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Original article

Preparation and Evaluation of Poly (ε-caprolactone) Nanoparticles-in-Microparticles by W/O/W Emulsion Method

*[1]Mitra Jelvehgari, [1,2]Jaleh Barar, [1,2]Hadi Valizadeh, [1]Nasrin Heidari

Abstract

Objective(s)
Theophylline, a xanthenes derivative, is still widely used as an effective bronchodilator in the management of asthmatic patients. It is used both as a prophylactic drug and to prevent acute exacerbations of asthma. The aim of study was to formulate and evaluate effect of the microencapsulation of theophylline loaded nanoparticles on the reduction of burst release.

Materials and Methods
Microparticles (simple and composite) and nanoparticles were prepared by using water-in-oil-in-water ($W_1/O/W_2$ double-emulsion solvent diffusion/evaporation method), taking different ratios of drug/polymer. Solvent systems consist of ethyl acetate and dichloromethane for microspheres and nanospheres, respectively. In the current study formulations were characterized by loading efficiency, yield, particle size, zeta potential, X-ray diffraction (XRD) and differential scanning calorimetry (DSC).

Results
In microparticles, the best drug to polymer ratio was 0.8:1 (F_3). F_3 formulation had minimum burst effect (37.81%), high loading efficiency (95.88%). In nanoparticles, F_4 formulation (0.4:1 drug/polymer ratio) showed high production yield (40.8%), loading efficiency (99.05%), low particle size (756 nm) and minimum burst effect compared with other nanoparticle formulations. The drug loaded composite microspheres (F_9) showed minimum burst effect, acceptable release and mean particle size 17.696 μm. The XRD and DSC showed stable character of theophylline in the drug loaded microspheres. The drug release was found to be diffusion and erosion controlled.

Conclusion
The burst was significantly lower with composite microparticles and may be explained by lower diffusion of the drug from double polymeric wall formed by the nanoparticles matrix followed by another diffusion step through the microparticle polymeric wall.

Keywords: Burst Effect, Composite Microparticle, Double-Emulsion, Ethylcellulose, Nanoparticle, Poly (ε-caprolactone)

1- Department of Pharmaceutics, Faculty of Pharmacy, Tabriz University of Medical Sciences, Tabriz, Iran
*****Corresponding author: Tel: +98-411-339-2617; Fax: +98-411-4798; e-mail: jelvehgri@tbzmed.ac.ir
2- Nanotechnology Center, Tabriz University of Medical Sciences, Tabriz, Iran

Introduction

Theophylline, a xanthine derivative, is still widely used as an effective bronchodilator in the management of asthmatic patients. It is used both as a prophylactic drug and to prevent acute exacerbations of asthma (1, 2). A popular method for the encapsulation of water-soluble drugs within water insoluble polymers is the double-emulsion solvent evaporation method (3). The water-soluble drug is dissolved in water and this solution is emulsified in an organic solution of the polymer to be used for the wall material. This primary emulsion is then emulsified in an aqueous phase to form a W/O/W emulsion. After evaporation of organic solvent an aqueous suspension of microparticles is produced. However, most of the microencapsulation techniques have been used for lipophilic drugs, since hydrophilic drugs showed low loading efficiencies. Other workers have used water in oil in water (W/O/W) emulsification in the solvent evaporation process to encapsulate highly water-soluble drugs (3, 4). A further effect of partitioning is the accumulation of drug crystals on the surface of microspheres which produces burst release of the drug on administration (5, 6). One major persistent problem in the development of commonly marketed injectable polymeric delivery systems is the burst release of drug when slow release is needed for some weeks or months. First burst release is one of critical problems in the improvement of controlled release formulations containing drug entrapment microparticles and nanoparticles, particularly with low molecular weight drugs. Hassan *et al* (7) and Lee *et al* (8) have suggested that encapsulation of nanoparticles into polymeric microparticles is a way to reduce the burst. This burst release, is useful in the drug permeation within dermal applications, but is a disadvantage for slow release formulations with drugs that are toxic at high strengths or those that require being released for a long time. Burst release is frequently resulted in polymeric delivery systems, it is not predictable and usually hard to control, but can be stopped by altering the drug dispersion in the polymer matrix (9) or by improving more complicated drug delivery systems. Recent results show that liposoms encapsulated in dextran (10) and alginate microcapsules enable the controlled drug release and removing the burst release (11). Double-walled microspheres (12), double-layered minipellets (13) and coated microspheres (14, 15) have developed to decrease the initial burst and supply drug prolonged release models. Microparticles prepared with blends of polymers characterized by different properties may also modify the release of the drug compared with microparticles prepared from a single polymer. For ibuprofen, the burst was higher for ethylcellulose microparticles than microparticles prepared with a blend of ethylcellulose and polystyrene when they were compared (16). Currently, the encapsulation of hydrophilic drug inside biodegradable polymer as poly (-ϵ-caprolactone)-based were improved by using solvent evaporation methods (17). The use of poly-ϵ-caprolactone (PCL), aliphatic semi-crystalline polyester, has been resulted in controlled drug release. When caprolactone was used alone led to the controlled release of the drug encapsulated for more than a month (18). However, the degree of degradation of PCL will generally depend upon the nature of the polymer (hydrophobicity and semi-crystalline type), and is more than polymers based on poly (lactic acid) (PLA) derivatives. The degradation of this polymer (PCL) includes a bulk erosion procedure (19-20).

