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Preparation of Diclofenac Sodium Composite Microparticles with Improved Initial Release Property

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Abstract. *The aim of this study is the evaluation of the effect of microencapsulation of nanoparticles in composite microparticles on the reduction of burst release. Microparticles (simple and composite) and nanoparticles were prepared by using water-in-oil-in-water ($W/O_1/W_2$ double-emulsion solvent diffusion/evaporation method), using different drug/polymer ratios. For preparation of the composite microparticle, nanoparticle suspension was used as the internal phase. In this investigation, the microparicle, nanoparticle and composite microparticle formulations prepared were characterized by loading efficiency, yield, particle size, zeta potential, XRD (X-ray Diffractometry), FTIR (Fourier Transform Infrared Spectroscopy), DSC (Differential Scanning Calormetry) and drug release. The best drug of the polymer ratio in the microparticle and nanoparticle were F_3 (0.4:1) and NP_1 (0.1:1), which showed 26.89% and 9.07% of entrapment, loading efficiency 94.2 %, 99.44% and mean particle size 13.114 µm and 756 nm, respectively. The drug loading microparticle, COM_3 (nanosuspension with 0.2.:1 drug/polymer ratio), showed 28.56% of entrapment, loading efficiency 99.96% and mean particle size 13.013 µm. The burst was significantly lower with composite microparticles and may be explained by the slower diffusion of the drugs through the double polymeric wall formed by the nanoparticle matrix, followed by another diffusion step through the microparticle polymeric wall.*

Keywords: *Composite microparticle; Nanoparticle; Diclofenac sodium; Burst release; Poly(ε-caprolactone).*

INTRODUCTION

Several techniques can be used to prepare polymeric microparticles, providing controlled drug delivery [1]. Most commonly, organic solvent evaporation and/or extraction methods are applied. Depending on the solubility of the drug, simple or multiple emulsion techniques, e.g. oil-in-water (O/W) and water-in-oil-in-water (W/O/W) methods, are used [2]. A popular method for the encapsulation of water-soluble drugs within water insoluble polymers is the double-emulsion solvent diffusion method [3]. In these works, 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. The organic solvent diffuses into the external water phase and evaporates at its surface. The main disadvantage of this method is its limited ability to encapsulate hydrophilic drugs, as partitioning into the aqueous phase of the emulsion readily occurs [3,4]. A further effect of partitioning is the accumulation of drug crystals on the surface of microparticles, which produce a burst release of the drug upon administration [5,6]. Burst release is often observed with microparticulate systems; it is unpredictable and generally difficult to control, but may be prevented by changing the drug distribution within the polymer matrix [7] or by developing more sophisticated drug delivery systems. Examples of the latter are liposomes encapsulated inside dextrin [8], and alginate microcapsules, allowing the release of the drug in a controlled way and eliminating the burst effect [9]. Double-walled microparticles [10],

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double-layered minipellets [11] and coated microparticles [12,13] have all been developed to reduce the initial burst and provide sustained release profiles of the drug. Indeed, the release of ibuprofen from microparticles prepared with a blend of ethylcellulose and polystyrene was prolonged over 24 h with a reduced burst, compared with microparticles prepared with ethylcellulose alone [14].

Recently, solvent evaporation methods were developed to incorporate a hydrophilic drug within biodegradable, poly(-ε-caprolactone)-based microparticles [1].

The aliphatic semi-crystalline polyester, poly-ε-caprolactone (PCL), has been used in the field of controlled drug release. When used alone, PCL produces controlled release over extended periods of up to 1 month [15]. However, due to its hydrophobic and semi-crystalline nature, the degradation of PCL is much slower than established polymers based on poly(lactic acid) (PLA) derivatives. PCL polymer tends to produce drug-loaded microparticles with an initial burst drug release. The degradation of these polyesters involves a bulk erosion process [5,16,17].

Since bulk erosion can accelerate the diffusion and release of the drug, the drug release mechanism based on these polyesters is quite complicated. An unpredictable release profile brought about by the bulk erosion may lead to a lack of control in drug release [5,16,17]. Polyesters such as PCL have a very slow degradation rate, especially at low temperatures; it is a challenge to design a microsphere-controlled-release matrix based on these polyesters for marine fish applications [5].

The size and release properties of microparticles are key considerations when designing microsphere delivery systems [5], since the release kinetics of the drug dominantly depend on the polymer nature. The physical states of the polymer and drug (e.g. crystalline, amorphous, glassy, rubbery, and molecularly dispersed) are of major importance for the underlying drug release mechanism [1,2].

