:

$[Cu(II)\text{-}Gly\text{-}DL\text{-}Asp]^+$ = $4.0 \times 10^{-4}$ mol $dm^{-3}$

[ninhydrin] = $10.0 \times 10^{-3}$ mol $dm^{-3}$

*p*H = 5.0

Temperature = 70 °C

| $10^5$ [14-s-14] (mol $dm^{-3}$) | 14-6-14 | | | 14-5-14 | | | 14-4-14 | | |
|---|---|---|---|---|---|---|---|---|---|
| | $10^4 k_\psi$ ($s^{-1}$) | $10^4 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ | $10^4 k_\psi$ ($s^{-1}$) | $10^4 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ | $10^4 k_\psi$ ($s^{-1}$) | $10^4 k_{\psi cal}$ ($s^{-1}$) | $\frac{k_\psi - k_{\psi cal}}{k_\psi}$ |
| 0 | 18.3 | -- | -- | 18.3 | -- | -- | 18.3 | -- | -- |
| 5.0 | 19.0 | -- | -- | 19.3 | -- | -- | 22.0 | -- | -- |
| 15.0 | 19.1 | 18.1 | +0.05 | 19.5 | 18.7 | +0.04 | 26.2 | 24.0 | +0.08 |
| 20.0 | 19.5 | 18.5 | +0.05 | 19.7 | 19.3 | +0.02 | 29.2 | 28.2 | +0.03 |
| 30.0 | 19.8 | 19.2 | +0.03 | 20.5 | 19.9 | +0.03 | 29.8 | 29.3 | +0.02 |
| 50.0 | 21.6 | 21.4 | +0.01 | 24.8 | 24.9 | 0.00 | 30.2 | 30.4 | - 0.01 |

*contd…*

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 70.0 | 22.8 | 22.9 | 0.00 | 25.9 | 26.2 | - 0.01 | 35.9 | 36.4 | - 0.01 |
| 100.0 | 25.6 | 26.1 | - 0.02 | 27.2 | 27.0 | +0.01 | 41.6 | 42.1 | - 0.01 |
| 150.0 | 27.7 | 27.3 | +0.01 | 29.8 | 29.4 | +0.01 | 44.3 | 45.6 | - 0.03 |
| 200.0 | 29.0 | 28.9 | 0.00 | 31.5 | 32.3 | - 0.02 | 45.3 | 46.6 | - 0.03 |
| 300.0 | 32.8 | 33.0 | - 0.01 | 34.1 | 35.1 | - 0.03 | 45.7 | 47.3 | - 0.03 |
| 500.0 | 33.4 | 34.1 | - 0.02 | 35.3 | 36.5 | - 0.03 | 45.4 | 48.7 | - 0.07 |
| 700.0 | 33.5 | 35.5 | - 0.06 | 35.9 | 37.1 | - 0.03 | 47.5 | 50.5 | - 0.06 |
| 1000.0 | 39.4 | -- | -- | 39.9 | -- | -- | 52.1 | -- | -- |
| 1500.0 | 44.4 | -- | -- | 45.8 | -- | -- | 54.7 | -- | -- |
| 2000.0 | 45.0 | -- | -- | 47.8 | -- | -- | 55.0 | -- | -- |
| 2500.0 | 45.0 | -- | -- | 48.4 | -- | -- | 56.0 | -- | -- |
| 3000.0 | 46.9 | -- | -- | 49.9 | -- | -- | 58.1 | -- | -- |

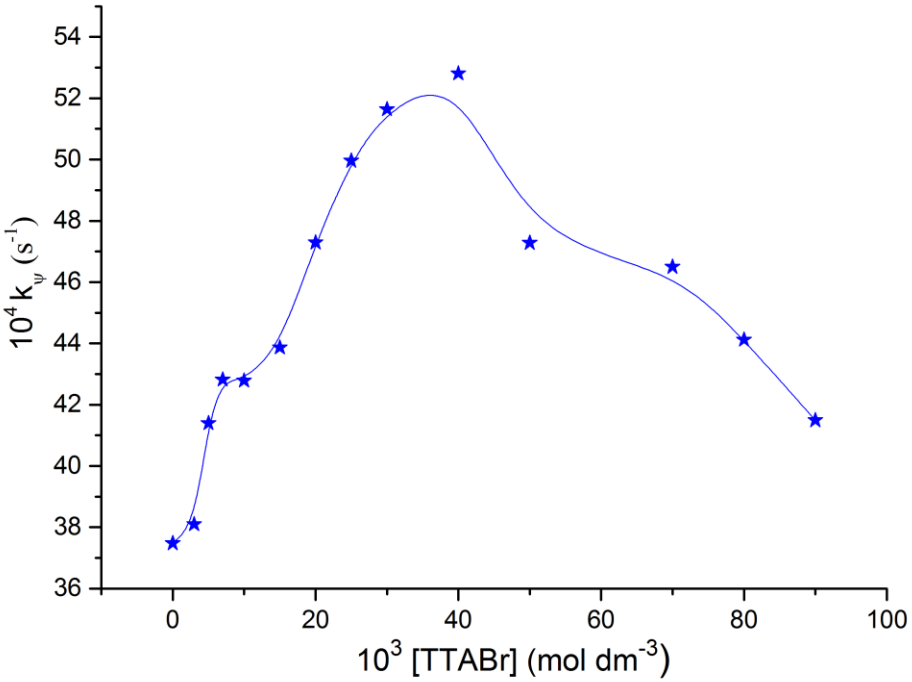



**Figure 4.9:** Influence of [TTABr] on the reaction rate of Hg(II)-Gly-L-Ala complex with ninhydrin. *Reaction conditions*: $[Hg(II)\text{-Gly-L-Ala}]^+ = 2.0 \times 10^{-4}$ mol $dm^{-3}$, [ninhydrin] $= 6.0 \times 10^{-3}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

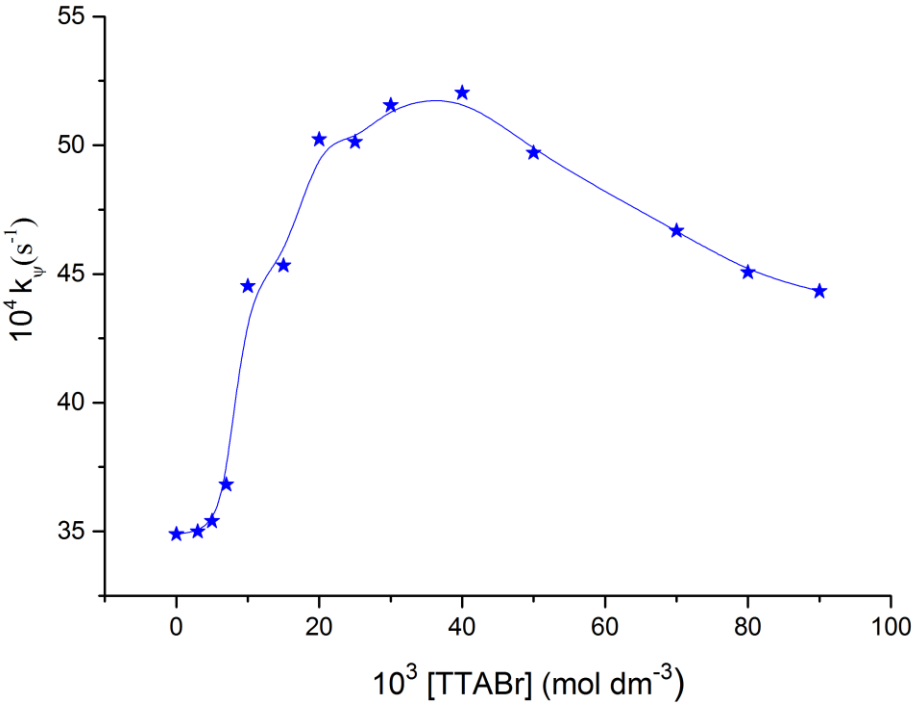



**Figure 4.10:** Influence of [TTABr] on the reaction rate of Hg(II)-Gly-DL-Asp complex with ninhydrin. *Reaction conditions*: $[Hg(II)\text{-}Gly\text{-}DL\text{-}Asp]^+$ = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

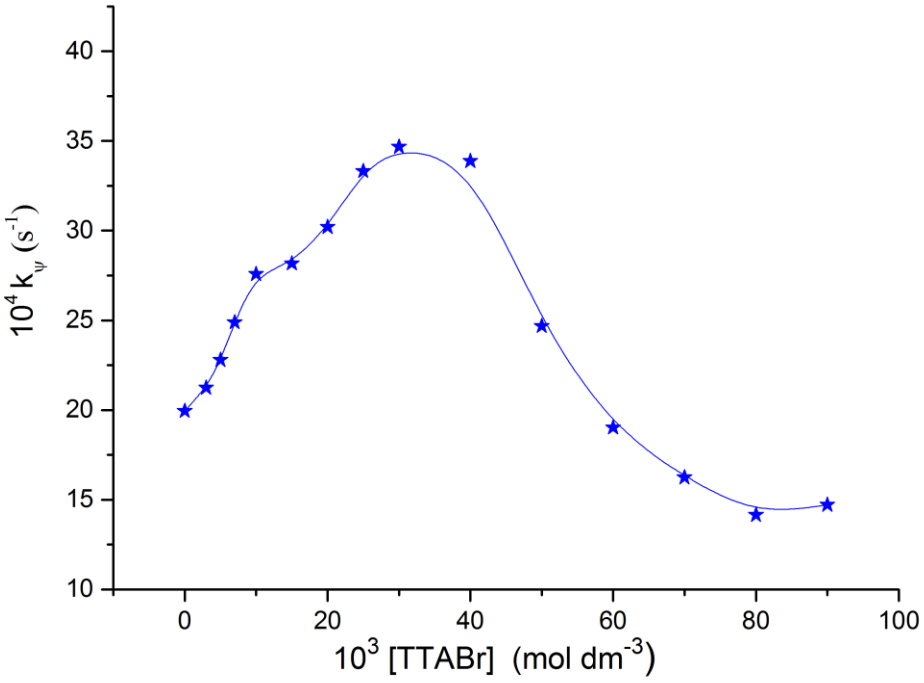



**Figure 4.11:** Influence of [TTABr] on the reaction rate of Cu(II)-Gly-L-Ala complex with ninhydrin. *Reaction conditions*: $[Cu(II)\text{-Gly-L-Ala}]^+$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

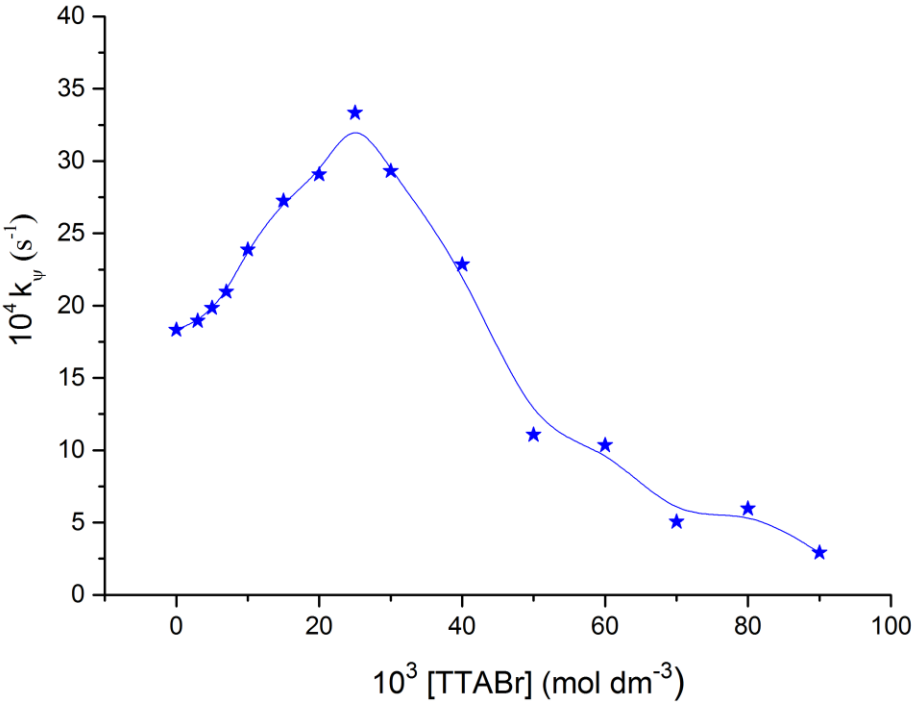



**Figure 4.12:** Influence of [TTABr] on the reaction rate of Cu(II)-Gly-DL-Asp complex with ninhydrin. *Reaction conditions*: $[Cu(II)\text{-Gly-DL-Asp}]^+$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

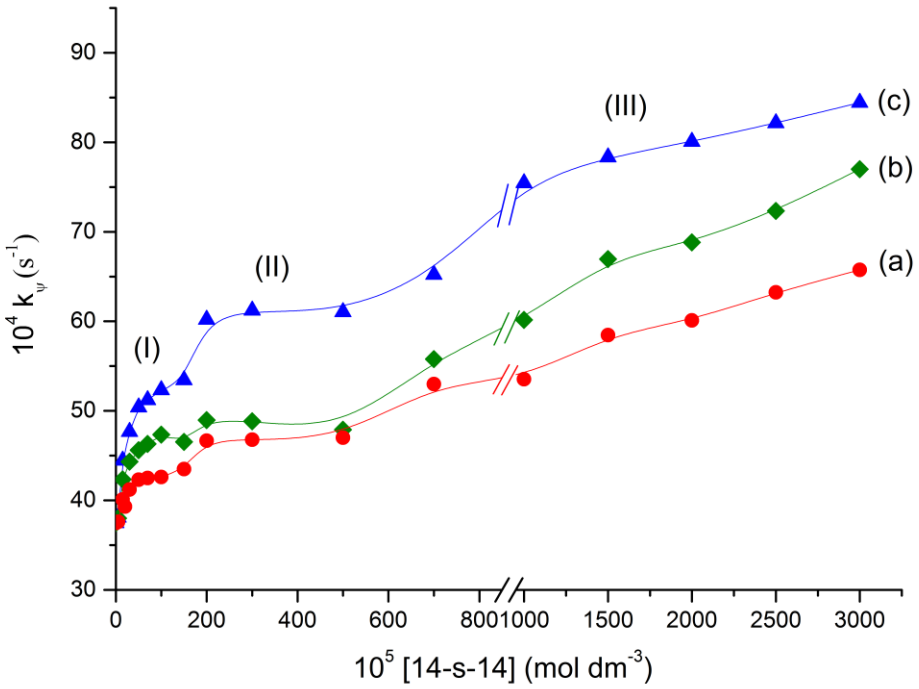



**Figure 4.13:** Influence of [geminis] on the reaction rate of Hg(II)-Gly-L-Ala complex with ninhydrin. *Reaction conditions*: $[Hg(II)\text{-}Gly\text{-}L\text{-}Ala]^+$ = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C. (a) 14-6-14; (b) 14-5-14; (c) 14-4-14.

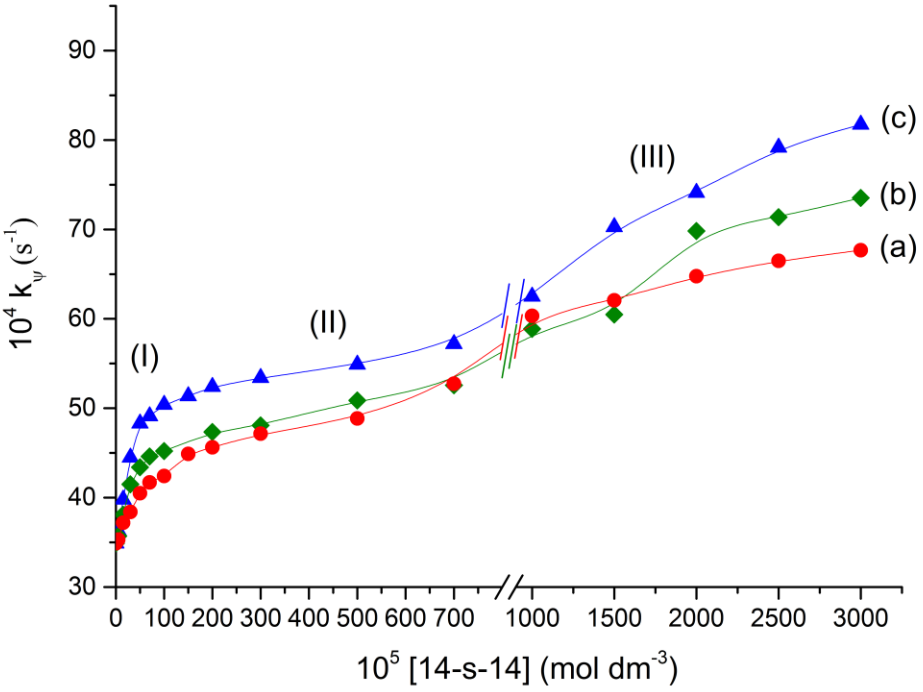



**Figure 4.14:** Influence of [geminis] on the reaction rate of Hg(II)-Gly-DL-Asp complex with ninhydrin. *Reaction conditions*: $[Hg(II)\text{-Gly-DL-Asp}]^+$ = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C. (a) 14-6-14; (b) 14-5-14; (c) 14-4-14.

