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ORIGINAL ARTICLE

Eudragit-coated albumin nanospheres carrying inclusion complexes for oral administration of indomethacin

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Abstract Oral administration of indomethacin (IN) as well as drugs with low aqueous solubility usually results in poor absorption and bioavailability. The aim of this study was to prepare enteric-coated bovine serum albumin (BSA) nanospheres carrying cyclodextrin complex for IN delivery. Inclusion complex composed of IN and β-cyclodextrin (CD) was prepared by spray-drying. Indomethacin alone and its inclusion complex were incorporated into albumin nanospheres using a coacervation method followed by thermal cross-linking. Then nanosphere suspensions were spray-dried. The inclusion complex and the nanospheres were characterized by FT-IR spectroscopy and DSC analysis. Phase-solubility diagrams and stability constants were determined at pH 2.0 and 7.4 and at different temperatures (10, 25 and 37 °C). Swelling ability of nanospheres were evaluated as well as the in vitro release behaviour at pH 2.0 and 7.4. The nanospheres were coated with Eudragit® L-100 (EudL) or S-100 (EudS) using spray-drying to give protection in the stomach. The results showed that IN solubility can be increased by complexation with β-CD or protein/drug interaction with albumin nanospheres. The inclusion complex loaded into BSA nanospheres provided a zero order drug release kinetic. The coating process with EudL and EudS allowed to obtain a negligible release at acidic pH without limiting drug availability at pH 7.4.

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Keywords Nanoparticles · Inclusion complex · Enteric-coated · Spray drying · Oral administration

Introduction

Indomethacin is a non-steroidal anti-inflammatory drug, commonly used in the treatment of osteo and rheumatoid arthritis [1, 2]. Recently, Hull et al. [3] investigated indomethacin's (IN) use as an anticancer agent against various in vitro and in vivo models of colorectal cancer. However, oral administration of IN as well as drugs with low aqueous solubility usually results in poor absorption and bioavailability. So, many investigations have been carried out to increase the water solubility of IN by means of complexation with cyclodextrin or association with hydrophilic polymers. As regard the use of cyclodextrins, Wongmekiat et al. [4] showed the ability of α-, β- and γ-cyclodextrin to improve IN solubility and Salustio et al. [5] investigated the inclusion of IN in cyclodextrins using two different preparation methods. Cyclodextrins are cyclic oligosaccharides with a lipophilic cavity and an hydrophilic outer surface with 6 (α), 7 (β) and 8 (γ) glucose units with different solubilities. These compounds have the ability to form inclusion complexes by taking up the whole molecule or rather some non-polar parts in its hydrophobic cavity [6, 7]. The complexes improve aqueous solubility, chemical stability (due to hydrolysis, oxidation, photodecomposition and dehydration of the drug) and bioavailability of drugs [8, 9]. As regards the use of hydrophilic polymers, fast releasing IN microparticles were prepared encapsulating co-freeze-dried indomethacin/polyvinylpyrrolidone (IN/PVP) particles into molten stearic acid, by means of a ultrasonic spray-congealing technique [10]. Finally, the development of nanoparticles based on the hydrophilic

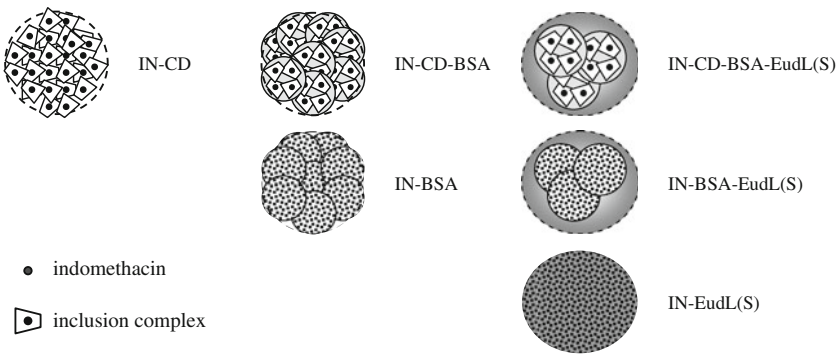

Fig. 1 Schematic description of the different microspheres produced by spray-drying

polymer albumin and different cyclodextrins [11] were found to improve solubility and release of lipophilic drugs such as progesterone.

