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Original article

An investigation into the role of surfactants in controlling particle size of polymeric nanocapsules containing penicillin-G in double emulsion

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ABSTRACT

Preparation, characterization and drug release behavior of loaded polybutyl adipate (PBA) nanocapsules with penicillin-G are described here. The nanocapsules were produced using a double emulsion solvent evaporation technique, using dichloromethane as an organic solvent and Tween and Span as surfactants. In this process, a mixture of glycerin and water was used instead of the traditional stabilizer system in the preparation of double emulsion. The influence of surfactants on the property of nanocapsules was discussed in detail. The effects of Span and Tween to modify the size of the nanocapsules were different. The mean diameters of penicillin-G loaded nanocapsules ranged from 75 nm to 638 nm and were dependent on the types and content of the surfactants. The encapsulation efficiencies and drug release rates were also affected by the surfactants in the preparation process. It was found that the encapsulation efficiencies of penicillin-G enhanced up to 76.8% with the increase in Span and Tween contents. Increasing Span concentration as an inner surfactant results in the remaining of penicillin-G mostly sealed in the inner aqueous phase and increasing Tween concentration as the outer surfactant enhanced the viscosity of external water phase, which decreased the rate of penicillin-G diffusion from the inner water phase to the outer water phase. Interestingly, the in vitro drug release profiles exhibited a significant burst release, followed by a lag phase of little or no release. Penicillin-G loaded nanocapsules with low concentrations of both surfactants tend to have higher burst release. Under optimum formulation conditions, the encapsulation of penicillin-G can reach up to 60% and the burst release can also fall below 45%. In this case, the fact that the nanocapsules have only 130 nm diameter will be important.

1. Introduction

The science of drug delivery may be described as the application of chemical and biological principles to control the *in vivo* temporal and spatial locations of drug molecules for clinical benefit. When drugs are administered, only a very small fraction of the dose actually hits the relevant receptors or sites of action, and most of the dose is actually wasted either by being taken up into the "wrong" tissue, removed from the "right" tissue too quickly, or destroyed en route before arrival. Scientists researching drug delivery seek to address these issues in order to (1) maximize drug activity and (2) minimize side effects [1]. In the pharmaceutical field, several advantages of drug delivery systems with nano size range have been shown including increasing solubility, enhancing dissolution rate and improving bioavailability. The main purpose of nanotechnology is the design of miniaturized drug carrier systems to achieve adequate stability, improved absorption, controlled release, quantitative transfer and therefore the expected pharmacodynamic activity. A variety of chemical techniques have been developed for the synthesis of nanoparticles in recent years [2]. Nanoparticles can be prepared using different kinds of materials, for example, biodegradable and biocompatible polymers, phospholipids, surfactants and lipids. Nanocapsules have a polymeric shell; the active substances are usually dissolved in the inner core, but may also be adsorbed at their surface. In recent years, an increasing interest has developed on the nanoencapsulation of active ingredients. Different technologies can be used for the formulation of these polymeric nanocapsules. Synthetic polymers have the advantage of high purity and reproducibility over the natural polymers. Among those, the polymers in the polyesters family are of interest because of their biocompatibility and biodegradability to nontoxic metabolites [3]. It was observed that the entrapment of hydrophilic drug substances inside the polymer capsules is a very difficult task using the nanoprecipitation method [4–6]. The reason for this is that the hydrophilic drug substances have very low affinity to the polymer. In addition, the interaction between the polymer and drug is weak, and the drug substance has a tendency to move from the organic phase to the outer aqueous phase and not in the precipitating nanoparticles. Nanoemulsions constitute the primary step in nanocapsule and nanosphere synthesis using nanoprecipitation [7].

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The nanoemulsions prepared displayed good stability and there was no phase separation after several weeks, although an increase in droplet size was noted with time. Nanoemulsions have uniform and extremely small droplet sizes. In addition, high kinetic stability, low viscosity and optical transparency make them very attractive systems for many industrial applications; for example, in the pharmaceutical field as drug delivery systems and in cosmetics as personal care formulations [8–12]. Multiple emulsions are systems in which the dispersed phase is itself an emulsion and they can be classified into two major types: water–oil–water emulsion (w/o/w) and oil–water–oil emulsion (o/w/o). The use of multiple emulsions can create delivery systems with novel encapsulation and delivery properties. Double emulsions are complex liquid dispersion systems known also as 'emulsions of emulsions', in which the droplet of one dispersed liquid is further dispersed in another liquid. The inner dispersed globule/droplet in the double emulsion is separated (compartmentalized) from the outer liquid phase by a layer of another phase [13–17]. The classic emulsification solvent evaporation (ESE) technique is commonly used for encapsulation of various substances [18–21]. It is well known that ESE is mainly a two-step process: the emulsification of a polymer solution containing the encapsulated substance, followed by particle hardening through solvent evaporation and polymer precipitation. During emulsification, the polymer solution is broken up in droplets by the shear stress produced either by homogenizer, sonicator or whirl mixer in the presence of a surface active agent (surfactant). This first step mainly determines particles' size distribution and it has been extensively investigated along with the influence of various process parameters [22–27]. Many potential applications for double emulsions are well documented and some have been patented.

