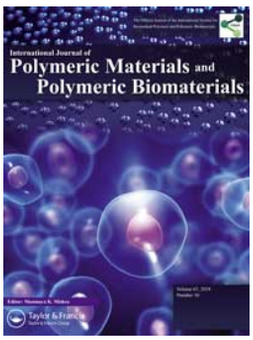

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Assessment of formulation parameters needed for successful vitamin C entrapped polycaprolactone nanoparticles

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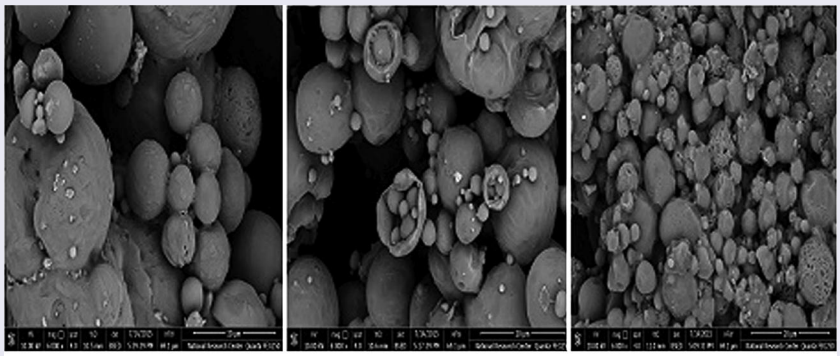
ABSTRACT

Vitamin C (Vit.C)-entrapped polycaprolactone (PCL) nanoparticles (Vit.C–PCNs) were prepared by encapsulation of Vit.C into PCL-based nanoparticles (PCNs) which were prepared using double emulsion method with two steps. First, the inner aqueous phase (W_1) was added to dichloromethane solution containing PCL with homogenization to form primary emulsion (W_1/O) which was emulsified with the outer aqueous phase (W_2) containing polyvinyl alcohol as stabilizer to attain the double emulsion ($W_1/O/W_2$). Versatile parameters were investigated to reach to the most successful formulation for Vit.C–PCNs, such as time, effect of speed of homogenization on drug encapsulation efficiency, etc.

GRAPHICAL ABSTRACT



Vitamin C (Vit.C) entrapped polycaprolactone (PCL) nanoparticles (Vit.C-PCNs) were prepared with different formulations by encapsulation of Vit.C into PCL based nanoparticles (PCNs) by using double emulsion technique. Versatile parameters were investigated to reach to the most successful formulation for Vit.C-PCNs such as time, effect of speed of homogenization during the 1st and 2nd steps of emulsification process on drug encapsulation efficiency (EE %), particles` size, zeta potential, size distribution and morphology. The SEM images showed that the all nanoparticles were in round, spherical shape with smooth surface and unimodel size distribution.

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Biopolymers; drug carriers; drug delivery; encapsulation; nanoparticles; polycaprolactone; vitamin C

1. Introduction

Vitamin C (Vit.C) which is also known as ascorbic acid is a water-soluble vitamin [1] and an indispensable required nutrient to retain the physiological processes for the human and animals [2,3]. It plays an important biological role as a reducing agent in various enzymatic reactions and as an important antioxidant that may reduce the risk of cancer using various mechanisms [4]. Also, Vit.C is playing an important role in collagen synthesis [5] and in brain particularly during development [6]. In general, the human should get external Vit.C [1] because of the lack of L-gulonolactone oxidase enzyme in the human body [7]. However, most of the animals and plants are capable of producing Vit.C from D-glucose and D-galactose. Vit.C is normally available in high concentrations in the immune cells, and is rapidly consumed in the body in case of any infection. However, deficiency of Vit.C is distinct below 23 µmol/ L of the plasma concentration [8] which affects up to 10% of adults in the developed countries [9] with certain subgroups such as smokers, people with low socioeconomic status, pregnant women, and their newborns who may display an even higher prevalence [10]. Severe deficiency of Vit.C is characterized by easy bleeding and bruising leading to gum recession, weakness, lethargy, and scurvy which results from the weakened connective tissues in the body. Thus, Vit.C is given to patients with scurvy to strengthen these tissues [11]. Accordingly, it was thought, in the current research, to