However, due to the gastrolesive effects of IN (e.g., gastric ulcers and gastric perforation) [12] a formulation with negligible release in the gastric tract and controlled release in the intestinal region would be desirable [1, 13]. Different approaches for bypass the gastric environment have been studied [14–16] including prodrugs and polymeric coating using pH-sensitive or bacterial degradable polymers [17, 18]. Some of these approaches include the coating of drug-loaded pellets or tablets with pH-sensitive polymers such as methacrylic acid copolymers [19–22] or degradable polymers such as guar gum [21, 23] and pectin and chitosan mixtures [24]. The pH-dependent polymers exploit the generally accepted view that pH of the human gastro-intestinal tract increases progressively from the stomach to the colon [25]. Most commonly used methacrylic acid copolymers are Eudragit® L and Eudragit® S, which dissolve at pH 6.0 and 7.0, respectively.

Recently we evaluated the use of bovine serum albumin nanoparticles, coated with stearic acid [26], for site-specific delivery of the hydrophilic drug vancomycin. This systems allowed to protect the drug from degradation in the environment of the upper gastrointestinal tract and to abruptly release it into the colon. Albumin is a natural protein extensively used to prepare drug carrier since it is biodegradable, biocompatible and bioadhesive. Moreover, protein crosslinking can be easily achieved by direct reaction between functional groups in the polypeptide side chains (self-crosslinking) providing particles relatively easy to prepare over a wide range of sizes [27]. The use of albumin particles with nanometric size can favour drug availability and absorption in the intestinal region due to the high surface area available for drug partition toward biological fluids and barriers.

Taking into account all these considerations, the aim of the present work was to develop a farmaceutical form able to limit IN release in the stomach avoiding its gastrolesive effects and enhance its availability in the intestinal environment. In order to obtain a suitable formulation, we evaluated the application of multistep processes in series to each other (drug complexation with cyclodextrins, encapsulation in hydrophilic nanoparticles, coating with pH-sensible polymers) and prepared Eudragit® based microparticulate carriers able to carry albumin nanostructures and/or drug-cyclodextrin complexes. Inclusion complex composed of IN and β-cyclodextrin (CD) was prepared by spray-drying. Indomethacin alone and its inclusion complex were incorporated into albumin nanospheres using a coacervation method followed by thermal cross-linking. Then nanosphere suspensions were directly recovered or coated with Eudragit®L-100 or Eudragit®S-100 using spray-drying. A standard formulation based on IN and EudL(S) was also prepared. For all the systems prepared, spray-drying was selected as final step in order to quickly and easily produce spherical particles with narrow size distribution [28, 29]. A representation of the different spray-dried drug delivery systems (microspheres) is illustrated in Fig. 1.

Materials and methods

Materials

Indomethacin, β-CD and bovine serum albumin (minimum 98% electrophoresis) were purchased from Fluka (Milan, Italy); Eudragit®S-100 and Eudragit®L-100 (Röhm GmbH & Co.) was kindly donated from Rofarma (Gaggiano, Milan, Italy). All the solvents employed were from Carlo Erba (Milan, Italy).