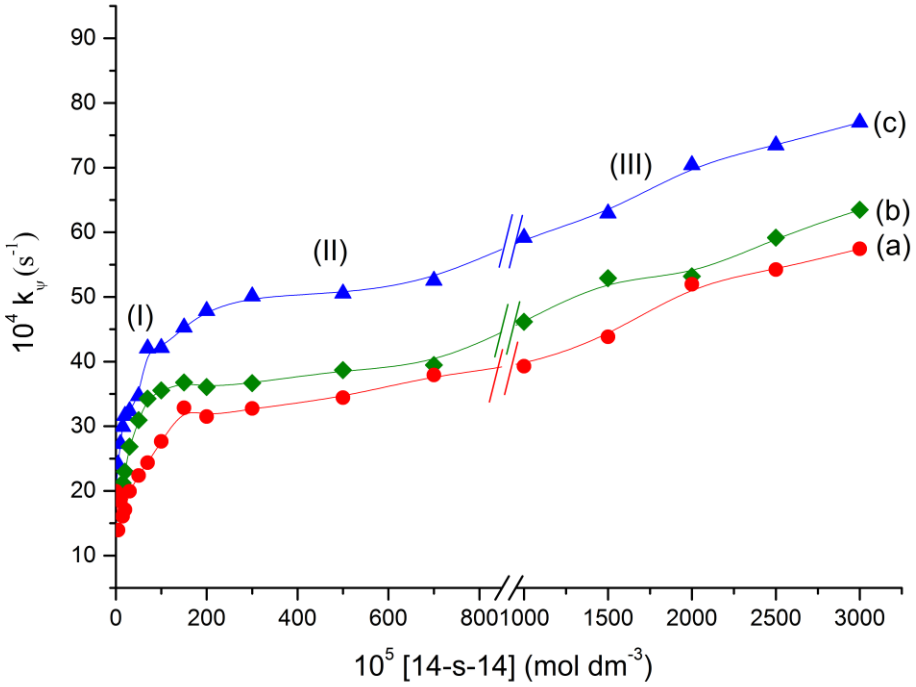



**Figure 4.15:** Influence of [geminis] on the reaction rate of Cu(II)-Gly-L-Ala complex with ninhydrin. *Reaction conditions*: $[Cu(II)\text{-Gly-L-Ala}]^+$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C. (a) 14-6-14; (b) 14-5-14; (c) 14-4-14.

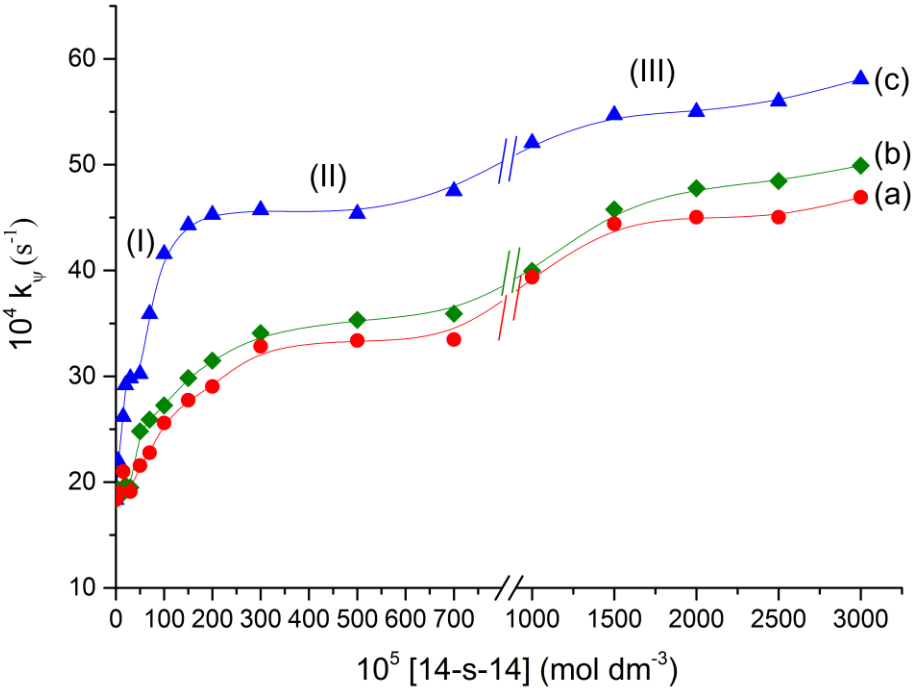



**Figure 4.16:** Influence of [geminis] on the reaction rate of Cu(II)-Gly-DL-Asp complex with ninhydrin. *Reaction conditions*: $[Cu(II)\text{-Gly-DL-Asp}]^+$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C. (a)14-6-14; (b) 14-5-14; (c) 14-4-14.

**Table 4.25:** Rate constants (k) for the reaction of Hg(II)-Gly-L-Ala complex with ninhydrin in the absence and presence of surfactants in aqueous-organic medium.

*Reaction conditions*

$[Hg(II)\text{-}Gly\text{-}L\text{-}Ala]^{+}$ = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x$10^{-3}$ mol $dm^{-3}$, [14-s-14] = 50.0 x$10^{-5}$ mol $dm^{-3}$, at *p*H = 5.0 and temperature = 70 $^{o}$C.

| % Solvent (v/v) | ($10^4$) k ($s^{-1}$) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DMSO | | | | | DO | | | | | AN | | | | |
| | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 |
| 0 | 37.5 | 47.3 | 42.3 | 45.6 | 50.4 | 37.5 | 47.3 | 42.3 | 45.6 | 50.4 | 37.5 | 47.3 | 42.3 | 45.6 | 50.4 |
| 3.0 | 38.9 | 50.5 | 43.6 | 49.0 | 51.5 | 38.4 | 49.8 | 43.2 | 48.8 | 51.2 | 39.9 | 51.5 | 44.5 | 49.8 | 54.1 |
| 5.0 | 40.8 | 52.4 | 44.9 | 50.0 | 52.8 | 43.6 | 50.1 | 44.7 | 49.1 | 52.7 | 42.7 | 53.4 | 46.4 | 49.7 | 55.2 |
| 8.0 | 41.3 | 55.5 | 45.5 | 51.5 | 55.8 | 46.2 | 52.2 | 47.0 | 50.2 | 53.6 | 44.9 | 54.2 | 48.0 | 51.9 | 58.2 |
| 10.0 | 37.3 | 51.2 | 45.5 | 50.4 | 54.6 | 48.3 | 54.8 | 49.9 | 52.4 | 55.3 | 47.7 | 55.7 | 50.1 | 53.5 | 58.1 |
| 14.0 | 36.6 | 47.7 | 43.1 | 48.5 | 52.7 | 50.0 | 56.5 | 51.0 | 52.7 | 58.0 | 48.9 | 54.8 | 51.4 | 53.8 | 58.4 |
| 16.0 | 36.4 | 47.0 | 42.7 | 46.2 | 50.8 | 50.4 | 56.4 | 51.1 | 52.6 | 58.1 | 48.4 | 56.2 | 52.0 | 54.2 | 60.0 |
| 18.0 | 37.6 | 46.0 | 42.0 | 44.6 | 50.7 | 51.0 | 57.0 | 52.6 | 52.8 | 58.0 | 48.9 | 58.2 | 51.7 | 54.3 | 59.7 |
| 25.0 | 35.9 | 44.9 | 40.2 | 43.6 | 47.7 | 51.4 | 58.8 | 52.9 | 54.8 | 60.0 | 50.3 | 58.9 | 53.5 | 55.4 | 61.3 |

**Table 4.26:** Rate constants (k) for the reaction of Hg(II)-Gly-DL-Asp complex with ninhydrin in the absence and presence of surfactants in aqueous-organic medium.

*Reaction conditions*

$[Hg(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x$10^{-3}$ mol $dm^{-3}$, [14-s-14] = 50.0 x$10^{-5}$ mol $dm^{-3}$, at *p*H = 5.0 and temperature = 70 $^{o}$C.

| % Solvent (v/v) | ($10^4$) k ($s^{-1}$) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DMSO | | | | | DO | | | | | AN | | | | |
| | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 |
| 0 | 34.9 | 50.9 | 40.5 | 43.4 | 48.3 | 34.9 | 50.9 | 40.5 | 43.4 | 48.3 | 34.9 | 50.9 | 40.5 | 43.4 | 48.3 |
| 3.0 | 35.9 | 51.2 | 41.2 | 44.9 | 51.9 | 35.0 | 51.0 | 42.2 | 46.2 | 51.1 | 38.3 | 51.2 | 43.4 | 46.9 | 52.6 |
| 5.0 | 36.9 | 52.3 | 41.9 | 45.5 | 53.6 | 35.4 | 52.9 | 40.9 | 46.8 | 51.3 | 38.9 | 53.4 | 45.7 | 48.9 | 55.1 |
| 8.0 | 34.8 | 53.0 | 40.0 | 43.6 | 51.8 | 36.0 | 53.5 | 42.0 | 50.0 | 54.8 | 39.2 | 56.6 | 44.3 | 51.0 | 58.0 |
| 10.0 | 35.0 | 52.2 | 42.4 | 45.0 | 50.9 | 36.5 | 54.0 | 43.1 | 50.3 | 54.7 | 40.3 | 57.6 | 48.3 | 50.7 | 58.2 |
| 14.0 | 35.8 | 53.3 | 41.8 | 44.0 | 52.1 | 37.0 | 55.2 | 44.1 | 52.4 | 56.9 | 41.4 | 55.8 | 47.1 | 52.6 | 58.8 |
| 16.0 | 36.4 | 56.9 | 43.8 | 45.7 | 55.1 | 38.0 | 57.5 | 45.3 | 53.8 | 57.0 | 42.0 | 56.5 | 49.1 | 53.4 | 59.2 |
| 18.0 | 37.4 | 56.2 | 42.7 | 45.7 | 55.3 | 38.1 | 56.7 | 45.4 | 53.9 | 58.0 | 41.9 | 57.5 | 49.7 | 54.9 | 60.8 |
| 25.0 | 41.2 | 57.5 | 44.0 | 47.3 | 55.8 | 39.6 | 58.0 | 44.7 | 55.8 | 60.0 | 42.7 | 59.9 | 49.4 | 58.0 | 62.0 |

**Table 4.27:** Rate constants (k) for the reaction of Cu(II)-Gly-L-Ala complex with ninhydrin in the absence and presence of surfactants in aqueous-organic medium.

*Reaction conditions*

$[Cu(II)\text{-}Gly\text{-}L\text{-}Ala]^{+}$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x$10^{-3}$ mol $dm^{-3}$, [14-s-14] = 50.0 x$10^{-5}$ mol $dm^{-3}$, at *p*H = 5.0 and temperature = 70 $^{o}$C.

| % Solvent (v/v) | ($10^4$) k ($s^{-1}$) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DMSO | | | | | DO | | | | | AN | | | | |
| | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 |
| 0 | 19.9 | 30.2 | 22.4 | 30.9 | 41.7 | 19.9 | 30.2 | 22.4 | 30.9 | 41.7 | 19.9 | 30.2 | 22.4 | 30.4 | 41.7 |
| 3.0 | 33.3 | 55.5 | 34.8 | 48.6 | 60.9 | 21.0 | 42.8 | 23.3 | 31.6 | 44.0 | 22.2 | 44.8 | 30.8 | 39.4 | 47.1 |
| 5.0 | 50.5 | 59.8 | 48.3 | 53.6 | 62.6 | 22.6 | 51.6 | 25.8 | 38.6 | 48.2 | 25.4 | 60.6 | 35.3 | 54.8 | 57.1 |
| 8.0 | 55.0 | 64.2 | 57.9 | 61.7 | 70.7 | 27.0 | 61.5 | 30.0 | 57.0 | 65.9 | 31.2 | 67.7 | 34.4 | 61.0 | 76.0 |
| 10.0 | 57.0 | 62.0 | 60.2 | 66.8 | 68.6 | 31.0 | 59.7 | 38.2 | 61.9 | 68.9 | 36.5 | 80.4 | 41.7 | 68.4 | 83.8 |
| 14.0 | 58.9 | 61.8 | 57.0 | 61.2 | 66.2 | 42.0 | 77.0 | 46.1 | 68.9 | 80.9 | 50.5 | 90.0 | 62.1 | 86.9 | 96.5 |
| 16.0 | 54.7 | 57.9 | 56.2 | 60.2 | 62.0 | 44.3 | 82.0 | 51.5 | 77.2 | 85.5 | 58.0 | 95.0 | 70.2 | 101.0 | 105.9 |
| 18.0 | 48.0 | 57.9 | 51.7 | 55.4 | 61.5 | 46.9 | 90.0 | 53.6 | 80.1 | 92.6 | 64.0 | 110.0 | 71.1 | 103.7 | 117.3 |
| 25.0 | 38.4 | 48.2 | 42.0 | 44.8 | 51.0 | 66.0 | 96.9 | 70.0 | 87.1 | 104.3 | 70.0 | 116.0 | 75.0 | 110.0 | 128.0 |

**Table 4.28:** Rate constants (k) for the reaction of Cu(II)-Gly-DL-Asp complex with ninhydrin in the absence and presence of surfactants in aqueous-organic medium.

*Reaction conditions*

$[Cu(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] = 20.0 x$10^{-3}$ mol $dm^{-3}$, [14-s-14] = 50.0 x$10^{-5}$ mol $dm^{-3}$, at *p*H = 5.0 and temperature = 70 $^{o}$C.

| % Solvent (v/v) | ($10^4$) k ($s^{-1}$) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DMSO | | | | | DO | | | | | AN | | | | |
| | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 | Aq. | TTABr | 14-6-14 | 14-5-14 | 14-4-14 |
| 0 | 18.3 | 29.1 | 20.6 | 23,0 | 30.2 | 18.3 | 29.1 | 20.6 | 23.0 | 30.2 | 18.3 | 29.1 | 20.6 | 23.0 | 30.2 |
| 3.0 | 23.4 | 33.2 | 25.6 | 29.0 | 38.5 | 22.3 | 31.6 | 26.1 | 32.3 | 37.2 | 25.8 | 43.2 | 31.0 | 36.8 | 50.7 |
| 5.0 | 28.5 | 38.4 | 30.3 | 33.5 | 43.0 | 30.0 | 37.0 | 33.2 | 34.9 | 37.4 | 37.8 | 47.7 | 41.9 | 43.9 | 54.8 |
| 8.0 | 34.3 | 43.2 | 37.5 | 41.7 | 49.0 | 39.0 | 47.0 | 42.4 | 48.6 | 54.9 | 49.8 | 53.2 | 50.0 | 52.0 | 56.0 |
| 10.0 | 36.5 | 50.4 | 39.3 | 47.0 | 52.8 | 43.0 | 53.9 | 45.6 | 51.3 | 58.4 | 51.6 | 57.0 | 53.1 | 55.2 | 61.6 |
| 14.0 | 41.7 | 54.4 | 48.6 | 51.4 | 61.0 | 47.0 | 60.0 | 51.0 | 56.4 | 63.5 | 55.6 | 63.0 | 59.9 | 61.9 | 67.0 |
| 16.0 | 47.6 | 59.3 | 52.5 | 56.5 | 61.5 | 50.0 | 62.0 | 54.4 | 60.4 | 66.9 | 56.0 | 69.0 | 62.3 | 64.3 | 67.4 |
| 18.0 | 50.4 | 63.3 | 54.4 | 60.2 | 67.0 | 53.0 | 66.7 | 55.3 | 61.8 | 69.4 | 57.0 | 73.0 | 62.3 | 68.4 | 75.5 |
| 25.0 | 53.9 | 73.6 | 56.8 | 65.0 | 76.7 | 59.0 | 71.5 | 64.7 | 67.9 | 75.9 | 63.0 | 80.0 | 68.0 | 71.0 | 84.0 |

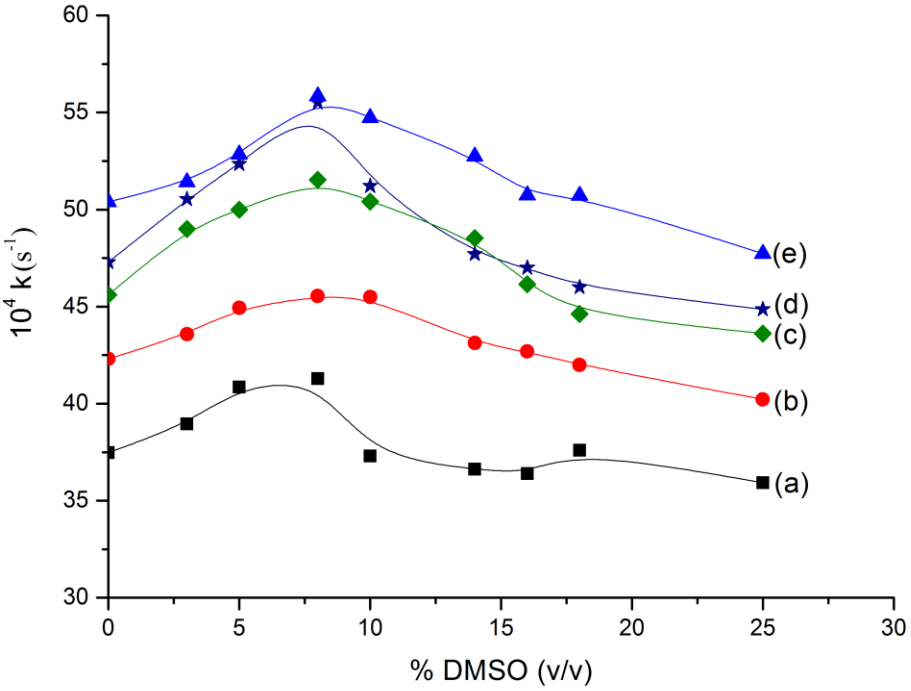



**Figure 4.17:** Influence of composition of DMSO on the rate reaction of Hg(II)-Gly-L-Ala complex with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions*: $[Hg(II)\text{-}Gly\text{-}L\text{-}Ala]^+ = 2.0 \times 10^{-4}$ mol $dm^{-3}$, [ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$, [TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$, [14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