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enhance the efficiency of commercial Vit.C in the future uses using biodegradable polymeric nanoparticles (PNs). PNs are solid carriers with a main size of less than1 μ m, which are capable to dissolve, entrap, encapsulate or attach active ingredients to its matrices [12]. PNs have been extensively studied as drug carriers in the pharmaceutical field [13] due to their unique features, such as the increased stability, the capability to protect drugs, unique ability to create controlled release, and adjustable surface properties [14]. Currently, the most important areas of applications for PNs in the optical diagnostics are in biomarker analysis, cancer diagnosis, diagnostic imaging, and immune assays [15]. Recently, biodegradable PNs have been involved in the potential drug delivery devices [4]. PNs are prepared from preformed polymers by emulsification-solvent evaporation, salting-out, dialysis, nanoprecipitation, and supercritical fluid technology. In addition, PNs are synthesized by polymerization of monomers using micro-, mini-emulsion, surfactant-free emulsion, and interfacial polymerization [12,14]. In general, a few synthetic polymers are of great interest in the design of carrier based drug delivery especially linear poly esters such as poly ε-caprolactone (PCL) [16], polyglcolide [17], poly D,L-lactide-*co*-glycolides [18], and polylactides [19]. In the present study, PCL was selected as valuable polymer as it is used in several medical applications as drug delivery and tissue engineering because of its high degree of crystallinity as semicrystalline linear aliphatic polyester, hydrophobicity [20,21], biocompatibility and biodegradability nature, miscibility with a variety of other polymers and high permeability for many drugs [21,22]. Degradation of PCL is very slow compared with many other polymers [22]. Also, PCL has flexible mechanical properties that may be defined through Young's modulus, elasticity, tensile strength, and elongation at break value which are suitable for paramedical applications, wound dressing, contraception, and dentistry [23]. Herein, the nanoparticles were prepared by double emulsification method which is usually used to encapsulate both hydrophilic and lipophilic substances [24] in complex hetero-dispersed system called "emulsion of emulsion," that can be classified into two major types: water–oil–water emulsion ($W_1/O/W_2$) and oil–water–oil emulsion ($O_1/W/O_2$) [25,26]. Thus, the dispersed phase itself is an emulsion and the inner dispersed globule/droplet is isolated from the outer liquid phase by a layer of another phase. Double emulsions are usually prepared in a two-step emulsification process using one surfactant to stabilize the external interface of the oil globules for $W_1/O/W_2$ emulsions [27]. Versatile factors may affect the stability of the double emulsion such as: method of preparation, type of oil phase, polymer concentration, type and concentration of emulsifiers, etc [28,29].. The composition of the double emulsions and their properties make them promising systems with potential applications in the pharmaceuticals, food industry and in cosmetics [30,31]. However, the inherent thermodynamic instability still limits the applications of multiple emulsions. Recently, great efforts have been made to improve the properties of the double emulsions, especially with respect to increasing the emulsion stability, decreasing, and homogenizing the emulsion droplets' size [27]. So, the current paper was dedicated to studying various parameters that may influence both of the formation of (Vit.C–PCNs) and its' efficiency as Vit.C carrier such as the effect of stirring speed for first emulsion and stirring time for first and second emulsion of emulsification process on the colloidal properties of the final particles prepared by double emulsion [29]. Also, the investigated parameters included drug encapsulation efficiency, particles size, zeta potential, and morphology of the prepared nanoparticles [25]. In addition, the drug crystallinity in the nanoparticles and the interaction between drug and polymer were evaluated by Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD) and thermal analyses (TA).

2. Experimental

2.1. Materials

Polycaprolactone (PCL; $C_6H_{10}O_2$; $M_{wt} = 14{,}000$ g/ mol), polyvinyl alcohol (PVA; $M_{wt} = 30{,}000$ g/ mol; 87–89% hydrolyzed), dichloromethane (DCM), and Vit.C ($C_6H_8O_6$ or L-(+) Ascorbic acid, 99%) were delivered from Sigma–Aldrich, Germany. Sodium acetate (CH_3COONa) was purchased from RFCL Company, India. All other chemicals—otherwise mentioned—were provided from Sigma–Aldrich, Germany and were used as received. Acetate buffer was prepared by diluting 6 mL of glacial acetic acid in 500 mL distilled water to form stock A, then, 8.2 g of sod acetate were dissolved in 500 mL distilled water to form stock B. Afterward, 48 mL of stock A were added to 452 mL of stock B, and then the volume was completed to 1,000 mL using distilled water to obtain the acetate buffer.

2.2. Methods

2.2.1. Preparation of polycaprolactone nanoparticles and vitamin C entrapped polycaprolactone nanoparticles

Two-step emulsification process was applied where the primary emulsion (W_1/O) was dispersed as small droplets in the outer aqueous phase (W_2) using the digital high-speed homogenizer (T-10; model: Unidrive X1000D-CAT, Germany). PVA was used as stabilizer [25] where 0.5% PVA solution was prepared by dissolving 2.5 g of PVA in 500 mL distilled water with stirring and heating at 60°C for 40 min to obtain a clear solution. To obtain the primary emulsion (W_1/O); 2 g of PCL were dissolved in 10 mL of DCM with stirring till forming a clear solution. For preparation of Vit. C–PNCs; 50 mg of Vit.C were dissolved in 1 mL of acetate buffer, then, 5 mL of PCL solution were added. This mixture was properly homogenized at definite speed and time using homogenizer (T-10). In the second step, the primary emulsion (W_1/O) was added to the outer aqueous phase (W_2) containing 0.5% PVA solution as stabilizer with homogenization to achieve the double emulsion ($W_1/O/W_2$) [25]. The obtained double emulsion ($W_1/O/W_2$) was subjected to evaporation under vacuum using rotary evaporator (Heidolph type VV2000, type WB2000; Germany) until the whole organic solvent was removed [29].

The nanoparticles were collected and separated from the free drug in the nanoparticulates' suspension by cooling centrifuge [32] (Union 32 R, Hanil, Korea) at 6,000 rpm and

at temperature of 4°C for 45 min. Then, the produced nanoparticles' pellets were washed again using acetate buffer. The supernatant solution was used for determination of the drug encapsulation efficiency. The drug-free nanoparticles (PCNs) were prepared by the same way using only the acetate buffer solution.

