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# Eco-friendly Route for Encapsulation of 5-Fluorouracil into Polycaprolactone Nanoparticles

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THE MAIN objective of this study was to allocate and evaluate the most optimum conditions to prepare the 5-Fluorouracil (5-FU) entrapped polycaprolactone (PCL) nanoparticles (5-FU–PCNs) by eco-friendly route. The inner aqueous phase ($W_1$) was added to 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 (PVA) as stabilizer to attain the double emulsion ($W_1$/O/$W_2$). The different parameters were investigated to reach the most successful formulation for 5-FU–PCNs, such as polymer concentration, effect of stabilizer concentration ratio(0.5%, 1% and 3%) on drug encapsulation efficiency, etc. The encapsulation efficiency of PCNs was in the range of 14-65.6%. The prepared nanoparticles showed the spherical shape having an average size of 183-944nm.The prepared systems were elucidated by fourier transform infrared spectroscopy (FTIR), UV-spectroscopy, photon correlation spectroscopy (PCS), X-ray diffraction (XRD), differential scanning calorimetry (DSC), thermal gravimetric analysis (TGA),scanning electron (SEM) and atomic-force microscopy (AFM).

The optimized ratios of the different parameters of double emulsion process were founded. SEM and AFM confirmed the complicated morphology and the formation of the spherical micro- and nanocapsules.

**Keywords:** Polycaprolactone, Nanoparticles,5-Fluorouracil, Encapsulation Efficiency, Structure, Morphology, Thermal Characterization.

## Introduction

5-Fluorouracil (5-FU or 5-fluoro-2,4-pyrimidinedione) is anticancer drug. 5-FU is an antimetabolite of the pyrimidine analogue type, with a broad spectrum of activity against solid tumors of (the gastrointestinal tract, pancreas, ovary, liver, brain, breast, etc.), alone or in combination with chemotherapy regimes [1]. Due to its structure, 5-FU interferes with nucleoside metabolism and can be incorporated into RNA and DNA, leading to cytotoxicity and cell death [2]. Biodegradable polymeric nanoparticles (BPNs) are solid carriers with a main size of less than 1 μm, which are capable to entrap, encapsulate or attach active ingredients to its surface [3]. BPNs have been extensively studied as drug carriers in the pharmaceutical field [4] due to their unique features [5], such as the increased stability, the capability to protect drugs, unique ability to create controlled release, and adjustable surface properties [6]. In addition, several lines of investigation have demonstrated that such carriers allow an adjustable drug release profile by altering molecular weight and degradation rate of polymers [7]. Currently, the most important areas of applications of BPNs in the optical diagnostics are biomarker analysis, cancer diagnosis,

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diagnostic imaging, and immune assays [8]. In the present study, polycaprolactone (PCL) was selected as a valuable polymer for preparation of BPNs as it is used in several medical applications as drug delivery and tissue engineering because of its high degree of crystallinity and hydrophobicity [9]. Herein, the PCL nanoparticles (PCNs) were prepared by double emulsification method which is usually used to encapsulate both hydrophilic and lipophilic substances in complex hetero-dispersed system called "emulsion of emulsion" [10, 11]. The current work was dedicated to studying the parameters that may influence both of the formation of 5-FU–PCNs and its' efficiency, such as:(i) the effect of concentration of PCL for first emulsion and (ii) concentration of stabilizer ratio for second emulsion of emulsification process on the colloidal properties of the final particles. Also, drug encapsulation efficiency, particles size, structure and morphology of the prepared particles were investigated. In addition, the drug crystallinity in the 5-FU–PCNs and the interaction between drug and polymer were evaluated by fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), thermal analyses (TA), scanning electron (SEM) and atomic-force microscopy (AFM).

The optimized ratios of the different parameters of double emulsion process were founded. SEM and AFM confirmed the complicated morphology and the formation of the spherical micro-and nanocapsules.

*Experimental*

*Materials*

Polycaprolactone (PCL; $C_6H_{10}O_2$; $M_w$ = 14,000 g/mol), polyvinyl alcohol (PVA; $M_w$ = 30,000 g/mol; 87-89% hydrolyzed; m.p.200$^{O}$C), dichloromethane (DCM), and 5-Fluorouracil (5-FU; $C_4H_3FN_2O_2$; 5-Fluoropyrimidine-2,4-dione, ≥99%) were delivered from Sigma–Aldrich, Germany. Acetic acid ($CH_3COOH$) was obtained from SHAM laboratory chemical; acetic acid is of pure grades. All other chemicals –otherwise mentioned- were provided from Sigma–Aldrich, Germany and were used as received.

*Methods*

*P*reparation of 5-Fluorouracil entrapped polycaprolactonenanoparticles (5-FU-PCNs)

In the present study, the biodegradable nanoparticulate systems were attained by loading the active anticancer drug (5-FU) into PCL (oil phase), which was encapsulated by PVA (water phase, $W_1$) acting as the polymer emulsifier (stabilizer) [3, 10]. Stock solutions of 1%, 2% and 3 wt.% PCL (stocks A, B and C, correspondingly) were prepared by slowly adding of 1.05 g, 2.10 g and 3.15 g PCL, respectively, into 100 mL DCM until fully dissolved with stirring till forming a clear solution. 5-FU (10 mg) was then added to generate drug-loaded nanoparticles.In order to obtain the primary emulsion ($W_1$/O); 0.5% Acetic acid (1ml) was added to the former in order to aid the dissolution of 5-FU. All samples (drug-loaded and control nanoparticles) were left to stir on a magnetic stirrer for 45 min. Four PVA stock solutions (0.5%, 1%, 2% and 3 wt.% stocks A, B, C and D) were prepared by slowly adding 2.5 mg, 5 mg, 10 mg and 15 mg PVA, respectively, into 500 mL distilled water with stirring and heating at 60 $^{o}$C for 40 min to obtain a clear 0.5% PVA solution. A set of twelve nanoparticulate formulations was attained by slowly adding the PCL oil phase (stock A or B or C) into the PVA. In the second step, the primary emulsion ($W_1$/O) was added in the outer aqueous phase ($W_2$) containing stocks A, B, C and D) PVA solutions as stabilizer with homogenization to achieve the double emulsion ($W_1$/O/$W_2$)[3,12]. This mixture was properly homogenized at definite speed and time by using homogenizer (T-10) then left to stir on a magnetic stirrer for 24 h to allow DCM evaporation thus producing a cloudy suspension ofnanoparticles. After the removal of DCM, the nanoparticles were collected and separated from the free drug in the nanoparticulates' suspension followed by freeze-drying. Drug-free nanoparticles (PCNs) were prepared by the same way using only the buffer solution and were used as blank.