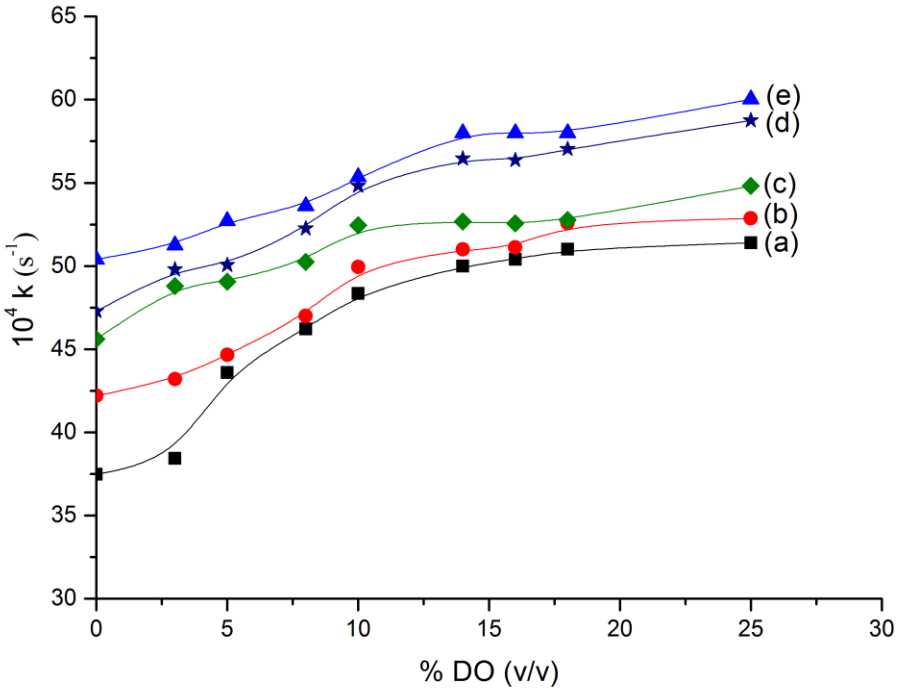



**Figure 4.18:** Influence of composition of DO on the rate reaction of Hg(II)-Gly-L-Ala complex with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions*: $[Hg(II)\text{-}Gly\text{-}L\text{-}Ala]^+ = 2.0 \times 10^{-4}$ mol $dm^{-3}$, [ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$, [TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$, [14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

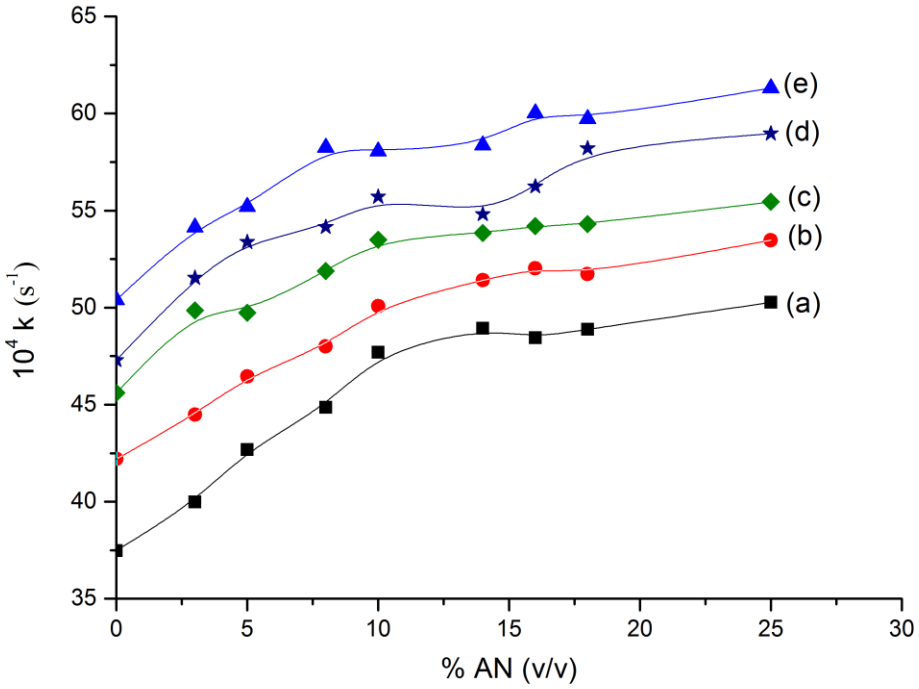



**Figure 4.19:** Influence of composition of AN on the rate reaction of Hg(II)-Gly-L-Ala complex with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions*: $[Hg(II)\text{-}Gly\text{-}L\text{-}Ala]^+ = 2.0 \times 10^{-4}$ mol $dm^{-3}$, [ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$, [TTABr] =$20.0 \times 10^{-3}$ mol $dm^{-3}$, [14-s-14] =$50.0 \times 10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

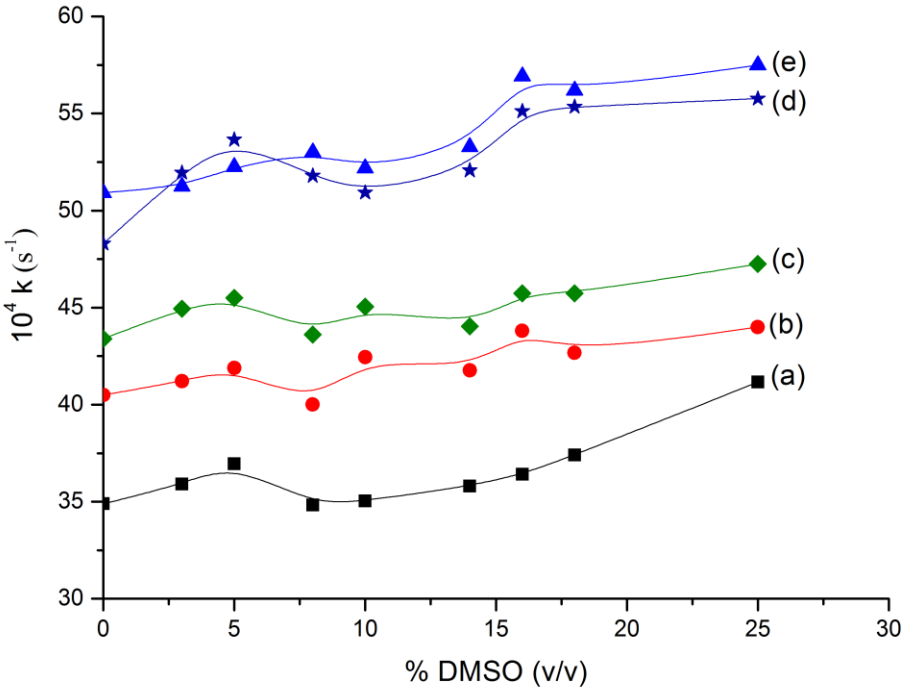



**Figure 4.20:** Influence of composition of DMSO on the rate reaction of Hg(II)-Gly-DL-Asp complex with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions*: $[Hg(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] =20.0 x$10^{-3}$ mol $dm^{-3}$, [14-s-14] =50.0 x$10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

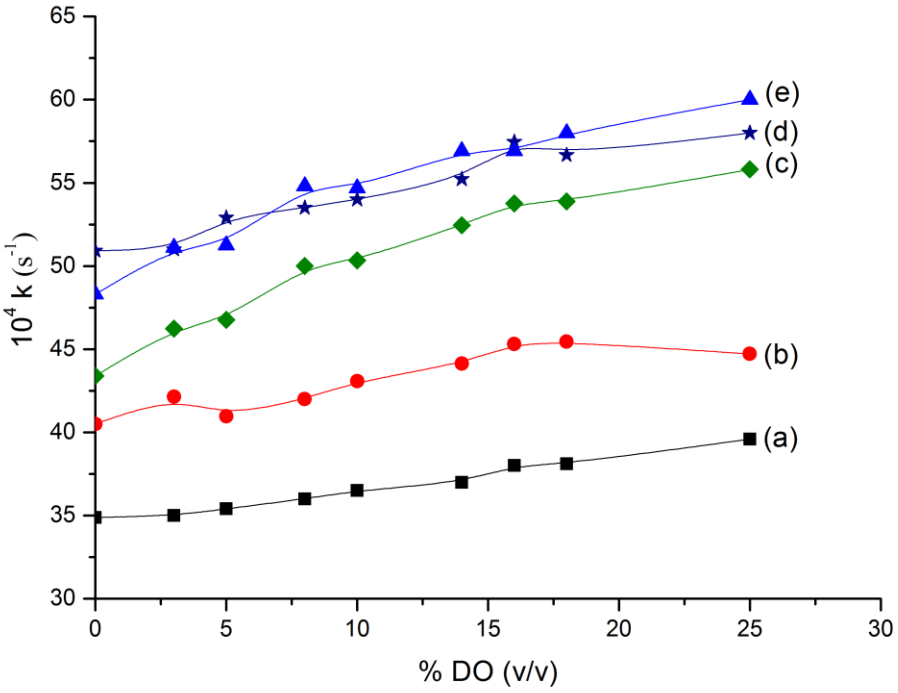



**Figure 4.21:** Influence of composition of DO on the rate reaction of Hg(II)-Gly-DL-Asp complex with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions*: $[Hg(II)\text{-}Gly\text{-}DL\text{-}Asp]^+ = 2.0 \times 10^{-4}$ mol $dm^{-3}$, [ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$, [TTABr] =$20.0 \times 10^{-3}$ mol $dm^{-3}$, [14-s-14] =$50.0 \times 10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

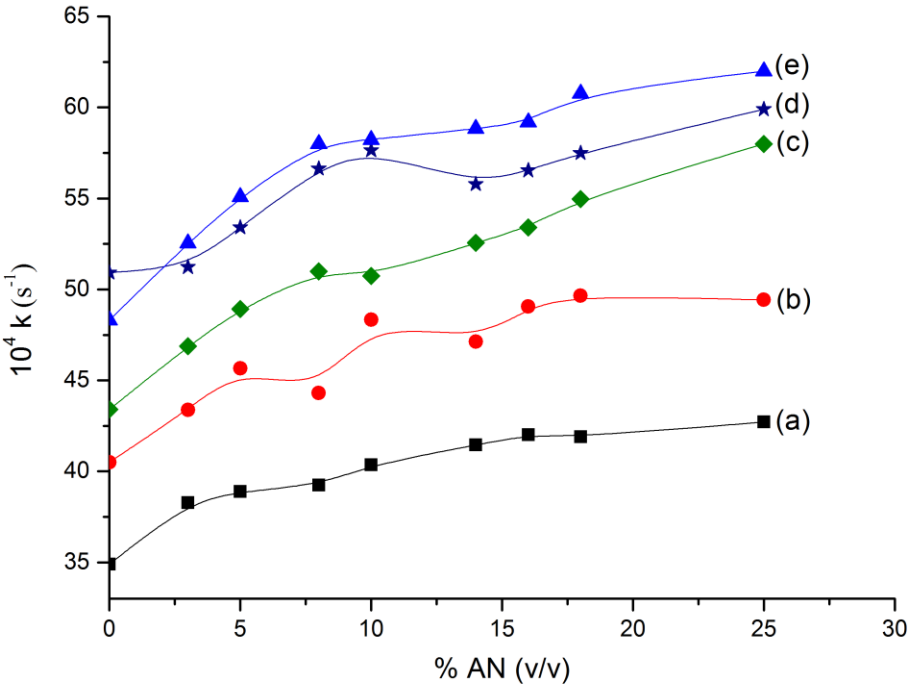



**Figure 4.22:** Influence of composition of AN on the rate reaction of Hg(II)-Gly-DL-Asp complex with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions*: $[Hg(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] =20.0 x $10^{-3}$ mol $dm^{-3}$, [14-s-14] =50.0 x $10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

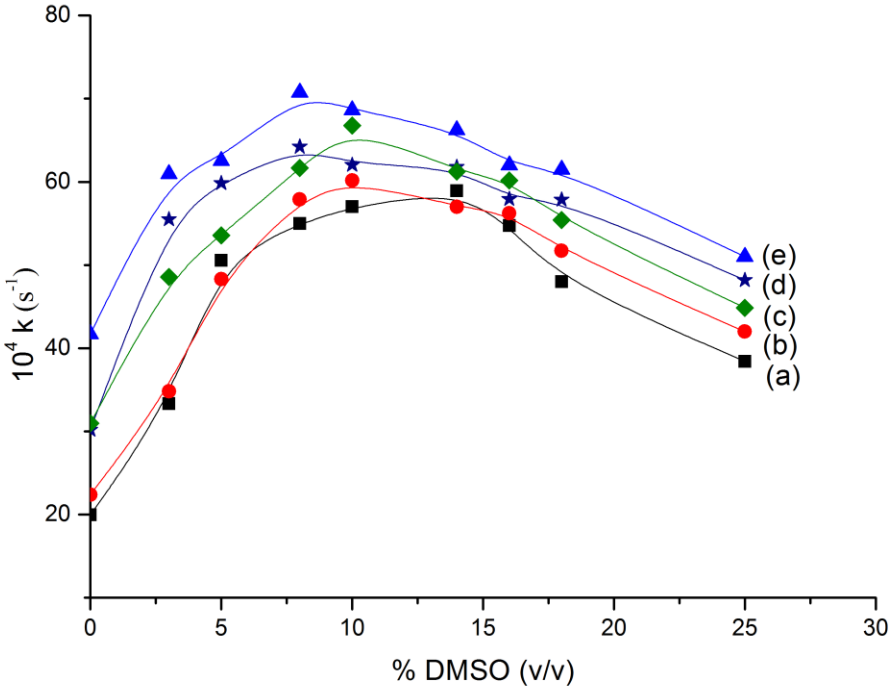



**Figure 4.23:** Influence of composition of DMSO on the rate reaction of Cu(II)-Gly-L-Ala complex with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions*: $[Cu(II)\text{-Gly-L-Ala}]^+ = 4.0 \times 10^{-4}$ mol $dm^{-3}$, [ninhydrin] = $10.0 \times 10^{-3}$ mol $dm^{-3}$, [TTABr] =$20.0 \times 10^{-3}$ mol $dm^{-3}$, [14-s-14] =$50.0 \times 10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

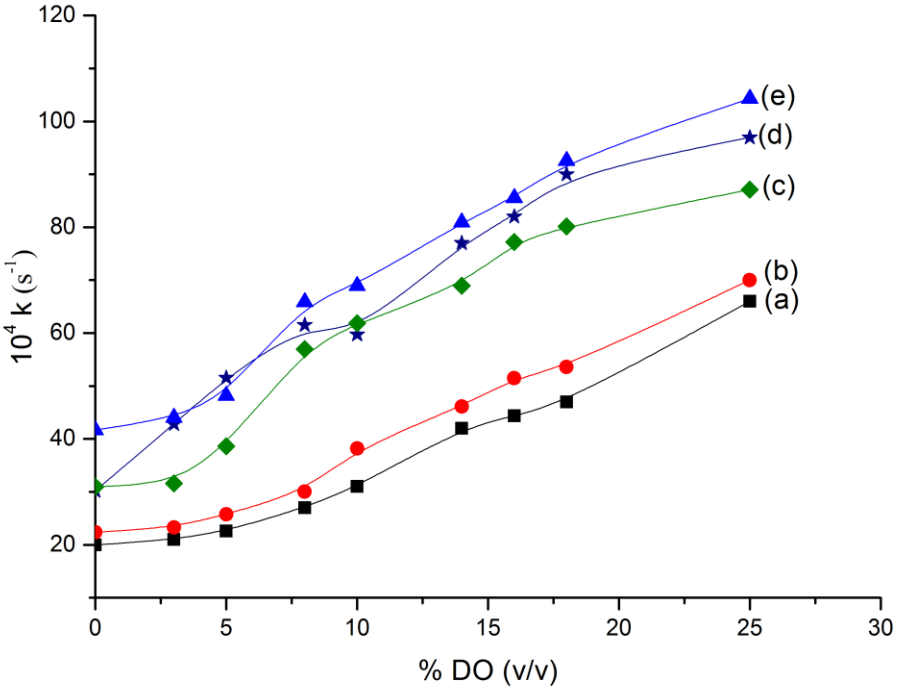



**Figure 4.24:** Influence of composition of DO on the rate reaction of Cu(II)-Gly-L-Ala complex with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions*: $[Cu(II)\text{-Gly-L-Ala}]^+ = 4.0 \times 10^{-4}$ mol $dm^{-3}$, [ninhydrin] = $10.0 \times 10^{-3}$ mol $dm^{-3}$, [TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$, [14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

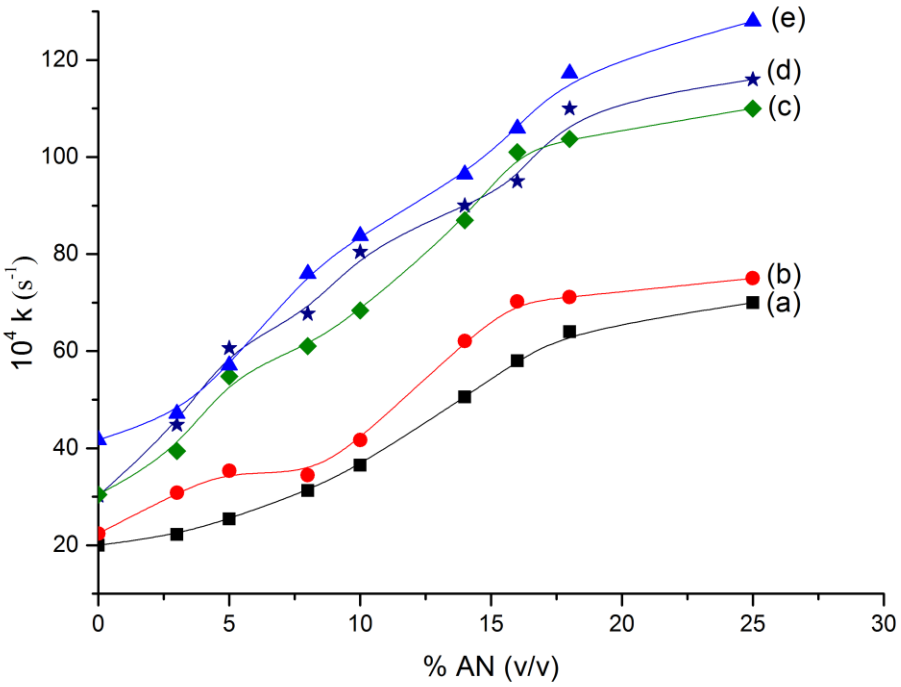



**Figure 4.25:** Influence of composition of AN on the rate reaction of Cu(II)-Gly-L-Ala complex with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions*: $[Cu(II)\text{-Gly-L-Ala}]^+ = 4.0 \times 10^{-4}$ mol $dm^{-3}$, [ninhydrin] = $10.0 \times 10^{-3}$ mol $dm^{-3}$, [TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$, [14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