In this study, polybutyl adipate (PBA) nanoparticles were prepared by a modified precipitation method as a biocompatible and biodegradable polymer. The degradation of this polymer occurs via hydrolytic ester-bond cleavage, leading to a decrease in their molecular weight. The entrapment of the penicillin-G was strongly dependent on the hydrophilicity of the polymer. The work presented here developed the modified w/o/w multiple emulsion method to prepare penicillin-G nanocapsules in PBA. Double emulsions are prepared in a two-step emulsification process using two surfactants: a hydrophobic one (Span) designed to stabilize the interface of w/o internal emulsion and a hydrophilic one (Tween) for the external interface of the oil globules for w/o/w emulsions. Generally, the characteristics of nanocapsules such as their particle size, morphology, drug content and in vitro release profiles were affected by different experimental conditions such as the type and concentration of surfactants in the inner and outer water phase. It was postulated that the surfactants play a double role in the emulsions: film former and barrier to the drug release at the internal interface, and steric stabilizers at the external interface. Therefore, regulating the amounts of emulsifiers improves encapsulation efficiency and slows down the release of drug. The interest of these nanoparticles as a carrier for penicillin-G was evaluated in terms of the drug entrapment efficiency and release profiles over 48 h.

2. Materials and methods

2.1. Materials

Penicillin-G was purchased from Jaberebne Hayyan Pharma. Co from Iran. Span 60 (sorbitan monostearate, HLB = 4.7), Tween 60 (polyoxyethylene sorbitan monostearate, HLB = 14.9), Span 20 (sorbitan monolaurate, HLB = 4.3), Tween 20 (polyoxyethylene sorbitan monolaurate, HLB = 16.7) and Glycerin were obtained from Merck Chemical Co. Adipoyl chloride and 1,4-butandiol were obtained from Fluka Chemical Co. Diethyl ether was purchased from Guandong Guango Chemical Co. All other reagents were of analytical grade. Deionized water was used in all experiments.

2.2. Preparation of PBA

5.52 g (4.40 ml, 3.01×10^{-2} mol) adipoyl chloride and 2.72 g (2.68 ml, 3.01×10^{-2} mol) 1,4-butandiol and a stirring bar were placed into a 25 ml round bottomed flask. The mixture was heated at 85 °C for 24 h until no more HCl was released. The viscous solution was poured in 40 ml diethyl ether and the resulting homopolymer was filtered off and then dried under vacuum to give 5 g (60.68%) of white PBA powder, $M_n = 7633$ g/mol, $M_w = 10\,000$ g/mol and $M_w/M_n = 1.31$.

2.3. Preparation of nanocapsules containing penicillin-G

Penicillin-G is frequently used as a model of water-soluble drug for polymer loading. The PBA nanocapsules containing penicillin-G were prepared as follows: 1.0 mg of penicillin-G was dissolved in 0.16 ml of deionized water. Then the solution was emulsified with 1.51 ml dichloromethane solution of 25 mg PBA containing different amounts of Span for 15 s with 50% power by using a prob sonicator to form the w/o primary emulsion. Next, 5 ml of an aqueous phase containing 50% glycerin and Tween was immediately poured into this primary emulsion and sonicated under the same conditions for 10 s to produce a w/o/w emulsion. The system was magnetically stirred at room temperature until all the dichloromethane evaporated. The produced nanocapsules were collected by centrifugation. The collected nanocapsules were washed two times with deionized water and freeze-dried to extract fine nanoparticles from aqueous medium.

2.4. Ultrasonic generator

The equipment employed in this search was a 20 kHz ± 500 Hz (200 W) ultrasonic generator, SONOPULS Ultrasonic homogenizer, Model HF-GM 2200 (BANDELIN electronic GmbH & Co. KG) and a titanium microtip MS-73 with diameter 3 mm as the probe.

2.5. Size and morphology of the PBA nanocapsules

Particle size of nanocapsules was determined with Zetasizer nano ZS (Malvern Instruments Ltd, United Kingdom). The sample was diluted to the appropriate concentration with deionized water, which was filtered previously with a 0.45 μm Millipore filter, to avoid any contamination.

A drop of nanoparticle suspension containing 2 wt% phosphotungstic acid was placed on a copper grid with Formvar® film and dried before measurement by Zeiss CEM902A transmission electron microscope (TEM) at the acceleration voltage of 80 keV. Then it was stained negatively with sodium solution (1 wt%/vol). The surface morphology was studied by Cambridge S-360 SEM scanning electron microscopy (SEM). Micrographs of nanocapsules' suspension showed a spherical shape of the dispersed particles.

2.6. Drug loading, nanoparticles' yield, encapsulation efficiency and in vitro drug release of penicillin-G loaded nanocapsules

In vitro release studies of penicillin-G from the drug-loaded PBA were performed by diffusion technique. The encapsulation efficiency and drug loading content were measured by ultraviolet absorption at their maximum wavelength on a Carry 100 Bio spectrophotometer. By using UV data, the encapsulation efficiency and drug loading content were obtained from equations (1) and (2), respectively. Nanoparticles' yield was obtained gravimetrically from equation (3). For drug release, 10 mg /10 ml of the

nanocapsule suspension (pH = 7.4) was placed into pre-swelled dialysis bag that was immersed into 100 ml PBS solution (Phosphate Buffer Saline, pH = 7.4) at 37 °C. At predetermined time intervals, 4 ml of the solution was collected from the released media and replaced with fresh PBS. Then the released penicillin-G was analyzed by UV/vis spectrophotometer.

$$\text{Encapsulation efficiency}(\%) = \frac{\text{Weight of the drug in nanoparticles}}{\text{Weight of the feeding drug}} \times 100 \quad (1)$$

$$\text{Drug loading content}(\%) = \frac{\text{Weight of the drug in nanoparticles}}{\text{Weight of the nanoparticles}} \times 100 \quad (2)$$

$$\text{Nanoparticle yield}(\%) = \frac{\text{Weight of the nanoparticles}}{\text{Weight of the feeding polymer and drug}} \times 100 \quad (3)$$

3. Results and discussion

3.1. Synthesis of PBA

Polymer selection is one of the most important step in the manufacture of nanocapsules. PBA ($M_w = 10\ 000$ g/mol) was synthesized by the polycondensation reaction from adipoyl chloride and 1,4-butandiol, with a ratio of 1:1 mol of adipoyl chloride to 1,4-butandiol (Scheme 1) in accordance with our previously reported procedure [28].

