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Preparation of Nanoparticles in Drug Delivery System Using Guar Derivatives and Dialysis Method

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Abstract To develop a new form of controlled release dosage for administering for indomethacin (IND), two formulations of IND-loaded nanoparticles were designed based on polysaccharide (guar) derivatives. Nanoparticles prepared by the dialysis method were characterized with respect to morphology, size distribution, drug content, and *in vitro* drug release. Morphological studies by scanning electron microscopy (SEM) indicated that guar acetate (GA) nanoparticles were spherical in shape and had a smooth surface. The particle size distributions of formulation I (40 mg of GA) and formulation II (80 mg of GA) were shown to be 250.78±185.13 nm and 718±145.90 nm in distilled water (20°C), respectively. The drug loading efficiencies of nanoparticles were approximately 26% and 31% for formulations I and II, respectively. The differential scanning calorimetry (DSC) results indicated that the IND was perfectly distributed within GA nanoparticles. We also found, from the X-ray diffractometry analysis, that a decrease in the degree of crystallinity of the drug occurred in the nanoparticles. No changes between the original IND and the released IND from GA nanoparticles were detected by FT-IR. Using guar acetate, it is possible to design nanoparticles which allow the controlled release of IND over an extended period of time.

Key words: Guar acetate, Nanoparticle, Indomethacin, Drug release

Recently, the use of polysaccharides as matrices for the controlled release of drug has received considerable attention. In several studies, the use of polysaccharides was proposed since its ingestion had no adverse dietary, physiological, or toxic effects in animals and humans [1, 7, 10]. For this research, we chose guar gum, which is a kind of polysaccharide, as the basic material. Guar gum is a galactomannan, composed of linear chains of D-mannopyranosyl units with side branching units of D-galactopyranose attached by (1→6) linkages. It is derived from the seed of the guar plant, *Cyanaposis tetragonolobys*. It is nonionic in nature and has a molecular weight of approximately $2.2\sim3\times10^5$. Guar gum is widely used as a thickener in many food products.

IND is one of the most potent non-steroid anti-inflammatory drug (NSAID) for the treatment of patients as rheumatoid arthritis, osteoarthritis, and acute gouty arthritis. As is the case for many of the NSAID, IND acts by suppressing prostaglandin synthesis in the tissues via inhibition of cyclooxygenase activity [5]. Even though IND is very potent, its use may be curtailed by the adverse effects that are frequently associated with the gastrointestinal tract and the central nervous system (mainly frontal headaches) [6]. The side effects are, in part, a result of high plasma levels following the administration of the conventional nonformulated product. These adverse effects could be overcome by encapsulation and entrapment of the active ingredient, according to a report by Albin *et al.* [1]. These methods would protect the gastrointestinal mucus from direct exposure to IND and lower plasma levels by retarding and controlling the release rate. Another factor requiring the formulation of a slow-release product is the short $t_{1/2}$ of this drug (usually 2.5~3 h) [11]. To avoid high local concentrations of IND in the gastrointestinal tract, we have developed a nanoparticle system. Such a system will allow a more uniform dispersal in the gastrointestinal tract, and the release of indomethacin should thus be more homogeneous and less harmful to the gastric mucosa.

In this study, nanoparticles of guar acetate containing IND were prepared by dialysis in an attempt to develop a controlled-delivery system for IND. The influence of the polymer concentration and processing conditions on the physical and physicochemical properties of these nanoparticles was examined. Finally, the relationship between the characteristics and the release profiles of the drug was also investigated.

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MATERIALS AND METHODS

Chemicals

Guar gum and IND were purchased from Sigma Chemical Co. (U.S.A.). Formamide, dimethylsulfoxide (DMSO), and pyridine (chemical grade) were purchased from Junsei Chemical Co. (Japan). Acetic anhydride was purchased from Lancaster Synthetic Co. (UK).

Preparation of GA from Guar Gum

GA was prepared as follows: 2 g of guar gum were suspended in 20 ml of formamide and dissolved by vigorous stirring at 50°C. Sixty ml of pyridine and 150 ml of acetic anhydride were added and the mixture was stirred at 54°C for 48 h. GA was obtained from reprecipitation in 200 ml of water using a modified version of the method reported by Motozato *et al.* [9]. Prepared GA was identified by FT-IR analysis (Nicolet 520P).