The morphology and drug distribution within microparticles and a fundamental understanding of the relationship between these key characteristics and release mechanisms is essential to yield useful products [5]. For example, within an amorphous polymer, the diffusion coefficient of a drug is much higher, compared to that within a crystalline polymer [1,2].

The major objective of the present study was to incorporate a hydrophilic drug within non-degradable microparticles using non-water soluble polymers and an appropriate organic solvent for the preparation of these composite microparticles. The microparticles containing nanoparticles, called composite microparticles, were characterized physicochemically (encapsulation efficiency, mean particle size, release kinetic) and compared with nanoparticles and simple microparticles prepared by the same double emulsion method. In addition, the drug crystalline in the microparticles and the interaction between the drug and polymer were evaluated by powder X-Ray Diffraction analysis (XRD) and Differential Scanning Calorimetry (DSC), respectively.

MATERIALS AND METHODS

Diclofenac sodium (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 0.5-0.8%) (4.48-6.77% ammonium methacrylate units by dry weight), polyvinyl alcohol (PVA) (Mw 95000-110000 Da), was supplied by Aldrich, USA. Ethylcellulose powder (viscosity 7 Cp) (Aldrich, USA), ethyl acetate, methylene chloride, buffer phosphate and buffer phosphate saline (pH 7.4) were obtained from Merck (Darmstadt, Germany). All solvents and reagents were of analytical grade.

Methods

Preparation of Particles (Simple Microparticle)

Diclofenac sodium-loaded ethylcellulose was prepared by the $W_1/O/W_2$ emulsion solvent extraction method using different ratios of drug to polymer (0.1:1, 0.2:1 and 0.4:1). [7,18]. In the first step (W_1/O emulsion), an aqueous solution (1 ml) of the 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 a homogenizer with 22000 rpm. After 2 minutes, 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. Blank microparticles (without the drug) were prepared under the same conditions as without the drug. Microparticles were collected by vacuum filtration (Heidolph, USA) and freeze-drying [18].

Preparation of Nanoparticles (NP)

Diclofenac sodium-loaded PCL were prepared by the $W_1/O/W_2$ solvent evaporation method using different ratios of drug to polymer (0.1:1, 0.2:1 and 0.4:1). Briefly, 1 ml of aqueous internal phase was emulsified for 15 s in 5 ml of methylene chloride (containing 125 mg of PCL and 125 mg ethylcellulose) using a

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 the oil-in-water emulsion. Three to four ml of NP suspension were 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 nanoparticles were redispersed in 3ml of purified water before freeze-drying. After lyophilization, the dried nanoparticles were resuspended in 2ml of purified water shortly before preparing the composite microparticles. Blank nanoparticles (without the drug) were prepared under the same conditions without the drug [18,19].

Preparation of Composite Microparticle

Microparticles containing diclofenac sodium PCL and ethylcellulose nanoparticles, called composite microparticles, were prepared under the same conditions as the simple microparticle preparation method, but in primary emulsion (W_1/O), PCL and ethylcellulose 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 (without drug) were prepared under the same conditions without drug [18].

Determination of Loading Efficiency and Production Yield (%)

The drug concentration in polymeric particles was determined spectrophotometrically (UV-160, Shimadzu, Japan) at 224.6 nm by measuring the amount of non-entrapped diclofenac sodium 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 diclofenac sodium solution (aqueous solution of 0.1% PVA).

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

$$\text{Loading efficiency (\%)} = \left(\frac{\text{actual drug content in microparticles}}{\text{theoretical drug content}}\right) \times 100.$$

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 experiments were performed in triplicate.

Particle Size Analysis

A laser light scattering particle size analyzer (SALD-2101, Shimadzu, Japan) was used to determine the particle size of the drug, and nanoparticle and microparticle formulation. 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. Zeta potential is not measurable directly but it can be calculated using theoretical models and an experimentally-determined electrophoretic mobility or dynamic electrophoretic mobility. Mobility is defined as the velocity of a particle per electric field unit and is measured by applying an electric field to the dispersion of particles and measuring their average velocity. This velocity can be determined by measuring the dropper shift of laser light scattered off the moving particles [20].

X-Ray Powder Diffractometry (X-RPD)

X-ray diffraction analysis was performed with a Siemens D5000 (Munich, Germany) using nickel-filtered CuKα radiation (a voltage of 40 KV and a current of 20 mA). The scanning rate was 2°/min over a 2θ range of 20-60° and with an interval of 0.02°.

Differential Scanning Calorimetry (DSC)

Typically, about 5mg of the sample were weighed into an aluminum pan, which was crimped non-hermetically, and heated in the differential scanning calorimeter (DSC 60, Shimadzu, Japan) from 30 to 300°C at a rate of 10°C per min.