*Characterization of 5-FU-PCNsnanosystem*

*Encapsulationefficiency (EE,%)*

To determine the EE,% of 5-FU in the prepared compositions of 5-FU–PCNs, the collected supernatant solution, after centrifugation step, was collected and properly diluted using acetic acid (0.5%). The amount of unentrapped 5-FU was estimated spectrophotometrically at λ = 265.2 nm using the regression equation of a calibration curve plotted using appropriate concentrations of the drug dissolved in 0.5% acetic acid [13]. The measurements were performed with reference to the supernatant of the drug- free nanoparticles as blank. The amount of encapsulated 5-FU was determined by subtracting the amount of free drug in the collected supernatant aftercentrifugation from the added amount in the beginning of the preparation using the following equation [14].

*Determination of particle size*

The particles sizesof 5-FU–PCNs samples were determined by photon correlation spectroscopy (PCS) 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).

*X-ray diffraction (XRD)*

X-ray diffraction is an important technique in case of drug delivery systems, which can form the crystalline phase. Therefore, it was used to confirm the physical state of the prepared5-FU–PCNs samples, and the initial PCL, PVA, and the 5-FU. Accordingly, the crystalline forms of PCNs and 5-FUand selected 5-FU-PCNs formulations of the prepared nanoparticles were examined in case of several ratio PCL:PVA asF2:0.5, F3:0.5, F1:1, F2:1& F3:1, respectively.The X-ray diffraction studies were performed on a SEIFERT XRD 3003 TT diffractometer (GE, Germany) equipped with a primary monochromator (Cu$K_\alpha$ radiation, $\lambda$ = 1.5406Å, 2$\Theta$ =3°–40°).

*Thermal analysis*

Thermal analysisis useful for evaluating thermal properties and drug–polymer interactions to assess the influence of recipients and micro- or nano- encapsulation process on the physicochemical characteristics of the pharmaceutical materials. Differential scanning calorimetry(DSC) and thermal gravimetric analysis (TGA) are the most frequently used thermo-analytical techniques [15].

*Differential scanning calorimetry (DSC)*

Thermal stability of freeze-dried 5-FU–PCNs was investigated through DSC using thermal analyzer DSC–SDT (Simultaneous DSC-TGA) Q600 V20.9 Build 20, USA, in the range from room temperature to 500°C at a heating rate of 10°C/min under inert nitrogen atmosphere ($N_2$) using reference alumina. The samples weight was between 2.5 and 12 mg.

*Thermal gravimetric analysis (TGA)*

Thermal behavior for freeze-dried 5-FU–PCNs samples was recorded using thermal analyzer 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.

*Fourier transform infrared spectroscopy (FTIR)*

The chemical composition of the prepared freeze-dried PCNs or 5-FU entrapped PCNs nanoparticles (5-FU–PCNs formulations) 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 $cm^{-1}$.

*Atomic-force microscopy (AFM)*

The fine surface morphology of the synthesized nanosystems was investigated in the contact mode at the room temperature by the AFM method on the device NT-206 ("ODO Microtestmashiny", Belarus) using NSC11/AlBS types silicon probes with the radius of tip curvature less than 10 nm and stiffness of cantilever 3 N/m produced by "Mikromash" (Estonia).

*Scanning electron microscopy (SEM)*

The morphology of the prepared freeze-dried nanoparticles was investigated by scanning electron microscopy (SEM) using Quanta FEG 250 (FEI Company, Holland) device. 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 cathodesputteringwith gold for 3 min.

## Results and Discussion

*Preparation of PCNs and 5-FU–PCNs*

PCNs and 5-FU–PCNs were prepared by double emulsion technique. Different parameters were studied such asthe speed of homogenization, the effect of stirringtime, different concentrations of PCL (1%, 2%, and 3%,) respectively and different concentrations of stabilizer PVA (0.5%, 1%, 2%, and 3%) for 2nd emulsion were investigated. Double emulsion $W_1/O/W_2$ 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 [16]. Also, stable emulsion was achieved using homogenization speeds 20000 rpm at 5 min for 1st emulsion & 21000 rpm at 15 min for 2nd emulsion was selected for preparation of the PCNs which provide lowest particle size. [3] At the 2nd emulsion step, excess of outer aqueous phase ($W_2$) was used in order to facilitate the diffusion of organic solvent from the PCL particles to outer aqueous phase. Figure (1) represents the two steps process for nanoparticles preparation by double emulsion.The effect of different parameters on the characteristics of the prepared particles via double emulsification was studied by fixing concentrations of PCL (1%, 2%, and 3%,) at the stirring time 5 min at speed of

homogenization 20,000 rpm for first emulsion where Four different concentration of PVA were tested as(0.5%, 1%, 2%, and 3%)at the stirring time 15min at 21,000 rpm as fixed speed of homogenization for second emulsionas shown in Table 1.