Preparation of IND-loaded GA Nanoparticles

40 mg of drug were dissolved in 5 ml of DMSO and subsequently 40 mg, 80 mg, 120 mg, and 160 mg of GA were added. This solution was dropped slowly into 20 ml of water, then dialyzed in double-distilled water using the dialysis membrane (M.W. cut-off: 12,000). The water was replaced five times in 5 h, followed by two more times in the next 4 h. The resulting solution was freeze-dried.

Scanning Electron Microscopic (SEM) Studies

The observation of GA nanoparticles was performed using scanning electron microscope (SEM, JEOL, JSM-5400, Japan). One drop of aqueous solution of GA nanoparticles was placed on the copper grid and dried in an air-blowing system, then coated with gold/paladium prior to observation.

Proton Correlation Spectroscopy (PCS) Measurements

For particle size measurements, zetasizer 3000 (Malvern Instruments, U.K.) with a He-Ne laser beam was used at a wavelength of 633 nm and 25°C (scattering angle of 90°). A nanoparticle solution (concentration: 0.1 wt%) was prepared by the filtration method and measured without filtering.

Differential Scanning Calorimetry (DSC) Analysis

The thermal properties of IND-loaded GA nanoparticles were examined by DSC (Polymer Laboratories Thermal Science, U.K.). Samples (10 mg) were transferred into a previously weighed aluminum crucible. DSC was carried out at a heating rate of 10°C/min.

Powder X-ray Diffractometry (XRD) Studies

Samples were exposed to CuKα radiation (40 kV × 30 mA) in a wide angle X-ray diffractometer (D/MAX-1200, Rigaku, Japan). The instrument was operated in the step scan mode, in increments of 0.02° (2θ).

Release of IND from GA Nanoparticles

The release rates of IND from GA nanoparticles were studied in phosphate-buffered saline (PBS) at pH 7.2 (0.1 M). Seven mg of IND-loaded GA nanoparticles and 1 ml of test solution were placed in dialysis membrane (M.W. cut-off: 12,000) bags, which in turn were immersed in 10 ml of test solution. The media was then incubated at 37°C. At predetermined time intervals, the sample solution was withdrawn and placed in fresh medium. The samples were assayed for IND concentration in the medium by UV-spectrophotometer (UV-VIS spectrophotometer, Shimadzu UV-1201, Japan) at 319 nm.

RESULTS AND DISCUSSION

Observation of GA Nanoparticles

In the present work, we chose to explore the possibility of developing polysaccharide (guar gum) nanoparticles in an attempt overcome several adverse effects associated with the use of the currently available dosage forms of IND. Since guar gum is hydrophilic polysaccharide, it is necessary to change it into a hydrophobic substance for use in a drug delivery system. We prepared GA by substituting a hydroxyl group in sugar (composition sugar of guar gum) with an acetyl group. The GA was analyzed with FT-IR (Fig. 1): the spectra confirmed the creation of an acetate group, as indicated by the peaks at 1749 cm^{-1}, 1379 cm^{-1}, and 600 cm^{-1}, representing C=O stretching, CH_3 deformation, and O-C=O bonds, respectively.

GA nanoparticles containing IND were prepared by a dialysis method using different formulation conditions (polymer concentration). The morphological structure of

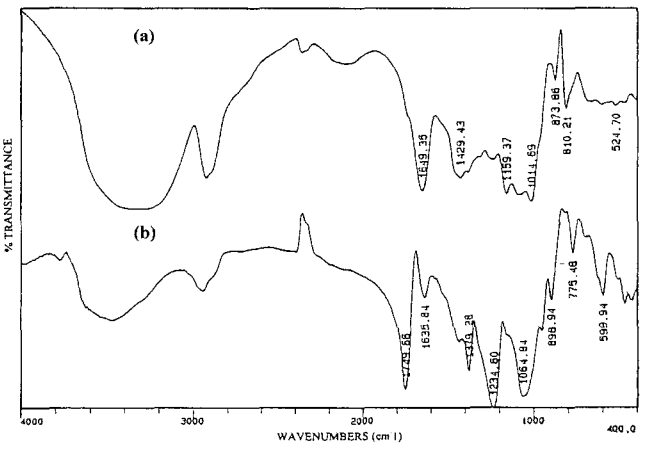

Fig. 1. Comparison of FT-IR spectra of guar gum (a) and GA (b).

the nanoparticles was observed by SEM. The shapes of IND loaded GA nanoparticles (formulation I: 40 mg of guar acetate concentration, and II: 80 mg of guar acetate concentration) were completely spherical in form and had smooth surfaces [Figs. 2(a) and (b)]. However, no shape was observed at concentrations above 120 mg of guar acetate. No drug crystals were detected on the surface of formulations I and II. Photographs also illustrated the influence of polymer concentration on the nanoparticle size.