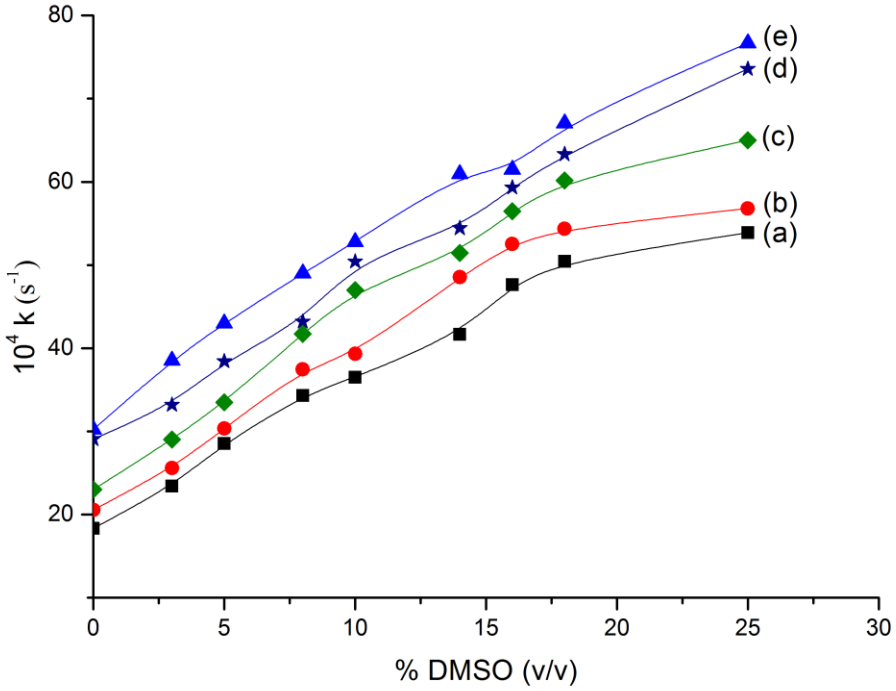



**Figure 4.26:** Influence of composition of DMSO on the rate reaction of Cu(II)-Gly-DL-Asp complex with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions*: $[Cu(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] =20.0 x $10^{-3}$ mol $dm^{-3}$, [14-s-14] =50.0 x$10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

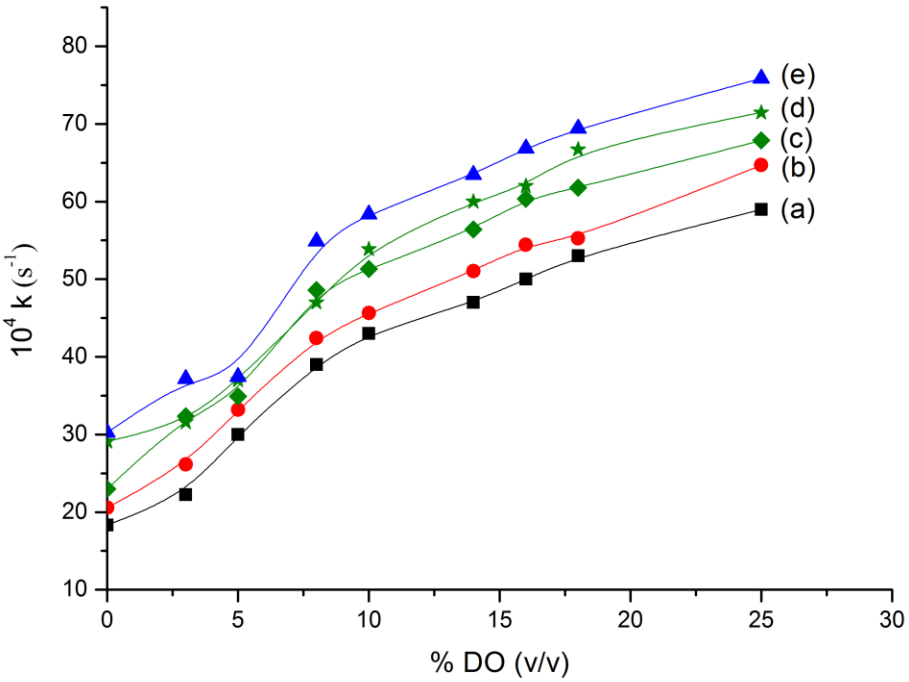



**Figure 4.27:** Influence of composition of DO on the rate reaction of Cu(II)-Gly-DL-Asp complex with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions*: $[Cu(II)\text{-}Gly\text{-}DL\text{-}Asp]^+ = 4.0 \times 10^{-4}$ mol $dm^{-3}$, [ninhydrin] = $10.0 \times 10^{-3}$ mol $dm^{-3}$, [TTABr] =$20.0 \times 10^{-3}$ mol $dm^{-3}$, [14-s-14] =$50.0 \times 10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

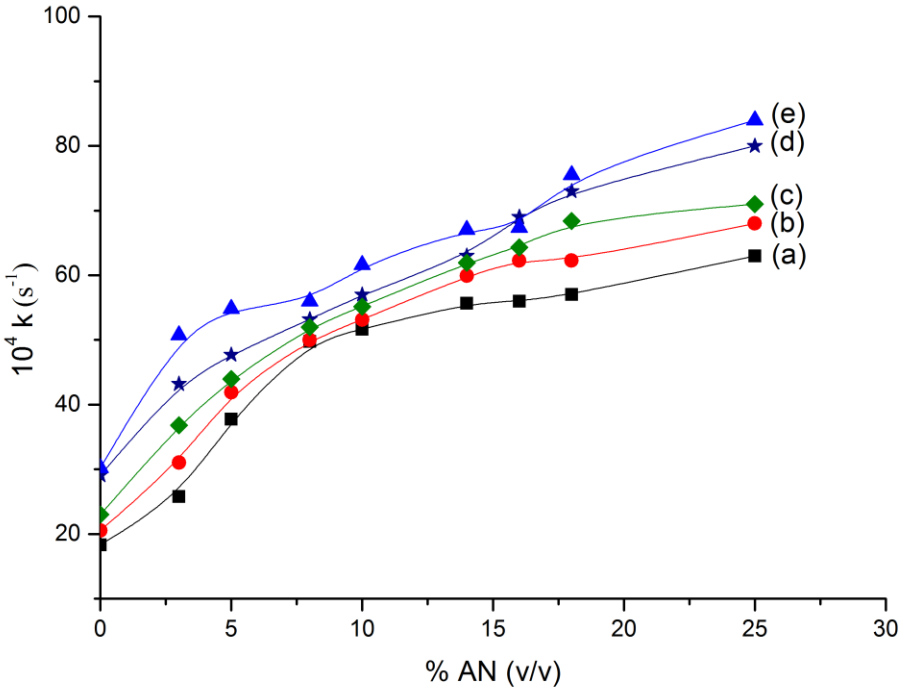
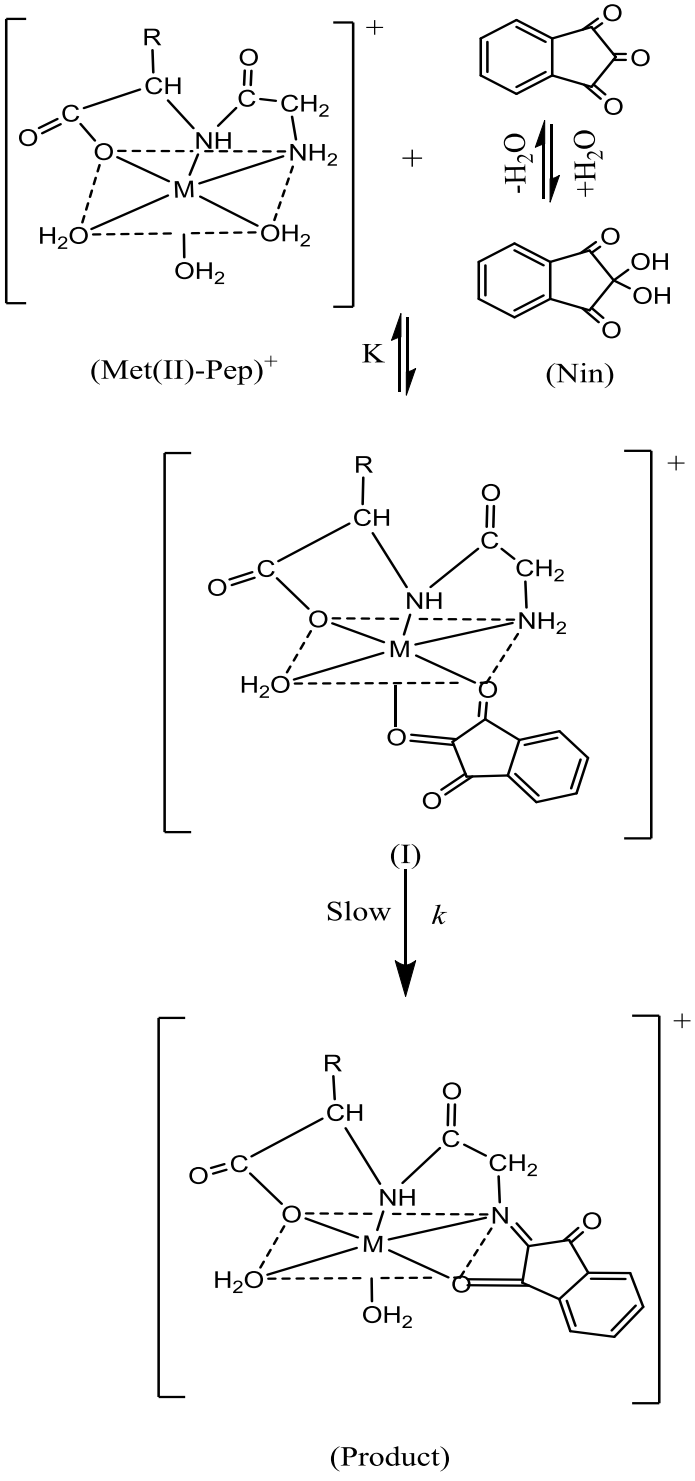



**Figure 4.28:** Influence of composition of AN on the rate reaction of Cu(II)-Gly-DL-Asp complex with ninhydrin in: (a) aqueous; (b) 14-6-14; (c) 14-5-14; (d) TTABr; (e) 14-4-14. *Reaction conditions*: $[Cu(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = 4.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 10.0 x $10^{-3}$ mol $dm^{-3}$, [TTABr] =20.0 x $10^{-3}$ mol $dm^{-3}$, [14-s-14] =50.0 x $10^{-5}$ mol $dm^{-3}$, *p*H = 5.0, temperature = 70 °C.

## 4.3 Discussion

### 4.3.1 Reactions in Absence of Surfactants

Before we discuss the kinetic results of the condensation reaction between metal (II)-dipeptide complexes and ninhydrin in micellar system and pertaining interpretations, it is necessary to shed light on the mechanism of the reaction in aqueous medium. Amino acids/dipeptides behave as a tridentate ligand with metal(II) and an equimolar mixture containing metal(II) and amino acid/dipeptide gives 1:1 complex [17,18, 39-44]. The reaction of α-amino acids/dipeptides with ninhydrin is an example of nucleophilic addition reaction. In these reactions lone-pair electrons of amino group are necessary for the nucleophilic attack on the carbonyl group of ninhydrin which gives the Schiff base (imine). In the case of metal(II)-dipeptide complexes $(Met(II)\text{-}Pep)^{+}$ (Scheme 4.1), the lone pair of electrons is not free due to coordination to the metal(II). Therefore, it is not possible for metal(II)-dipeptide complex to react with ninhydrin such as the nucleophilic addition reaction. The reaction proceeds through the formation of an inner sphere complex (I) (a ternary labile complex of $[Met(II)\text{-}Pep]^{+}$–ninhydrin) which is a feature of a CLAM (Combination-of-Ligands-Attached-to-the-same-Metal-ion) reaction [45,46]. In these types of reactions the dipeptide and ninhydrin are connected together with one metal ion. The presence of metal ions brings the reactive groups together and provides a better chance for their combination within its coordination sphere. In order to confirm the cleavage of –COOH groups with Met(II), we have experimentally tested that no $CO_2$ is evolved in the present case. Clearly, metal ion inhibits the cleavage of–COO group by reducing its escaping tendency and by enhancing the electrophilic character of >C=O group. On the basis of the above discussion, and the observed rate data, it can be concluded that the reaction takes place in two kinetically distinguishable steps: Firstly, a fairly rapid ternary labile complex formation between $[Met(II)\text{-}Pep]^{+}$ and ninhydrin takes place and secondly, a slower condensation of amino group to carbonyl group gives the product.




**Scheme 4.1**: Mechanism of $[Met(II)\text{-}Pep]^+$ complex–ninhydrin reaction. M: Hg(II) or Cu(II). R: $-CH_3$ for Gly-L-Ala, $-CH_2COOH$ for Gly-DL-Asp.

On the basis of observed rate law d[product]/dt=$k_{obs}$ [Met(II)-Pep]$^{+}$ and the proposed mechanism (Scheme 4.1), the rate equation (4.2) is derived;

$$k_{obs} = kK\,[Nin] / (1 + K[Nin]) \quad (4.2)$$

where [Nin] is the total concentration of ninhydrin.

On inverting Eq. (4.2), we get

$$1/k_{obs} = 1/kK\,[Nin] + 1/k \quad (4.3)$$

Concerning Eq. (4.3), a plot of $1/k_{obs}$ *vs.* 1/[Nin] should be linear with positive intercept (=1/*k*) and slope (= 1/*k*K). The respective values of rate constant $k_{obs}$, and equilibrium constant K were thus evaluated in aqueous medium. The calculated values of rate constants ($k_{cal}$), obtained by substituting *k* and K (in Eq. 4.2), are in close agreement with the $k_{obs}$ (given in parentheses, Tables 4.9-4.12) which supports the proposed mechanism and confirms the validity of the rate Eq. (4.2).

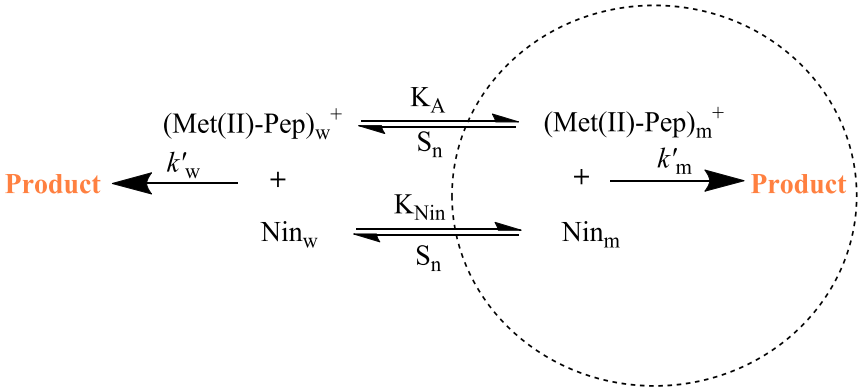
### 4.3.2 Reactions in Presence of TTABr/14-s-14 Surfactants

The reactions of [Met(II)-Pep]$^{+}$ complexes with ninhydrin in presence of TTABr/14-s-14 surfactant micelles (Tables 4.17- 4.24, Figures 4.9- 4.16) follow the same pattern as described above with the same first- and fractional- order kinetics –as in aqueous medium- with respect to [Met(II)-Pep]$^{+}$ complex and [ninhydrin], respectively. The addition of TTABr/14-s-14 micelles (as well as organic solvents) leads to increase the absorbance as it is shown in Figures 2.14- 2.33. Moreover, in presence of surfactants, the kinetic results are similar with those obtained in aqueous media except the substrate effect in which Michaelis–Menten behavior has been observed, *i.e.*, the binding of the substrate with the surfactant.

Electrostatic interactions and *hydrophobic* character are the two main factors in the micellar catalysis which increase the concentration of reactants into small volume. The dipeptides form part of the inner coordination shell of a cationic metal complex

(which give the complex some *hydrophobicity*). From purely electrostatic considerations, ninhydrin (due to presence of an electron cloud) can be assumed to reside predominantly in the Stern layer. The micelles thus help in bringing the reactants together which may now orient in a manner suitable for the condensation. At higher concentration of TTABr and above the critical micelle concentration (CMC), the decrease in $k_\psi$ values can be explained by Berezin's model [47] which involves solubilization of the reactants in both media. Because all the substrate has been incorporated into the micellar phase and when water bulk of the substrate is incorporated into the micelles, addition of more TTABr generates more cationic micelles, which simply take up the ninhydrin molecules into the Stern layer, and thereby deactivate them; because a reactant molecule in one micelle should not react with the other in another micelle [48]. The other reason of decrease in $k_\psi$ could be a result of counter ion ($Br^-$) inhibition due to competition with lone pair electrons in nitrogen atom of ninhydrin. Moreover, partitioning a *hydrophilic* species between the bulk phase and micellar phase decreases $k_\psi$ [49]. In case of gemini micelles, it can be seen that $k_\psi$–[gemini] profiles have three zones (Figures 4.13-4.16). Rate constant ($k_\psi$) first increases (zone I), remains constants up to certain concentration (zone II) (zones I and II behavior is like to conventional surfactant micelles) and then increase again (zone III). Gemini micelles provide much better environment for the $[Met(II)\text{-}Pep]^+$ complex–ninhydrin reaction as compared to the analogous monomeric (TTABr) micelles. The reason for that is the nature of the spacer which decreases the water content in the aggregates making the environment less polar and thus the $k_\psi$ increases.