2.3. Characterization of PCNs and Vit.C–PCNs

2.3.1. Encapsulation efficiency

To determine the EE% of Vit.C in the prepared nanoparticles of (Vit.C–PCNs), the collected supernatant solution, after centrifugation step, was collected and properly diluted using hydrochloric acid (0.1 N). The amount of unentrapped Vit.C was estimated spectrophotometrically at 243 nm using the regression equation of a calibration curve plotted using appropriate concentrations of the drug dissolved in 0.1 N hydrochloric acid [33]. The measurements were performed with reference to the supernatant of the drug-free nanoparticles as blank. The amount of encapsulated Vit.C was determined by subtracting the amount of free drug in the collected supernatant after centrifugation from the added amount in the beginning of the preparation using the following equation [14,34].

$$\text{EE\%} = \frac{\text{Total drug} - \text{Free drug}}{\text{Total drug}} \times 100 \qquad (1)$$

2.3.2. Determination of particle size and zeta potential

The particles' size and Zeta-potential for Vit.C–PCNs samples were determined by photon correlation spectroscopy using the Malvern Zeta-sizer (Nano ZS, Malvern Instruments Ltd., Malvern, UK). Each sample was diluted with distilled water and transferred to a 4 mL quartz cuvette where it was measured at room temperature (i.e., 25°C).

2.3.3. X-ray diffraction

X-ray diffraction is an important technique in case of drug delivery. Therefore, it was used to confirm the physical state of the present PCL, PVA, and the drug in the polymer matrix. Accordingly, the crystalline forms of PCL and Vit.C and the prepared nanoparticles were examined. XRD measurements were acquired with X-ray diffractometer (Bruker D8 Advance, USA) which was operated at KV = 40 and mA = 40 using Cu Kα as a radiation source where $\lambda = 1.54$ Å.

2.3.4. Thermal analyses

Thermal analyses (TA) are useful for evaluating drug–polymer interactions to assess the influence of excipients and micro- or nanoencapsulation process on the physicochemical properties of the pharmaceutical materials. Differential scanning calorimetry (DSC) and thermal gravimetric analysis (TGA) are the most frequently used thermo-analytical techniques [35].

2.3.4.1. Differential scanning calorimetry. Thermal stability of freeze-dried Vit.C–PCNs was investigated through DSC using thermal analyzer (i.e., DSC–SDT (Simultaneous DSC-TGA) Q600 V20.9 Build 20, USA) in the range from room temperature to 500°C at heating rate of 10°C/min under inert nitrogen atmosphere (N_2) using reference alumina. The sample weight was between 2.5 and 12 mg.

2.3.4.2. Thermal gravimetric analysis. Thermal behavior for freeze-dried Vit.C–PCNs samples was recorded using thermal analyzer (i.e., TGA–SDT Q600 V20.9 Build 20, USA) in the range from room temperature to 700°C at a heating rate of 10°C/min under inert nitrogen atmosphere (N_2) using reference Alumina.

2.3.5. Fourier transform infrared spectroscopy

The chemical composition of the prepared freeze-dried PCNs or Vit.C-entrapped PCL nanoparticles (Vit.C–PCNs) were assessed using FTIR (Jasco, FT/IR 6100, Japan). The spectra were recorded on KBr disc, at 4 cm^{-1} with resolution from 4,000 to 400 cm^{-1}.

2.3.6. Transmission electron microscopy

The shape of the prepared nanoparticles and their dimensions on the nanoscale were confirmed by transmission electron microscopy (TEM). Measurements were attained using JEOL 2001T, TEM microscopy, high resolution transmission (Japan). TEM was adjusted at magnification power = 200 kV and maximum resolution = 1.4 Å. Samples were prepared by drop casting the suspended polymer with 1% phosphotengestic acid dye onto carbon-coated copper grids, and then they were left to dry.

2.3.7. Scanning electron microscopy

The morphology of the prepared freeze-dried nanoparticles was investigated by scanning electron microscopy (SEM) (Quanta FEG 250-FEI Company, Holland). Freeze-dried nanoparticles were deposited on a flat aluminum holder and were dried at room temperature. The concerned sample in each case was finally coated under vacuum by cathodic sputtering with Gold for 3 min.

3. Results and discussion

3.1. Preparation of PCNs and Vit.C–PCNs

PCNs and Vit.C–PCNs were prepared by double emulsion technique where different parameters were studied such as the speed of homogenization and the effect of stirring time. Double emulsion W1/O/W2 was prepared by two-step emulsification process using PVA as stabilizer where PVA is the most commonly used stabilizer because of its low toxicity, good solubility in water, and its availability in large range of molecular weights [36]. Also, stable emulsion was achieved using 0.5% PVA whereas no emulsion was observed and phase separation occurred at lower PVA concentrations [25]. At the second step, excess of outer aqueous phase (W_2) was used to facilitate the diffusion of organic solvent from the PCL particles to outer aqueous phase. The effect of different parameters on the characteristics of the prepared particles via double emulsification was studied by fixing the stirring time of first emulsion at 5, 15, and 5 min where five different speeds of homogenization were tested as 4,000, 8,000, 12,000, 16,000, and 20,000 rpm at 21,000 rpm as fixed speed of homogenization for second emulsion at stirring time of 15, 5, and 5 min,

respectively, for the second emulsion with stable concentration of PVA (e.g., 0.5%) as shown in Table 1.