Fourier-Transform Infrared Spectroscopy (FTIR)

The infrared spectrum of drug, microparticles containing the drug, were obtained in potassium bromide discs (0.5% w/w) using a FTIR (Bowmen Hartmann & Brann, Canada) spectrophotometer.

In Vitro Release Study

Microparticles

Dissolution was carried out, using a USP basket method at 37°C and 100 rpm, in 900 milliliters of

phosphate buffer (pH 7.4). Microparticles (25, 50 and 100 mg drug, respectively) were placed in the apparatus and 5ml aliquots of the medium were withdrawn at preset times over 2 h and replaced by 5 ml of fresh medium. The samples were filtered through 0.45 μm filters and used for the spectroscopic determination of the drug. Drug concentration in the samples was measured by UV spectrophotometric analysis at 224nm. Each experiment was repeated three times.

Nanoparticles and Composite Microparticles

Nanoparticles or composite microparticles containing 25, 50 and 100 mg freeze-dried were suspended in 20 ml of saline phosphate buffer (pH 7.4). The particles suspension was gently stirred (200 rpm) at 37°C into a water bath. Three ml of suspension were withdrawn at appropriate intervals (0.25, 0.5, 0.45, 1, 2, 3, 4, 5, 6, 8 and 24 h) and each sample was centrifuged at 42,000 × g for 10 min. The filtrate (diclofenac sodium) was replaced by 3 ml of fresh buffer [19,21]. The amount of diclofenac sodium in the release medium was determined by UV at 207 nm.

RESULTS AND DISCUSSION

A W/O/W multiple emulsion solvent evaporation/extraction method is mostly used for the encapsulation of a water-soluble drug and, therefore, is the method of choice for the water-soluble diclofenac sodium drug. Several research workers have investigated the utilization of ethyl cellulose as a retardant polymer to encapsulate highly water soluble drugs using the emulsion solvent evaporation method [6,7] and spherical crystallization technique [8].

The use of water in the oil emulsion solvent evaporation technique is one method used to modify the drug release of highly water soluble drugs. The use of the w/o/w double emulsion method to microencapsulate diclofenac, using ethycellulose as a retardant polymer and PVA as an emulsifying agent, was reported [9].

The primary requirement of this method to obtain microspheres is that the selected solvent system for the polymer be immiscible with a non-aqueous processing medium [18]. Ethylacetate is an organic solvent which is polar, water miscible and oil immiscible. Poly-ε-caprolactone dissolved in methylene chloride was used for the manufacture of nanoparticles, whereas ethylcellulose dissolved in ethylacetate, a non-solvent of PCL, was used for the preparation of microparticles.

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,
2. The fine dispersion of the aqueous drug solution into the organic polymer solution to form a W/O emulsion [22].

Diclofenac sodium 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 diclofenac sodium solution was added to the organic phase. One method of ensuring the high entrapment efficiency of water-soluble active ingredients is to use a hydrophobic processing medium into which the hydrophobic macromolecule is unlikely to migrate. It is supposed that diclofenac sodium is concentrated at the interfaces (either internal water in oil or external oil in water). Therefore, a significant amount of the drug is assumed to be adsorbed at the outer surface. With increasing drug concentration, there is a saturation of the outer surface. The encapsulation efficiency of the drug depended on the solubility of the drug in the solvent and continuous phase. 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 is evaporated, the higher the encapsulation efficiency will be.

In all formulations, the mean amount of drug entrapped in prepared nanoparticles and composite microparticles was near to the theoretical value, since the drug loading efficiency is almost 100% (Table 1). The encapsulation efficiency of the drug depended on the solubility of the drug in the solvent and continuous phase. Youan et al. reported similar observation [23]. The entrapment efficiency of polypeptides was increased by enhancing the viscosity builders [24].

In simple microparticles prepared by the extraction method, the amount of drug entrapped in microparticles was lower than the theoretical value. This indicated that some free drug crystals were lost in the process of encapsulation. As the ratio of drug to polymer increased, the amount of free drug loss decreased (Table 1), so that at the ratio of drug to polymer, 0.4:1, the amount of drug entrapment was 26.89% , which was very close to the theoretical value (28.57%).

Using higher amounts of the drug caused a slight increase in the viscosity of the dispersed phase. The entrapment efficiency of polypeptides was increased by enhancing the viscosity builders [22]. Generally, increasing the drug-polymer ratio increased the production yield (in the nanoparticles and composite microparticles, Table 1). When the ratio of drug/polymer increased from 0.1:1 to 0.4:1, the production yield was decreased ($p < 0.05$). The reason for decreased

Table 1. Effect of drug/polymer ratio on drug loading efficiency, production yield and particle size of diclofenac sodium microparticles, nanoparticles and composite microparticles.