The *pseudophase* kinetic model [38,50-53] was successfully applied in our study to describe the catalytic effects of [TTABr]/[14-s-14] on $[Met(II)\text{-}Pep]^+$ complex–ninhydrin reactions. According to this model, the total volume of micelles can be treated as separate phase uniformly distributed in the aqueous phase; the reaction occurs in both phases as Scheme 4.2.




**Scheme 4.2:** The *pseudophase* kinetic model for the reaction of $(Met(II)\text{-}Pep)^+$ complex with ninhydrin in aqueous and in micellar system.

where the symbols have the same meaning as in Chapter three, $(Met(II)\text{-}Pep)^+$ represents the metal(II) (Hg(II) or Cu(II)) coordinated with dipeptide (Gly-L-Ala or Gly-DL-Asp).

Although several kinetic equations based on this general Scheme have been developed, the most successful appears to be that of Berezin's model for bimolecular reactions [47] and further development by Bunton [51,54] who suggested an Eq. (4.4), which takes into account both (1) the solubilization of the reactants into the micelles, and (2) the mass action model

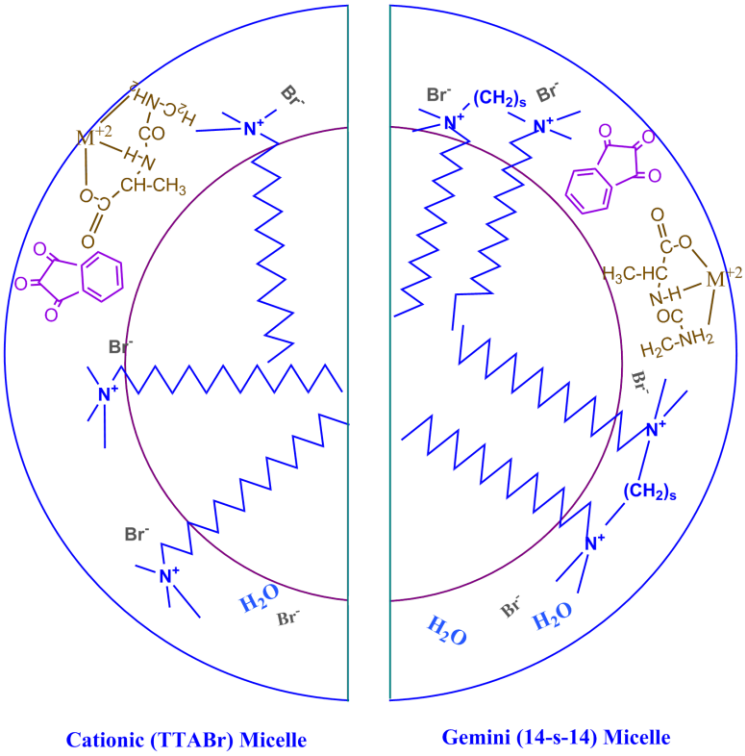
$$k_\psi = \{k_w[Nin] + (K_A k_m - k_w)\, M_N^S\, [S_n]\} / 1 + K_A\, [S_n] \qquad (4.4)$$

$K_A$ is the binding constant of the complex to the micelles. The values of $k_w$, $k_m$ and $K_A$ are calculated as described in Chapter III. The best fit values are given in Tables 4.29-4.32. The validity of the proposed mechanism is confirmed by a close agreement between the observed and calculated $k_\psi$ values (Tables 4.17-4.24).

The high $K_A$ values indicate that the substrate is strongly bound to micelles. A higher value of $K_A$ is observed in case of metal(II)-Gly-L-Ala complex as compared to metal(II)-Gly-DL-Asp complex. A possible explanation for the difference between $K_A$ could be related to the fact that the Gly-DL-Asp molecule is more hydrophilic (less hydrophobic) than Gly-L-Ala molecule because of its side chain effect (the former

contains –COOH group). The decreased hydrophobicity seems responsible for a lower concentration of the metal ion-Gly-DL-Asp complex in the Stern layer of micelles.

Due to the different properties of micellar *pseudophase*, it is not possible to precisely locate the exact site of the reactions but, at least, localization of the reactants can be considered. It is believed that most ionic micelle mediated reactions take place either inside the Stern layer's water rich-region (close to the surface of the micelle) or at the interface between the bulk water solvent and micellar surface [19,38]. Cordes concluded in his excellent review [55] that the water activity at the surface of ionic micelles is similar to that in the aqueous *pseudophase*. Explanation of the catalytic effect of TTABr/14-s-14 on the $(Met(II)^{-}Pep)^{+}$ complexes and ninhydrin reaction can be made using electrostatic considerations as follows. Ninhydrin, as it contains electron clouds [15], can be assumed to reside predominantly in the Stern layer. The micellar surface can repel or attract ionic species due to electrostatic interaction whereas *hydrophobic* interaction can bring about the incorporation of reactants into micelles. As TTABr/14-s-14 are cationic micelles, their surface attracts ninhydrin closer which increases the local molarities in the Stern layer. For the $[Met\ (II)–Pep]^{+}$ complex, despite of bearing positive charge, the removal of water molecules from the inner solvation shell of metal by coordinated dipeptide gives the complex some *hydrophobic* character and, due to this hydrophobic nature, the complex gets incorporated into the cationic micelles. As a result, the $[Met(II)–Pep]^{+}$ complex and ninhydrin are brought close together into a small volume, *i.e.* the Stern layer, by the help of micelles. Then they orient in manner suitable for the condensation (a possible arrangement-although highly schematic-could be as illustrated in Figure 4.29).




R= $-CH_3$ for Gly-L-Ala or $-CH_2COOH$ for Gly-DL-Asp

**Figure 4.29:** Schematic model representing probable location of reactants for the cationic/dicationic (TTABr/14-s-14) micellar catalyzed condensation reaction between Met(II)-Pep complex and ninhydrin.

Effect of temperature on micellar-catalyzed reactions of metal(II)-dipeptide complexes with ninhydrin in the presence of 20.0 x $10^{-3}$ mol $dm^{-3}$ [TTABr] or 50.0 x $10^{-5}$ mol $dm^{-3}$ [14-s-14] was carried out to evaluate activation parameters *i.e.*, free energy ($E_a$), activation enthalpy $\Delta H^{\#}$ and activation entropy ($\Delta S^{\#}$). It was found that the Eyring equation is applicable to the micellar systems. The difference of activation parameters in cationic micelles (*i.e.*, TTABr/14-s-14) as compared to water is as expected, because incorporation of the reactants into the cationic micelles reduces $\Delta H^{\#}$. The large negative value of $\Delta S^{\neq}$ (Tables 4.13-4.16) in presence of surfactants indicates the formation of more ordered activated complex in surfactant media. Small values of $E_a$ and $\Delta H^{\#}$ in the

presence of micelles indicate the catalytic effect on the reaction as compared to aqueous medium.

#### *4.3.2.1 Influence of s-Value of Gemini Surfactants*

It was noted that the spacer chain length (s-values) affects the reaction rate constant ($k_\psi$) values, with maximum increase obtained at s = 4 (in comparison to s =5 and s =6) under the identical kinetics experimental conditions (Figures 4.30-4.33). It is known that variation of spacer chain length alters the CMC and morphology of gemini micellar aggregates [56-59] and this alteration is supported by microviscosity and SANS data [60], while the length and type of the spacer moiety dictate the conformation of the gemini molecules [61,62]. Thus, because of the spacer greatly affecting the surfactant morphology, the *pseudo*-first order rate constant values ($k_\psi$, $s^{-1}$), obtained in the present studies, are consistent with the expectation being maximum when using 14-4-14 micelles.

#### *4.3.2.2 Influence of Organic Solvents*

According to the '*hydrophobic* or *solvophobic* interaction', which is the main driving force behind aggregation, addition of organic solvents to aqueous solutions of surfactants modifies the ability of the surfactant molecules to avoid contact with the bulk phase, and thereby changes various micellization parameters which affect the rate of reaction [63]. The rate of an elementary chemical reaction has been turned to change by orders of magnitude when the solvent is changed [64-67]. Solvent effects on kinetics and chemical reactions are usually correlated in terms of "solvent polarity", which sums up all the specific and nonspecific interactions of the media with initial and transition states [68,69]. However, the interpretation of the kinetic results on the basis of the solvent polarity on the medium sometimes is not possible. Several attempts has been directed towards understanding such solvent effects, and a great deal of progress has been made [70-78].

Tables 4.25-4.28 and Figures 4.17-4.28 summarize our study on the effect of organic solvents on $(Met(II)\text{-}Pep)^+$–ninhydrin reactions. Solvent can affect the 3D

structure of bulk water in several ways depending on the category of organic solvents: (1) 1,4-dioxane (DO) (non polar) and acetonitrile (AN) (polar) which form hydrogen bonds with water, and (2) dimethyl sulfoxide (DMSO) which forms hydrates with water [79]. Although the studied solvents increase the absorbance and intensity of the Met(II)-Pep complex–ninhydrin reactions (Figures 2.14- 2.33) each solvent has been found to postpone micellization of the surfactants (Tables 2.8- 2.11), due to different reasons [80]. In case of DO and AN, the decrease in the number of micelles is due to the formation of strong hydrogen bonds between water and DO or AN molecules. The effect of DMSO on TTABr/14-s-14 micellization can be explained on the basis of strong interaction with water and stoichiometric hydrate ($DMSO.2H_2O$) formation which results in increased structuring of the solvent system and the inhibiting effect of DMSO on the formation of micelles [78,79,81-83]. An increase in the orderliness of the DMSO–$H_2O$–TTABr/14-s-14 system takes place as the composition of DMSO is increased.

Despite all the three solvents inhibiting the micellization in TTABr/14-s-14 micelles, the reaction still shows some catalytic effect in presence of these solvents, especially at low concentrations. This can be due to the relative participation of water and organic solvents in acid-base equilibria and hydrogen bonding. Our observations indicate that there is no major change in the *p*H of the working condition in presence of these solvents. Here, with metal ions complexation, it is not preferable to take organic solvent at high concentration because (1) a high concentration speeds up the reaction and becomes difficult to measure rate constant under experimental range sensitivity, (2) avoiding the side effect on the mechanism, (3) probable binding occurs between micelles and metal ions and thus abstracting metal(II) ions from $(Met(II)\text{-}Pep)^+$ complex which renders the dipeptide free and gives side reaction with ninhydrin, and (4) a side reaction between a buffer and organic solvent occurs (as it occurred between a DO (higher than 25.0%) and a buffer which turns the solution turbid). For these reasons, we didn't pass beyond 25.0% (v/v) concentration.

**Table 4.29:** Rate and binding constants for the reaction of Hg(II)-Gly-L-Ala complex with ninhydrin.

*Reaction conditions*:

| | | |
|---|---|---|
| $[Hg(II)\text{-}Gly\text{-}L\text{-}Ala]^+$ | = | $2.0 \times 10^{-4}$ mol $dm^{-3}$ |
| [ninhydrin] | = | $6.0 \times 10^{-3}$ mol $dm^{-3}$ |
| [TTABr] | = | $20.0 \times 10^{-3}$ mol $dm^{-3}$ |
| [14-s-14] | = | $50.0 \times 10^{-5}$ mol $dm^{-3}$ |
| *p*H | = | 5.0 |
| Temperature | = | 70 °C |

| Constants | Surfactant | | | |
|---|---|---|---|---|
| | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| $10^2 k_m$ ($s^{-1}$) | 5.4 | 7.6 | 6.8 | 8.2 |
| $10\ k_w$ ($mol^{-1}dm^3\ s^{-1}$) | 6.2 | 6.2 | 6.2 | 6.2 |
| $K_A$ ($mol^{-1}dm^3$) | 70.0 | 142.0 | 135.0 | 118.0 |
| $K_{Nin}$ ($mol^{-1}\ dm^3$) | 55.8 | 58.7 | 56.4 | 54.5 |

**Table 4.30:** Rate and binding constants for the reaction of Hg(II)-Gly-DL-Asp complex with ninhydrin.

*Reaction conditions*:

$[Hg(II)\text{-Gly-DL-Asp}]^{+}$ = $2.0 \times 10^{-4}$ mol $dm^{-3}$
[ninhydrin] = $6.0 \times 10^{-3}$ mol $dm^{-3}$
[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$
[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$
*p*H = 5.0
Temperature = 70 °C

| Constants | Surfactant | | | |
|---|---|---|---|---|
| | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| $10^3 k_m$ ($s^{-1}$) | 4.2 | 8.6 | 7.4 | 9.2 |
| $10\ k_w$ ($mol^{-1}dm^3\ s^{-1}$) | 5.8 | 5.8 | 5.8 | 5.8 |
| $K_A$ ($mol^{-1}dm^3$) | 62.0 | 120.0 | 112.0 | 100.0 |
| $K_{Nin}$ ($mol^{-1}\ dm^3$) | 37.4 | 42.3 | 40.2 | 39.8 |

**Table 4.31:** Rate and binding constants for the reaction of Cu(II)-Gly-L-Ala complex with ninhydrin.

*Reaction conditions*:

| | | |
|---|---|---|
| $[Cu(II)\text{-}Gly\text{-}L\text{-}Ala]^+$ | = | $4.0 \times 10^{-4}$ mol $dm^{-3}$ |
| [ninhydrin] | = | $10.0 \times 10^{-3}$ mol $dm^{-3}$ |
| [TTABr] | = | $20.0 \times 10^{-3}$ mol $dm^{-3}$ |
| [14-s-14] | = | $50.0 \times 10^{-5}$ mol $dm^{-3}$ |
| *p*H | = | 5.0 |
| Temperature | = | 70 °C |

| Constants | Surfactant | | | |
|---|---|---|---|---|
| | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| $10^2 k_m$ ($s^{-1}$) | 1.9 | 3.1 | 2.9 | 2.8 |
| $10\ k_w$ ($mol^{-1}dm^3\ s^{-1}$) | 2.0 | 2.0 | 2.0 | 2.0 |
| $K_A$ ($mol^{-1}dm^3$) | 66.0 | 132.0 | 124.0 | 112.0 |
| $K_{Nin}$ ($mol^{-1}\ dm^3$) | 64.6 | 70.6 | 70.2 | 68.4 |

**Table 4.32:** Rate and binding constants for the reaction of Cu(II)-Gly-DL-Asp complex with ninhydrin.

*Reaction conditions*:

$[Cu(II)\text{-}Gly\text{-}DL\text{-}Asp]^{+}$ = $4.0 \times 10^{-4}$ mol $dm^{-3}$
[ninhydrin] = $10.0 \times 10^{-3}$ mol $dm^{-3}$
[TTABr] = $20.0 \times 10^{-3}$ mol $dm^{-3}$
[14-s-14] = $50.0 \times 10^{-5}$ mol $dm^{-3}$
*p*H = 5.0
Temperature = 70 °C

| Constants | Surfactant | | | |
|---|---|---|---|---|
| | TTABr | 14-6-16 | 14-5-14 | 14-4-14 |
| $10^3 k_m$ ($s^{-1}$) | 4.0 | 9.2 | 8.4 | 8.8 |
| $10 k_w$ ($mol^{-1}dm^3 s^{-1}$) | 1.8 | 1.8 | 1.8 | 1.8 |
| $K_A$ ($mol^{-1}dm^3$) | 60.0 | 116.0 | 98.0 | 94.0 |
| $K_{Nin}$ ($mol^{-1}$ $dm^3$) | 24.3 | 34.4 | 32.6 | 30.8 |

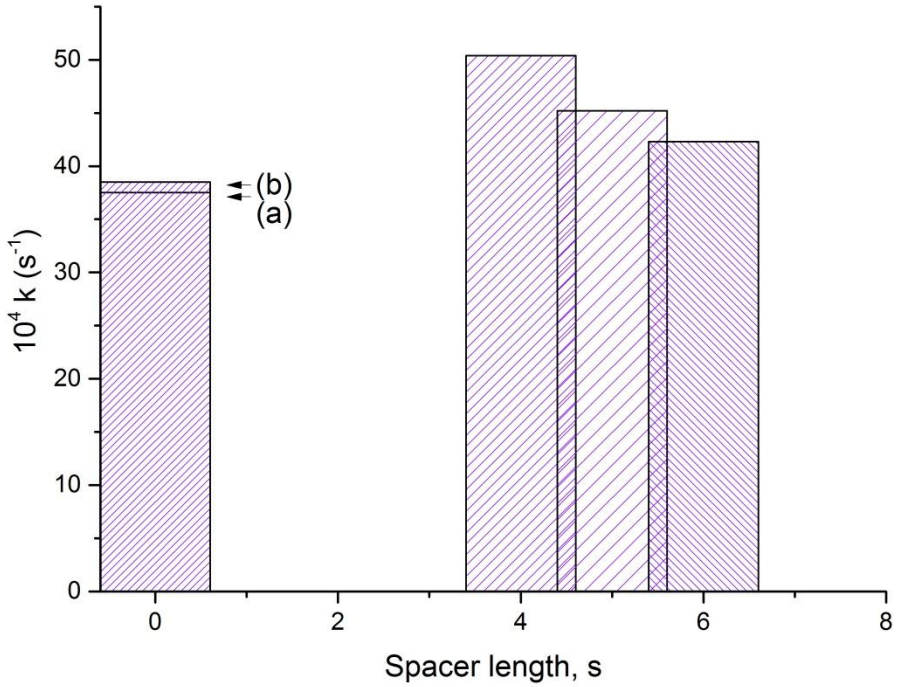



**Figure 4.30:** Spacer length (s = 4, 5, 6) effect on the reaction rate of Hg(II)-Gly-L-Ala complex (2.0 x $10^{-4}$ mol $dm^{-3}$) with ninhydrin (6.0 x $10^{-3}$ mol $dm^{-3}$), (a) in aqueous, and (b) [TTABr] = 50.0 x $10^{-5}$ mol $dm^{-3}$, at *p*H=5.0 and temperature = 70 $^{o}$C. Others are for [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ with the respective s values.