Size and release properties of microspheres are the key considerations to design suitable microsphere delivery systems (5). Since the release kinetics of drug dominantly depends on polymer nature, the physical states of the polymer and drug (e.g. crystalline, amorphous, glassy, rubbery, and molecularly dispersed) are of major importance for underlying drug release mechanism (1, 17). Morphology and drug distribution within microspheres, fundamental understanding of the relationship among these key characteristics and release mechanisms are essential to yield useful products (21). For example, within an amorphous polymer the diffusion coefficient of a drug is much higher compared to that within a crystalline polymer (1, 17).

The aim of the present work was to encapsulate, using the W/O/W emulsion method, polymeric nanoparticles into polymeric microparticles by biodegradable polymer, poly (-ε-caprolactone)-based microparticles and water insoluble polymer (ethylcellulose). In addition, drug crystallinity in the microspheres, interaction between drug and polymer were evaluated by powder X-ray diffraction analysis (XRD) and differential scanning calorimetry (DSC), respectively.

Materials and Methods

Materials

Theophylline (Merck, Germany), poly (ε-caprolactone) (MW 40,000 Da) was supplied by Aldrich, USA. An acrylic polycationic nonbiodegradable polymer (copolymers of acrylic acid esters with a low content of quaternary ammonium groups (o.5-0.8%) (4.48-6.77% ammonium methacrylate units by dry weight), poly (vinyl alcohol) (PVA) (MW 95000-110000 Da) was supplied by Aldrich, USA, ethylcellulose powder (viscosity 7 Cp) (Aldrich, USA), ethyl acetate, methylene chloride, hydrochloric acid, potassium hydrogen phosphate and sodium hydroxide were obtained from Merck (Darmstadt, Germany). All solvents and reagents were of analytical grade.

Experimental Methods

Preparation of particles (classical microparticle)

Theophylline-loaded ethylcellulose microparticles were prepared by the $W_1/O/W_2$ emulsion solvent extraction method, using different ratios of drug to polymer (0.4:1, 0.6: 1 and 0.8: 1 as shown in Table 1) (8, 9). In the first step, an aqueous solution (1 ml) of drug used as the internal aqueous phase was emulsified into an organic solution of the polymer (ethylcellulose 250 mg) in ethyl acetate (5 ml) by homogenizer with 22000 rpm. At 2 min later, the primary emulsion was poured into 20 ml of 0.1% PVA aqueous solution in order to obtain a $W_1/O/W_2$ pre-emulsion. After magnetically stirring for 1 min (1000 rpm) at room temperature, this pre-emulsion was added to 400 ml of a 0.1% PVA aqueous solution and stirred mechanically (three-blended propeller, 1600 rpm) for 10 min to form the final $W_1/O/W_2$ emulsion and allow microparticle hardening. Upon solvent extraction, the polymer precipitated and microparticle cores were solidified. Microparticles were collected by vacuum filtration (Heidolph, USA) and freeze-drying (9). Blank microparticles were prepared under the same conditions, without drug.

Preparation of nanoparticles (NP)

Theophylline -loaded PCL and ethylcellulose microparticles were prepared by the $W_1/O/W_2$ solvent evaporation method taking different ratios of drug to polymer (0.4:1, 0.6: 1 and 0.8: 1 as shown in Table 2). Briefly, 1 ml of aqueous internal phase was emulsified for 15 sec in 5 ml of methylene chloride (containing 125 mg of PCL and 125 mg ethylcellulose) using homogenizer with 22000 rpm. This primary emulsion was poured into 40 ml of a 0.1% PVA aqueous solution while stirring using a homogenizer for 1 min under the same conditions in order to create the water-in-oil-in-water emulsion. Three to four ml of NP suspension was obtained after solvent evaporation under reduced pressure (Evaporator, Heidolph, USA). Nanoparticles were separated from the bulk suspension by centrifugation (Hettich universal 320R, USA) at 42,000×g for 20 min. The supernatant was kept for drug assay as described later and the sediment

Table 1. Theophylline polymeric microspheres prepared by double-emulsion solvent extraction ($W_1/O/W_2$).

Formulations	Drug/ Polymer ratios	Initial emulsion (W_1/O)				Secondary aqueous phase (W_2)
		Initial aqueous phase (W_1)		Organic phase (O)		
		Water (ml)	Theophylline (mg)	Ethylcellulose (mg)	Ethyl acetate (ml)	Polyvinyl alcohol (0.1%) (ml)
F_1	0.4:1	1	100	250	5	420
F_2	0.6:1	1	150	250	5	420
F_3	0.8:1	1	200	250	5	420

nanoparticles were redispersed in 3 ml of purified water before freeze-drying. After lyophilization, the dried nanoparticles were resuspended in 2 ml of purified water shortly before preparing the composite microparticles. Blank nanoparticles were prepared under the same conditions, without drug (8, 22).