3.2. Encapsulation efficiency

In general, EE% indicates the amount of retained drug in the particles at the end of the process. Also, efficiency of the nanoparticles (NPs) indicates the amount of active substance in a known mass of NPs. Thus, encapsulation efficiency (EE%) depends on various parameters related to drug, polymer, emulsification method (i.e., homogenization time and speed), surfactant, and additives in the internal water phase and external water phase [37]. Table 1 shows the encapsulation efficiencies of the prepared (Vit.C–PCNs) using various speeds of homogenization of the first emulsion. The results showed that all formulations exhibited EE% values ranged from 44.82 up to 98.86%. It was found that by increasing the stirring speed of first emulsion for 5 min (i.e., 4,000–20,000 rpm), keeping the other parameters constant including the parameters at 5 min for the second emulsion in case of F1C-F5C, there was a general decrease in the observed EE% values from 92.68% at 4,000 rpm for F1C to 49.57% at 16,000 rpm for F4C. However, a very slight increase was observed in EE % at 20,000 rpm. Similar results were recorded by increasing stirring time from 5 to 15 min for both the first and second emulsion preparation steps. Also, Table 1 shows that increasing the stirring time for the first emulsion from 5 to 15 min led to lower EE%. This finding agreed with the previous reports on the possible reduction in the encapsulation efficiency by increasing the ultrasonification time from 1 to 5 min [38]. However, it was found that increasing the stirring time for the second emulsion from 5 to 15 min led to a clear increase in EE%. Such observation was obvious at higher speeds of the first emulsification step. Accordingly, EE% increased with 12% at 8,000 rpm of the first emulsification step in case of F2C compared to F2A, while a remarkable increase with 42% occurred at a speed of 20,000 rpm for the first emulsification step in case of F5C compared to F5A. That indicated the importance of maintaining a proper control on the stirring time of the second emulsification step in achieving high drug EE%.

3.3. Determination of particle size

The particle size is an important parameter which influences the biopharmaceutical feature of the carrier. Hence, the particle size is evaluated as a function of the formulation parameters. Consequently, the particles' size for the prepared nanoparticulates' formulations were measured in each case as shown in Table 1. The results showed decreasing particles' size with increasing the stirring speeds of first emulsion from 4,000 to 20,000 rpm, keeping stirring time at 5 min for both first and second emulsion as in case of F3C which exhibited a particle size of 912.9 nm at 12,000 rpm whereas in case of F5C at 20,000 rpm, the particle size was reduced to 122.2 nm. The recorded results were compatible to literature as the stirring speed is a governing factor in determining the particles' size prepared via double emulsification technique [39,40] where by increasing the stirring speed of first emulsion, the particles' size was reduced. It was found that increasing the time of stirring for first emulsion to 15 min led to an obvious decrease in the particles' size until 12,000 rpm as previously reported in the literature where this reduction in the particle size was referred to supply of input power for longer period of time [29].

However, at higher homogenization speeds of the first emulsion (i.e., 16,000 and 20,000 rpm), the particles' size increased which was attributed to possible particles' aggregations. Also, increasing stirring time for the second emulsion from 5 to 15 min led to a remarkable decrease in the particles' size with stirring speeds up to 12,000 rpm for the first emulsion. A slight change in the particles' size was observed at higher stirring speeds of the first emulsion. In general, it was mentioned in the literature that the stirring time of the second emulsion can be a significant factor to prevent aggregation and

Table 1. Encapsulation efficiency (EE%), Particle size and zeta-potential for the prepared (Vit.C–PCNs) formulations.

	Primary emulsion's parameters (first step)		Double emulsion's parameters (second step)			
Samples*	Speed of homogenization (rpm)**	Stirring time (min)***	Stirring time (min)***	EE% (±mean SD)	Mean particle size (nm)	Zeta potential (mV)
F1A	4,000	5	15	91.76 (±0.25)	1,021	−4.14
F1B		15	5	68.33 (±8.19)	281.7	−1.53
F1C		5	5	92.68 (±9.50)	2,423	−16.4
F2A	8,000	5	15	82.17 (±5.99)	103.60	−1.80
F2B		15	5	55.74 (±5.76)	1,455	−22.4
F2C		5	5	70.71 (±8.11)	1,662	−13.8
F3A	12,000	5	15	81.27 (±2.70)	90.25	−2.81
F3B		15	5	51.71 (±5.02)	246.8	−8.11
F3C		5	5	55.57 (±6.26)	912.9	−5.95
F4A	16,000	5	15	78.6 (±7.45)	321.80	−11.7
F4B		15	5	44.82 (±9.76)	1,666	−21.7
F4C		5	5	49.57 (±9.18)	306.2	−2.97
F5A	20,000	5	15	93.2 (±2.54)	150.5	−5.66
F5B		15	5	60.96 (±9.93)	1,208	−21.2
F5C		5	5	51.8 (±1.05)	122.2	−0.920