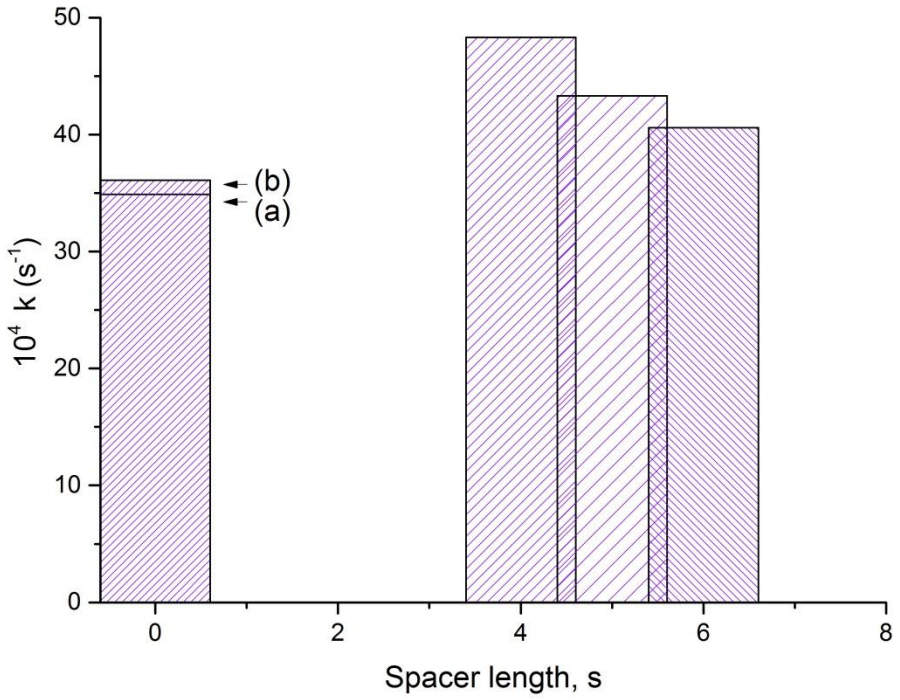



**Figure 4.31:** Spacer length (s = 4, 5, 6) effect on the reaction rate of Hg(II)-Gly-DL-Asp complex (2.0 x $10^{-4}$ mol $dm^{-3}$) with ninhydrin (6.0 x $10^{-3}$ mol $dm^{-3}$), (a) in aqueous, and (b) [TTABr] = 50.0 x $10^{-5}$ mol $dm^{-3}$, at *p*H=5.0 and temperature = 70 $^{o}$C. Others are for [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ with the respective s values.

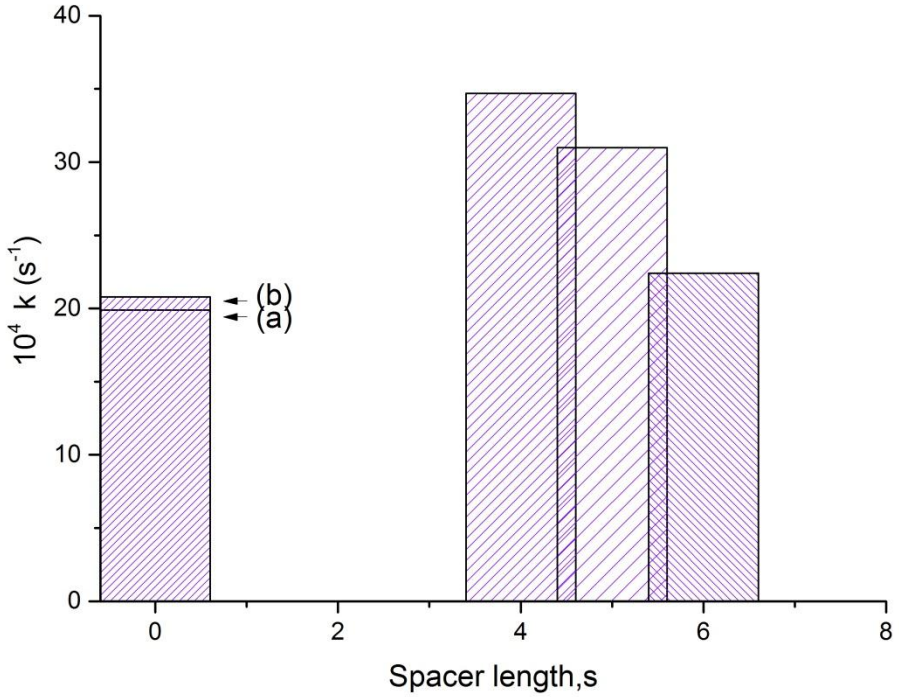



**Figure 4.32:** Spacer length (s = 4, 5, 6) effect on the reaction rate of Cu(II)-Gly-L-Ala complex (4.0 x $10^{-4}$ mol $dm^{-3}$) with ninhydrin (10.0 x $10^{-3}$ mol $dm^{-3}$), (a) in aqueous, and (b) [TTABr] = 50.0 x $10^{-5}$ mol $dm^{-3}$, at *p*H=5.0 and temperature = 70 $^{o}$C. Others are for [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ with the respective s values.

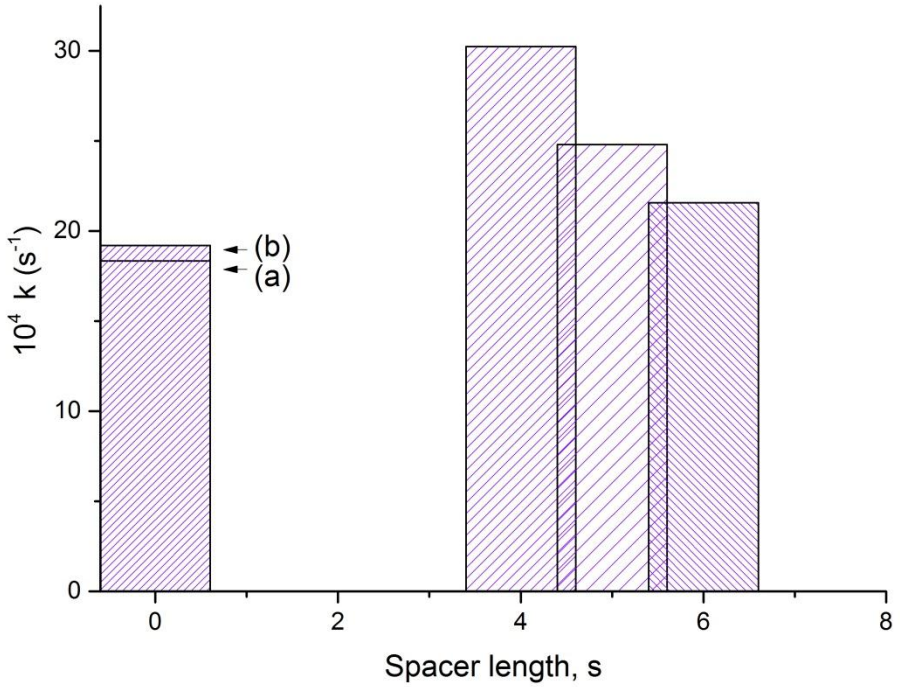



**Figure 4.33:** Spacer length (s= 4, 5, 6) effect on the reaction rate of Cu(II)-Gly-DL-Asp complex (4.0 x $10^{-4}$ mol $dm^{-3}$) with ninhydrin (10.0 x $10^{-3}$ mol $dm^{-3}$), (a) in aqueous, and (b) [TTABr] = 50.0 x $10^{-5}$ mol $dm^{-3}$, at *p*H=5.0 and temperature = 70 $^{o}$C. Others are for [14-s-14] = 50.0 x $10^{-5}$ mol $dm^{-3}$ with the respective s values.

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# CATIONIC MICELLAR EFFECT ON THE REACTION BETWEEN DIPEPTIDE GLYCYL-ALANINE (GLY-ALA) AND NINHYDRIN WITH AND WITHOUT SALT ADDITIVES

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**Keywords:** surfactant, dipeptide (Gly-Ala), ninhydrin, catalysis, micelles, TTAB, CTAB, CPC, salts

The effect of cationic conventional surfactants myristyltrimethylammonium bromide (TTAB), cetyltrimethylammonium bromide (CTAB), and cetylpyridinium chloride (CPC) on the interaction of dipeptide glycyl-alanine (Gly-Ala) with ninhydrin has been studied spectrophotometrically under different conditions. The reaction rates are higher in the presence of surfactants but the reaction order remains the same in both the media (first- and fractional-order with respect to [Gly-Ala] and [ninhydrin]). Quantitative kinetic analyses of $k_\psi$–[surfactant] data were performed on the basis of pseudo-phase model of the micelles (proposed by Menger and Portnoy and developed by Bunton and Romsted) and Piszkiewicz model wherein the micellar binding constants $K_S$ for Gly-Ala and $K_N$ for ninhydrin with surfactant micelles were evaluated. The catalytic efficiency in TTAB increased by added electrolytes which had been discussed in detail.

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## Introduction

Surfactants are amphiphiles that contain polar or ionic head groups and apolar tails. They form association colloids, known as micelles, when they self-associate at concentrations above the critical micelle concentrations. In ionic head micelles, for example, the aqueous solution-micelle interfacial region contains the ionic head groups, the Stern layer of the electrical double layer with the bound counterions, and water. The remaining counterions are contained in the Gouy-Chapman portion of the double layer that extends further into the aqueous phase.[1] A micelle or a micellar aggregate constitutes an inhomogeneous microreaction environment, which is highly dynamic, in the sense that it is in rapid equilibrium with the constituent monomers in aqueous phase. So that, a micelle is not a separate phase, like aqueous phase, although it does provide microreaction medium, which is called pseudophase, in which micellar mediated reactions occur. Micellar catalysis of numerous reactions is an area of current research because of the parallel behaviour of macromolecules and enzymes.[2]

The ninhydrin (triketohydrindene hydrate)–mediated colour formation is the most widely used method for detection and quantitative estimation of amino acids/peptides.[3] The so-called *'ninhydrin reaction'* forms a product known as *'Ruhemann's purple'* which is attributed to be anion of diketohydrindylidenediketohydrindamine (DYDA), and this product can be quantitatively measured at 570 nm. To improve the sensitivity, however, modifications in the method are continuously being made.[3,4] In this regard studies by our group had shown success toward increased sensitivity of ninhydrin-amino acid reaction by involving surfactant micelles, solvents and complexation with metal cations.[5-10] As studies on ninhydrin-peptide reaction are limited,[11-13] systematic kinetic and mechanistic studies of the Gly-Ala-ninhydrin reaction in absence and presence of cationic micelles of myristyltrimethylammonium bromide (TTAB), cetylpyridinium chloride (CPC), and cetyltrimethyl-ammonium bromide (CTAB) at different temperatures have been performed. Also, it is found that various added salts can affect the overall course of the reaction. Therefore, the investigation concerns the reaction in aqueous and micellar media with and without salts.

## Experimental Section

### Materials and Methods

The surfactants (TTAB, ≥99%, Sigma, India; CPC, 99%, Merck, Germany; CTAB, 99%, Merck, Germany), glycyl-L-alanine (≥99% (NT), Aldrich, Switzerland), ninhydrin (99%, Merck, India), sodium acetate anhydrous (≥ 99%, Merck, India), acetic acid glacial (99-100%, Merck, India), sodium nitrate purified (99 %, Merck, India), sodium sulphate (≥ 98%, Merck, India), sodium phosphate (96%, Aldrich, USA) , sodium salicylate (99.5%, CDH, India), sodium benzoate (99.5%, Merck, India), sodium tosylate (70-80%, (HPLC), Fluka, Switzerland), and sodium oxalate (≥ 99.5%, S.D. Fine-chem Ltd., India) were used as received. Demineralized double-distilled water was used throughout the work (specific conductivity (Λ): $(0.8 – 2.1)\times10^{-6}$ $S^{-1}cm^{-1}$). Stock solutions of the reactants and the surfactants were prepared in acetic acid – sodium acetate buffer which was prepared by mixing acetic acid (0.2 mol $dm^{-3}$) and sodium acetate (0.2 mol $dm^{-3}$) up to desired volume.[14] The pH measurements were made using a digital Systronics pH meter model MK-VI (Ahmedabad-India) in conjugation with a combined electrode (glass-saturated calomel electrode) and standardized using WTW buffer solutions

(Germany). A Systronics conductivity meter model 306 (Ahmedabad-India) with platinized electrodes was used for the conductivity measurements.

#### Kinetic measurements

For each set of kinetic experiments, the requisite volumes of Gly-Ala, buffer and surfactant solutions (when required) were taken in a three-necked reaction vessel (also fitted with a double-surface water condenser), which was then kept in an oil bath at the experimental temperature. The reaction was started by adding a requisite volume of thermally equilibrated ninhydrin solution; zero-time was taken when half of the ninhydrin solution had been added. Pure $N_2$-gas (free from $O_2$ and $CO_2$) was bubbled through the reaction mixture for stirring as well as to maintain an inert atmosphere. Pseudo-first-order conditions were maintained in all the kinetic runs by using excess of ninhydrin over Gly-Ala concentration (≥10 times). The absorbance of the product DYDA was measured at 570 nm ($\lambda_{max}$-vide infra) at definite time intervals with a Shimadzu UVmini-1240 Spectrophotometer. Other details regarding kinetic methodology were the same as described elsewhere.[5-13]

#### Determination of CMC

The critical micellar concentration (CMC) values of the TTAB, CPC, and CTAB solutions under the experimental conditions were determined conductometrically. The values in the presence and absence of reactants have been obtained from the break points of nearly two straight line portions of the specific conductivity *vs.* concentration plots.[15] Experiments were carried out under different conditions, i.e., solvent being water, water + Gly-Ala, water + ninhydrin or water + Gly-Ala + ninhydrin and the respective CMC values are recorded in Table 1.

#### Viscosity measurements

Using Ubbelohde viscometer the viscosity measurements were made at 70 ± 0.1 °C. The method of viscosity measurements was the same as reported elsewhere.[16]

## Results and Discussion

#### Spectra of the product

The UV-visible spectra of the product formed by the reaction between Gly-Ala and ninhydrin in the buffer solution were recorded in the absence and presence of surfactant micelles (Figure 1). We see that the absorbance is higher in presence of micelles than in aqueous medium with no shift in $\lambda_{max}$(570 nm), i.e., the wave length of maximum absorbance remains the same in both aqueous and micellar media. It is, therefore, concluded that the purple-coloured product of Gly-Ala reaction with ninhydrin to be the same in aqueous and micellar systems.

**Table 1. T**he CMC values for CPC, CTAB, and TTAB at 30 ºC and 70 ºC using electrical conductivity technique.

| System | CMC·$10^3$ mol $dm^{-3}$ 30 ºC | CMC·$10^3$ mol $dm^{-3}$ at 70 ºC |
|---|---|---|
| Pure CPC | 1.06 | 1.40 |
| CPC + Ninhydrin | 1.26 | 1.35 |
| CPC + Gly-Ala | 1.05 | 1.29 |
| CPC + Gly-Ala+ Ninhydrin | 1.19 | 1.31 |
| | | |
| Pure CTAB | 0.98 | 1.27 |
| CTAB + Ninhydrin | 1.29 | 1.41 |
| CTAB + Gly-Ala | 0.93 | 1.09 |
| CTAB + Gly-Ala+ Ninhydrin | 1.07 | 1.38 |
| | | |
| Pure TTAB | 3.90 | 5.11 |
| TTAB + Ninhydrin | 4.32 | 5.40 |
| TTAB + Gly-Ala | 3.80 | 4.30 |
| TTAB + Gly-Ala+ Ninhydrin | 4.25 | 5.53 |

The kinetics of the reaction of glycyl-alanine and ninhydrin was, therefore studied under varying experimental conditions spectrophotometrically by following the appearance of purple colour at 570 nm. The results are described below.

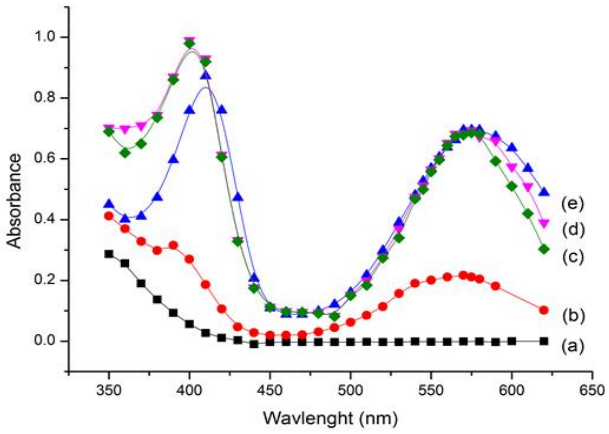



**Figure 1.** Spectra of reaction product of ninhydrin (6.0 x $10^{-3}$ mol $dm^{-3}$) with Gly-Ala (2.0 x $10^{-4}$ mol $dm^{-3}$), surfactant (20 x $10^{-3}$ mol $dm^{-3}$), pH = 5.0 and temperature = 70 °C in the absence of surfactant immediately after mixing the reactants (a) in the absence of surfactant (b), in the presence of TTAB (c), CTAB (d), and CPC (e), spectra (b) to (e) were recorded after the completion of the reaction

#### Dependence of Reaction Rate on pH

To find out the sensitivity of the reaction on the pH, the kinetic experiments were performed at pH varying from 4.0 to 6.5 while all other parameters were kept fixed in aqueous as well as in micellar media (Figure 2.).