Preparation of composite microparticle

Microparticles containing theophylline PCL and ethylcellulose nanoparticles, called composite microparticles were prepared under the same conditions of simple microparticles preparation method, but in primary emulsion (W_1/O): PCL and ethylcelulose NP suspension (2 ml) used as the internal aqueous phase (instead of 2 ml of drug aqueous solution) was emulsified in an organic solution of polymer in ethyl acetate. Blank composite microparticles were prepared under the same conditions without drug (8).

Viscosity measurement

A Brookfield rotational digital viscometer DVLV-II was used to measure the viscosity (cPs) of the internal and external phases at 25 ºC. The spindle number 1was rotated at 100 rpm.

Particle size analysis

A laser light scattering particle size analyzer (SALD-2101, Shimadzu, Japan) was used to determine the particle size of the drug, nanoparticles and microparticle formulations. Samples were suspended in distilled water contained in a 1 cm cuvette and stirred continuously during the particle size analysis. Each sample was measured in triplicate.

Zeta potential analysis

Zeta potential is an abbreviation for electrokinetic potential in colloidal systems. Zeta potential is electric potential in the interfacial double layer (DL) at the location of the slipping plane versus a point in the bulk fluid away from the interface (22).

Determination of drug loading, loading efficiency (%) and production yield

The drug concentration in polymeric particles was determined spectrophotometrically (UV-160, Shimadzu, Japan) at 271.6 nm by measuring the amount of non-entrapped theophylline in the external aqueous solution (indirect method) before freeze-drying. In the case of nanoparticles, the external aqueous solution was obtained after centrifugation of the colloidal suspension for 20 min at 42,000×g. A standard calibration curve was performed with the theophylline solution (aqueous solution of 0.1% PVA).

The loading efficiency (%) was calculated according to the following equation:

Loading efficiency (%) = (actual drug content in microparticles/theoretical drug content)× 100

The production yield of the microparticles was determined by calculating accurately the initial weight of the raw materials and the last weight of the polymeric particles obtained. All the experiments were performed in triplicate.

X-ray powder diffractometry (X-RPD)

X-ray diffraction analysis was performed with an apparatus (Siemens D5000, Munich, Germany), using nickel-filtered CuKα radiation radiation (a voltage of 40 KV and a current of

Table 2. Theophylline nanoparticle formulations prepared by double-emulsion solvent evaporation method (W_1/O/W_2).

Formulations	Drug/polymer ratio	Initial emulsion (W_1/O)					Secondary aqueous phase (W_2)
		Initial aqueous phase (W_1)		Organic phase (O)			
		Water (ml)	Theophylline (mg)	Ethylcellulose (mg)	Poly (ε-caprolactone) (mg)	Methylene chloride (ml)	Polyvinyl alcohol (0.1%) ml
F_4	0.4:1	1	100	125	125	5	40
F_5	0.6:1	1	150	125	125	5	40
F_6	0.8:1	1	200	125	125	5	40

20 mA). The scanning rate was 2˚ /min over a range of 20-60˚ and with an interval of 0.02˚.

Differential Scanning Calorimetry (DSC)

Differential scanning calorimetry (DSC) plots were obtained, using Dynamic DSC (DSC 60, Shimadzu, Japan). An empty pin-holed aluminum pan was used as a reference. Both the reference pan and the sample pan were allowed to equilibrate isothermally for 5 min at 0 ˚C. In an open aluminum pan under a 10 ml/min stream of nitrogen purge, samples of 5 mg were heated from room temperature to 300 ˚C at a heating rate of 10˚C/min.

In vitro release studies

Microparticles

Theophylline dissolution patterns from microparticles were obtained under sinking conditions. USP rotating basket method was used for all microsphere formulations. A set amount of microspheres (100, 150 and 200 mg drug) was added to 900 ml dissolution medium (pH 1.2 HCl solution), preheated and maintained at 37±1 ˚C in a water bath, then stirred at 100 rpm. Aliquots (5 ml) of the solution were withdrawn at appropriate intervals (0.25, 0.5, 0.45, 1, 2, 3, 4, 5, 6, 8, 24 hr) through a 0.45 μm filter and replaced with an equal volume of fresh test fluid to keep the volume constant. After 2 hr, 17 ml of 0.2 M phosphate buffer stock, pre-equilibrated at 37 ˚C, were added to the dissolution vessel. The pH was immediately adjusted, if necessary, with 0.2 N HCl or 0.2 N NaOH to pH 7.4. Samples were suitably diluted with the same fluid, and drug concentration was measured by UV analysis at 207.6 nm (for acidic medium) or 208 nm (for alkaline buffer). Each experiment was performed in duplicate and a close reproducibility was attained.