The structure of PBA was characterized by spectroscopic methods (^{1}H NMR and FT-IR) and its thermal properties were evaluated by DSC analysis [28].

3.2. Preparation of double emulsion (w/o/w)

In this study, polymeric nanocapsules were prepared by means of a modified w/o/w double emulsion without general stabilizers through sonication process in both steps. The primary emulsion (w/o) was prepared by introducing dissolved penicillin-G in water, containing low-HLB surfactant (Span) in methylene chloride and PBA as the oil phase. In the second step, the primary emulsion (w/o) was re-emulsified in an aqueous solution of a high-HLB surfactant (Tween) and glycerin to produce w/o/w multiple emulsion (Scheme 2).

The stabilizer plays the role of dispersing emulsion drops and preventing the emulsion drops from coagulation and precipitation in the emulsion system. The traditional stabilizers for the preparation of micro- and nanocapsules are usually a type of macromolecular substances such as gelatin, poly(vinyl alcohol) and so on. They are not easily washed from the micro- and nanocapsules. As a result, they may cause trouble during purification of the product and lead to reduced yield and entrapment efficiency of the drug [29]. Whereas glycerin is not harmful, it is not also necessary to be washed from the nanocapsules. Therefore, this method would make the purifying of the produced nanocapsules easier [30]. Multi-hydroxyl structure of glycerin could accelerate the diffusion of organic solvent from nanodroplets to the external aqueous phase. Thus the solvent can be evaporated immediately to form nanocapsules after addition of water.

3.3. Characterization of nanocapsules

Morphology of the nanoparticles was studied by transmission electron microscopy (TEM). PBA nanocapsules containing penicillin-G were dispersed in water and air-dried on a metal stub and then were investigated by transmission electron micrograph. It could be observed that separate nanocapsules have spherical shapes with particle size below 90 nm (Fig. 1).

Particle size was determined by photon correlation spectroscopy using a zetasizer (Malvern Instruments, UK). Nanocapsules prepared by double emulsion were diluted with double-distilled and filtered water (0.45 μm, Millipore filter). Particle size of the prepared nanocapsules is below 90 nm even with double emulsion method for a typical sample shown in Fig. 2.

3.4. UV studies

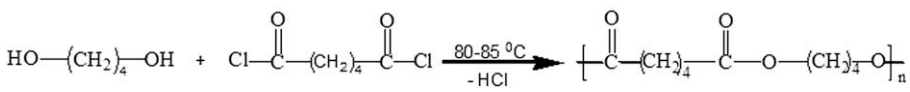
The encapsulation efficiency of nanoparticles was measured from UV absorbance spectra and on the basis of Beer–Lambert law and absorbance superposition as below:

$$A = \varepsilon c b \quad (4)$$

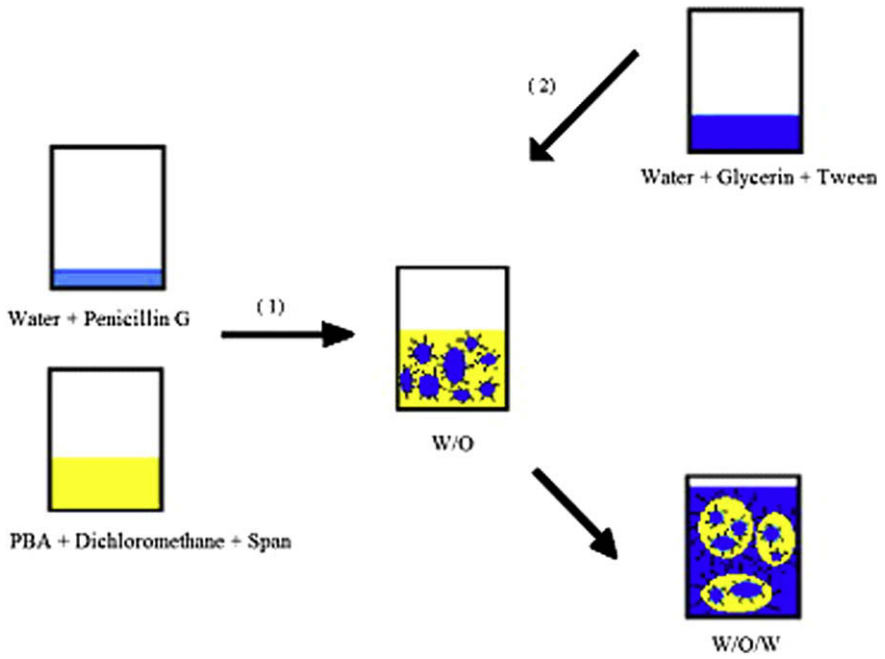
where A is the absorption intensity, c is the drug concentration (penicillin-G), b is the path length of the radiation through the absorbing medium and ε is a proportionality constant, so-called molar absorptivity. According to Beer–Lambert law, absorbance of the absorbent component is directly proportional to its concentration. Therefore, absorbance of the drug-loaded nanoparticles varies with the content of penicillin-G. The absorption intensity grows up by increasing the loaded drug. A predetermined quantity of loaded penicillin-G nanoparticles was dissolved in 25 ml chloroform as an aprotic and polar solvent and its absorption was compared with the chloroform solution of pure drug precisely (Fig. 3). It could be seen that loaded and free drugs exhibit similar absorptions at 280 nm. According to the molecular structure of penicillin-G, the main chromophoric groups are esteric and amidic functional groups, absorption intensities of which were very weak, due to their forbidden transition ($n \rightarrow \pi^*$). The strong absorption peak at 280 nm is related to $n \rightarrow \sigma^*$ allowed electron transition of sulfur in C–S–C bond. The presence of hydrogen bonding between drug and polyesteric segments is the major factor for the remaining drug in nanoparticles' cavity. However and in spite of drug encapsulation, no shift in λ_{max} was observed for the loaded drug in comparison with the free one. This is due to the fact that functional groups capable of formation of hydrogen bonding have weak absorption intensity in the UV–vis region. On the other hand, those without any hydrogen bonding have significant absorption in this region.