Solubility studies

Solubility studies were performed according to the method reported by Higuchi and Connors [26]. Indomethacin in amounts that exceeded its solubility, was weighed carefully (50 mg) into a 50-mL Erlenmeyer flask to which 20 mL of aqueous solutions containing β-CD at various

concentrations (0–0.016 mol/L) were added. The flasks were sealed and equilibrated by shaking at different temperatures (10, 25 and 37 °C) and at two different pH values (2.0 and 7.4). When equilibrium had been reached (4 days), the samples were filtered through a 0.22 μm filter (Albet-Jacs, Valencia, Spain) and the concentration of IN was measured spectrophotometrically at 320 nm (UV–VIS, Shimadzu, Kyoto, Japan). The apparent 1:1 stability constants, K_s, were calculated from the initial straight line portion of the phase solubility diagram according to the following equation (Eq. 1) [30, 31]:

$$K_s = \text{slope}/[S_o(1 - \text{slope})] \quad (1)$$

where S_o is the saturation concentration of the drug measured without cyclodextrin (intercept of the line).

The change in enthalpy (ΔH°) on complexation was determined using the Van't Hoff equation (Eq. 2)

$$\ln K_2/K_1 = \Delta H[T_2 - T_1/RT_2T_1] \quad (2)$$

The Gibbs free energy changes (ΔG°) were determined using the following equation (Eq. 3):

$$\Delta G^\circ = -RT\ln K \quad (3)$$

And the change in entropy (ΔS°) upon complexation was determined by the Eq. 4:

$$\Delta S = (\Delta H - \Delta G)/T \quad (4)$$

Indomethacin–β-CD inclusion complex

Indomethacin–β-CD inclusion complex was prepared by spray-drying. Suspension of IN and β-CD was prepared in the molar ratio 1:1. Briefly, IN was dispersed in 100 mL of water containing β-CD and mixed for 24 h at room temperature. The suspension was filtered through a 0.22 filter (Albet-Jacs, Valencia, Spain). The filtrate was spray-dried (Büchi, Milan, Italy). The drying conditions were as follow: inlet temperature, 110 °C; outlet temperature 50 °C; air flow rate 600 normal litre per hour (NL/h).

Albumin nanospheres containing IN or its inclusion complex

First, 3.0 g of BSA were dissolved in 100 mL of water. Pure drug and its inclusion complex were dispersed in this solution. Second acetone was added dropwise to this solution (stirred continuously with a magnetic stirrer at room temperature) to produce a ratio 2:1 (v/v) acetone:BSA solution. Magnetic stirring was continued at 70 °C for 30 min to evaporate the acetone and yield an aqueous suspension of albumin nanospheres. Then the nanosphere suspension was spray-dried (Büchi, Milan, Italy) using the conditions previously described.

Coating process

Pure drug, IN–cyclodextrin inclusion complex and BSA nanospheres were coated with Eudragit®S-100 or Eudragit®L-100. Pure drug or IN–cyclodextrin inclusion complex and BSA nanospheres were added to an Eudragit solution (acetone/water mixture; 1:1, v/v). The samples were spray-dried (Büchi, Milan, Italy) obtaining gastroresistant microspheres. The drying conditions were as follow: inlet temperature, 100 °C; outlet temperature 45 °C; air flow rate 600 NL/h.

SEM analysis

The morphology of microspheres was observed using a scanning electron microscope (LEO 420, LEO Electron Microscopy Ltd., Cambridge, England). Samples were mounted onto brass stubs and sputter-coated with gold in an argon atmosphere prior to examination under scanning electron microscopy (SEM). The morphology of the nanoparticles was also characterized. Nanosphere suspensions were reconstituted in deionised water and drops were placed on aluminium stubs, allowed to dry and finally coated with gold–palladium as described above.

FT-IR analysis

Infrared (IR) spectra were recorded with a Jasco FT-IR 410 (Jasco Corporation Tokyo, Japan) spectrophotometer. The samples were prepared by processing compressed KBr disks.

DSC analysis

Differential scanning calorimetric (DSC) analysis were performed using a Perkin-Elmer DSC 6 (Perkin Elmer, Beaconsfield, UK). The samples, weighting 7–10 mg, were placed into the DSC under a nitrogen flux (20 mL/min) and heated from 25 to 275 °C at a scanning rate of 10 °C/min.