Nanoparticles and composite microparticles

Nanoparticles or freez dried composite microparticles containing 100, 150 and 200 mg were suspended in 20 ml of phosphate buffer (pH 7.4). The suspension was stirred (200 rpm) at 37 °C into a water bath. Then 1 ml of suspension was withdrawn at appropriate intervals (0.25, 0.5, 0.45, 1, 2, 3, 4, 5, 6, 8, 24 hr) and, each sample was centrifuged at 42,000×g for 10 min. The filtrate (theophylline) was replaced by 1 ml of fresh buffer. The amount of theophylline in the release medium was determined by UV at 208 nm (8).

Statistical analysis

Results were evaluated using a one-way ANOVA (SPSS version 14), where $P< 0.05$ was taken to represent a statistically significant difference (23).

Results

A W/O/W multiple emulsion solvent evaporation/extraction method is mostly used for the encapsulation of water-soluble drug and therefore, was the method of choice for the water-soluble theophylline drug. In microparticles prepared by extraction method, the amount of drug entrapped in microspheres was lower than the theoretical value. In all formulations, the mean amount of drug entrapped increased from 0.4:1 to 0.8:1, the production yield increased ($P< 0.05$). Size of microspheres increased with an increase in the drug concentration ($P< 0.05$) (Table 3).

Table 3. Effect of drug: polymer ratios on drug loading efficiencies, production yield s and particle sizes of theophylline microparticles, nanoparticles and composite microparticles.

Mean particle size (μm±SD)	Drug loading efficiency (%±SD)	Mean drug entrapped (%±SD)	Theoretical drug content (%)	Production yield (%±SD)	Polymer/ drug ratio	Formulation code	Process variable
18.29±0.31	79.24±3.56	22.67±3.75	28.57	39.06±2.11	0.4:1	F_1	
19.48±0.42	94.31±8.45	35.32±2.51	37.50	41.20±3.14	0.6:1	F_2	Microparticle
26.44±0.31	95.88±7.06	42.61±1.38	44.44	47.79±2.19	0.8:1	F_3	
0.756±0.16	99.05±7.18	28.3±3.11	28.57	40.85±7.21	0.4:1	F_4	
0.857±0.253	99.07±5.46	37.5±5.15	37.50	3.46±41.25	0.6:1	F_5	Nanoparticle
0.959±0.29	98.29±6.32	43.68±3.35	44.44	5.32±45.91	0.8:1	F_6	
8.69±0.29	98.80±5.23	16.47±1.21	16.67	36.67±3.02	0.2:1	F_7	Composite microparticle
17.47±0.671	97.36±6.43	22.47±1.48	23.08	36.12±4.58	0.3:1	F_8	
17.70±0.59	95.03±8.12	27.15±1.27	28.57	35.48±5.08	0.4:1	F_9	

A volume-based size distribution of drug, polymer, and drug in prepared nanoparticles and composite microparticles was near to the theoretical value, as the drug loading efficiency is almost 100%. As the ratio of drug to polymer increased the amount of free drug lost decreased (Table 3) so that at the ratio of drug to polymer 0.8:1 the amount of drug entrapment was 42.61% which was very close to the theoretical value (44.44%). Generally, increasing the drug-polymer ratio increased the production yield, when the ratio of drug–polymer loaded microparticles and nanoparticles indicated a log-probability distribution. Mean particle size of original theophylline and ethylcellulose was 51.206±0.474 µm and 2.53±0.179 µm, respectively. Mean particle size of F_3 was 26.44±0.31 µm. The data describing the particle sizes of the microspheres are given in Tables 3. As it can be seen, the sizes of particles were increased with an increase in the amount of the drug. The zeta potential of three nanosphere formulations, theophylline (-16.5 mV), ethylcellulose (-13.9 mV) and poly (ε-caprolactone) (+36 mV) are shown in Table 4. Blank nanoparticles had negative charge.

Drug-loaded nanoparticles indicated a positive charge, because poly (ε-caprolactone) was a polycationic polymer and changed the charge of nanoparticles. The endothermic peak of the pure drug was observed at about 271.41 °C (Figure 1) and ethylcellulose showed an amorphous state. Indeed, in the thermogram of the nanoparticles containing poly (ε-caprolactone), there was endothermic peak at 59.12 ˚C. However, in the thermogram of the microparticles (simple and composite) and nanoparticles there was endothermic peak of the drug melting, but in the thermogram of the composite microparticles, there was no endothermic peak of the poly (ε-caprolactone). In the thermogram of nanoparticles, there was no endometric peak of theophylline. The X-ray diffraction patterns of pure drug and poly (ε-caprolactone) showed that the pure drug and poly (ε-caprolactone) are crystalline in nature (Figure 2).