*Encapsulation efficiency (EE,%)*

In general, encapsulation efficiency (EE,%) indicates the amount of retained drug in the particles at the end of the process. Thus, EE,%depends on various parameters related to drug, polymer concentration [3,17], stabilizer concentration, emulsification method (i.e., homogenization time and speed), and additives in the internal water phase and external water phase [18]. Table 1 shows the encapsulation efficiency (EE,%), particle sizesfor the prepared 5-FU–PCNs formulationsusing various PCL concentration of the first emulsion.The results showed that all formulations exhibited EE,% values ranged from 14 up to 65.6%. Table 1 shows that increasing concentration of PCL of 1st emulsion (1% to 2%), at constant concentration of stabilizer (PVA, 1%) and with keeping the other parameters constant for the 1st and 2nd emulsion (F1:1; F2:1; F3:1), there was a general increase in observed of the EE,% values with increasing concentration of PCL (from 20.5% for F1:1 to 29% for F2:1, and to 40.7% for F3:1).Similar results were recorded by increasing PCL concentration of 1st emulsion (1% to 2%), at constant concentration of stabilizer (PVA, 2%) as (F1:2-F3:2), there was a general increase in the values of EE,% observed with increasing concentration of PCL (from 23.1%; F1:2, to 37.9; F2:2, to F3:2; 65.6%) respectively. It was found that decrease in EE,% values was observed with increasing stabilizer (PVA) concentration of 2nd emulsion at constant concentration of PCL of 1st emulsion (1%), keeping the other parameters constant for the 1st and 2nd emulsion (F1:0.5; F1:1) (i.e. from 34%; F1:2 to 20.5%; F1:1). A clear increase in the encapsulation efficiency (EE,%) values were observed with increasing concentration of PVA

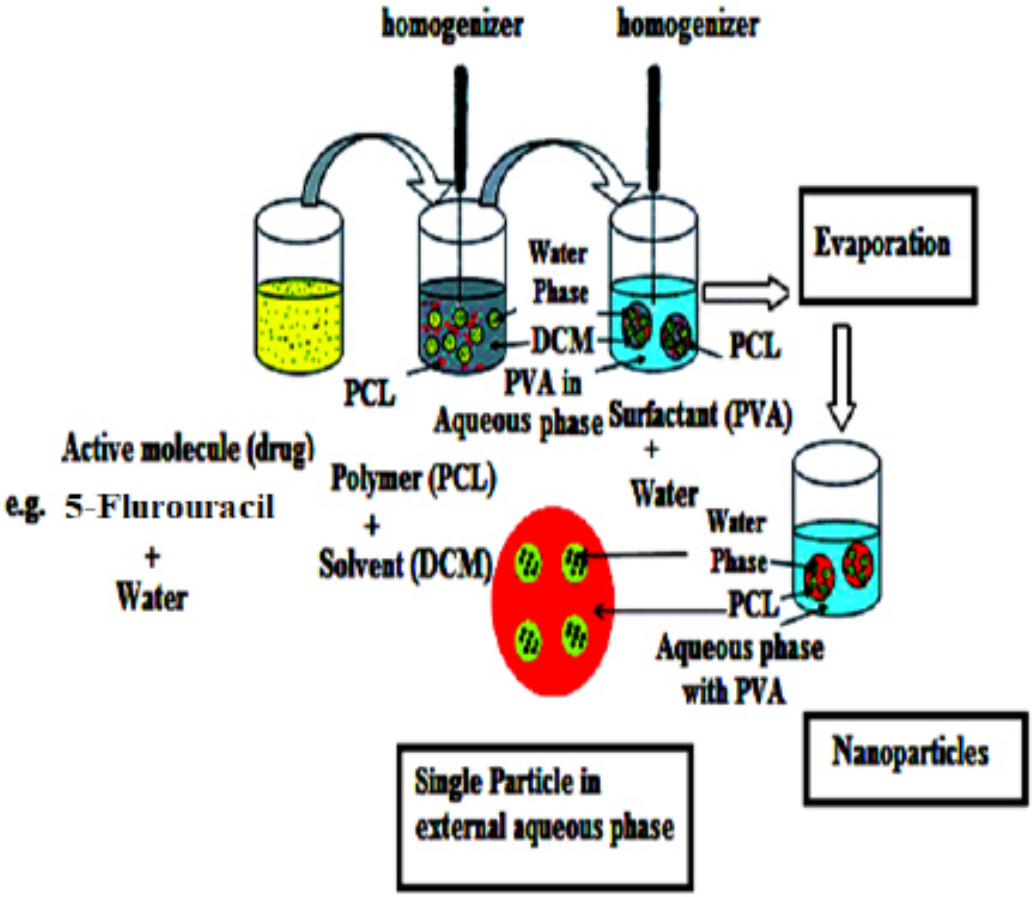


**Figure 1.** Illustration of a double emulsion method

(from 20.5%; F1:1 to 23.1%; F1:2). Similarly, increasing the stabilizer PVA concentration of 2nd emulsion at constant concentration of PCL of 1st emulsion (2%), keeping the other parameters constant for the 1st and 2nd emulsion (F2:0.5-F2:1) has led to increase in EE,% values observed with increasing concentration of PVA (from 29%; F2:1 to 45.4%; F2:0.5). A clear increase in the encapsulation efficiency (EE,%) values were observed with increasing concentration of PVA (from 29%; F2:1 to 34%; F2:3).In conclusion, the encapsulation efficiency increased with increasing concentration of PVA.