Determination of drug loading

For the determination of drug content in the different spray-dried microspheres, samples of 100 mg were weighed and transferred into a 10 mL volumetric flask and brought to volume with water/ethanol mixture (1:1, v/v). The suspension obtained was sonicated for 20 min, left at room temperature for 120 min and then centrifuged at 3400×*g* for 20 min. The supernatant was finally diluted in the mobile phase employed for HPLC analysis and the drug was detected using a Shimadzu (model SPD-10A) liquid chromatograph connected to a UV–Vis detector (SPD-10AV) and to a computerized integration system

ChromatoPlus (Shimadzu, Kyoto, Japan). Manual injections were made using a Rheodyne 7125 injector with a 20 µL sample loop. Separations were obtained on a C18 Phenomenex Luna (3 µm, 15 cm) column at room temperature using 70:30 (v/v) acetonitrile:phosphate buffer pH 3.5 mixture at a flow rate of 1.0 mL/min. Ultraviolet absorption was read at 280 nm.

Swelling studies

To evaluate the swelling behavior in alkaline and acidic environments, the spray dried samples containing albumin nanospheres (IN–BSA and IN–CD–BSA) were suspended in pH 7.4 or 2.0 buffers and their size was monitored for 60 min. Particle size distributions were measured by photo correlation spectroscopy (PCS) using an instrument (Brookhaven 90-PLUS) with an He–Ne laser beam at a wavelength of 532 nm (scattering angle of 90 °C) [32]. Nanospheres suspensions were used for particle size measurement without filtering.

In vitro release studies

In vitro release studies were performed in pH 2.0 and 7.4 phosphate buffers for 60 min, using USP4 "Flow-Through-Cell" apparatus (Erweka, Milan, Italy). The cells were held at 37 °C, and fitted with glass microfiber filters to prevent loss of sample. The drug was detected by HPLC using the method previously described.

In order to understand the mechanism of drug release from nanospheres, the cumulative percentage drug release data was fitted into the power law equation (Eq. 5) given by Ritger and Peppas [33]:

$$M_t/M_\infty = Kt^n \quad (5)$$

where M_t/M_∞ is percentage of drug released at any time t; K is release rate constant incorporating the structural and geometric characteristics of the polymeric system and the drug; and n is the diffusion exponent indicative of the release mechanism of the drug.

Results and discussion

Solubility studies

The phase-solubility studies for complex formation between IN and CD at different pH (2.0 and 7.4) and at different temperatures (10, 25 and 37 °C) were performed. The solubility of IN increased linearly with the concentration of CD, providing a characteristic A_L type solubility diagram (not reported) [30, 31]. Table 1 lists the 1:1 apparent stability constants of the inclusion complex (K_s).

Table 1 Thermodynamic parameters of the inclusion process of IN in β-CD

pH	Temperature (°C)	K_s (M^{-1})	ΔG (J/mol)	ΔH (J/mol)	ΔS (J/mol K)
2.0	10	358.31	−13714.9	−21510.8	−27.53
	25	185.43	−13302.7		
	37	163.60	−12971.6		
7.4	10	108.63	−10886.7	−13842.8	−10.44
	25	63.88	−10730.1		
	37	66.89	−10604.9		

The stability constant decreased with increasing temperature, probably due to the decrease in the interaction forces, such as Van der Waals and hydrophobic forces [27]. Moreover the K_s values were higher at pH 2.0 than at pH 7.4, according to the ionization of IN (pK_a 4.5). In fact at acidic pH the complexation of IN in the hydrophobic cavity of cyclodextrin is facilitated by the presence of the undissociated form of the drug.