Vit.C, Vitamin C; PCNs, polycaprolactone nanoparticles; PVA, polyvinyl alcohol.
*All samples were prepared using 0.5% PVA as a stabilizer and 21,000 rpm as homogenization speed for the second emulsion. **Samples containing numbers 1–5 refer to a homogenization speed for first emulsion as 4,000, 8,000, 12,000, 16,000, and 20,000 rpm, respectively. ***Samples containing letter A refer to stirring time of 5 min for first emulsion and 15 min for second emulsion. Samples containing letter B refer to stirring time 15 min for first emulsion and 5 min for second emulsion. Samples containing letter C refer to stirring time of 5 min for both first and second emulsion.

large particles' size [25]. Therefore, in the current work, it might be possible that short stirring time of 5 min was not sufficient for dispersion of polymer particles and proper formation of the nanoparticles at lower stirring speed in case of the first emulsion [25]. However, increasing the stirring time to 15 min for either the first or second emulsion was suitable for the process of double emulsion leading to production of smaller particles.

3.4. Determination of zeta potential

The recorded values of the zeta potential indicate the potential stability of the colloidal system. Herein, the negative surface charges of zeta potential were observed between −1.53 and −22.4 mV for all the prepared formulations as seen in Table 1. These results were in agreement with other reports using the same polymer (PCL) and stabilizer (PVA) as was mentioned by Ozturk et al. who had zeta potential values up to − 16.73 mV in the nanoparticles prepared using PCL and PVA at different concentrations [41]. Other similar results were reported elsewhere in the literature [42,43]. This behavior was attributed to the preferential surface localization of the drug on the nanoparticles' surface. Therefore, the observed negative values for zeta potential in our work were attributed to the negative charge of Vit.C as a weak acid with pK_a of 4.2 [44].

3.5. X-ray diffraction

The XRD patterns of pure PCL, PVA, and Vit.C were represented as in Figure 1a. PCL showed two sharp peaks at around 21.5° and 23.8° which were referred to scattering from crystalline region [45]. Vit.C showed multiple sharp peaks representing the crystalline nature of the drug around 10.5° and 25.3°. PVA showed multiple sharp signals revealing its' amorphous nature. Figure 1b–d represents the XRD patterns for different samples of Vit.C–PCNs as indicated in Table 1 by preparing the first and second emulsion at different stirring time intervals and different speeds of homogenization. Two peaks were observed at 2θ of 21.2° and 23.6° which were attributed to the crystalline nature of PCL, while the distinguished peaks for the drug disappeared revealing that the drug particles were dispersed at the molecular level in the polymer matrix as similarly mentioned in the literature [46].

3.6. Thermal analyses

Both TGA and DSC techniques usually provide qualitative and quantitative information about the physical and chemical properties of the drug in the nanoparticles.

3.6.1. Differential scanning calorimetry

Differential scanning calorimetry is essentially used to measure enthalpy changes according to the changes in the physicochemical properties of the material as a function of time or temperature. The final melting temperatures and enthalpy changes were elucidated from DSC thermograms as indicated in Figure 2. Pure PCL polymer showed T_m at 60.66°C while T_m of PVA and Vit.C recorded 290.52 and 189.37°C, respectively. The recorded T_m of PCL was nearly similar to the reported

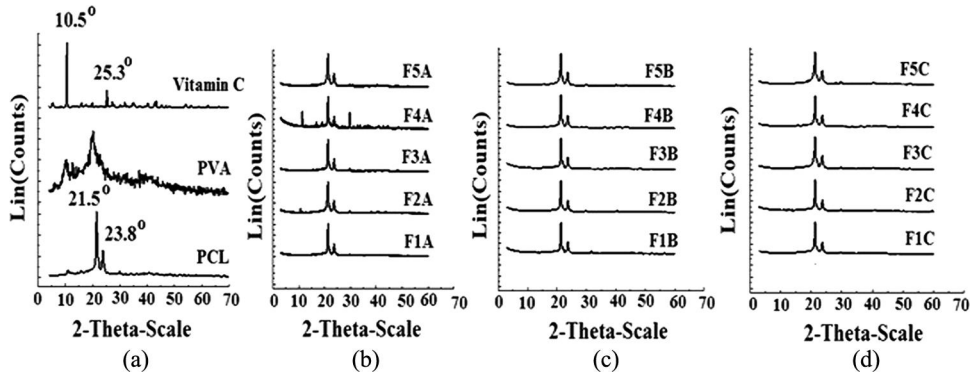

Figure 1. XRD patterns of (a) PVA, PCL, Vit.C & (b), (c), (d): PCL nanoparticles emulsion for F1A, F2A, F3A, F4A, F5A & F1B, F2B, F3B, F4B, F5B and F1C, F2C, F3C, F4C, F5C, respectively according to Table 1.