3.5. Effect of preparation conditions on the size of nanocapsules

In order to find out optimum conditions for the expected controlled release system, two kinds of surfactants were used to



Scheme 1. Polycondensation reaction of adipoyl chloride and 1,4-butandiol.



Scheme 2. Preparation of w/o/w double emulsion in two steps.

prepare drug-loaded nanocapsules and their effects on the characteristics of the above produced carriers were investigated. It was found that the drug encapsulation efficiency and the average particle size were affected by changing the kind and concentration of these two surfactants. Scheme 3 shows the molecular structure of Span 20, Span 60, Tween 20 and Tween 60, which were used as internal and external surfactants in our double emulsion procedure. It can be seen that the presence of polyethylene glycol tail (R groups) as hydrophilic domains and hydrocarbon chain (R′ groups) as hydrophobic ones could differ in their surface characteristics. Due to this difference, the size of nanoparticles and their encapsulation efficiency changed significantly. We used Span 20 (in the inner oil phase) together with Tween 20 (in the outer aqueous phase) and also Span 60 together with Tween 60. One possible explanation, which may account for the changes in nanocapsule diameter with Span and Tween concentration, was based on surfactant packing parameters. There are two interfaces: one is between the inner water and oil phases and the other is between the oil and outer water phases too.

Mitchell and Ninham thought that aggregation of surfactants is controlled by a balanced molecular geometry [31]. They defined a critical packing parameter, P_c, as the ratio of volume to surface area (equation (5)).

$$P_c = v/(a_o l_c) \tag{5}$$

where a_o is the minimum interfacial area occupied by the head group, v is the volume of the hydrophobic tail and l_c is the maximum extended chain length of the tail in the micelle core. The parameter v varies with the number of hydrophobic groups, chain unsaturation, chain branching, and chain penetration by other compatible hydrophobic groups, whereas a_o is mainly controlled by electrostatic interactions and head group hydration. P_c is a useful quantity since it allows the prediction of aggregate shape and size. When Span 20 and Tween 20 are used, a small P_c is obtained owing to the shorter chain length of the hydrocarbon tail. So the obtained nanocapsules have large mean sizes in contrast to the system containing Span 60 and Tween 60.

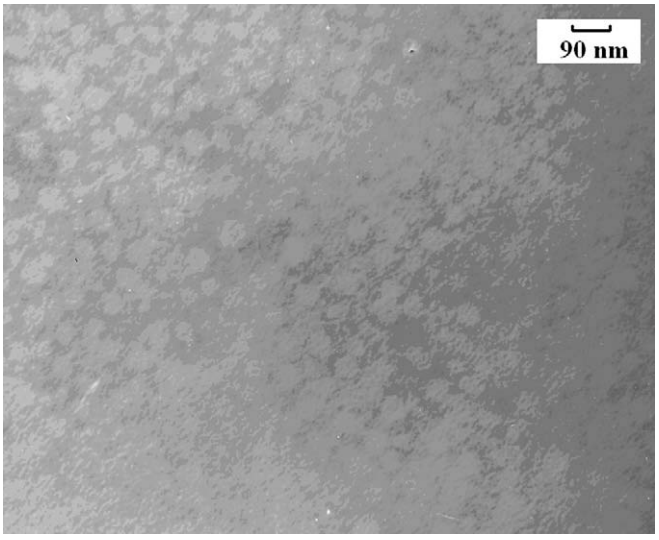


Fig. 1. TEM of PBA nanocapsules with Span 60 and Tween 60 as surfactants (7 and 2 wt%, respectively).

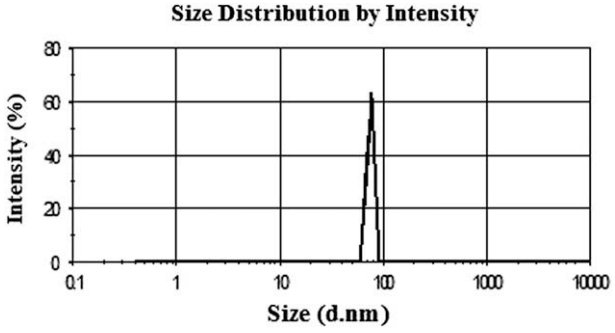


Fig. 2. Particle size distribution of PBA nanocapsules with Span 60 and Tween 60 as surfactants (7 and 2 wt%, respectively) by zetasizer.