SEM analysis

SEM was used to observe the shape and surface morphology of the different samples (Figs. 2, 3). SEM images of reconstituted nanospheres in deionised water (Fig. 2) showed individualized spherical shaped particles. Moreover, the mean diameter was consistent with that measured by PCS indicating that spray drying did not cause irreversible aggregation of the nanospheres which are discrete entities. Figure 3 shows that all the spray-dried samples are homogeneously round particles. Moreover, IN–CD (a) and IN–CD–BSA (b) presented a smooth surface, while IN–CD–BSA–EudL (c) and IN–CD–BSA–EudS (d)

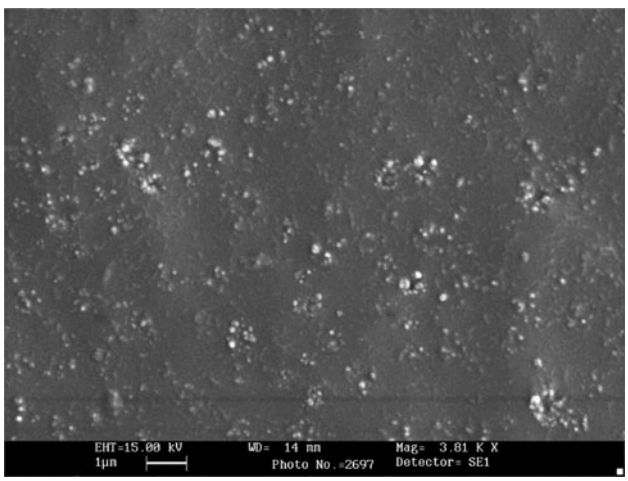

Fig. 2 SEM images of reconstituted IN–BSA nanospheres

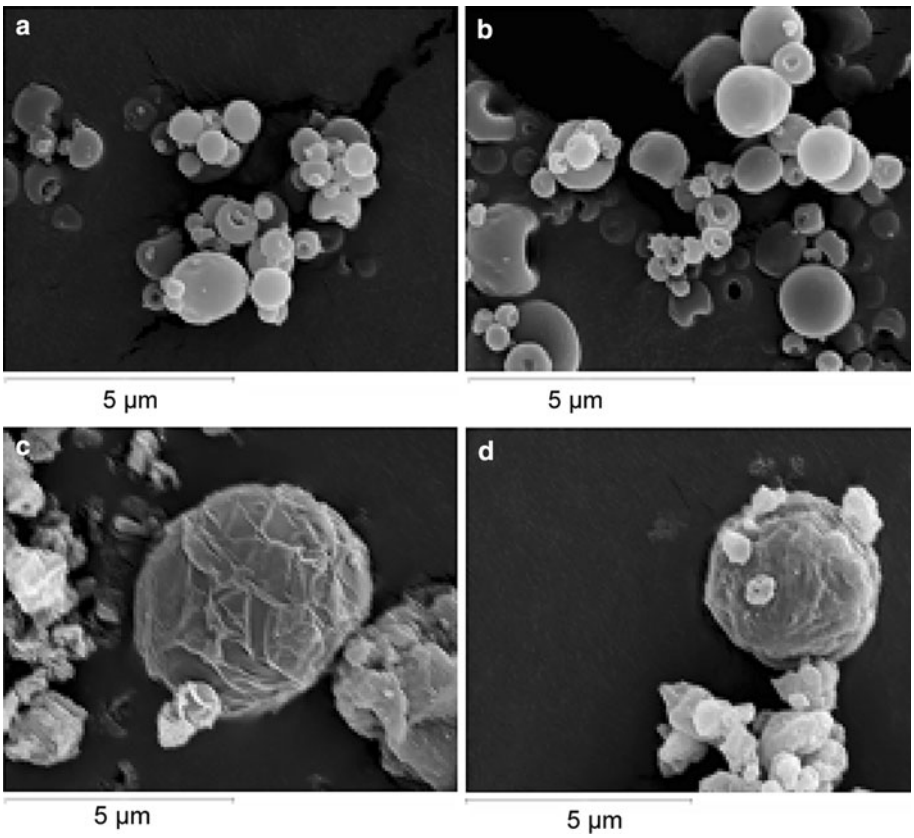

Fig. 3 SEM images of spray-dried samples: uncoated IN–CD (*a*), uncoated IN–CD–BSA (*b*), EudS-coated IN–β-CD–BSA (*c*) and EudL-coated IN–CD–BSA (*d*)

appeared as with a rugged surface due to the presence of the coating material.