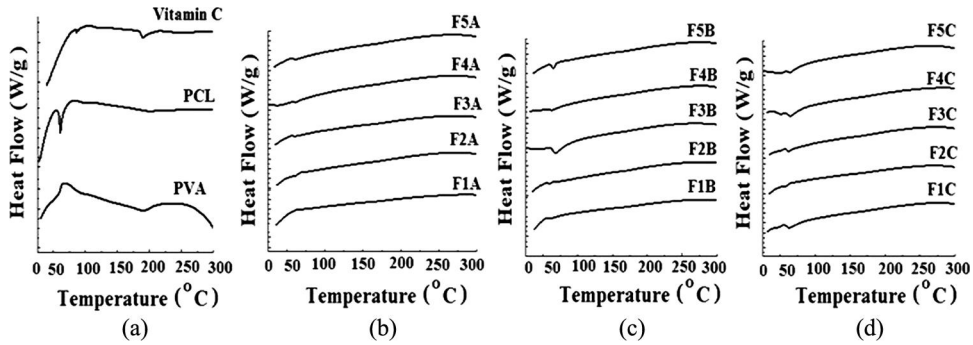

Figure 2. DSC thermograms of (a) PVA, PCL, Vit.C and (b), (c), (d): PCL nanoparticles emulsion (PCNs) for F1A–F5A, F1B–F5B, and F1C–F5C, respectively according to Table 1. *Note*: DSC, differential scanning calorimetry; PVA, polyvinyl alcohol; PCL, polycaprolactone; Vit.C, Vitamin C; PCNs, polycaprolactone nanoparticles.

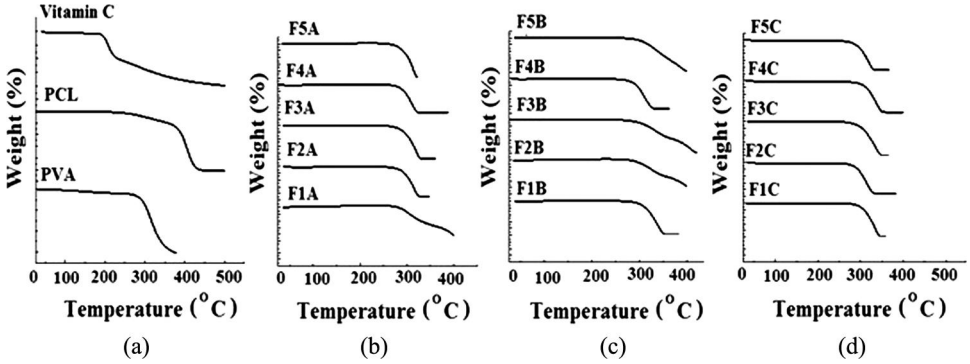

Figure 3. TGA thermograms of (a) PVA, PCL, Vit.C and (b), (c), (d): PCL nanoparticles emulsion (PCNs) for F1A–F5A, F1B–F5B, and F1C–F5C, respectively according to Table 1. *Note*: TGA, thermal gravimetric analysis; PVA, polyvinyl alcohol; PCL, polycaprolactone; Vit.C, Vitamin C; PCNs, polycaprolactone nanoparticles.

theoretical value [47] which confirmed that the crystallization and the melting behavior of PCL were not changed by the double emulsion method [48]. DSC thermograms for different samples of Vit.C–PCNs as shown in Table 1, Figure 2 revealed disappearance of the distinguished band for Vit.C at $T_m = 189.37°C$. Therefore, Vit.C–PCNs demonstrated molecularly dispersed drug in the nanoparticles as previously mentioned in the literature [49].

3.6.2. Thermal gravimetric analysis

Thermal gravimetric analysis normally provides information on mass loss as a function of temperature. TGA thermograms of pure PCL, PVA, and Vit.C were represented as shown in Figure 3a. PCL was thermally stable until 170°C where the thermal decomposition occurred in two steps at 320 and 500°C with weight loss of 20.21 and 81.61%, respectively. Thermal degradation of PCL took place through the rupture of the polyester chain via ester pyrolysis reaction with the release of CO_2, H_2O, and formation of carboxylic acid groups according to Fukushima et al. [48]. As shown in Figure 3b–d, it was noticed that all formulations of Vit C-PCNs decomposed at 320°C instead of 170°C for pure Vit C which was attributed to the presence of PCL that increased the thermal stability of Vit.C–PCNs compared to pure Vit.C and PCNs [50]. Both of PCNs and Vit.C–PCNs showed low thermal stability compared to pure PCL which was referred to the fact that the nanoparticles have a greater superficial area with higher reactivity than the polymer which led to faster thermal decomposition. A similar observation was noted in the literature [48] which confirmed the results obtained from XRD.