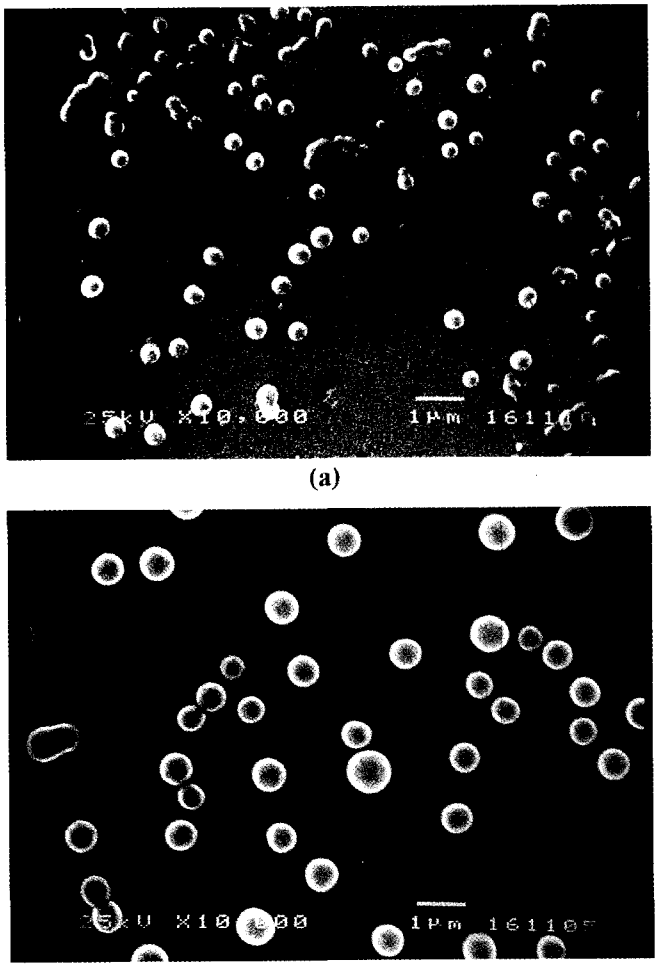

Fig. 2. Photographs of GA [formulations I (a) and II (b)] by SEM (×10,000).

The particle size distributions of formulations I and II were 250.78±185.13 nm and 718±145.90 nm in distilled water, respectively [Figs. 3(a) and (b)], as measured by proton correlation spectroscopy. When the polymer concentration was increased, the nanoparticles became smaller. The range of particle diameter, also depended on the polymer concentration.

Table 1 shows the drug load ratio, loading efficiency, and the yield of GA nanoparticles. In formulation I, the drug loading ratio and the drug loading efficiency in GA nanoparticles were shown to be approximately 19 and 26% (w/w), respectively, while in formuation II, they were shown to be approximately 17 and 31% (w/w), respectively.