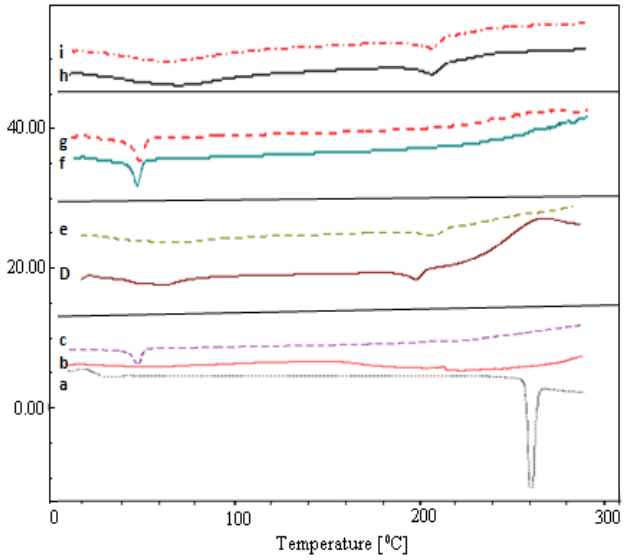

Figure 1. DSC thermograms of a) theophylline; b) ethylcellulose; c) poly (ε-caprolactone); d) simple microparticles; e) blank microparticles; f) nanoparticles; g) blank nanoparticles; h) composite microparticles; i) blank composite microparticles.

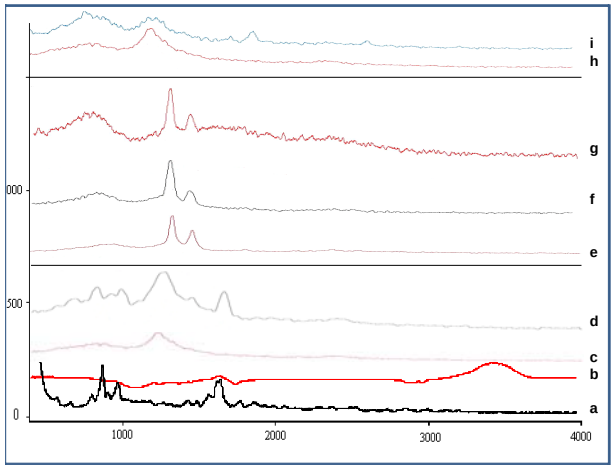

Figure 2. X-ray diffractions of a) theophylline; b) ethylcellulose; c) blank microparticles; d) simple microparticles; e) blank nanoparticles; f) poly (ε-caprolactone); g) nanoparticles; h) blank composite microparticles; i) composite microparticles.

Table 4. Effect of drug to polymer ratio of theophylline on the zeta potential of the nanoparticles.

Samples	Zeta potential (mV)
Theophylline	-16.5
Ethylcellulose	-13.9
Poly (ϵ-caprolactone)	+36
F_4	-11.4
F_5	-12.3
F_6	-12.5
Blank nanoparticle	- 4.8

In vitro release studies

The *in vitro* release of theophylline from microparticles and nanoparticles exhibited an initial burst effect, which may be due to the presence of some drug particles on the surface of the microparticles and nanoparticles. The release profiles are illustrated in Figure 3. Theophylline always shows an expected increase in dissolution after the change of pH from 1.2 to 7.4. For microparticles, dissolution of theophylline at pH 7.4 strongly showed an initial burst effect. The results indicated that some factors such as a drug-polymer ratio governed the drug release from these microparticles. The results indicated that some factors such as a drug-polymer ratio governed the drug release from these microparticles. Drug release rates were decreased with increasing amounts of theophylline in the formulation (Figure 3A). PCL-loaded nanoparticles of each formulation displayed an immediate and important initial drug release in the first 15 min, followed by duration of 20-35% release in 24 hr which was obtained (Figure 3B). The initial percentage of drug release and dissolution profiles was very different for all types of microparticles compared with nanoparticles, as shown in Figure 3C. However, composite microparticles tend to reduce the initial burst effect especially compared with microparticles prepared from ethylcellulose alone (simple microparticles).