3.7. Fourier transform infrared spectroscopy

The Fourier transform infrared spectroscopy spectra as in Figure 4 showed the characteristic peaks of Vit.C, PCL, blank PCNs (i.e., free from Vit.C; B1), and Vit.C–PCNs (F5A), respectively. The characteristic peaks for the PCL polymer appeared at 1636.3 and 1729.8 cm^{-1} which were referred to the stretching vibration of C=O bond [51,52]. The FTIR spectra showed strong distinguished peak for Vit.C at 3411.4 cm^{-1} which confirmed the O-H stretch but the shoulder peak that appeared at 3316.9 cm^{-1} indicated the overtone of C=O stretch. The presence of strong absorption peak at 1025.9 cm^{-1} confirmed the C–O–C stretching and C–O–H bending bands that were attributed to the medium and weak peaks appeared instead of shoulder peak of blank PCNs (B1) at 2940.9 and 2867.64 cm^{-1} which were ascribed to the C-H asymmetric and symmetric stretching vibrations [53]. Shoulder peaks at 1729.8 and 1632.4 cm^{-1} were attributed to the absorption of the residual acetate and carbonyl groups. Weak peak at 1467.5 cm^{-1} indicated the presence of CH_2 bending. The FTIR spectra showed the characteristic medium and weak peaks for Vit.C–PCNs (F5A) at 2947.6 and 2867.6 cm^{-1} which were related to the C-H asymmetric and symmetric stretching vibrations, respectively. Weak peak at 1470.4 cm^{-1} was

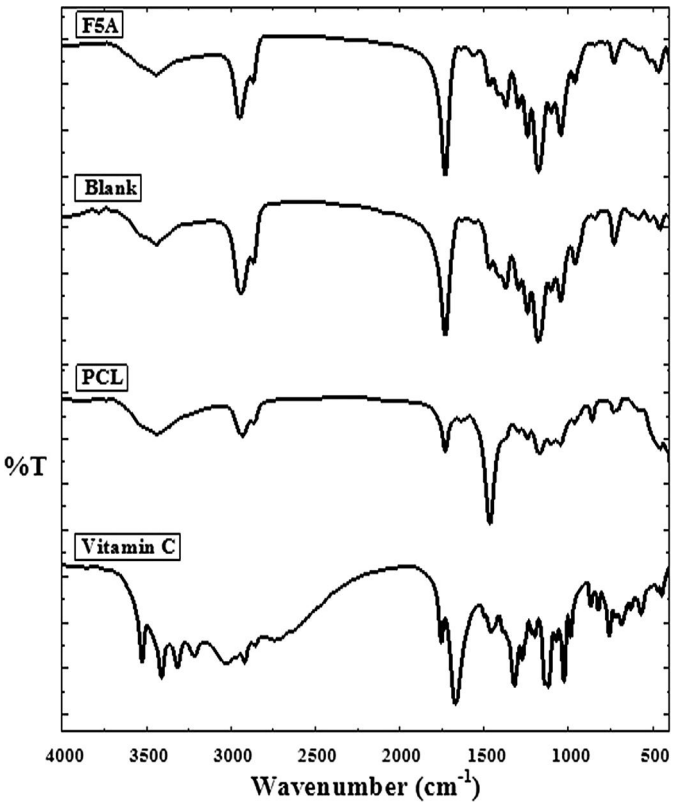

Figure 4. FTIR spectra of Vit.C, PCL, blank PCNs (B1), Vit.C–PCNs (F5A), respectively. *Note*: FTIR, Fourier transform infrared spectroscopy; Vit.C, Vitamin C; PCL, polycaprolactone; PCNs, polycaprolactone nanoparticles.

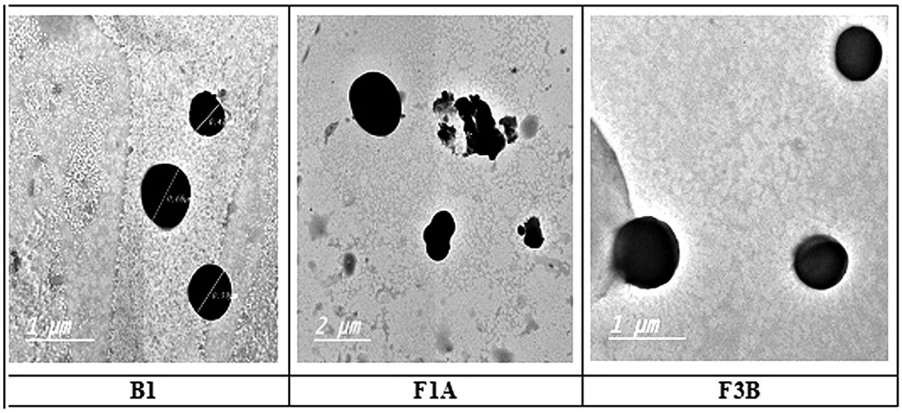

Figure 5. TEM photos of PCNs emulsion for different samples as B1 (blank sample). F1A and F3B. *Note*: TEM, transmission electron microscopy; PCNs, polycaprolactone nanoparticles.

referred to CH_2 bending band. That peak showed the interaction between PCL and Vit.C and it confirmed the encapsulation of the drug by the PCNs.

3.8. Transmission electron microscopy

Figure 5 shows TEM micrographs of selected Vit.C–PCNs formulations namely; B1 as blank drug free formulation, F1A and F3B. As shown in the micrographs, the nanoparticles were present, and well identified in a nearly perfect sphere to oval shape. The particles were dark stained having a smooth surface with noticeable aggregations or agglomerations.