*Determination of particle size*

The particles size is an important parameter, which influencesthe biopharmaceutical feature of the carrier. Hence, theparticles size is evaluated as a function of the formulation parameters. Consequently, the particles size for the prepared nanoparticulates formulations were measured in each caseas shown in Table 1. An increase in the particles size of the formulations with increasing the concentration of PCL of 1st emulsion, keeping both the concentration of PVA for 2nd emulsion (0.5%), and the other parameters constant for the 1st and 2nd emulsion (F1:0.5; F2:0.5). Particle size values of the prepared (5-FU-PCNs) observed until concentration of PCL (from 239 nm; F1:0.5 to 509.5 nm; for F2:0.5). A clear decrease in the values of particle size of the prepared (5-FU-PCNs) was observed with increasing concentration of PCL (from 509.5; for F2:0.5 to

**TABLE 1. Encapsulation efficiency (EE, %), particle sizes for the prepared 5-FU–PCNs formulations.**

| Samples* name | Primary emulsion's parameters (first step) | Double emulsion's parameters (second step) | EE,% (±mean SD) | Mean particle size (nm) |
|---|---|---|---|---|
| | **PCL Conc.** (%) * | **PVA Conc.** (%) ** | | |
| **F 1:0.5** | **1** | 0.5 | 34 (±9.41) | 239.4 |
| **F 1:1** | | 1 | 20.5 (±11.32) | 675.5 |
| **F 1:2** | | 2 | 23.1(±9.51) | 367.2 |
| **F1:3** | | 3 | 14 (±5.62) | 500.5 |
| **$F_B$** | | | | 1178 |
| **F 2:0.5** | **2** | 0.5 | 45.4 (±0.93) | 509.2 |
| **F 2:1** | | 1 | 29 (±0.64) | 465.6 |
| **F 2:2** | | 2 | 37.9 (±7.81) | 1344 |
| **F2:3** | | 3 | 34 (±4.31) | 693.4 |
| **F 3:0.5** | **3** | 0.5 | 18.8 (±7.65) | 485.6 |
| **F 3:1** | | 1 | 40.7 (±6.81) | 183 |
| **F 3:2** | | 2 | 65.6 (±5.83) | 484.4 |
| **F3:3** | | 3 | -- | 944.2 |

5-FU, 5-Fluorouracil; PCNs,polycaprolactone nanoparticles; PVA, polyvinyl alcohol.

*All samples were prepared using 10 mg/ml of 5-FU at 20,000 rpm as homogenization speed for the first (for 5 min) and second emulsion (for 15 min), respectively.

**Samples containing first numbers 1–3 refer to a concentration of PCL for first emulsion as 1%, 2%, and 3 wt.%, respectively.

***Samples containing second numbers 0.5–3 refer to a concentration of stabilizer PVA for second emulsion as 0.5%, 1%, 2%, and 3%, respectively.

$F_B$ is the prepared polymeric nanoparticles free from 5-FUat concentration of PCL for first emulsion – 1 wt.%, and concentration of PVA for second emulsion – 1 wt.%.

485.6 nm; for F3:0.5). Similarly, an increase in the particle size of the formulations was observed with increasing the concentration of PCL of 1st emulsion, and concentration of PVA for 2nd emulsion (2%), and the other parameters constant for the 1st and 2nd emulsion (F1:2-F2:2). The value of particle size observed until concentration of PVA (from 267.2 nm; F1:2 to 1344 nm; F2:2). A clear decrease in the particle size values was observed with increasing PCL concentration (from 1344; F2:2 to 484.4 nm; F2:3).The particles size of the prepared 5-FU-PCNs increased. According to Ortiz et al. [19], by increasing conc. of PCL of 1st emulsion. This was in good agreement with our findings where, for example, it was found that by increasing concentration of PCL for 1st emulsion, a remarkable decrease in the particle size value was observed. However, at higher concentration of PCL of the 1st emulsion (i.e.2 % and 3%), the particle size increased which could be attributed to possible particle aggregations.The results showed decreasing particles size with increasing the PVA concentrations of second emulsion from 0.5% to 3%, keeping the other parameters constant including the parameters in case of F2:0.5 – F2:3, a particles size of 509.2 nm at 0.5% PVA whereas in case of F2:0.5 the particle size was reduced to 465.4 nm in case of F2:1. A decrease in particles size agrees with the results obtained by Ozturk et al. [13]. However, a very slight increase was observed in particle size at concentration of PVA (3%), the particles size increased which was attributed to possible particles' aggregations. The recorded results were compatible to literature as the stabilizer concentration is a governing factor in determining the particles size prepared via double emulsification technique [12, 13] where by increasing the stabilizer concentration in the 2nd emulsion, the particles size was reduced. Since PVA is a high molecular weightpolymer, the presence of PVA in the outer most water phase ($W_2$) may increase the viscosity of the dispersion phase, resulting in anincreased difficulty to reduce the emulsion particles to smaller size [12]. Also, it was found that the particles size of the prepared PCNs without 5-FU as drug ($F_B$; 1178 nm) was high as compared with the prepared 5-FU-PCNs (F3:1; 500.5nm) which were prepared at 1% concentration of PCL of 1st emulsion and 3% of PVA of 2nd emulsion. That means that the drug entrapment of 5-FU into the prepared PCNs will affect the particles size.

*X-ray diffraction (XRD) study*

The XRD patterns of pure PCL, PVA and 5-FUsamples are represented in Figure 2a. PCL showed two sharp peaks at around 2θ = 21.5°and 23.8° that were referred to scattering from crystalline phase[20]. 5-FU displays multiple sharp peaks between 2θ = 28.4° and 31.8° representing the highly crystalline nature of the drug [21]. PVA showed two diffuse reflections and several low intensity reflections revealing itslow crystalline nature. Figure 2b represent the XRD patterns for different samples of 5FU-PCNs as indicated in Table 1 by preparing the 1st and 2nd emulsion at different concentration of stabilizer PVA, and concentration of PCL. Two peaks were observed at 2θ = 21.2° and 23.6° which were attributed to the crystalline phase of PCL, while the distinguished peaks for the drug disappeared revealing that the drug particles are dispersed at the molecular level in the polymer matrix as similarly mentioned in the literature [22].