FT-IR analysis

Figure 4 shows the infrared spectra of IN, CD and IN–CD complex, BSA, IN–BSA and IN–CD–BSA. The peaks at around 1721 and 1689 cm^{-1} were assigned to carbonyl group of IN. In the spectrum of IN–CD complex, the bands of carbonyl groups disappeared suggesting the inclusion of the two carbonyl groups into the CD cavity [34]. The presence of albumin in the nanospheres hided the IN peaks and weakly masked the characteristic absorption of the cyclodextrin (1164 cm^{-1}, 1083 cm^{-1}, C–H, C–O stretching vibration), indicating protein ability to encapsulate the pure drug and the complex [11].

DSC analysis

Differential scanning calorimetry (DSC) was performed on the pure drug, on the inclusion complexes, on the nanospheres and on the nanospheres carrying inclusion complex. The thermogram of IN showed the characteristic endothermic peak at 162.5 °C, corresponding to drug melting. The thermal behaviour of CD and BSA, showed no phenomena in the same temperature interval (Fig. 5). The IN–CD complex showed disappearance of the

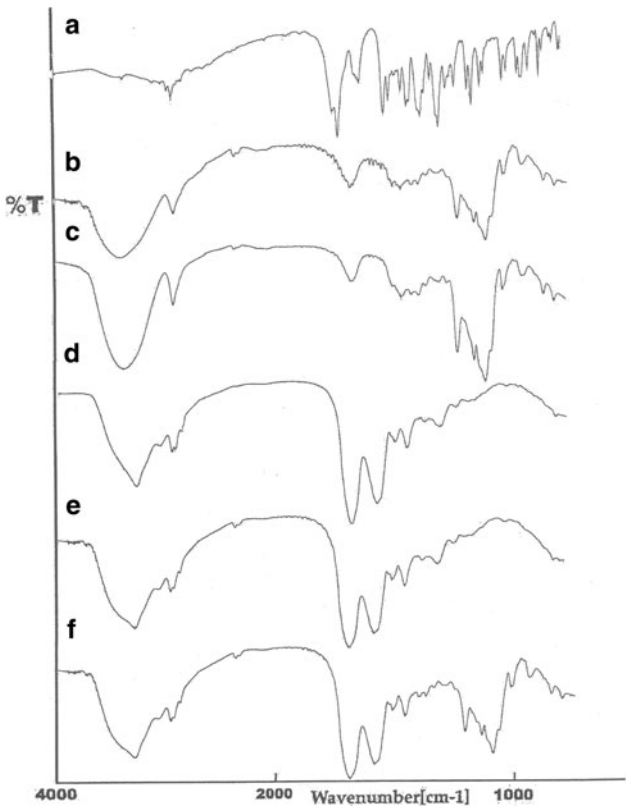

Fig. 4 FT-IR spectra of indomethacin (*a*), β-CD (*b*), IN–CD complex (*c*), BSA (*d*), IN–BSA (*e*) and IN–CD–BSA (*f*)