Process Variable	Formulation Code	Drug: Polymer Ratio	Production Yield (%±SD)	Theoretical Drug Content (%)	Mean Drug Entrapped (%)	Drug Loading Efficiency (%±SD)	Mean Particle Size (μm±SD)
Microparticle	F_1	0.1:1	52.08 ± 3.67	9.09	5.94 ± 3.75	65.35 ± 6.73	34.63 ± 0.51
	F_2	0.2:1	43.05 ± 3.87	16.67	12.47 ± 2.51	74.81 ± 9.08	18.72 ± 0.486
	F_3	0.4:1	30.50 ± 3.08	28.57	26.89 ± 1.38	94.20 ± 7.89	13.11 ± 0.51
Nanoparticle	NP_1	0.1:1	58.18 ± 7.21	9.09	9.07 ± 1.45	99.82 ± 8.98	3.54 ± 0.56
	NP_2	0.2:1	56.01 ± 3.46	16.67	16.58 ± 0.75	99.71 ± 6.74	1.24 ± 0.53
	NP_3	0.4:1	69.33 ± 5.32	28.57	28.49 ± 0.35	99.44 ± 6.32	0.76 ± 0.19
Composite microparticle	COM_1	0.05:1	77.93 ± 1.32	4.76	4.71 ± 0.21	98.97 ± 4.23	9.35 ± 0.39
	COM_2	0.1:1	83.98 ± 3.56	9.09	9.07 ± 0.78	99.78 ± 6.43	13.42 ± 0.71
	COM_3	0.2:1	88.91 ± 6.78	16.67	16.51 ± 1.56	99.04 ± 7.12	13.01 ± 0.69

production yield at high drug/polymer ratios (as simple microparticles) could be due to the decreased diffusion rate of solvents (ethyl acetate) from concentrated solutions into initial emulsion. The size of microparticles was found to increase with an increase in the concentration of the drug (Table 1).

It can be attributed to that fact that with the higher diffusion rate of non-solvent to the polymer solution, a smaller size of microcapsules is easily obtained [25,26]. As can be seen, the sizes of particles were decreased with an increase in the amount of the drug. These results are correlated with those observed by Kumar et al. and Ubrich et al. [18,27].

Indeed, they stated that the nanoparticle size decreased with increasing drug concentration in the internal aqueous phase (Figure 1). Since the particle size is related to a great extent to the stability of the first emulsion, they claimed that the drug can act

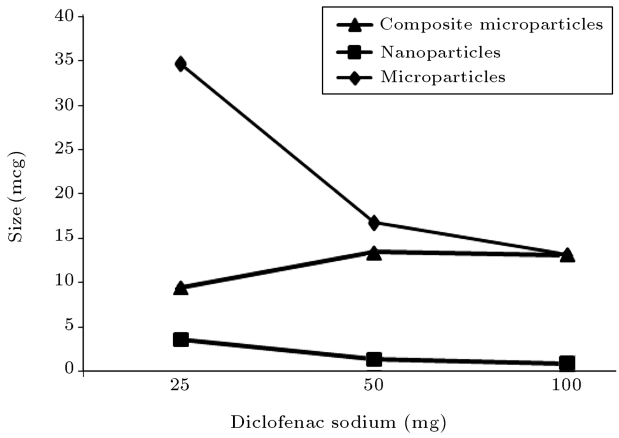

Figure 1. Comparison of the size of macroparticles, nanoparticles and composite microparticles prepared by $W_1/O/W_2$ emulsification with diclofenac sodium (25, 50 and 100 mg).

as a surfactant by stabilizing the first emulsion, and consequently, hampering the fast coalescence of the droplets. The chemical structure of diclofenac sodium, and a hydrophilic molecule circled with a hydrophobic acidic group may confer a relative amphiphilicity to the drug, which could behave as a surfactant.

The zeta potential of three nanoparticle formulations were negative; diclofenac (-35.9 mv), ethylcellulose (-13.9 mv) and poly(ε-caprolactone) (+36 mv). Blank nanoparticles had a negative charge (-4.8 mv).

Drug-loaded nanoparticles indicated a positive charge, because poly(ε-caprolactone) is a polycationic polymer and it changed the charge of nanoparticles. Addition of a cationic polymer can lead to the flocculation of yeast cells, thus forming macroscopic flocs [28]. Flocculation occurs by two main mechanisms: (a) formation of macromolecular bridges between the particles, and (b) a surface and charge reduction due to the adsorption of highly charged polyelectrolytes on oppositely charged particles [28]. 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 potential zeta (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 [29].