*Fourier transform infrared spectroscopy (FTIR)*

The FTIR spectra in Figure 3 showed the characteristic peaks of pure 5-FU, PVA, PCL, blank PCNs (free from 5-FU; FB), and 5-FU–PCNs formulations (F3:0.5, F3:1, F3:3 and F2:1), respectively. The characteristic peaks for the PCL appeared at 1636.3 and 1729.8 $cm^{-1}$which were referred to the stretching vibration of C=O bonds [23]. Spectral peaks of 5-FU were observed at 3026.7–2929.3 $cm^{-1}$ due to –C–H stretching bands at 1348 $cm^{-1}$ due to pyrimidine vibration. The –C–O and –C–N vibrations were observed at 1179.2, 1655.5 and 1245 $cm^{-1}$ [24].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 (free from 5-FU, $F_B$) at 2940.9 and 2867.64 $cm^{-1}$ which were ascribed to the C-H asymmetric and symmetric stretching vibrations [25]. 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 5-FU–PCNs 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-1wasreferred to CH2 bending band. That peak showed the interaction between PCL and 5-FU and it confirmed the encapsulation of the drug by the PCNs.

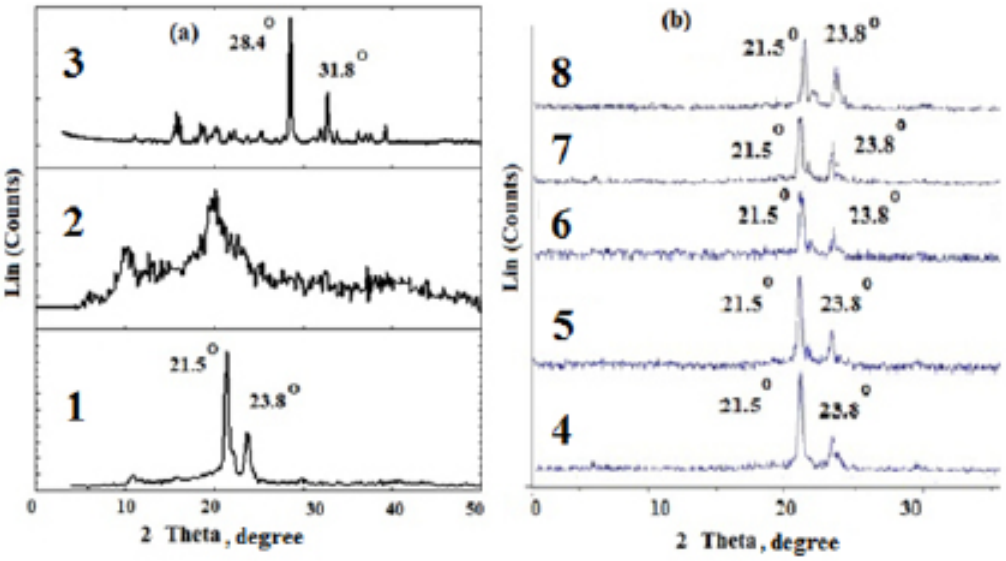


**Figure 2.** XRD patterns of (a) pure PCL (1), PVA (2), 5-FU (3), and (b) 5-FU–PCNs formulations F2:0.5 (4), F3:0.5 (5), F1:1 (6), F2:1 (7) & F3:1 (8), respectively.

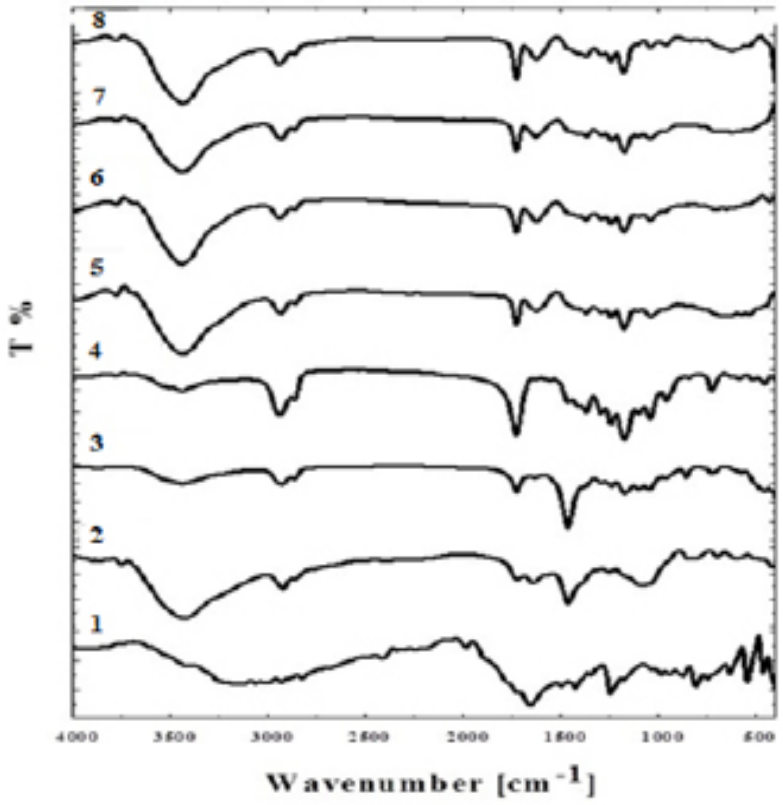


**Figure 3.** FTIR spectra of pure 5-FU (1), PVA (2), PCL (3), blank PCNs ($F_B$) (4), and 5-FU-PCNs for F3:0.5 (5), F3:1 (6), F3:3 (7) & F2:1 (8), respectively.

*Thermal analyses*

Both TGA and DSC techniques usually provide qualitative and quantitative information about the thermal properties of the nanoparticles.

*Differential scanning calorimetry (DSC)*

Differential scanning calorimetry is essentially used to measure enthalpy changes according to the changes in the thermalproperties 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 4. Pure PCL showed $T_m$= 60.66°C while $T_m$ of PVA recorded at 290.52°C.The DSC curve of 5-FU exhibits endothermic peak at the temperature of 282.80°C corresponding to its melting point [26].The recorded $T_m$ of PCL was nearly similar to the reported theoretical value [27] which confirmed that the crystallization and the melting behavior of PCL were not changed by the double emulsion method [28]. DSC thermograms of different samples of 5-FU–PCNs as shown in Figure 4revealed disappearance of the distinguished band for 5-FU at $T_m$=282.80°C. Therefore, 5-FU–PCNs demonstrated molecularly dispersed drug in the nanoparticles as previously mentioned in the literature [29].