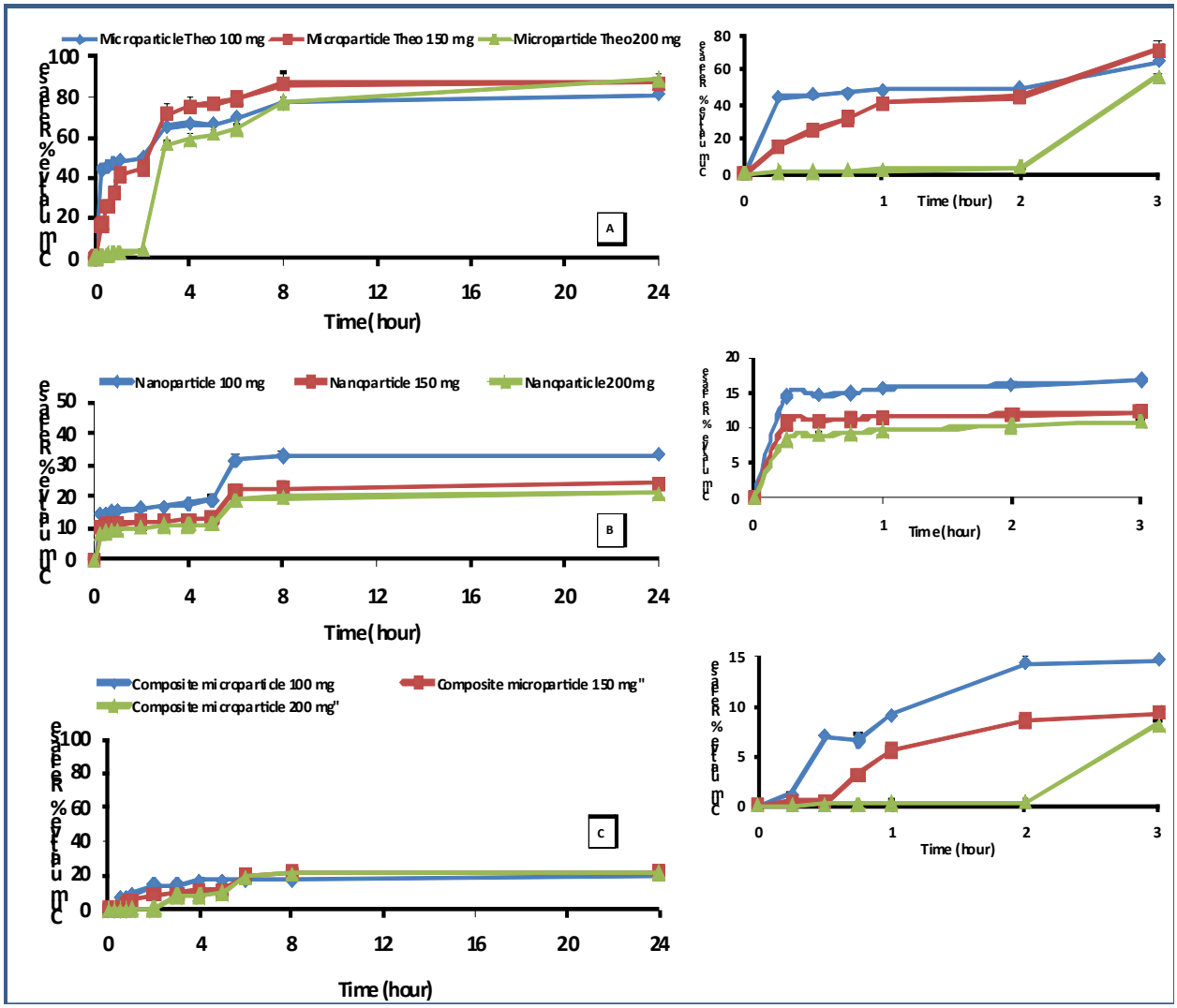

Figure 3. Cumulative percent release of theophylline from A) simple microspheres; B) nanoparticles; C) composite microparticles prepared with different drug-to-polymer ratio.

Discussion

Drug entrapped in microspheres indicated that some free drug crystals were lost in the process of encapsulation and lost the drug when immigrated to aqueous phase. The encapsulation efficiency of the drug was depended on the solubility of the drug in the solvent and continuous phase. A similar observation was reported by Youan *et al* (24). Important prerequisites for high encapsulation efficiencies by the W/O/W method are: (1) the insolubility of the drug in the external phase from the internal aqueous phase, and (2) the fine dispersion of the aqueous drug solution into the organic to form a W/O emulsion (25, 26). Theophylline is insoluble in organic solvents used to dissolve the polymer (methylene chloride or ethyl acetate) and thus cannot partition from the internal into the external aqueous phase via diffusion through the organic polymer solution. In order to obtain a fine dispersion, the aqueous theophylline solution was added to the organic. The encapsulation efficiencies increased from about 30% without shaking to more than 90% (7). Despite the hydrosolubility of theophylline, which favors the leakage of the drug into the external aqueous phase, entrapment efficiencies were rather high. It is assumed that theophylline is localized at the interfaces (either internal water in oil or external oil in water). Therefore, a significant amount of drug is supposed to be adsorbed at the outer surface. With increasing theophylline concentration, there is a saturation of the outer surface. At that time, the drug will dissolve more in the aqueous core and the encapsulation efficiency will also consequently increase (8). In addition, the removal of the organic solvent under reduced pressure favors its fast evaporation followed by the polymer precipitation, thus reducing the migration of the drug to the external phase. Indeed, the faster the solvent evaporation is, the higher the encapsulation efficiency will be. One possible explanation could concern the increase of the internal phase viscosity due to the different theophylline concentrations studied which could reduce the leakage of the drug towards the external aqueous phase (8).