The drugs have been dispersed in a crystalline or amorphous form or dissolved in the polymeric matrix during formulation of the microparticles. Any abrupt or drastic change in the thermal behavior of either

the drug or polymer may indicate a possible drug-polymer interaction. The DSC curve of the diclofenac is seen in Figure 2 (curve A), an endothermic peak and an exothermic peak at 63 and 281.4°C, respectively. The curve of the ethylcellulose is presented as an exothermic peak at 211°C (Figure 2; Curve C); this peak corresponds to the oxidative degradation of ethylcellulose. The DSC poly(ε-caprolactone) is shown in Figure 2 (Curve C). In this curve, a melting endometric peak at 59.12°C was observed. The DSC curve of the drug-loaded ethylcellulose classical microsphere, nanosphere and composite formulations is given in Figure 2 (Curves D, F and H). It was shown that the drug and poly(ε-caprolactone were crystalline and the ethylcellulose was in an amorphous state.

The X-ray diffraction spectrum pure drug shows that the pure drug and poly(ε-caprolactone is crystalline in nature (Figure 3). However when it was incorporated into the polymer matrix, the principal peaks of the drug existed or could be observed with less intensity by the matrix materials. This confirms the results obtained from DSC experiments. FTIR spectra are assigned as follows:

1. Diclofenac sodium: amide band II at 3257.39 cm^{-1} and C=O stretching 1570 cm^{-1}.
2. Ethylcellulose and poly(ε-caprolactone): C=O stretching band at 2982.12 and 2976.71 cm^{-1}, respectively.
3. Microparticle simple, nanoparticle, composite microparticle: amide band at 2942, 2948 and 2934 cm^{-1}, respectively.
4. Microparticle simple, nanoparticle, composite microparticle: C=O stretching band at 1735, 1730, 1733 cm^{-1}, respectively (Figure 4).

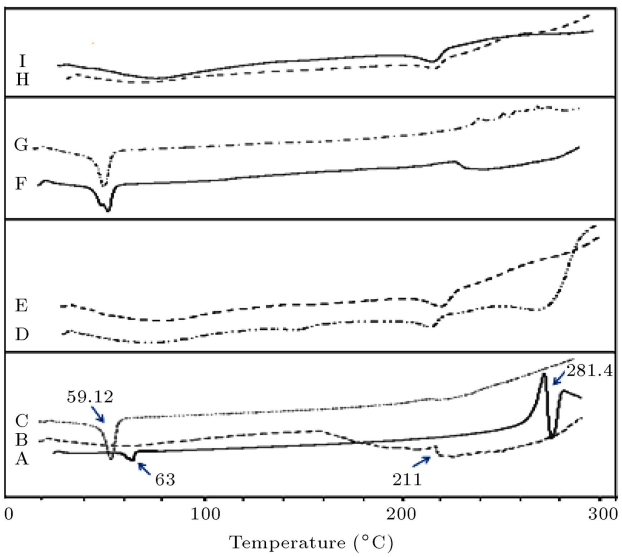

Figure 2. DSC thermogram. A: diclofenac sodium; B: ethylcellulose; C: poly(ε-caprolactone); D: microparticles simple; E: blank microparticles; F: nanoparticles; G: blank nanoparticles; H: composite microparticles; and I: blank composite microparticles.