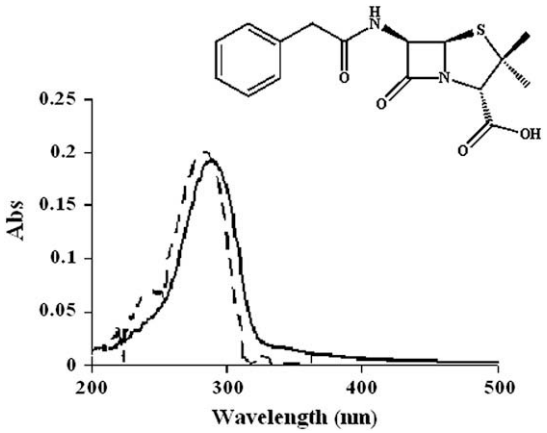
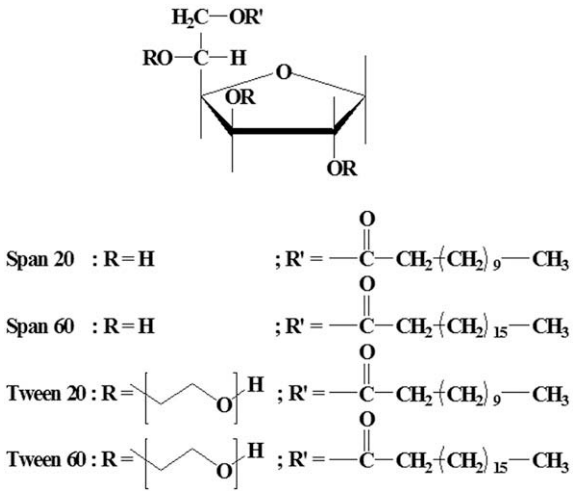


Fig. 3. UV absorption of penicillin-G (—) and penicillin-G loaded nanoparticles (– –) dissolved in chloroform.



Scheme 3. Chemical structure of Span 20, Span 60, Tween 20, and Tween 60.

3.5.1. *Effect of span concentration on average particle size of the nanocapsules*

Particle size of the nanocapsules was decreased when the concentration of Span 60 and Span 20 increased at constant Tween 60 and Tween 20 concentration, respectively (Fig. 4a and b). In this section, the properties of the produced nanocapsules with 1.4, 2.8, 4.2, and 7 wt% Span 20 and 60 as internal surfactants in which the amount of Tween 20 and 60 remained unchanged at 4 wt% (Fig. 4a) and 8 wt% (Fig. 4b) were investigated.

Fig. 4a shows that at 4 wt% of Tween, nanocapsules' sizes were decreased from 621 nm to 356 nm for Span 20 and from 337 nm to 131 nm for Span 60, when the Span concentration in dichloromethane increased from 1.4 to 7 wt%. Similarly, by increasing Span 20 and Span 60 concentrations from 1.4 to 7 wt% at 8 wt% of Tween (Fig. 4b), the particle sizes reduces from 638 nm to 416 nm and from 356 nm to 240 nm, respectively. It could be observed that the particle size depends on the balance between type and concentration of the internal surfactant. It was considered that the higher concentration of Span at a constant concentration of Tween results in a higher hydrophobic group (v in equation (5)) and consequently, a larger P_c that makes the formation of smaller w/o/w emulsion droplets and nanoparticles easier.

3.5.2. *Effect of Tween concentration on the average particle size of nanocapsules*

Concentration of Tween in the external water phase is known to be an affecting key factor on the size of nanocapsules. When the concentration of Span 60 and Span 20 was kept constant, the particle size of nanocapsules increased with the increase in Tween 60 and Tween 20 concentrations. In the present study, 2, 4, 8 and 16 wt% Tween 20 and 60 solutions were used in the external water phase to examine the effect of the above mentioned parameters on the characteristics of the nanocapsules. The results are summarized in Fig. 5 and b. The size of the produced nanocapsules at 2–16 wt% Tween 20 and Tween 60 concentrations, when 4.2 wt% Span was present in the internal aqueous phase, was 419–480 and 247–267 nm, respectively (Fig. 5a). For w/o/w emulsions prepared with 7 wt% Span, addition of 2–16 wt% of Tween 20 and 60 to the external aqueous phase increased the emulsion droplets sizes from 242 to 478 nm for Tween 20 and from 75 to 258 nm for Tween 60 (Fig. 5b). Evidently, a significant increase in particle size could be observed by increasing the concentration of Tween 20 and 60 at a constant concentration of Span. Higher Tweens' concentration causes an increase in the nanoparticle size. This is because of the increased concentration of Tweens that promotes the head group hydration (a_o in equation (5)) and therefore reduces P_c, resulting in greater emulsion droplets. These emulsion droplets are gradually solidified to form nanocapsules during evaporation of the solvent from the emulsion droplets. Tween has a large interfacial area owing to its head group (a_o) (in contrast to Span) because of its ethylene oxide chain. So with the increase in Tween concentration at a constant amount of Span, the value of the packing parameter (P_c) decreases and the mean size of nanocapsules rises up. On the contrary, P_c increases with increasing the concentration of Span at a constant amount of Tween and the mean size of nanocapsules comes down. There is only a small fraction of Tween in the inner interface between inner water and oil. Hence, the effect of Tween on the nanocapsule size will be less than Span [32].