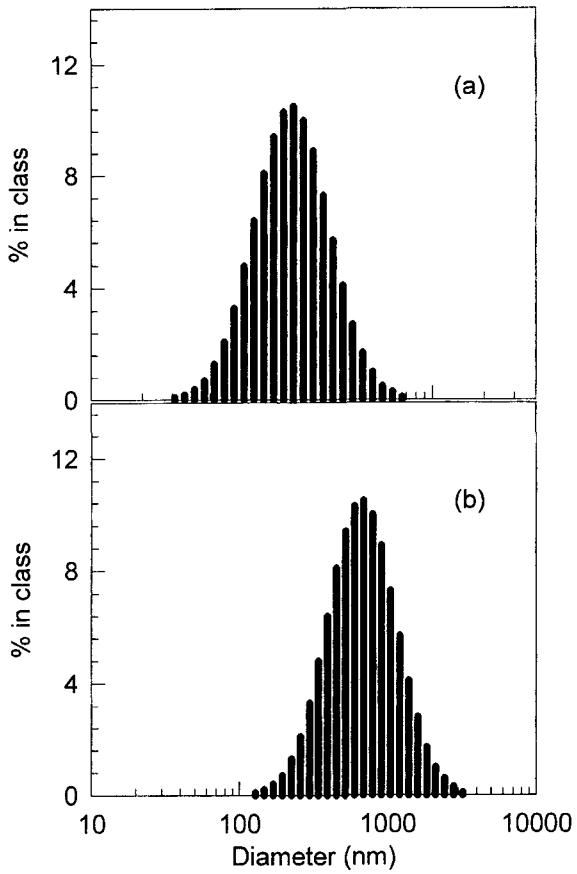

Fig. 3. The particle size distributions of formulations I (a) and II (b) in distilled water at 20°C. These results indicate the mean intensity diameter based on five repeated measurements.

Table 1. IND loading content, loading efficiency in GA nanoparticles, and the yield of nanoparticles at an initial drug concentration (n=3) of 40 mg.

Initial amount of GA (mg)	Drug loading ratio (wt%)±SD	Loading efficiency (wt%)±SD	Yield of nanoparticles (wt%)±SD
40 (formulation I)	19±3.9	26±5.3	67±2.4
80 (formulation II)	17±4.0	31±7.2	60±3.4

*The drug loading ratios (wt%) were calculated by loaded drug weight (per mg)÷nanoparticle weight (per mg)×100. The loading efficiencies (wt%) were measured as total loaded drug weight (at total obtained nanoparticles)÷initial drug weight×100.

These results indicated that the drug loading efficiency increased with loading polymer concentration, however, the drug loading content decreased with loading polymer concentration. On the other hand, the yield (defined as the ratio of the weight of nanoparticles to the initial weight of polymer and drug employed) was, in both cases, higher than 60%.

Physical State of the Drug in GA Nanoparticles

A second step in the development of these new IND formulations was the characterization of the solid dispersion state of the drug in the polymer matrix. Since IND is completely soluble in the guar acetate solution (DMSO) and only slightly soluble in the aqueous phase, it could be formulated within the nanoparticles during polymer formation, in either crystalline or amorphous form, or it could be present in a molecularly dispersed state within the guar acetate matrix. DSC and X-ray analysis were performed in order to characterize the physical state of the polymer and drug in nanoparticles. Thermal characterization of GA nanoparticles was conducted with a differential scanning calorimeter (DSC). Melting endotherms of samples are shown in Table 2. The enthalpy for the phase transition of GA and IND is approximately 13 and 47 (mcal/mg), respectively. With formulations I and II, the enthalpies for the phase transition were 33 and 23 (mcal/mg), respectively. These results indicate that the level of enthalpy increased with increasing IND concentration.

Figure 4 displays DSC thermograms of GA (a), IND (b), formulations I (c) and II (d). GA exhibited the characteristic peaks at 129°C (a). The DSC curve of pure IND showed only one endothermic peak at 163°C (b). With formulation I (c), the endothermic peak appeared at 153°C and the characteristic peaks of GA and IND disappeared. Formulation II (d) showed similar trend. However, with the physical mixture of GA and IND (containing 15% of the drug), the endothermic peak appeared at 129.6 and 160°C, respectively (data not shown). The melting temperature depression absorbed in Figs. 4 (c) and (d) may be due to the decrease of crystal perfectness

Table 2. DSC analysis of GA, IND, and samples from formulations I and II (n=3).

Sample	Melting endotherm*		
	$T_o \pm SD$ (°C)	$T_p \pm SD$ (°C)	$\Delta H \pm SD$ (mcal/mg)
GA	118±1.3	130±2.3	13±1.2
IND	159±2.1	164±0.9	47±3.2
Formulation I	146±1.1	153±1.2	33±1.6
Formulation II	144±0.5	152±1.1	23±0.8

*T_o is the onset temperature when melting begins, T_p is the critical point temperature, ΔH is the energy required for phase transition, and SD is the standard deviation.