*Thermal gravimetric analysis (TGA)*

Thermal gravimetric analysis normally provides information on mass loss as a function of temperature. TGA thermograms of pure PCL, PVA, and 5-FU were represented as shown in Figure 5a. PCL was thermally stable until 170°C where thethermal decomposition occurred in two steps at 320 and 500°C with weight loss of 20.21 and 81.61%, respectively.Thermal degradation of PVA took place through the rupture of the polyester chains via ester pyrolysis reaction with the release of $CO_2$, $H_2O$, and formation of carboxylic acid groups according to Fukushima et al. [28]. 5-FU have thermal decomposition occurred in two steps. The degradation step takes place in the range of 225-500°C with weight loss of 0.6, 85.77%, respectively, due to the loss of water.As shown in Figure 5b, it was noticed that all formulations of 5-FU-PCNs decomposed at 320°C instead of 225°C for pure 5-FU which was attributed to the presence of PCL that increased the thermal stability of 5-FU–PCNs compared to pure 5-FU and PCNs [30] .Both of PCNs and 5-FU–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 [28] which confirmed the results obtained from XRD.

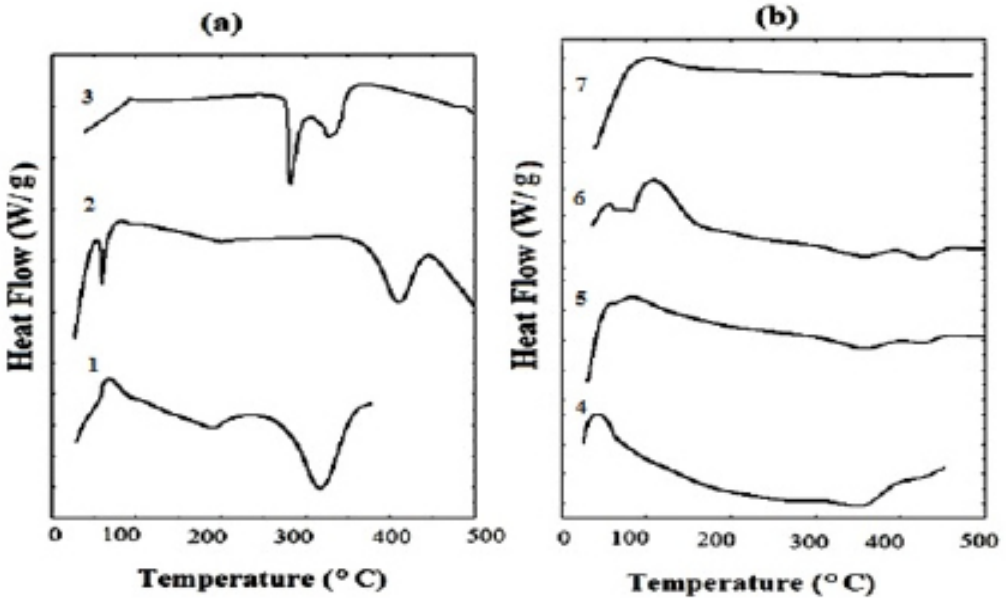


**Figure 4.** DSC thermograms of (a): PVA (1), PCL (2), 5-FU (3) and (b):5-FU–PCNs formulations F3:0.5 (4), F3:1 (5), F3:3 (6) and F2:1 (7), respectively, according to Table 1

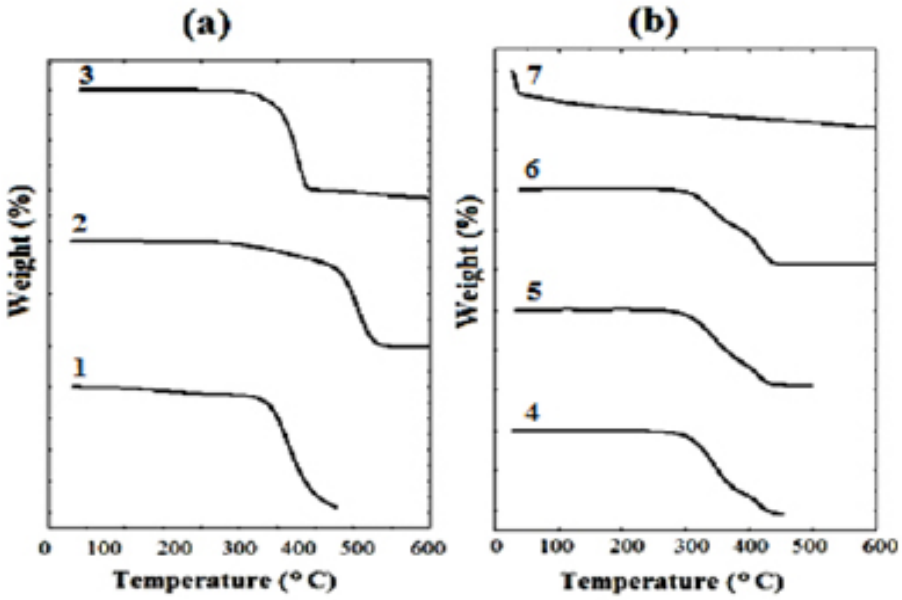


**Figure 5.** TGA thermograms of (a): 5-FU (3), PCL (2), PVA (1) and (b):5-FU–PCNs formulations F3:0.5 (4), F3:1 (5), F3:3 (6) and F2:1 (7), respectively.