3.5.3. *Effect of kind of Span and Tween on average particle size of nanocapsules*

Here, the ability of Span and Tween on controlling the w/o/w emulsion droplets size was investigated. The influence of Span 60

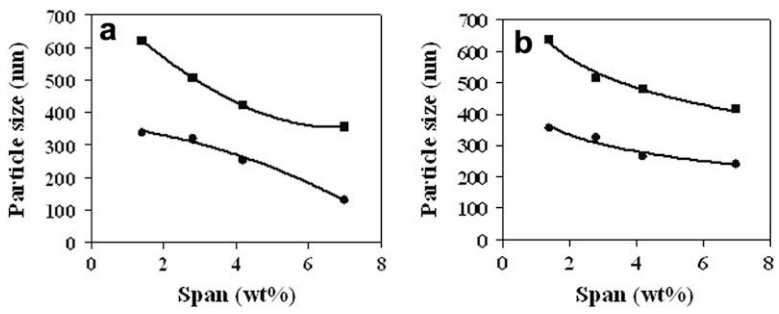


Fig. 4. Influence of Span 20 (■) and Span 60 (●) concentrations from 1.4 to 7 wt% on the particle size of nanocapsules prepared from double emulsions at 4 wt% (a) and 8 wt% (b) of Tween 20 and 60, respectively.

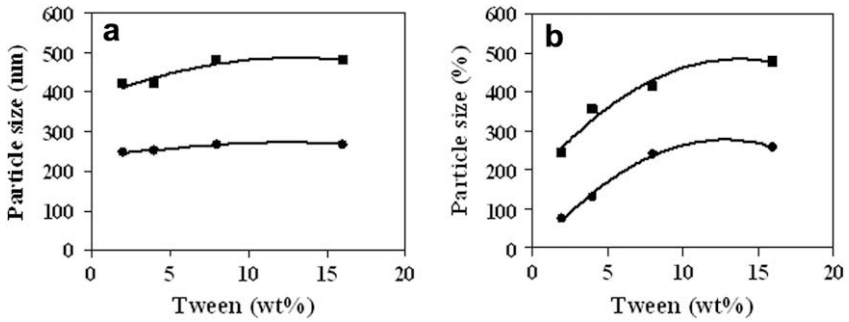


Fig. 5. Influence of Tween 20 (■) and Tween 60 (●) concentrations from 2 to 16 wt% on the particle size of nanocapsules prepared from double emulsions, at 4.2 wt% (a) and 7 wt% (b) of Span 20 and 60, respectively.

was much greater than Tween 60. In fact, the emulsifying power of Span and Tween 20 versus Span and Tween 60 at different weight ratios was studied (Figs. 4a and b and 5a and b) and it was found that the size of the prepared nanocapsules from Span 60 and Tween 60 was markedly smaller than that of Span 20 and Tween 20. Span 20 and Tween 20, in comparison with Span 60 and Tween 60, have shorter chain length at their hydrophobic tail (i.e. smaller v in equation (2)) and thus a small P_c and consequently large mean size of nanocapsules would be obtained.

3.6. Effect of preparation conditions on encapsulation efficiency

Double emulsions stabilized with Span and Tween as surfactants were used for entrapping penicillin-G as a hydrophilic drug. Double emulsions in which penicillin-G was entrapped in the inner aqueous phase show high yields of encapsulation in most tested conditions. The preparation route, for instance, in the well-known 'two-step' emulsification method is suitable for multiple globules formation, stabilized with biopolymeric emulsifiers with high efficiency. Moreover, the analytical tool for determining the amount of penicillin-G in the inner aqueous phase was ultraviolet spectrophotometer. The encapsulation efficiencies of penicillin-G in PBA nanocapsules are shown in Figs. 6 and 7. The encapsulation efficiency was modified by changes in the surfactant selection. The obtained data showed that encapsulation efficiencies of penicillin-G enhanced with the increase in Span and Tween contents. The best encapsulation efficiency reached to 76.8% at 4.2 wt% of Span 60 and 16 wt% of Tween 60 used in the typical procedure. Size of nanocapsules plays an important role in the drug encapsulation efficiency and affects on the drug release. The preparation conditions and some characteristics of the drug-loaded nanoparticles are given in Table 1.

3.6.1. Effect of Span concentration on the encapsulation efficiency

The encapsulation efficiency of w/o/w emulsions prepared by varying Span 60 concentration (1.4–7 wt%) in the oil phase is shown in Fig. 6. Yield of encapsulation of double emulsions increases from 21.85% at 1.4 wt% of Span 60 to 59.22% at 7 wt% of Span 60, while the Tween concentration in the external aqueous phase remained constant. When the overall Span 60 concentration increased, an increase in the encapsulation yield of the double emulsion was observed (Fig. 6). This increase in the yield of encapsulation indicates that the penicillin-G remained mostly sealed in the inner aqueous phase. These results show that by providing a hydrophilic condition, created by introducing Span 60 as the internal surfactant, might improve residence of penicillin-G in the inner phase. This is probable through the influence of polar groups of the surfactant molecule (hydroxyl groups) at the water–oil interface on penicillin-G functional groups. In fact the same parameters, that could decrease the mean size, could also increase the encapsulation efficiency.