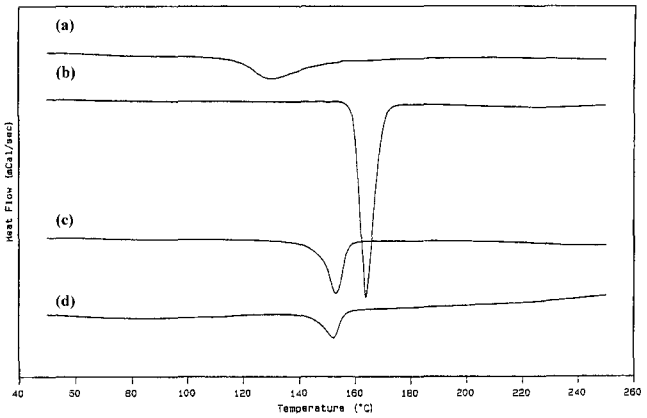

Fig. 4. DSC thermograms of (a) GA, (b) IND, (c) formulation I, and (d) formulation II.

and crystal size of IND, which are the result of the experimental conditions during formulation. Consequently, these data suggest that the IND forms a fine dispersion in the co-polymer matrix [12].

In order to confirm the physical state of the drug in nanoparticles, powder X-ray diffraction studies were carried out for GA (a), IND (b), a physical mixture of the drug (15%) and GA nanoparticles (c), formulation I (d) and II (e) (Fig. 5). GA exhibited an amorphous aspect, while IND was shown to have a crystalline structure. For the physical mixture of the drug (15%) and GA, both the crystalline structure of IND and the amorphous phase of GA was observed. However, in formulation I, a polymorphic change occurred with a decrease in the degree of crystallinity of the drug in the nanoparticles. A similar behavior was observed for formulation II (Fig. 5). These results indicated that the drug was not present in its crystalline state in the formulation developed, suggesting that IND is dispersed in an amorphous form in the polymer network. Such findings were reported previously for other hydrophobic drugs, such as hydrocortisone [2].

This was also consistent with the report by Dash [3] and Davis [4]. A change in the crystal habit of the drug occurs only in the micro- or nano- formulations. In addition, Miyamotto [8] reported that the crystallinity of IND decreased when pullulan was added, and the absorption of IND in the intestinal tract was facilitated. Therefore, we assumed that formulations I and II will facilitate the absorption of IND in the intestinal tract. Once we concluded that a drug-polymer molecular dispersion existed, it was important to determine whether or not this physical dispersion can promote a chemical interaction between the drug and the polymer. To investigate this possibility, the nanoparticle formulations developed were analyzed by FT-IR. No difference were observed in the IR patterns of the original IND and the released IND from nanopaticles