**Table 2.** Dependence of pseudo-first order rate constants ($k_{obs}$ or $k_\Psi$) on [Gly-Ala], [ninhydrin] and temperature at pH = 5.0.

| $10^4$[Gly-Ala], mol dm$^{-3}$ | $10^3$[Ninhydrin], mol dm$^{-3}$ | Temperature, °C | $10^5\,k_{obs}$[a], s$^{-1}$ | $10^5 k_\Psi$[b], s$^{-1}$ | | |
|---|---|---|---|---|---|---|
| | | | | TTAB | CTAB | CPC |
| 1.0 | 6.0 | 70 | 13.0 | 60.9 | 61.7 | 30.2 |
| 1.5 | | | 13.5 | 63.2 | 64.4 | 30.8 |
| 2.0 | | | 14.1 | 63.9 | 65.1 | 30.2 |
| 2.5 | | | 14.3 | 61.0 | 62.6 | 29.6 |
| 3.0 | | | 14.6 | 64.8 | 60.6 | 27.9 |
| 2.0 | 6.0 | 70 | 14.1 | 63.9 | 65.1 | 30.3 |
| | 10 | | 16.5 | 96.8 | 69.1 | 55.2 |
| | 15 | | 31.5 | 110 | 95.9 | 55.5 |
| | 20 | | 47.8 | 124 | 96.9 | 81.5 |
| | 25 | | 45.5 | 122 | 106 | 81.7 |
| | 30 | | 52.7 | 126 | 123 | 89.0 |
| | 35 | | 53.5 | 123 | 120 | 85.8 |
| | 40 | | 51.2 | 115 | 129 | 88.7 |
| 2.0 | 6.0 | 60 | 3.15 | 18.9 | 14.4 | 7.80 |
| | | 65 | 8.23 | 33.2 | 49.9 | 17.6 |
| | | 70 | 14.1 | 63.9 | 65.1 | 30.3 |
| | | 75 | 20.8 | 101 | 96.9 | 36.7 |
| | | 80 | 47.9 | 127 | 98.8 | 51.5 |

[a] in the absence of surfactant.[b] in the presence of [surfactant] = 20 x $10^{-3}$ mol dm$^{-3}$

It is observed that the optimum pH value is 5.0 and then the reaction rate becomes almost constant. Every elementary reaction of α-amino acids/dipeptides and ninhydrin depends upon the [$H^+$] because the reaction proceeds through the formation of an intermediate which has Schiff base linkage (>C=N-). The product of this reaction also has this type of linkage. Since the Schiff base formation is acid catalysed and pH 5.0 is the optimum pH, all subsequence kinetic runs were made at pH =5.0 (vide infra).

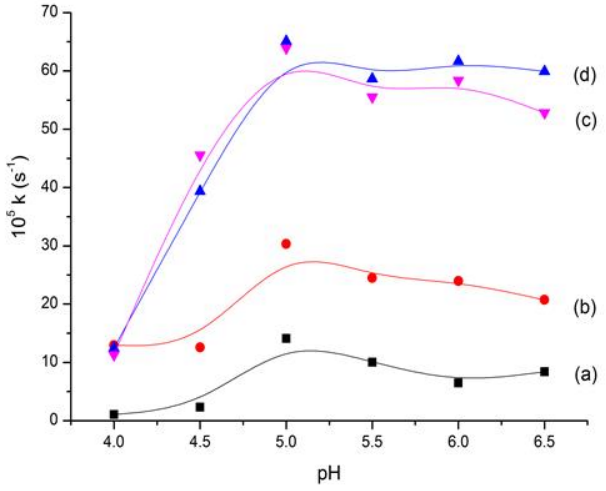



**Figure 2.** Plots of reaction rate constant vs. pH for the reaction of ninhydrin with Gly-Ala in the absence (a) and presence of [CPC] = 20 x $10^{-3}$ mol dm$^{-3}$ (b), [TTAB] = 20 x $10^{-3}$ mol dm$^{-3}$ (c), and [CTAB] = 20 x $10^{-3}$ mol dm$^{-3}$ (d). Reaction conditions: [Gly-Ala] = 2.0 x $10^{-4}$ mol dm$^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol dm$^{-3}$, temperature = 70 °C.

**Dependence of Reaction Rate on Gly-Ala Concentration**

To find the dependence on [Gly-Ala], the reaction was carried out under pseudo-first-order conditions of [ninhydrin] >> [Gly-Ala] in the range of (1.0 x $10^{-4}$ to 3 x $10^{-4}$ mol dm$^{-3}$ of [Gly-Ala] at constant [ninhydrin] of 6.0 x $10^{-3}$ mol dm$^{-3}$, temperature (70 °C) and pH (5.0). The $k_{obs}$ values are recorded in Table 2. As the values of rate constants ($k_{obs}$ and $k_\Psi$) were found to be independent of the initial concentration of Gly-Ala, the order of reaction with respect to [Gly-Ala] is unity in both the media.

**Dependence of Reaction Rate on Ninhydrin Concentration**

The effect of ninhydrin concentration was determined by carrying out a series of kinetic experiments at different concentrations of ninhydrin with fixed [Gly-Ala] (2.0 x $10^{-4}$ mol dm$^{-3}$), temperature (70 °C) and pH (5.0) constant (Table 2). The plots of rate constants *versus* [ninhydrin] (Figure 3) give non-linear profile and curved passing through the origin that indicates the order to be fractional with respect to [ninhydrin] in both the media.

**Dependence of Reaction Rate on Temperature**

A series of kinetic runs were carried out at different temperatures (60 to 80 °C), with fixed reactant concentrations both in the absence and presence of micelles (Table 2). The calculated rate constant values were found to satisfy the Arrhenius and Eyring equations. The activation energy ($E_a$) resulted from the slope of the lines of Figure 4. The activation enthalpy ($\Delta H^{\neq}$) and activation entropy ($\Delta S^{\neq}$) were calculated using linear least squares regression technique.

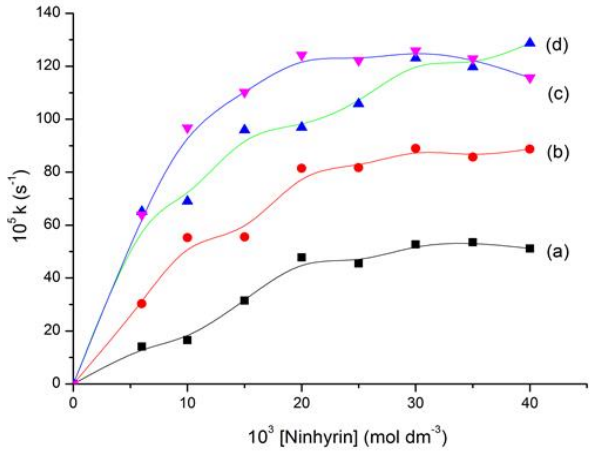



**Figure 3.** Effect of [ninhydrin] on the reaction rate of ninhydrin with Gly-Ala in the absence (a) and presence of CPC (b), TTAB (c), and CTAB (d). Reaction Conditions: [Gly-Ala] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [surfactant] = 20 x $10^{-3}$ mol $dm^{-3}$, pH = 5.0, temperature = 70 °C.

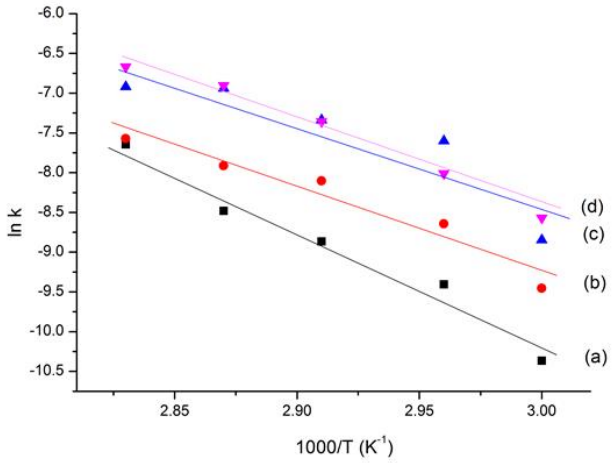
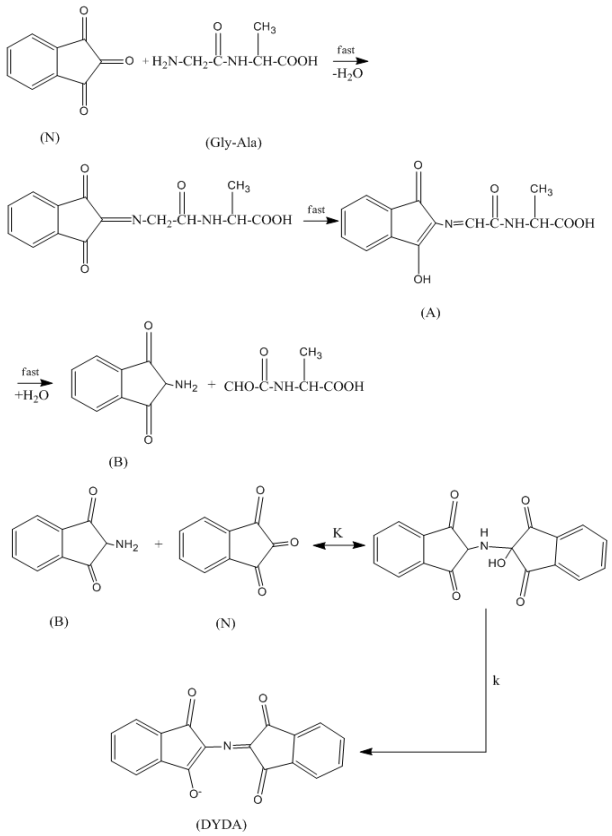



**Figure 4.** Arrhenius plot for the reaction of ninhydrin with Gly-Ala in the absence (a) and presence of CPC (b), CTAB (c), and TTAB (d). Reaction Conditions: [Gly-Ala] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, [surfactant] = 20 x $10^{-3}$ mol $dm^{-3}$, pH = 5.0.

### Reaction in Aqueous Medium

On the basis of several studies made on the kinetics of amino acid-ninhydrin (triketohydrindene hydrate) reactions it has been established that the scheme involves oxidation of the amino acid to carbon dioxide and an aldehyde possessing a carbon atom less than the amino acid with the simultaneous reduction of the tri-ketone to hydrindantin and the condensation of the hydrindantin with the ammonia liberated by the oxidation of the amino acid, forming the blue coloured ammonium salt of diketohydrindylidene-diketohydrindamine (DYDA). Further, the amount of the coloured reaction product depends mainly upon temperature, pH and reactant concentrations. In the present case, condensqation between carbonyl group of ninhydrin and amino group of Gly-Ala takes place.[17,18] The reaction starts through the attack of lone-pair of electrons of amino nitrogen (of Gly-Ala) to the carbonyl carbon (of ninhydrin) to give Schiff base A (Scheme 1). This Schiff base is unstable and hydrolyses to give 2-amino indanedione, B, which reacts slowly with another ninhydrin molecule to yield the product P (DYDA).




**Scheme 1.** Gly-Ala-ninhydrin reaction mechanism

On the basis of the observed rate law d[*P*]/d*t*= $k_{obs}$ [Gly-Ala]$_T$ and the proposed mechanism (Scheme 1), the following rate equation is derived

$$k_{obs} = \frac{kK[\text{Nin}]_T}{1+K[\text{Nin}]_T} \quad (1)$$

where [Nin]$_T$ = total concentration of ninhydrin.

Alternatively,

$$\frac{1}{k_{obs}} = \frac{1}{k} + \frac{1}{kK[\text{Nin}]_T} \quad (2)$$

which envisages a straight line between $1/k_{obs}$ and $1/[\text{Nin}]_T$ with a positive slope (=$1/kK$) and an intercept (=$1/k$). Indeed it was found so (Figure 5), and thus confirmed the validity of the proposed mechanism. From the intercept and slope, the respective values of *k* and *K* were evaluated, which are: 1.23 x $10^{-3}$ $s^{-1}$ and 6.87 $mol^{-1}dm^3$ respectively, in aqueous medium.

### Reaction in the Presence of Surfactant Micelles

To investigate the surfactant concentration effect on the reaction rate, [TTAB], [CTAB], or [CPC] were varied at constant [ninhydrin] (6.0 x $10^{-3}$ mol $dm^{-3}$), [Gly-Ala] (2.0 x $10^{-4}$ mol $dm^{-3}$) and pH 5.0 at 70 °C (Table 3). The rate constant ($k_\Psi$) increased *ca.*4-5x with increase in [surfactant] from (0 to 30 x $10^{-3}$) mol $dm^{-3}$; then the $k_\Psi$ decreased noticeably (Figure 6).

**Table 3.** Effect of [TTAB] on the pseudo-first-order rate constants ($k_\Psi$) for the reaction of ninhydrin with Gly-Ala at pH = 5.0, [Gly-Ala] =2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$ and temperature = 70 °C.

| $10^3$[Surfactant], mol $dm^{-3}$ | $10^5 k_\Psi$, $s^{-1}$ | | | $10^5 k_{\Psi cal}$[a], $s^{-1}$ | | | $(k_\Psi - k_{\Psi cal})/k_\Psi$ | | |
|---|---|---|---|---|---|---|---|---|---|
| | TTAB | CTAB | CPC | TTAB | CTAB | CPC | TTAB | CTAB | CPC |
| 0 | 14.1 | 14.1 | 14.1 | | | | | | |
| 1.0 | 15.7 | 14.0 | 15.2 | 14.3 | 13.0 | 14.0 | +0.09 | +0.07 | +0.07 |
| 3.0 | 18.7 | 15.3 | 17.6 | 17.6 | 17.9 | 16.4 | +0.06 | +0.07 | +0.06 |
| 5.0 | 26.5 | 34.5 | 18.4 | 23.4 | 31.5 | 17.4 | +0.11 | +0.09 | +0.05 |
| 7.0 | 33.3 | 39.1 | 21.4 | 26.9 | 39.4 | 22.3 | +0.19 | -0.007 | -0.04 |
| 10.0 | 40.5 | 49.9 | 26.8 | 43.9 | 46.8 | 27.1 | -0.08 | +0.06 | -0.01 |
| 12.0 | 43.0 | 52.8 | 27.7 | 50.2 | 50.1 | 29.4 | -0.17 | +0.05 | -0.06 |
| 15.0 | 47.6 | 53.2 | 28.6 | 56.1 | 55.1 | 31.2 | -0.16 | -0.04 | -0.09 |
| 20.0 | 63.9 | 65.1 | 30.3 | 61.1 | 57.7 | 34.9 | +0.04 | +0.11 | -0.15 |
| 30.0 | 59.4 | 63.6 | 32.6 | 62.8 | 62.0 | 38.2 | -0.05 | +0.03 | -0.17 |
| 40.0 | 48.6 | 53.2 | 31.3 | 63.2 | 64.3 | 39.9 | -0.30 | -0.21 | -0.28 |
| 50.0 | 45.3 | 50.3 | 31.2 | 64.3 | 65.3 | 40.2 | -0.42 | -0.29 | -0.29 |
| 60.0 | 36.6 | 49.8 | 30.7 | 64.4 | 68.7 | 42.1 | -0.76 | -0.38 | -0.37 |
| 70.0 | 30.4 | 35.9 | 28.3 | 65.7 | 70.1 | 44.1 | -1.16 | -0.95 | -0.56 |
| 90.0 | 20.9 | -- | -- | 66.3 | | | -2.17 | | |
| 100.0 | -- | 32.9 | 28.3 | | 72.7 | 46.9 | | -1.21 | -0.66 |

[a]calculated values using Eq. (3).

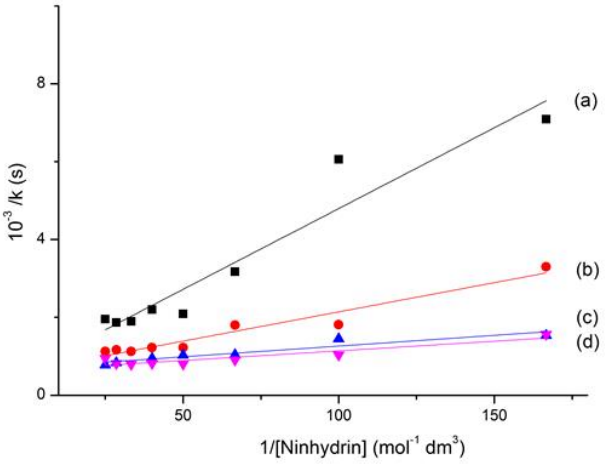



**Figure 5.** Plots of 1/*k versus* 1/[ninhydrin] for the reaction of Gly-Ala with ninhydrin in the absence (a) and presence of CPC (b), CTAB (c), and TTAB (d). Reaction Conditions: same as in Figure 3.

The existence of maximum in the $k_\Psi$ – [surfactant] profile shape can be explained by considering that in this case, the reaction takes place in the aqueous as well as in the micellar pseudophases. The increase in rate constant at low surfactant concentrations results in an acceleration of the reaction because the organic substrate incorporates into the micelles and the contribution of the reaction occurring in the small volume of the micellar pseudophase (the so-called Stern layer) increases. However, as [surfactant] increases, a diminution in the Gly-Ala ion concentration in the micellar pseudophase is provoked by the greater number of micellar aggregates present in the reaction media. This effect is the one responsible for the decrease in $k_\Psi$ observed at high surfactant concentrations.[19] Another reason for decreasing $k_\Psi$ could be a result of counterion inhibition.

It was mentioned[20,21] that the head group size of the surfactant is one of the factors that decides the packing of the surfactant monomers into a micelle; if so, we would expect difference of packing of the CPC, CTAB and TTAB surfactant monomers. Of course, with aromatic pyridinium ring in CPC, there would be delocalization of charge as well as less charge shielding in comparison to CTAB and TTAB. Additionally, there may be an orientational effect. This effect must be taken into account with the effect of side chain (R) of the dipeptide to describe the reaction rate. The nature of $k_\Psi$-[surfactant] profile has been found experimentally similar with rate being CTAB ≈ TTAB > CPC (Figure 6).