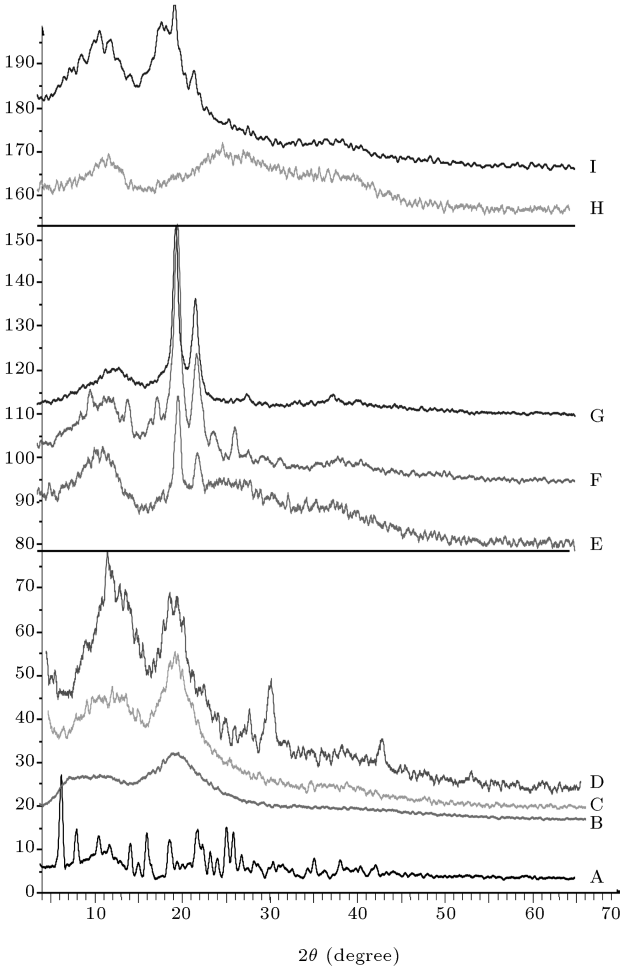

Figure 3. X-ray diffraction. A: diclofenac sodium; B: ethylcellulose; C: microparticles simple; D: blank microparticles; E: blank nanoparticles; F: nanoparticles; G: poly(ε-caprolactone); H: blank composite microparticles; and I: composite microparticles.

After diclofenac sodium was encapsulated into the microparticles and nanoparticles, the characteristic peaks for diclofenac sodium were shown by the stronger intensity peaks of matrix materials [30]. On the other hand, the C=O stretching bands of diclofenac sodium in polymeric systems (at 1570 cm^{-1}) were merged, thus, leading to a peak shifting from 1730 cm^{-1} to 1735 cm^{-1} obtained from DSC experiments.

The in vitro release profiles of diclofenac from microparticles and nanoparticles exhibited in Figure 5 also showed an initial burst effect, which may be due to the presence of some drug particles on the surface of the microparticles and nanoparticles. Due to its weakly acidic nature, diclofenac sodium always shows

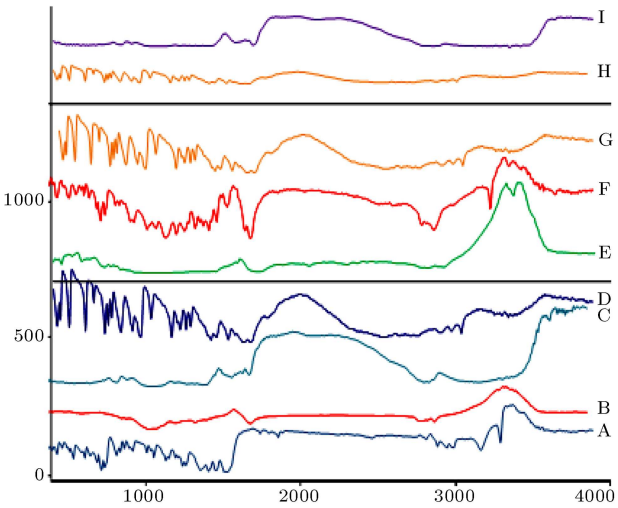

Figure 4. FTIR spectrum. A: diclofenac sodium; B: ethylcellulose; C: microparticles simple; D: blank microparticles; E: poly(ε-caprolactone); F: nanoparticles; G: blank nanoparticles; H: blank composite microparticles; and I: composite microparticles.

an expected increase in dissolution at pH 7.4. For microparticles, the dissolution of diclofenac sodium at pH 7.4 strongly showed an initial burst effect. 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 diclofenac sodium 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 enhances the permeability of the polymer to the drug (Figure 5) [31]. The results indicated that some factors, such as drug/polymer ratio, governed the drug release from these microparticles. In order to keep the total surface area of the microparticles constant and, thus get comparable results, the release studies were carried