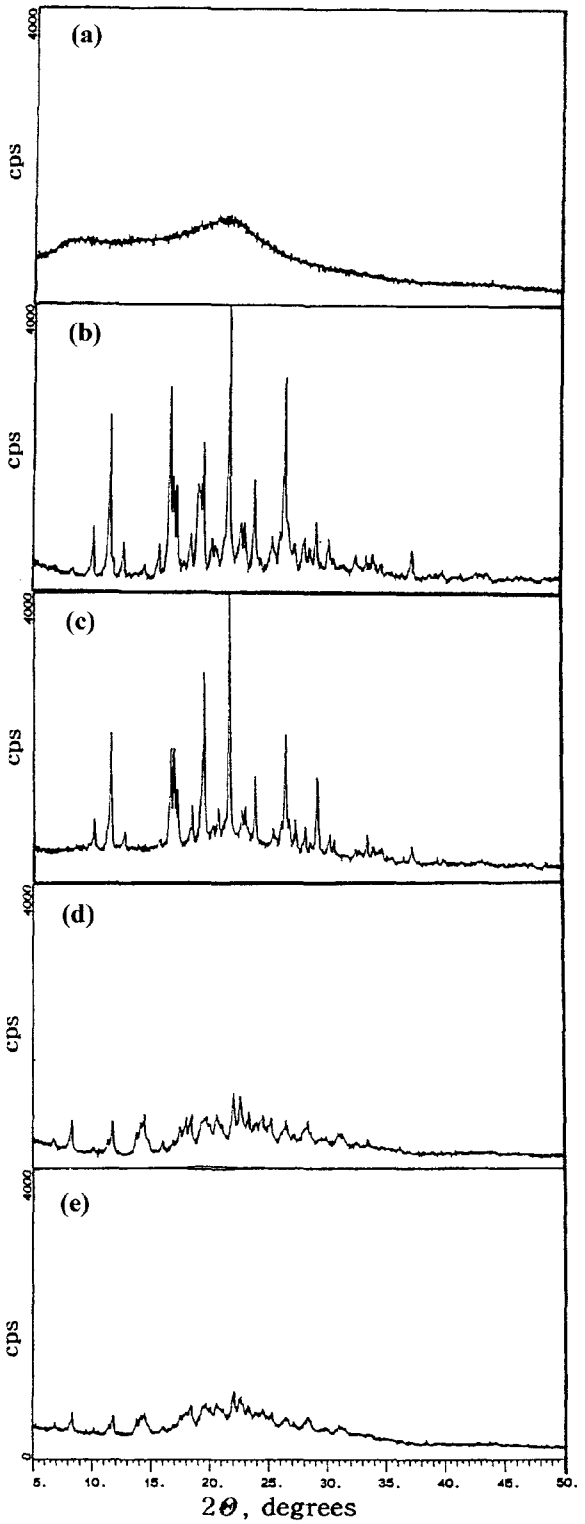

Fig. 5. Powder X-ray diffraction patterns of (a) GA, (b) IND, (c) physical mixture of 15 w/w% IND and GA, (d) formulation I, and (e) formulation II.

(Fig. 6). Thus, it was determined that no chemical interaction occurred between the components of the nanoparticles.

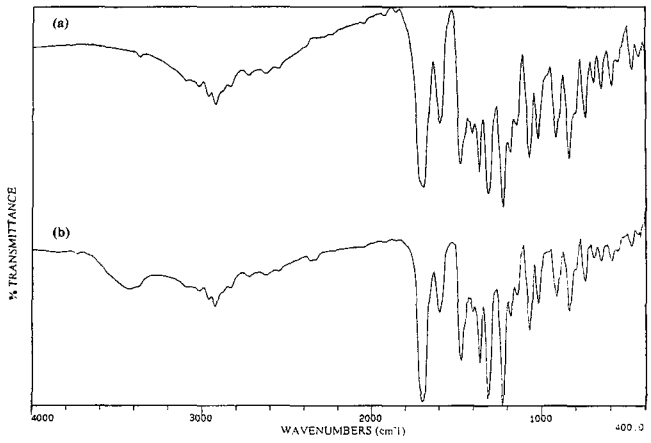

Fig. 6. FT-IR spectra of (a) original IND and (b) released IND from GA nanoparticles.

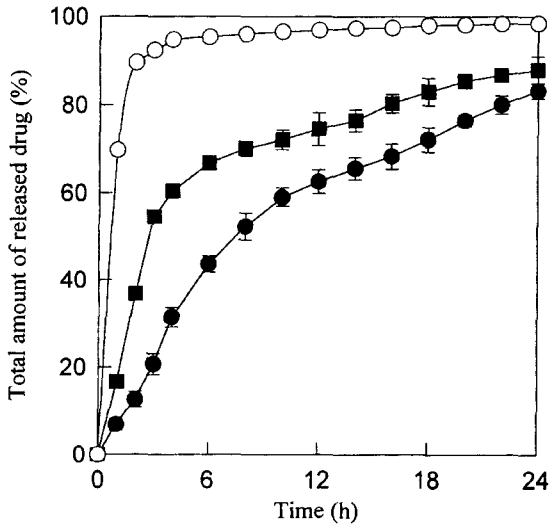

Fig. 7. Release of IND from formulation I and II at pH 7.2. Results were expressed as the mean values (±SD) of five experiments.
Control: nonformulated IND (—○—), formulation I (—■—), II (—●—).

IND Release from GA Nanoparticles

The retardation and control of the IND release rate are critical in order to avoid possible side effects during IND use. The release profiles from our formulations were compared with free indomethacin. When free and formulated IND were tested for release *in vitro*, 90% of nonformulated IND was released within 3h. For formulation I and II, 54% and 21% of IND were released within 3 h, while 87% and 83% were released after an additional 24 h, respectively.

In the case of two formulas, the initial release usually referred to as an initial burst, was found to be greatly affected by the nanoparticle size. Thus, a modest initial burst in the first 4 h followed by a slower release was observed with formulation I. Initial burst was greater for

formulation I with smaller particle size. This first phase corresponds to the release of the IND located on or near the surface of the delivery system which is thus available for immediate release. Consequently, formulation I (small nanoparticles) displayed more rapid initial release of the drug, compared to the larger particles due to their greater surface area per unit mass, as expected. We concluded that it was possible to control the drug release rate by changing the drug to polymer ratio.

Acknowledgments

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