3.9. Scanning electron microscopy

Scanning electron microscopy was used to examine the morphology of some samples of Vit.C–PCNs as F3B, F3C, F4A, F5A, and B2 as blank (i.e., Vit.C free sample of PCNs) as shown in Figure 6. The SEM images showed that the all nanoparticles were in round and spherical shape with smooth surface and unimodel size distribution. The measured particles were slightly smaller and some particles had pores on their surfaces because of the induced expected contraction by drying during evaporation of solvent. Also, some particles were connected with each other because of the surface tension of water on the particles during drying as was demonstrated by

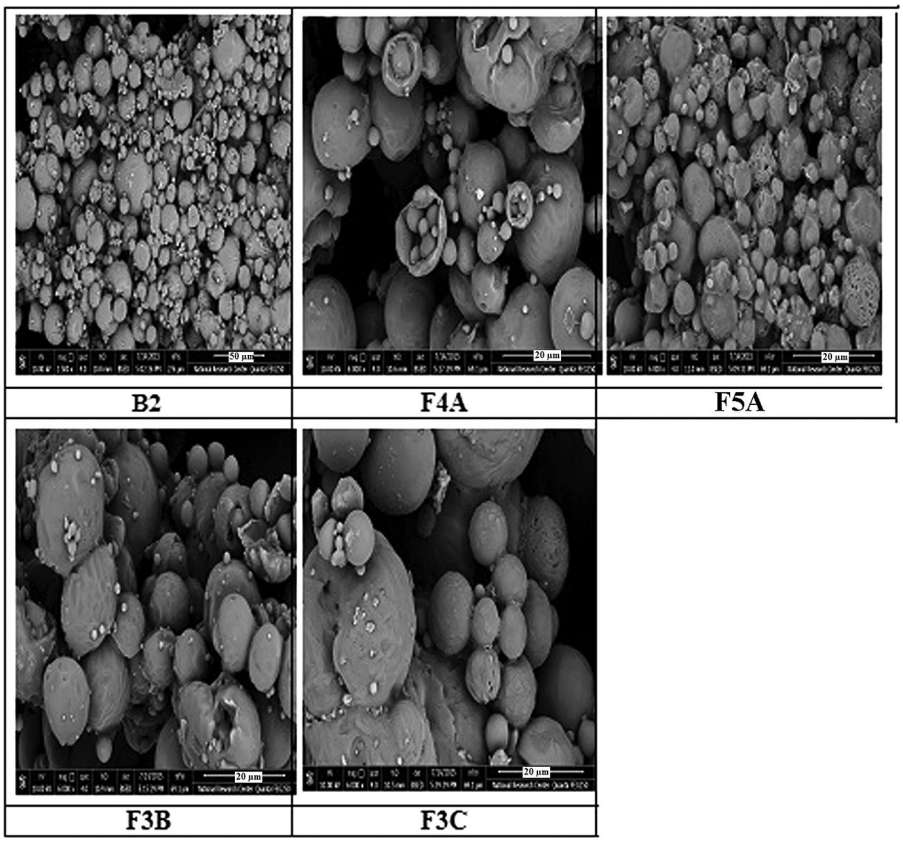

Figure 6. SEM images of samples B2 (blank sample), F4A, F5A, F3B, F3C at magnification 600×. *Note*: SEM, scanning electron microscopy.

Wu and Clark [54]. That contact among particles was attributed to the presence of PVA traces, which was not easily removed because of the sticky nature of PVA [25]. SEM illustrated that the nanoparticles prepared at 20,000 rpm, in case of first emulsion, had narrow size distribution compared to particles prepared at 16,000 rpm as in Figure 6. Also, the influence of stirring time could be noticed for both first and second emulsion. From the SEM images, it was clear that the stirring time of first emulsion had negligible effect on the morphology of particles. At the second emulsion, the particles were prepared at stirring time of 5 and 15 min, where the particles prepared at 5 min had regular morphology, but the particles prepared at 15 min had been well encapsulated the inner water phase and some particles had cup shaped morphology as shown in Figure 6. The homogeneity of particles' size increased when the second emulsion was agitated for long period of time.

4. Conclusion

Nanoparticulates' drug delivery systems represent a promising strategy for the biopharmaceutical industry as it has advantages over conventional drug delivery systems because of the expected improvement in solubility, bioavailability and permeability of the drugs encapsulated inside these particles. In the current paper, Vit.C–PCNs were prepared by double emulsion where several factors influencing the size of particles were investigated as the stirring time for first and second emulsion and stirring speed for first emulsion. A general decrease in drug EE% was observed with increasing the stirring speed from 4,000 up to 20,000 rpm, whereas the increase in stirring time exhibited an increase in the EE% in case of the second emulsification step. The increase in stirring time led to a decrease in the particle size of the prepared nanoparticles up to 12,000 rpm with more observed decrease in the particle size with increasing the stirring time, especially in the second emulsification step. DSC and XRD studies revealed molecularly dispersed drug in the nanoparticles in case of drug loaded nanoparticles. TGA studies in case of Vit.C–PCNs demonstrated no decomposition peak for the drug indicating enhanced stability effect for the prepared carriers. Generally, the obtained results from the present work would help in understanding the influence of various formulation parameters in the double emulsion method, leading to the preparation of an efficient formulation for nanoparticulate of appropriate characteristics for successful delivery of Vit.C and similar hydrophilic drugs.

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