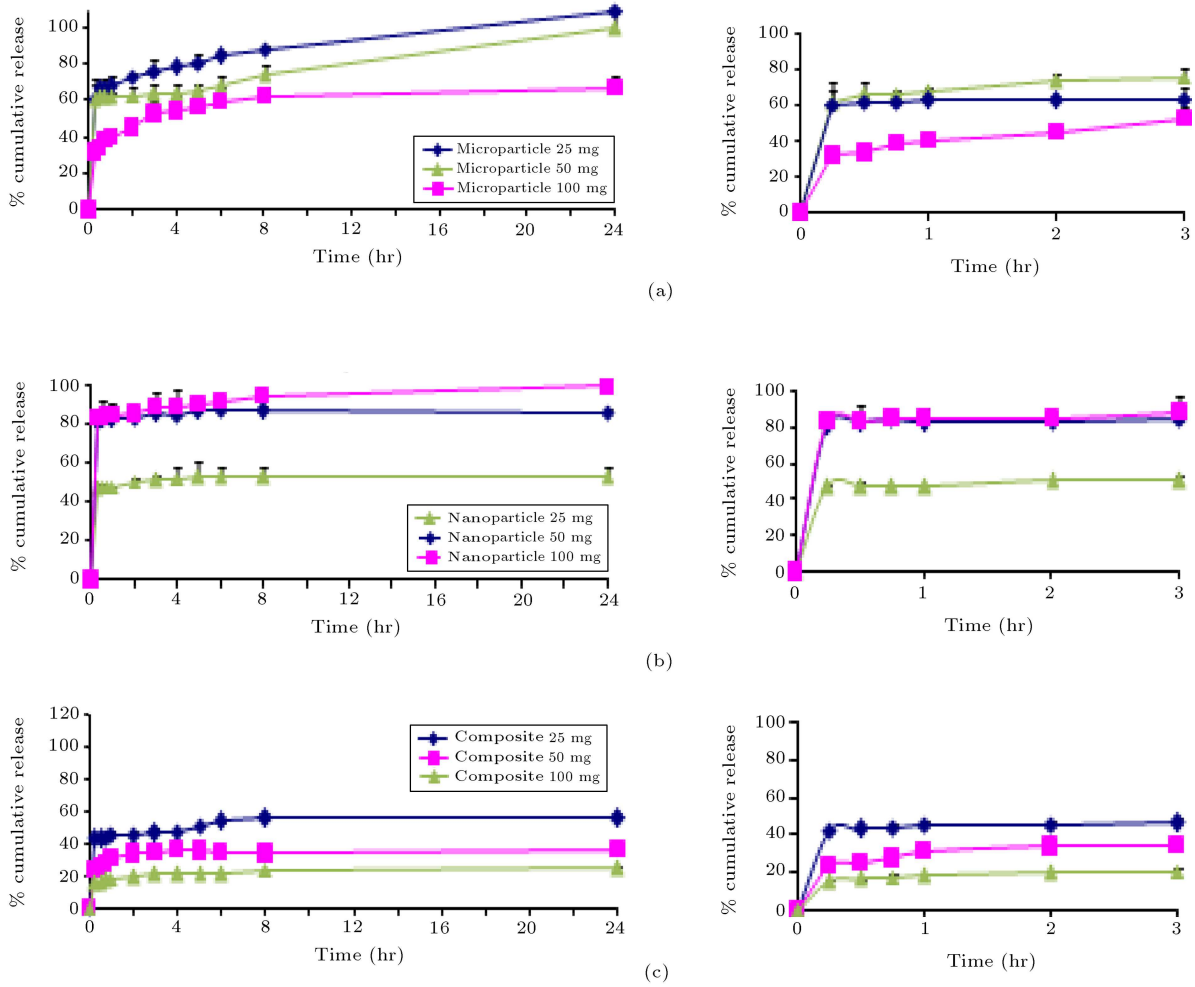

Figure 5. Cumulative percent release of diclofenac sodium from a) microparticles simple; b) nanoparticles and c) composite microparticles prepared with different drug/polymer ratio.

out using the same size fractions of microparticles containing equivalent amounts of diclofenac sodium from different batches. Drug release rates were decreased with increasing amounts of diclofenac sodium in the formulation (Figure 5a). Higher levels of diclofenac sodium corresponding to lower levels of the polymer in the formulation resulted in an increase in the drug release rate (F_1). As more drugs are released from the microparticles, more channels are probably produced, contributing to faster drug release rates. However, Figure 5a shows that the burst effect is lower when the drug/polymer ratio is 0.1:1 (F_1) compared with 0.4:1 (F_3). In the formulation F_3, an increase of the internal phase viscosity, due to the different diclofenac sodium concentrations, could reduce the leakage of the drug towards the external aqueous phase and decrease the burst effect (to compare with F_1 and F_2).

PCL-loaded nanoparticles of each formulation displayed an immediate and important initial drug release in the first 15 min, followed by a 50-80% release obtained over 24 h (Table 2, Figure 5). This immediate high release may 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 [32]. Indeed, it has been already 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 [1]. Although not all the encapsulated drug was released in 24 h (Table 2), 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 [18].

Table 2. Mean of diclofenac sodium released after 0.25 h and 24 h from microparticles simple, nanoparticles and composite microparticles.

Formulation	[a] $Q_{0.25}$	[b] Q_{24}
F_1	61.87 ± 12.11	109.5 ± 7.14
F_2	61.12 ± 8.2	100.54 ± 11.99
F_3	32.15 ± 2.63	67.01 ± 7.79
NP_1	47.77 ± 1.8	53.17 ± 5.34
NP_2	88.31 ± 1.15	89.42 ± 1.45
NP_3	83.24 ± 3.37	99.88 ± 8.1
COM_1	42.39 ± 0.64	55.16 ± 0.63
COM_2	23.46 ± 0.77	36.54 ± 0.57
COM_3	15.30 ± 0.64	24.64 ± 1.51

a: Amount of drug release after 0.25 h.

b: Amount of drug release after 24 h.