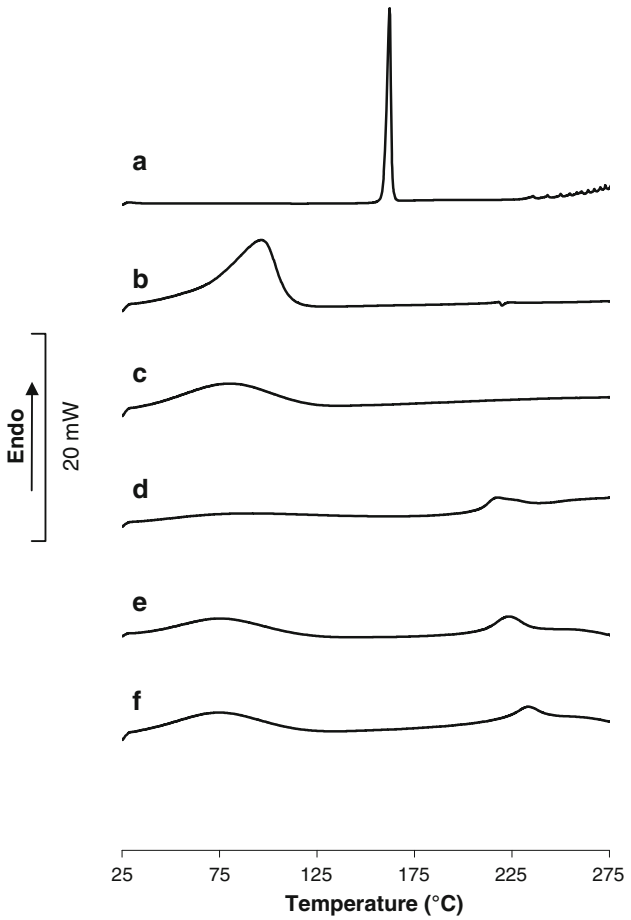

Fig. 5 DSC spectra of indomethacin (*a*), β-CD (*b*), IN–CD complex (*c*), BSA (*d*), IN–BSA (*e*) and IN–CD–BSA (*f*)

endothermal peak of IN, indicating that the drug can penetrate into the cyclodextrin cavity replacing the water molecules. This behaviour is also evident in the presence of albumin nanospheres or albumin nanospheres carrying inclusion complex: the disappearance of IN peak confirmed the strong drug interaction with albumin or albumin/beta-cyclodextrin carrier. Moreover, the disappearance of IN peak in all the samples can be attributed to the spray-drying method which can induce a polymorphic change from a crystalline form to an amorphous, as reported in previous works [35, 36].

Swelling studies

Immediately after nanoparticles reconstitution their size ranged from 170 to 400 nm with a polydispersity index lower than 0.20. Swelling ability of IN–BSA and IN–CD–BSA was determined at pH 2.0 and 7.4. Figure 6 reports the mean diameter calculated during the first 60 min after nanosphere suspension reconstitution. The IN–BSA nanospheres showed higher swelling ability than IN–CD–BSA

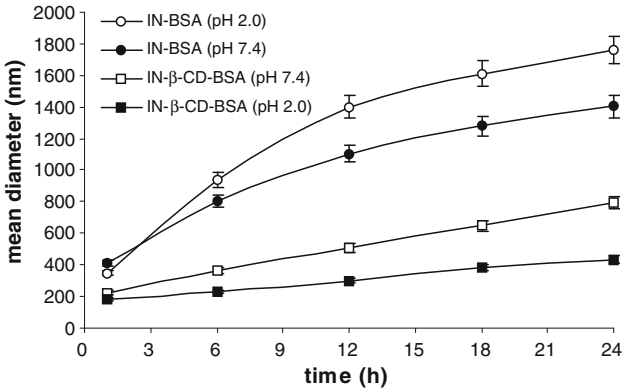

Fig. 6 Swelling profiles (mean diameter ± SD, $n = 5$) of IN–BSA and IN–β-CD–BSA nanospheres at pH 2.0 and 7.4

nanospheres at each pH analyzed. The presence of CD decreased the protein swelling capacity allowing a linear increase of nanosphere mean diameter; this can be explain due to reduced interactions of buffer solutions with albumin hydrophilic groups involved with hydroxyl groups of CD and to a control of water entry in the polymeric network. Moreover, the swelling ability was more evident at pH 7.4 than pH 2.0 according to the ionization of IN (pK_a of 4.5) whose presence in the nanospheres structure can influence water entry. In the case of IN–CD–BSA nanospheres, the environmental pH can also influence the amount of free (not complexed) IN in the nanoparticle structure. The higher nanosphere swelling at alkaline pH with respect to acidic pH can be attributed to the higher amount of free IN at pH 7.4, as confirmed by the lower stability constant of the complex at this pH.