*Scanning electron microscopy (SEM)*

Scanning electron microscopy was used to examine the morphology of some samples of 5-FU–PCNs as F3:0.5, F3:1, F2:1, and blank PCNs ($F_B$) (5-FU free sample of PCNs) as shown in Figure 6.SEM images showed that all nanoparticles have a spherical shape with practically smooth surface and comparatively narrowsize distribution. The particles were slightly smaller and some particles had pores on their surfaces because of the expected induced contraction by drying during evaporation of solvent. Also, some particles were connected with each otherbecause of the surface tension of wateron the particles during drying as was demonstrated by Wu and Clark [31]. That contact among particles was attributed to the presence of PVA traces, which was not easily removed because of the sticky nature of PVA [3,10].These images show that all the particles are spherical shape. From SEM images observations it was foundthat, the particles obtained with 3 % polymer (F3:1) havingnarrow size distribution and more regular shape as compare toparticles obtained from 2% polymer concentration (F2:1) as shown in Figure 6. This confirms that the polymer concentration has a significant factor that affects the characteristic of PCNs prepared by double emulsion method according to literature [12].

*Atomic–force microscopy (AFM) investigations*

AFM studies (Figure 7) showed that the 5-FU–PCNs compositions synthesized by the double emulsification method have a complex heterogeneous morphology with various shapes and internal content of PCNs. In addition, the formation of spherical micro- and nanocapsules was found when changing the composition. Figure 7 shows the AFM height images of a surface fragments of a 5-FU–PCNs thin film containing from 1 wt.% 5-FU obtained from an aqueous solution of the compositions - ratio PCL:PVA = 1:0.5; 2:0.5; 3:0.5; 1:1; 2:1; 3:1on mica. On the AFM images, a polymer phase, consisting of nanoparticles with sizes of 50-100 nm, and forming a highly porous film with holes sizes from 10 nm to several microns, is clearly visible.The values of arithmetic mean (Ra) and root mean square (Rq) surface roughness for samples PCL:PVA = 1:0.5; 2:0.5; 3:0.5; 1:1; 2:1; 3:1 are in the range of Ra = 0.6 nm and Rq = 0.7 nm, respectively. The height of the relief above the substrate surface does not exceed 3 – 5 nm (profiles not shown there), that is, the film is quite smooth and is formed, apparently, by a single layer of nanoparticles.

With an increase in the PCL content in the composition from 1 to 3 parts (PCL:PVA ratio from 1:0.5 to 3:0.5), micron-sized capsules on

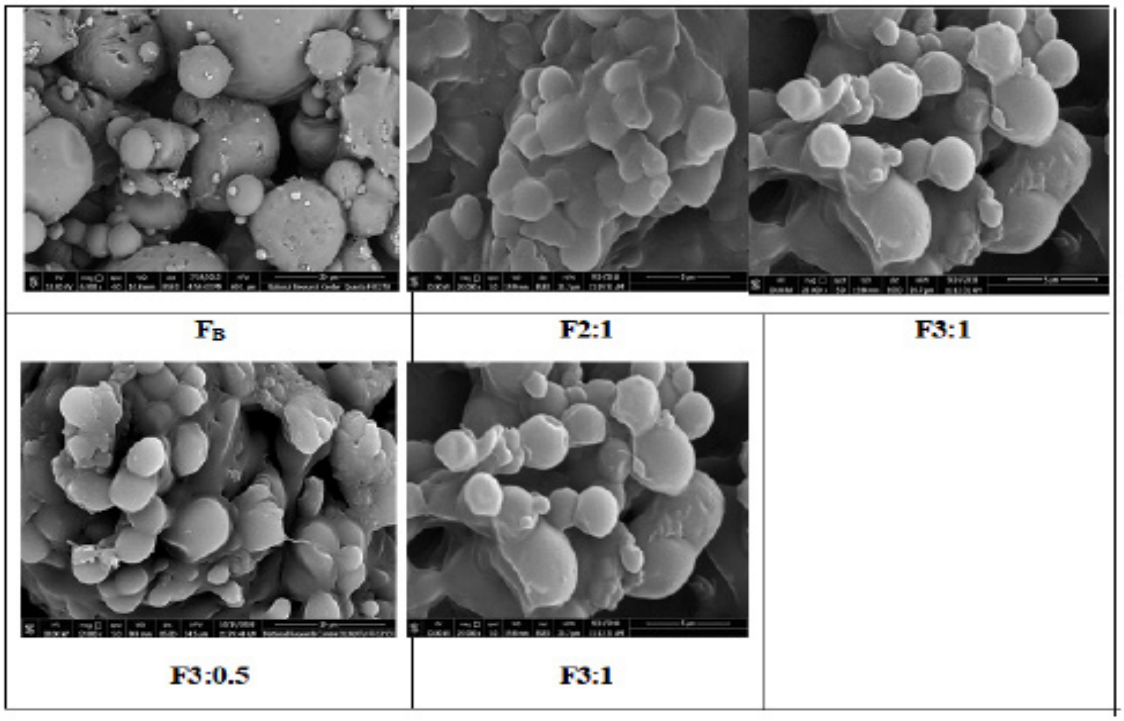


**Figure 6.** SEM images of samples blank PCNs ($F_B$), 5-FU-PCNs for F2:1, F3:1, F3:0.5, and F3:1, at a magnification of 20000×.