3.6.2. Effect of Tween concentration on the encapsulation efficiency

A significant increase in penicillin-G encapsulation efficiency from 24.62% to 76.8% was observed as the concentration of Tween increased from 2 wt% to 16 wt% in the external water phase (Fig. 7). That is, higher Tween concentration increased the viscosity of external water phase and decreased the rate of penicillin-G diffusion from the inner water phase to the outer water phase

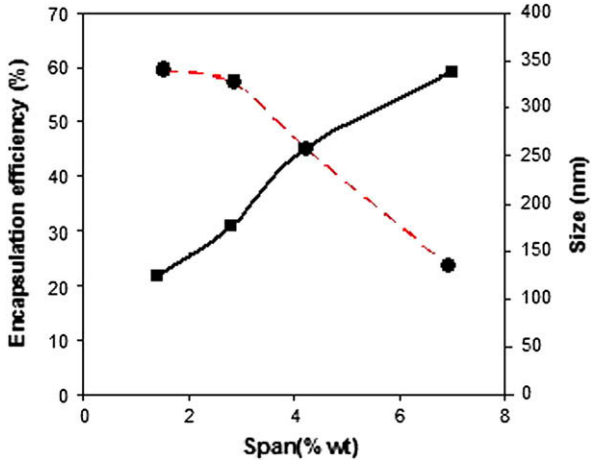


Fig. 6. The effect of the changing of Span 60 concentration from 1.4 wt% to 7 wt% at 4 wt% Tween 60 on encapsulation efficiency (■) and particle size (●) of nanocapsules.

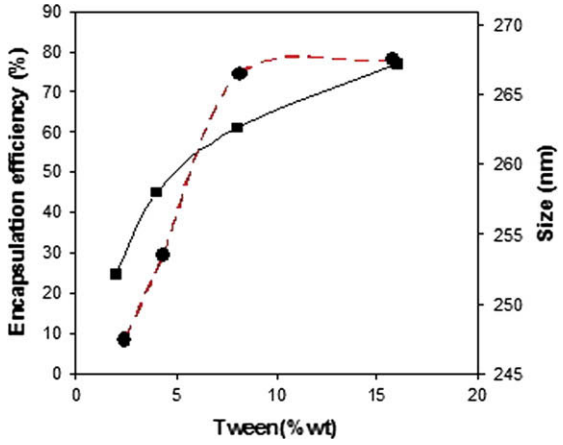


Fig. 7. The effect of the changing of Tween 60 concentration from 2 wt% to 16 wt% at 4.2 wt% Span 60 on encapsulation efficiency (■) and particle size (●) of nanocapsules.

Table 1
Some properties of the penicillin-G loaded PBA nanoparticles.

Sample	Span 60 (wt%)	Tween 60 (wt%)	Size (nm)	Encapsulation efficiency (%)	Drug loading (%)	Nanoparticle yield (%)
1	4.2	2	247	24.62	5.2	18.8
2	4.2	4	253	44.9	8.6	20.71
3	4.2	8	266	60.99	17.1	14.14
4	4.2	16	267	76.8	19.3	15.85
5	1.4	4	337	21.85	6.5	13.46
6	2.8	4	322	30.99	7.4	16.73
2	4.2	4	253	44.9	8.6	20.71
7	7	4	131	59.22	4.4	54.18

(i.e. hindering the mass transfer of penicillin-G to the surrounding region). Thus, the drug can be distributed more evenly in the interior of the nanocapsules and results in the increased encapsulation efficiency of penicillin-G. The increased penicillin-G entrapment efficiency can be also attributed to the increase in both oil phase viscosity and larger size of the oil droplets, which prevents leakage of the inner aqueous phase through the oil phase and into the outer aqueous phase (Fig. 7). Consequently, the influence of Tween content on the encapsulation efficiency is greater than that of Span. The encapsulation efficiencies were affected greatly by the content of Tween, whereas the effect of Span content was small. Particle size of the nanocapsules and viscosity of the system increased as the Tween content was increased.

3.7. *In vitro release studies*

The release rate of penicillin-G from PBA was studied with selection of nonionic surfactants (Tween and Span series). Many methods have been used to evaluate the drug release profiles from nanostructures. Among these techniques, the dialysis bag diffusion, bulk equilibrium reverse dialysis sac, centrifugal ultrafiltration, ultrafiltration at low pressure and centrifugation could be frequently considered. In vitro release profile of penicillin-G was determined by using dialysis bag technique here. This study investigates the effect of surfactants on the drug release rate from PBA polymer. Penicillin-G release rate was affected by degradation rate of the polymeric wall and drug encapsulation. The release profiles of nanocapsules prepared by PBA polymer are shown in Figs. 8 and 9 for constant Span and Tween, respectively. It was found that a significant initial burst release was obtained during the first day for samples with low surfactant concentration. In the case

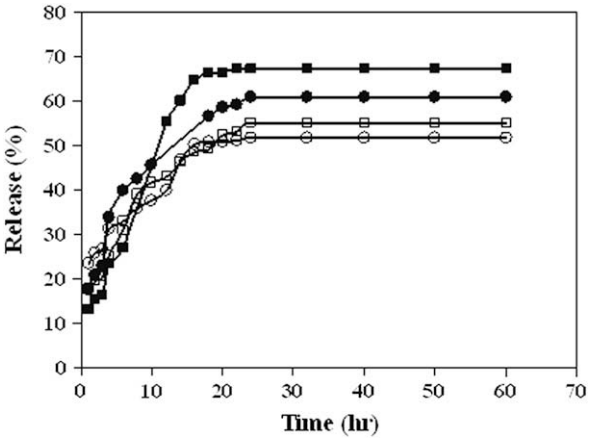


Fig. 8. The effect of the weight percent of the Span 60 in the internal phase on the release rate of penicillin-G from PBA nanocapsules: 1.4 wt% (■), 2.8 wt% (●), 4.2 wt% (□) and 7 wt% (○) at 4 wt% Tween 60.