The reason for increased production yield at high drug: polymer ratios could be due to decreased diffusion rate of solvent (ethyl acetate) from concentrated solutions into initial emulsion (Table 3). Size of microspheres can be attributed to the fact that with a higher diffusion rate of non-solvent to the polymer solution the smaller size of microcapsules is easily obtained (27, 28). It has already been reported that particle size was proportional to the viscosity of the dispersed phase (29-32). In fact viscosity of the dispersed phase was increased from F_1 (0.4:1) to F_3 (0.8:1). When the viscosity of the dispersed phase of these formulations was investigated it was found that particle sizes of microparticles were directly proportional to the apparent viscosity of dispersed phase. The results showed that the apparent viscosities of the different drug: polymer ratios (0.4:1, 0.6:1 and 0.8:1) were 8, 14 and 17 mPa.S (mega Pascal Second) respectively. When the dispersed phase with higher viscosity was poured into the dispersion medium, bigger droplets were formed with larger mean particle size.

Addition of a cationic polymer could lead to the flocculation of yeast cells thus forming macroscopic flocs (33). Flocculation occurs by two main mechanisms (a) formation of macromolecular bridges between the particles, and (b) surface and charge reduction due to the adsorption of highly charged polyelectrolytes on oppositely charged particles (33). In other words, zeta potential is the potential difference between the dispersion medium and the stationary layer of fluid attached to the dispersed particle. A value of zeta potential (positive) could be taken as the arbitrary value that separates low-charged surfaces from highly-charged surfaces. The significance of zeta potential is that its value can be related to the stability of colloidal dispersion. The zeta potential indicates the degree of repulsion between adjacent, similarly charged particles in dispersion. For molecules and particles that are small enough, a high zeta potential will confer stability, i.e. the solution or dispersion will resist aggregation (22).

The drug may have been dispersed in a crystalline or amorphous form or dissolved in the polymeric matrix during formation of the

microspheres. Any abrupt or drastic change in the thermal behavior of either the drug or polymer may indicate a possible drug-polymer interaction (34).

However, endothermic peak of the drug melting of the microparticles (simple and composite) and nanoparticles, suggests the crystalline state of the drug in the polymeric matrix but in the thermogram of the composite microparticles, presenting the amorphous state of the poly (ε-caprolactone) in the composite microparticles (Figure 1) (35). The thermogram of nanoparticles, showed that drug had been dispersed in amorphous form in the polymeric matrix during formation of the nanoparticles (Figure 1).

However, when they were incorporated into the polymeric matrix, the principal peaks of the drug existed or could be observed with less intensity. Figure 2 shows the amorphous state of the poly (ε-caprolactone) in the composite microparticles and confirms the results obtained from DSC.

In most cases, a biphasic dissolution profile was observed at pH 7.4: the initial rapid drug leakage generally ended very early and for the remaining time, nearly linear behavior was observed. It showed that the first portion of the curves is due to theophylline dissolution, which starts immediately after the beginning of the test for the portion of drug on the surface of microparticles. After such a phase, two phenomena can combine in enhancing the diffusion of the remaining dispersed drug into the bulk phase as well as the formation of pores within the matrix due to the initial drug dissolution; particle wetting and swelling which enhances the permeability of the polymer to the drug (36) (Figure 3). As more drugs are released from the microparticles, more channels are probably produced, contributing to faster drug release rates. However, Figure 3A shows that the burst effect is lower when the theophylline to polymer ratio is 0.4:1 (F_1) compared with 0.8:1 (F_3). In F_3 formulation, an increase of the internal phase viscosity due to the different theophylline concentrations could reduce the leakage of the drug towards the external aqueous phase and decrease the burst effect and release rate (compare with F_1 and F_2). This immediate high burst release might be due to the small diameter of nanoparticles leading to a large exchange surface and probably to a more porous structure owing to the solvent evaporation method, favoring the release of the encapsulated drug (2, 8). Indeed, it has already been demonstrated that the slow precipitation of microparticles after solvent evaporation leads to more porous particles compared to the fast polymer precipitation obtained after solvent extraction (8, 15). Although not all of encapsulated drug was released in 24 hr, the dissolution test was limited to this time because the aim of this research was to demonstrate the influence of the encapsulation of nanoparticles within microparticles on the initial burst release. Moreover, the burst release could also be explained by the imperfect encapsulation of the drug inside nanoparticles, resulting from the unstable nature of the emulsion droplets during the solvent removal step.

The incomplete release of the drug from each formulation could result from the rather short time of the release study. This initial release was followed by a rather stable plateau between 2 and 6 hr. The interactions between theophylline and the polymers may slow down the drug release or hamper its complete dissolution. Our results are consistent with Okada *et al* who reported an increase in Tg of PLGA microspheres (as observed with EC and PLGA nanoparticles) with increasing basic drug content, implying an incomplete and slower drug release (37). Although the increase in Tg correlated with a decrease in chain mobility was predominant (made the matrix more rigid).