The initial percent of drug release and dissolution profiles were very different with all types of microparticles, compared with nanoparticles, as shown in Figure 5. However, composite microparticles tend to reduce the initial burst effect, especially for microparticles prepared from ethylcellulose used alone (simple microparticles). Reduction in the initial burst effect can be described not only by the rather hydrophilic properties of diclofenac sodium, which prefers to diffuse towards the surrounding dissolution in aqueous media, but also to the high encapsulation ratio of PCL nanoparticles [5,18]. The 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 diclofenac sodium during the time of the experiment [2]. Therefore, any mechanism which is able to restrict this diffusion of diclofenac sodium towards water would be easily observed. This is, indeed, due to the slow diffusion of water into the lipophilic ethylcellulose matrix [18,32].

When PCL nanoparticles were encapsulated into the microparticles (Table 2), there was a large decrease in the burst release again (15.30-42.39%); this decrease is much more marked when $p < 0.05$. Therefore, the advantage of encapsulating nanoparticles in microparticles (composite microparticles) has been definitely demonstrated for a hydrophilic drug [3,18]. For nanoparticles, the burst was higher than composite micropaticles prepared with poly(ε-caprolactone ($32.15 \pm 2.63\%$ when compared to 15.3 ± 6.3). However, composite microparticles tend to reduce the initial burst effect. Indeed, for the composite microparticles, the total diclofenac released was much lower after 24 h, and a plateau of around 50% was obtained as early as 2 h (Table 2). The presence of ethylcellulose in the matrix of microparticles conferred a slower and more progressive release of drug during the time of the experiment. The burst of simple microparticles and composite microparticles was low (Table 2), but the drug was more slowly released from the composite (COM_3) from the simple microparticles (F_3) in the 24 h period of the dissolution test ($24.64 \pm 1.51\%$ versus $67.01 \pm 7.79\%$). On the other hand, the effect of burst is much more marked with nanoparticles. Indeed, due to the high hydrophilicity of the drug, this compound has a natural tendency to diffuse very rapidly towards an aqueous phase. The burst effect of simple microparticles was intermediate between nanoparticles and composite microparticles when taking into account the whole 24 h of the experiment. This is probably due to the slow diffusion of water into the lipophilic ethylcellulose matrix.

High correlation was observed for the Peppas model (Table 3). The data obtained were also put in the Korsemeyer-Peppas model in order to find the n

Table 3. Fitting parameters of the in vitro release data to various release kinetic models for nanoparticles and composite microparticles.

Order		NP_1	NP_2	NP_3	COM_1	COM_2	COM_3
Zero $f = kt$	K	0.0022	0.0020	0.0070	0.0055	0.0039	0.0035
	RSQ	0.3560	0.3844	0.8201	0.6046	0.3377	0.6305
	D(SS)%	107.3188	1089.5455	1059.9086	1015.0503	1038.4934	954.0298
First $\ln(1-f) = kt$	K	-0.0136	-0.0136	-0.2078	-0.01101	-0.0058	0.0044
	RSQ	0.4022	0.4022	0.9845	0.6151	0.3496	0.7001
	D(SS)%	1055.217	10356.4605	555.1089	995.6630	1013.6050	914.6610
Peppas $\ln f = \ln k + b \ln t$	b	0.0338	0.0321	0.0352	0.1118	0.072	0.0627
	K	0.0175	0.0175	0.0407	0.0679	0.1069	0.1120
	RSQ	0.8278	0.8278	0.9073	0.8519	0.8521	0.9714
	D(SS)%	18.1295	17.1465	19.6487	18.5987	55.5512	24.7681
Higuchi $f = kt^{0.5}$	K	0.0138	0.0138	0.0407	0.0345	0.0284	0.0221
	RSQ	0.6274	0.6274	0.9576	0.8011	0.5945	0.8573
	D(SS)%	1037.6844	1070.6649	1008.6019	884.3044	925.9099	886.0978

value which describes the drug release mechanism [22]. The n value of composite microparticles of different drug/polymer ratios was between $0 < n < 0.5$, indicating that the mechanism of the drug release was diffusion controlled (Table 3). The n value of nanoparticles was not calculated, because the primary release percent was more than 60% [33,34].

CONCLUSION

Generally, the differences observed with the composite microparticles may be explained by the heterogeneous composition of the polymeric matrix. A fast rate of solvent removal can also contribute to a heterogeneous distribution of the drug within the internal phase as it hardens, which would further explain the biphasic release profile [35]. Indeed, in order to be released into the external dissolution medium, diclofenac sodium 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 microparticales to dramatically change the burst effect and release profile of a drug in vitro.

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