The same first- and fractional-order kinetics for [Gly-Ala] and [ninhydrin], respectively, was followed in both aqueous and micellar media. Another thing, the absorption band of the product remains unchanged in the presence of TTAB, CTAB, or CPC micelles (Figure 1). Thus, we summarize that the reaction mechanism remains the same in the presence of conventional cationic micelles as that in aqueous medium.

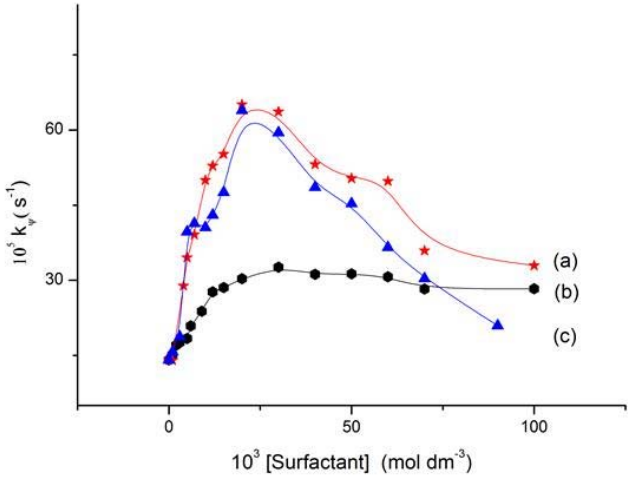
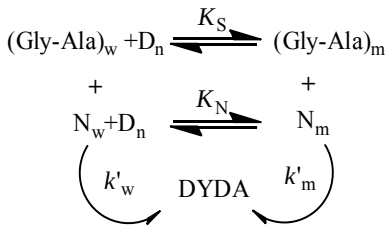



**Figure 6.** Effect of surfactant structure and concentration on the reaction rate for the interaction of ninhydrin with Gly-Ala. Reaction conditions: [Gly-Ala] = 2.0 x $10^{-4}$ mol $dm^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol $dm^{-3}$, pH = 5.0, temperature = 70 °C, in the presence of CTAB (a), CPC (b) and TTAB (c).

The rate increase for many reactions upon addition of surfactants has been explained on the basis of the following Scheme, proposed by Menger and Portnoy[22] and developed by Bunton[23] and Romsted.[24]




**Scheme 2.** The pseudo-phase model for the reaction of Gly-Ala with ninhydrin in micellar medium

Although several kinetic equations based on this general Scheme 2 have been developed, the most successful appears to be that of Romsted who suggested Equation (3), which takes into account the solubilization of both the reactants into micelles as well as mass action model

$$k_{\psi} = \frac{k_w\,[\mathrm{Nin}]_T + (K_S k_m - k_w)\,M_N^S\,[D_n]}{1 + K_S\,[D_n]} \qquad (3)$$

where $k_w$ and $k_m$ are the second order rate constants, referring to aqueous and micellar pseudo phases, respectively, $K_S$ is the binding constant of the Gly-Ala to the cationic micelles, and $[D_n]$ = [surfactant] – CMC. $M_N^S$ is the mole ratio of bound ninhydrin to the micellar head group, given by

$$M_N^S = \frac{[N_m]}{[D_n]} \qquad (4)$$

Values of $M_N^S$ were estimated by considering the equilibrium

$$N_w + D_n \overset{K_N}{\rightleftharpoons} N_m$$

$$K_N = \frac{[N_m]}{[N_w]([D_n]-[N_m])} \qquad (5)$$

and the mass balance

$$[\mathrm{Nin}]_T = [N_w] + [N_m] \qquad (6)$$

Calculation of $k_m$ and $K_S$ requires CMC under kinetic conditions which has been determined conductimetrically. For a given value of CMC, the $k_m$ and $K_S$ were calculated from Equation (3) using the non-linear least squares technique. Such calculations were carried out at different presumed values of $K_N$. The best value was considered to be the one for which the value of $\Sigma d_i^2$ (where $d_i = k_{\psi obsi} - k_{\psi\,cali}$) turned out to be a minimum. The fitting of the evaluated data ($K_S$, $k_m$ and $K_N$) to Equation 3 is evident from the calculated values of rate constants, $k_{\psi cal}$, recorded in Table 3.

The observed catalysis is due to the increased concentration of both ninhydrin and Gly-Ala in the Stern layer of micelles. Besides this, micelles also exert a medium effect influencing reactivity (the effect arises from a combination of cage, preorientation, microviscosity, polarity and charge effects).[25]

In order to calculate the dissociation constant of the micellized surfactant back to its components ($K_D$) and the index of cooperativity ($n$), the Piszkiewicz model,[26] analogous to the Hill model applied for the enzyme-catalysed reactions, was used. In the micellar systems, the value of n reflects the average number of surfactant molecules associated with each substrate molecule. The Piszkiewicz model relates $n$ and $K_D$ and its contribution to the rate is given by

$$k_{\Psi} = \frac{k'_m[D_n]^n + k'_w K_D}{K_D + [D_n]^n} \qquad (7)$$

On rearrangement, Equation (7) gives

$$\log\left(\frac{k_{\psi} - k'_w}{k'_m - k_{\psi}}\right) = n\log[D] - \log K_D \qquad (8)$$

According to Equation (8), a plot of $\log((k_{\psi}-k'_w)/(k'_m-k_{\psi}))$ versus log[D] should be a straight line with a positive slope (n). Such a plot has been realized in the CPC, CTAB, and TTAB catalysis of the present study (Figure 7).

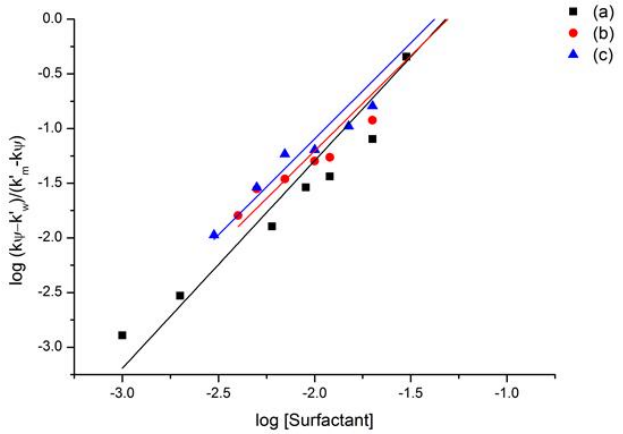



**Figure 7.** Piszkiewicz plot of log ($k_{\Psi}$ - $k'_w$/ $k'_m$ - $k_{\Psi}$) vs. log [Surfactant]. Surfactant = [CPC] (a), [CTAB] (b), and [TTAB] (c). Reaction Conditions: same as in Figure 6.

The $K_D$ and n are: 1.29 x $10^{-3}$, 1.9 (CPC), 3.49 x $10^{-3}$, 1.74 (CTAB), and 1.12 x $10^{-3}$, 1.76 (TTAB), respectively. A value of $n$ greater than unity indicates positive cooperativity, i.e., the binding of the first molecule of the substrate makes it easier for subsequent molecules to bind. The advantage of Equation (8) is that it does not require the knowledge of CMC of surfactant used.

Activation parameters such as activation energy ($E_a$), enthalpy of activation ($\Delta H^{\neq}$) and entropy of activation ($\Delta S^{\neq}$), are summarized in Table 4. Comparing the values with those obtained in aqueous medium we find that the presence of surfactants lowers the $\Delta H^{\neq}$ with a substantial negative $\Delta S^{\neq}$. This lowering occurs not only through the adsorption of both the reactants on the micellar surface but also through stabilization of the transition state. The fitting of the observed $k_\Psi$ at different temperatures to the equation was examined and it was found that the Eyring equation is applicable to the micellar media and the sensitivity of micelle structure to temperature is kinetically unimportant. A meaningful mechanistic explanation of the apparent values of $\Delta H^{\neq}$and $\Delta S^{\neq}$ is not possible because the $k_\Psi$ does not represent single elementary kinetic step; it is a complex function of true rate, binding and ionization constants.

**Table 4.** Thermodynamic parameters, and $k_m$, $K_S$ values for the reaction of Gly-Ala and ninhydrin at pH = 5.0 and temperature = 70 °C.

| Parameters and constants | Aqueous | TTAB | CTAB | CPC |
|---|---|---|---|---|
| $E_a$ (kJ mol$^{-1}$) | 127 | 98.1 | 87.9 | 90.0 |
| $\Delta H^{\neq}$ (kJ mol$^{-1}$) | 124 | 95.3 | 85.1 | 87.2 |
| $\Delta S^{\neq}$ (JK$^{-1}$mol$^{-1}$) | -306 | -297 | -299 | -305 |
| $\Delta G^{\neq}$ (kJ mol$^{-}$) | 216 | 184 | 174 | 178 |
| $10^3$ $k_m$ (s$^{-1}$) | | 0.74 | 8.62 | 5.54 |
| $10^3$ $k_w$ (mol$^{-1}$dm$^3$ s$^{-1}$) | | 23.5 | 23.5 | 23.5 |
| $K_S$ (mol$^{-1}$dm$^3$) | | 270 | 214 | 162 |
| $K_N$ (mol$^{-1}$dm$^3$) | | 45.5 | 59.1 | 61.3 |

**Salt Effect**

The salt effect on micellar catalysis should be considered in the light of competition between the reactant(s) and counterion for micellar binding sites as well as their effect on the aqueous solubility of substrates. Experimentally, for the title reaction, this effect was explored in the condition of [TTAB] (20 x $10^{-3}$ mol dm$^{-3}$), [ninhydrin] (6.0 x $10^{-3}$ mol dm$^{-3}$), [Gly-Ala] (2.0 x $10^{-4}$ mol dm$^{-3}$), pH (5.0) at 70 °C (Tables 5 and 6). Salts, as additives, in micellar systems acquire a special place due to their ability to modify the systems' properties.[27]

**Table 5.** Effect of inorganic salts on pseudo-first-order rate constants ($k_\Psi$) for the reaction of ninhydrin with Gly-Ala at pH = 5.0, [Gly-Ala] = 2.0 x $10^{-4}$ mol dm$^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol dm$^{-3}$, [TTAB] = 20.0 x $10^{-3}$ mol dm$^{-3}$and temperature = 70 °C.

| [Salt], mol dm$^{-3}$ | $10^5$ $k_\Psi$, s$^{-1}$ | | |
|---|---|---|---|
| | $NaNO_3$ | $Na_2SO_4$ | $Na_3PO_4$ |
| 0 | 63.9 | 63.9 | 63.9 |
| 0.05 | 67.9 | 90.4 | 48.2 |
| 0.1 | 98.1 | 82.4 | 23.6 |
| 0.2 | 100 | 62.2 | 13.1 |
| 0.3 | 104 | 48.2 | 11.4 |
| 0.4 | 106 | 45.8 | 8.10 |
| 0.5 | 99.9 | 43.9 | 4.30 |
| 0.6 | 99.4 | 42.9 | 1.42 |
| 0.7 | 105 | 42.3 | 0.08 |
| 0.8 | 98.3 | 41.4 | 0.06 |
| 0.9 | 95.9 | 41.0 | 0.04 |

**Table 6.** Effect of organic salts on pseudo-first-order rate constants ($k_\Psi$) for the reaction of ninhydrin with Gly-Ala at pH = 5.0, [Gly-Ala] =2.0 x $10^{-4}$ mol dm$^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol dm$^{-3}$, [TTAB]= 20.0 x $10^{-3}$ mol dm$^{-3}$and temperature = 70 °C.

| [Salt], mol dm$^{-3}$ | $10^5$ $k_\Psi$, s$^{-1}$ | | | |
|---|---|---|---|---|
| | NaSal | NaBenz | NaTos | $Na_2C_2O_4$ |
| 0 | 63.9 | 63.9 | 63.9 | 63.9 |
| 0.5 | 75.5 | 80.2 | 78.9 | 67.4 |
| 1.0 | 95.2 | 112 | 82.3 | 72.6 |
| 3.0 | 82.4 | 106 | 89.2 | 83.5 |
| 5.0 | 76.2 | 97.0 | 95.3 | 76.6 |
| 7.0 | 69.2 | 96.2 | 80.2 | 62.4 |
| 10.0 | 66.3 | 94.6 | 79.8 | 57.5 |
| 20.0 | 61.8 | 90.9 | 81.7 | 48.9 |
| 30.0 | 60.9 | 87.2 | 81.3 | 47.8 |
| 40.0 | 52.1 | 82.1 | 74.8 | 33.1 |
| 50.0 | 50.0 | 62.8 | 74.8 | 30.6 |
| 80.0 | 45.3 | 30.1 | 56.3 | 24.7 |

Figure 8 shows that the rate increases at low concentration of $NaNO_3$, and then becomes almost constant. However, in $Na_2SO_4$ a slight increase in the rate is observed, then a decrease which becomes almost constant. At low concentration range, the reactant solubility is affected and they are driven off toward the micellar surface. The increased concentration brings about increase in $k_\Psi$. When the salt concentration is high, the exclusion effect prevails with consequent decrease in $k_\Psi$. As regards $Na_3PO_4$, it shows a sharp decreasing effect.

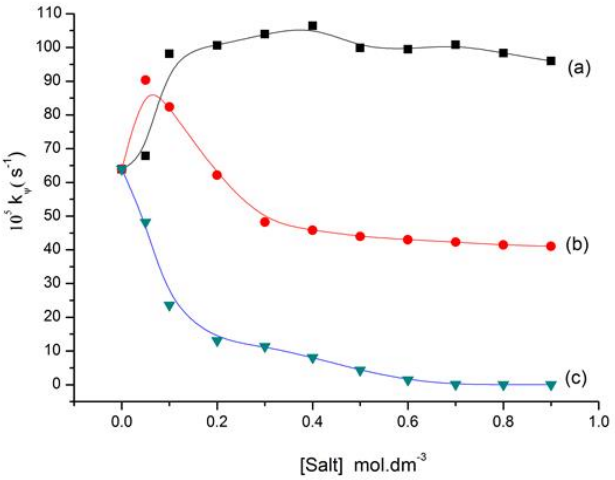



**Figure 8.** Effect of [inorganic salt] on the reaction rate for the interaction of ninhydrin with Gly-Ala in the presence of surfactant. Reaction Conditions: [Gly-Ala] = 2.0 x $10^{-4}$ mol dm$^{-3}$, [ninhydrin] = 6.0 x $10^{-3}$ mol dm$^{-3}$, [TTAB] = 20 x $10^{-3}$ mol dm$^{-3}$, pH = 5.0, temperature = 70 °C. $NaNO_3$ (a), $Na_2SO_4$ (b), $Na_3PO_4$ (c).

The main reason for this is the change in pH which equals ~ 12 which destroys the buffering effect. On the other hand, the biocompatible hydrophobic salts (the so-called *'hydrotropes'*) sodium salicylate (NaSal), sodium benzoate (NaBenz), sodium tosylate (NaTos), and sodium oxalate ($Na_2C_2O_4$) produce marked rate enhancement at low salt concentration, passing through a maximum as the [salt] was increased (Figure 9, Table 6).

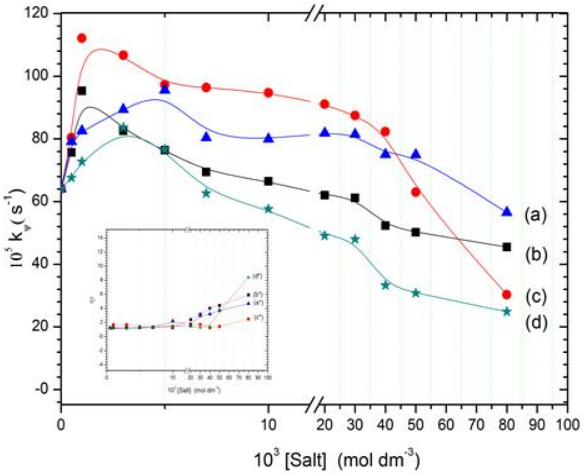



**Figure 9.** Effect of [organic salt] on the reaction rate (*a, b, c, d*) and on solution viscosity (*a*, b*, c*, d**) (inset) for the reaction of ninhydrin with Gly-Ala in the presence of surfactant. Reaction Conditions: same as in Figure 8. NaSal (*a*,*a**), NaBenz (*b*,*b**), NaTos (*c*,*c**), $Na_2C_2O_4$ (*d*,*d**).

The addition of these organic hydrophobic salts means that we are adding ionic species having hydrophobic character and, therefore, they can interact with micelles both electrostatically and hydrophobically.[28] Therefore, in addition to neutralization of micellar positive charge, they will restrict solubilization sites to hydrophobic substrates. Thus, they catalyse the reaction by virtue of increased concentration of reactants in the Stern layer. The decreased rate observed at relatively higher concentrations of added organic salts is a consequence of the adsorption of hydrophobic anions at the micellar surface and the exclusion of substrate from the micellar surface. The progressive withdrawal of the substrate from the reaction site (micellar surface) would slow down the rate, as was indeed observed. Another factor which could inhibit the rate is the possible micellar growth at higher [salt] as reflected by viscosity data (Figure 9).

In our case the change in morphology from spheroidal micelles to rod-shaped (as inferred by viscosity increase) [29] would have certain changes on the characteristics of the micelle. In rod-shaped micelles the counterions bind more tightly and, therefore, suppress the interactions at the micellar surface.

## Conclusions

Kinetic experiments between Gly-Ala and ninhydrin have been performed in aqueous and micellar media by studying the reaction spectrophotometrically at 570 nm. We found that the presence of conventional cationic micelles of TTAB, CTAB, and CPC accelerate the reaction and this is supported by comparing the values of activation parameters in both the media. Finally, we can conclude that interaction of Gly-Ala with ninhydrin in micellar media could successfully be treated using the pseudo-phase and Piszkiewicz models. Quantitative treatment of the kinetic data seems justified as $k_\psi$ and $k_{\psi cal}$ are in close agreement within the experimental error.

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