Usually when there are strong interactions between the drug and the polymer, the Tg is significantly decreased. In addition, although the melting temperature of the drug decreased significantly after encapsulation, its crystalline state was maintained, probably as very small and unstable crystals. It has to be noted that in the order studies, the melting temperature of the encapsulated drug was totally disappearing from the DSC runs, meaning a transformation to an amorphous state.

Reduction in the initial burst effect can be explained not only by the rather hydrophilic properties of theophylline which favors it's diffusion towards the surrounding aqueous media, but also by high encapsulation ratio of PCL nanoparticles (8, 17). Encapsulation of nanoparticles into microparticles also had a strong effect on the dissolution profiles. The presence of ethylcellulose in the matrix of microparticles conferred a slower and more progressive release of theophylline during the time of the experiment (1, 8). This is indeed, due to the slow diffusion of water into the lipophilic ethylcellulose matrix (2, 8).

However, in terms of burst release reduction there is a difference between simple and composite microparticles (Table 5). When PCL nanoparticles were encapsulated into the microparticles (Figure 3C), there was a large decrease in burst release again (0.23-1.16%), this decrease is much more marked ($P< 0.05$). Therefore, the advantage of encapsulating nanoparticles in microparticles (composite microparticles) has been definitely demonstrated for the hydrophilic drug (8, 17). The *in vitro* release profiles were fitted on various

Table 5. Mean of theophylline released after 0.25 hr and 24 hr from simple microparticles, nanoparticles and composite microparticles.

Formulation	$^{a}Q_{0.25}$	$^{b}Q_{24}$
F_1	0.44±44.32	4.05±81.10
F_2	0.14±16.31	3.40±86.84
F_3	0.03±12.39	2.55±89.47
F_4	0.04±14.54	0.36±33.32
F_5	0.05±10.64	1.82±24.15
F_6	0.14±8.33	1.77±21.18
F_7	0.08±1.16	0.82±20.31
F_8	0.02±0.4	0.54±21.87
F_9	0.02±0.23	1.98±22.01

a: Amount of drug release after 0.25 hr, b: amount of drug release after 24 hr.

Table 6. Parameters of the *in vitro* release data fitted to various release kinetic models for nanoparticles and composite microparticles.

Order		F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
Zero f=kt	K	0.0003	0.0005	0.0005	0.0003	0.0002	0.0001	0.0001	0.0001	0.0002
	RSQ	0.6159	0.5343	0.6216	0.5811	0.6075	0.6525	0.4336	0.5852	0.6233
	D (SS)%	982.3664	892.4038	841.2354	499.8675	513.8522	848.0612	901.9522	647.9950	629.9116
First Ln (1-f)=kt	K	0.0008	0.0022	0.0015	0.0003	0.0002	0.0001	0.0001	0.0002	0.0002
	RSQ	0.7287	0.8983	0.8636	0.5919	0.6197	0.6610	0.4535	0.5988	0.6335
	D (SS)%	822.1482	529.4631	569.0389	556.7263	513.0387	818.7032	879.9270	628.5015	604.6704
Peppas Lnf=lnk+blnt	B	0.0575	0.5044	0.5905	1.1543	1.0642	0.3587	0.5275	0.9765	0.8992
	K	0.3790	0.0451	0.0230	0.0002	0.0002	0.0170	0.0078	0.0005	0.0007
	RSQ	0.9778	0.9282	0.9827	0.8737	0.8556	0.8973	0.7257	0.8109	0.8324
	D (SS)%	2.4625	41.9376	51.4170	623.2251	637.9919	143.1441	419.5801	674.4713	566.7799
Higuchi $f=kt^{0.5}$	K	0.0126	0.0250	0.0251	0.0125	0.0088	0.0054	0.0052	0.0073	0.0072
	RSQ	0.8361	0.7878	0.8570	0.7652	0.7919	0.8149	0.6940	0.8033	0.8121
	D (SS)%	791.4635	450.3528	271.2409	4018.7570	3177.4460	372.2460	530.9801	1762.7658	1457.8144

kinetic models, in order to find out the mechanism of drug release (38, 39). The fit parameters to Higuchi, first-order, Peppas and zero-order equations are given in Table 6. The rate constants were calculated from the slope of the respective plots. High correlation was observed for the Peppas model. The data obtained were also put in Korsemeyer-Peppas model, in order to find out then value, which described the drug release mechanism. The n value of composite microspheres with different drug to polymer ratio was between $0< n<0.5$, indicating that the mechanism of the drug release was diffusion controlled

Conclusion

More generally, the differences observed with the composite microparticles might be explained by the heterogeneous composition of polymeric matrix. A fast rate of solvent removal can also contribute to a heterogeneous distribution of drug within the internal phase as it hardens which would further explain the biphasic release profile. Indeed, in order to be released into the external dissolution medium, theophylline has to diffuse first through the PCL nanoparticles followed by another diffusion step through the ethylcellulose. The diffusion pathway takes longer due to the hydrophobicity of this polymer. The overall dissolution profiles show the potential of composite microparticles to dramatically change the burst effect and release profile of drug *in vitro*.

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