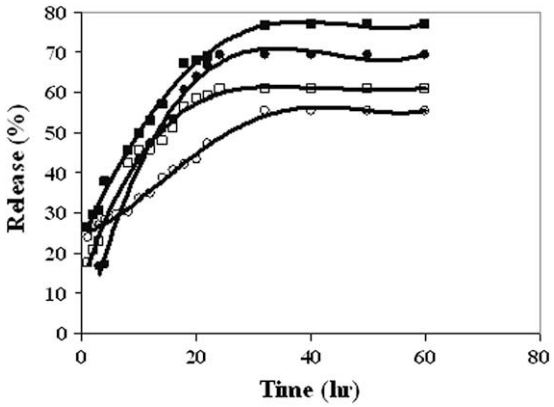


Fig. 9. The effect of the weight percent of the Tween 60 in the external phase on the release rate of penicillin-G from PBA nanocapsules: 2 wt% (■), 4 wt% (●), 8 wt% (□) and 16 wt% (○) at 4.2 wt% Span 60.

of the Span 60, 76.9% of the entrapped penicillin-G was released in the first day approximately.

It was assumed that the burst release phenomenon was due to the release of poorly entrapped and surface-associated penicillin-G or also its diffusion through the porous channels. Surface morphology (Figure 10) of the prepared PBA nanocapsules demonstrated that those with determined amount of surfactants possess a polyporous surface and "sponge-like" structure. So penicillin-G diffused readily through the channels and pores. The initial release of penicillin-G for 4.2–7 wt% of Span (Fig. 9) or 8–16 wt% Tween samples (Fig. 8) was apparently restricted, compared with those of low surfactant content ones. After the initial burst release, there was a lag time of little or no release. The lag time of the penicillin-G release profile depended on the degradation rate of the polymer [33]. Higher encapsulation efficiency and lower initial release are the most important parameters in the development of sustained release of nanocapsules containing water-soluble drugs. Nanocapsules containing penicillin-G and two surfactants with low concentrations tend to have lower encapsulation efficiencies and higher burst release. The encapsulation of penicillin-G can reach up to 60% under optimum formulation conditions and the burst release can also fall below 45%. In this case, the fact that nanocapsules have only 130 nm diameter will be important.

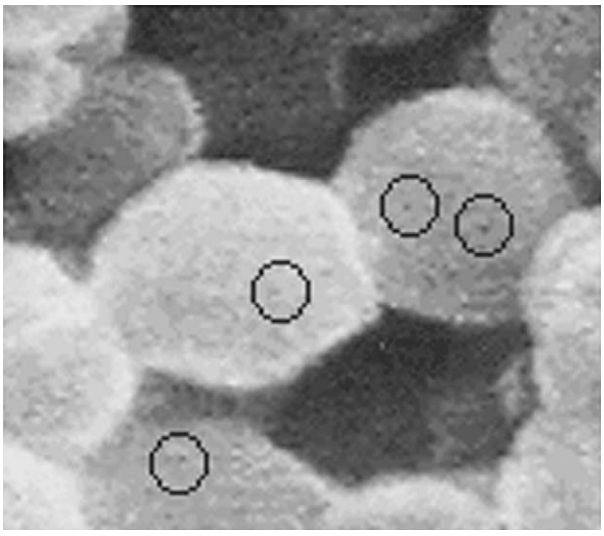


Fig. 10. Typical SEM image of the PBA nanocapsules prepared from double emulsions at 16 and 4.2 wt% of Tween 60 and Span 60, respectively.

4. Conclusion

In this study, polybutyl adipate (PBA) was used as model to investigate the drug loading and release properties of the obtained nanocapsules. Penicillin-G loaded nanocapsules had a spherical shape. Particle size of the products could be decreased up to 75 nm by application of two kinds of surfactants: Span and Tween series. The best penicillin-G encapsulation efficiency could reach to76.8%. This work developed a modified w/o/w double emulsion method for the preparation of penicillin-G loaded nanocapsules by PBA. It was found that by modifying the preparation conditions, such as type and concentration of surfactants, obtaining drug-loaded biodegradable carriers with a high encapsulation efficiency, spherical shape and nanoscale particles will be possible. Penicillin-G was successively encapsulated into the PBA nanocapsules with high drug loading and encapsulation efficiency using the w/o/w emulsion solvent evaporation technique. Addition of Span 20 and 60 to the internal and Tween 20 and 60 to the external aqueous phase of w/o/w emulsions had different effects on improving the properties of the resulting w/o/w emulsions. It was found that the size of nanocapsules prepared from Span 60 and Tween 60 was markedly smaller than that prepared from Span 20 and Tween 20. At a constant concentration of Span 60, a significant increase in the particle size and encapsulation efficiency can be observed by increasing the concentration of Tween 60. It was observed that the higher concentration of Span 60 at a constant concentration of Tween 60 results in higher encapsulation efficiency and makes the formation of small w/o/w emulsion droplets easier and produces smaller nanoparticles. For the sample with 7 wt% Tween 60 and 4 wt% Span 60, the release profile of penicillin-G was accompanied with a small initial burst release and a lag period. The initial burst release was due to the fast surface diffusion of penicillin-G. The lag period was associated with a separation between the initial diffusion and erosion-controlled release period. The length of lag period depends on the rate of polymer degradation. The differences in the formulations could lead to differences in the particle size, encapsulation efficiency and drug loading. Therefore, the initial burst release is influenced by the above formulation parameters.

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