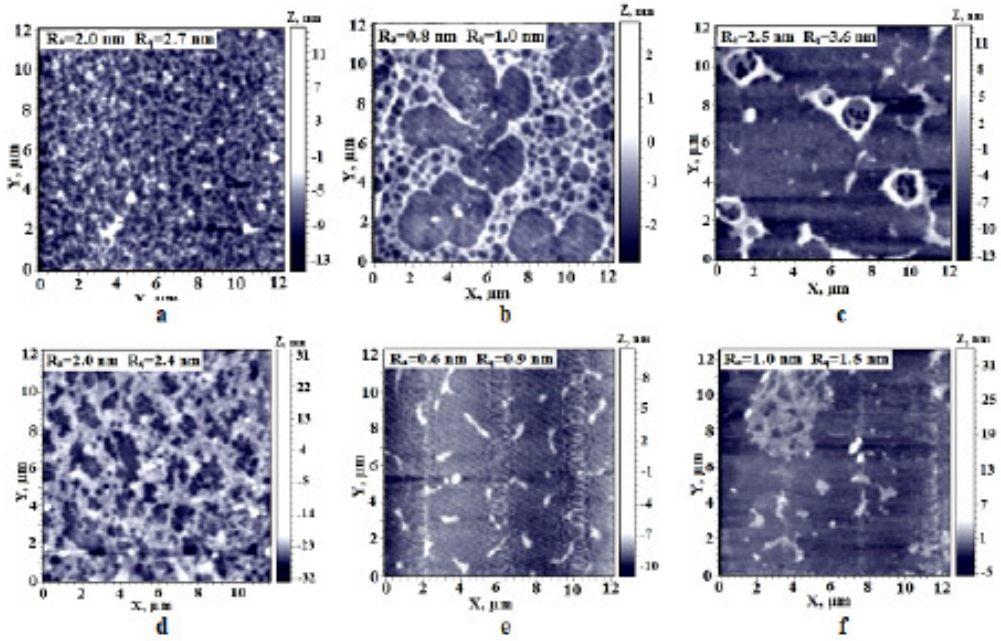


**Figure 7.** AFM height images of the surface of a double emulsion thin film 5-FU-PCL/PVA containing 1% by weight of a 5-FU preparation cast on mica; ratio of **PCL: PVA**: a- (1: 0.5), b-(2:0.5), c-(3:0.5), d – (1:1), e – (2:1), f – (3:1).

the surface of the substrate were found on the surface of the film obtained from the double emulsion solution (Figure7c). Capsule sizes vary from ~ 1 to 6 microns.The thickness of the shell of capsules with a diameter of ~ 5 microns is ~ 200-400 nm. The wall thickness of larger capsules is 400-500 nm. The surface roughness values are also significantly higher compared to the previous sample and are Ra = 3.7 nm and Rq = 4.7 nm, respectively.

An increase in the content of the PVA stabilizer in the samples also leads to a significant change in the nature of the morphology of the composite film. Thus, with the PCL:PVA ratio from 1:1 to 3:1 (Figures 7d-f), the film surface consists of spherical nanoparticles, forming an almost continuous coating on the substrate surface (nanopores were detected in some parts of the image), which is clearly visible both on the AFM image of the topography.The surface roughness values are small and are Ra = 0.5 nm and Rq = 0.8 nm. Figure 7d image clearly shows that the individual capsule consists of a PCNs with a diameter of about 50-100 nm.

The sample with a PCL:PVA ratio of 3:1 (Figure7d) exhibits different type of capsules, which can be called micro-containers. Comparison of AFM (Figure 7) and SEM (Figure 6) results confirmed complicate morphology and various shape of the 5-FU-PCNs double emulsion droplets and the formation of spherical micro- and nanocapsules.

## Conclusion

In the current paper, 5-FU–PCNs were prepared by double emulsion where several factors influencing the size of particles were investigated as concentration of PCL for first emulsion and concentration PVA for second emulsion. A general increase in observed of the EE,% values with increasing concentration of PCL from 1% to 3%. Also, increase in EE,% values was observed with increasing concentration of PVA of 2$^{nd}$ emulsion from 1% to 3% at constant concentration of PCL of 1$^{st}$ emulsion.The increase in PVA concentration led to a decrease in the particle size of the prepared nanoparticles. DSC and XRD studies revealed molecularly dispersed drug in the nanoparticles in case of drug loaded nanoparticles. TGA studies in case of 5-FU–PCNs demonstrated no decomposition peak for the drug indicating enhanced stability effect for the prepared carriers. Finally, the correlations between structural and morphological parameters and preparation conditions (composition, concentration of stabilizer) were evaluated. The estimation of 5-FU drug encapsulation efficiency into polymer matrix showed that the prepared PCNs can be effectively used for preparation of controlled release matrices for anticancer drugs. SEM and AFM confirmed the complicated morphology and the formation of the spherical micro- and nanocapsules.

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## Conflict of Interests

The authors declare that they have no conflict of interest.

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## كبسله دواء سرطان ٥-فلوريوراسيل داخل جزئيات البولى كابرولكتون بطريقة أمنة

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الهدف الاساسى من هذه الدراسة هو معايرة و تقيم افضل الظروف لتحضير جزئيات البولى كابرولاكتون النانومترية المحمله ب ٥-فلوريوراسيل(دواء لعلاج السرطان) بطريقة أمنة . تم استخدام طريقة الاستحلاب الثنائية بمرحلتيها الاولى والثانية لتحضير جزئيات البولي كابرولاكتون المحملة بدواء ال ٥ فلورسيل فى وجود تركيزات مختلفة من البولى فينيل الكحولى كعامل استحلاب ودراسة تاثيرة على ثبات وكفاءة وتكوين جزئيات البولى كابرولاكتون النانومترية وعلى كفاءة تحمل ٥-فلوريوراسيل. ولقد وجد ان معدل كفاءة التحمل لفلوريوراسيل داخلجزئيات البولى كابرولاكتون النانومترية المحضره تتراوح مابين ١٤-٦٥,٦٪ وان حجم .جزئيات البولى كابرولاكتون النانومترية المحمله ب ٥-فلوريوراسيل متواجد على شكل دائرى وحجمها يترواح من١٨٣-٩٤٤ نانومتر كما تم توصيف الجزئيات النانومترية المحضره باستخدام الاشعة تحت الحمراءو القياس الطيفى فى النطاق فوق البنفسجى وحيود الاشعة السينية والتحليل التفاضلى والتكمالى الحرارى والميكروسكوب الالكترونى الماسح الميكروسكوب القوى الذرى .وتم ايجاد افضل النسب المحضره من العوامل المختلفه التى تم دراستها لبلمرة الاستحلاب الثنائية. وتحققنا من خلال الميكروسكوب الالكترونى الماسحوالميكروسكوب القوى الذرى على الشكل الظاهرى للسطح وتكونجزئيات مايكرو او نانو كبسولة محتوية على الدواء .