In vitro release studies

For in vitro release profiles, Mo for each formulations was calculated from drug loading obtained as previously described. The results for drug loading analysis (drug loading %) were as follows: 0.91 ± 0.05, IN–EudL; 0.87 ± 0.06, IN–EudS; 9.92 ± 0.10, IN–CD; 1.65 ± 0.02, IN–CD–EudL; 1.62 ± 0.09, IN–CD–EudS; 1.64 ± 0.05, IN–BSA; 0.82 ± 0.05, IN–BSA–EudL; 0.79 ± 0.08, IN–BSA–EudS; 2.48 ± 0.05, IN–CD–BSA; 1.23 ± 0.01, IN–CD–BSA–EudL; 1.15 ± 0.06, IN–CD–BSA–Eud). The in vitro release profiles from uncoated samples are shown in Fig. 7. Drug release at pH 7.4 was higher than pH 2.0 according to IN ionization degree at these different pH values. Moreover, IN–CD, IN–BSA and IN–CD–BSA showed significantly higher release profiles compared to the corresponding commercial drug at each pH analyzed. This can be attributed to the increased water solubility following complexation and the reduced crystallinity of the spray-dried product. The diffusional exponent indicative of

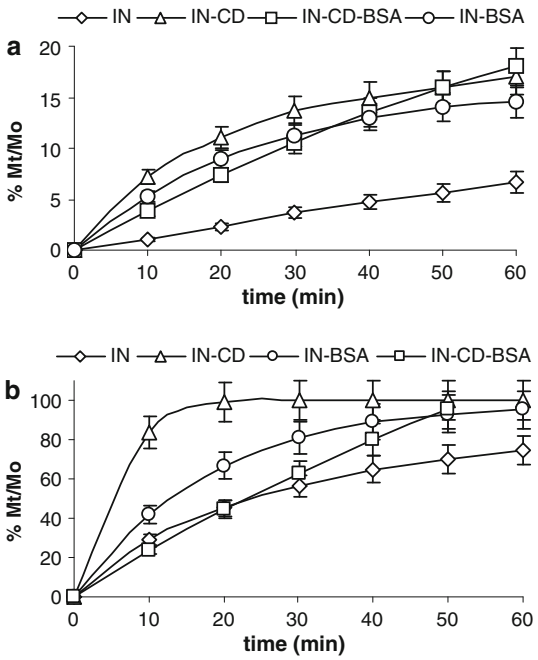

Fig. 7 Indomethacin release (% fractional amount ± SD, $n = 3$) at pH 2.0 (**a**) and pH 7.4 (**b**)

the release mechanism is reported in Table 2. The values of n from Peppas equation for IN–CD–BSA was about $\cong 1$ suggesting that nanosphere swelling plays a role in the control of drug release. In contrast, n values for IN–CD and IN–BSA ranged from 0.56 to 0.62 indicating a Fickian-type kinetic of release. This behaviour is in accordance with swelling studies as described in Fig. 6.

At pH 2.0, the presence of EudL as well as EudS determined negligible IN release from all formulations (% M_t/M_o lower than 8% after 60 min). It is due to their low solubility at acidic pH. In fact, EudL and EudS tend to dissolve at pH 6.0 and 7.0 respectively. For this reason, they are considered suitable coating materials for adequately protect the drug during its passage through the stomach and small intestine [37].

At pH 7.4, the presence of EudL as well as EudS decreased IN availability with respect to commercial IN.

Table 2 Diffusional exponent (n) indicative of the release mechanism

Sample	pH	Diffusional exponent (n)
IN–CD	2.0	0.5856
	7.4	0.6234
IN–BSA	2.0	0.5621
	7.4	0.6055
IN–CD–BSA	2.0	0.9368
	7.4	0.9472

Eudragit-coated albumin nanospheres carrying inclusion complexes for oral administration of IN

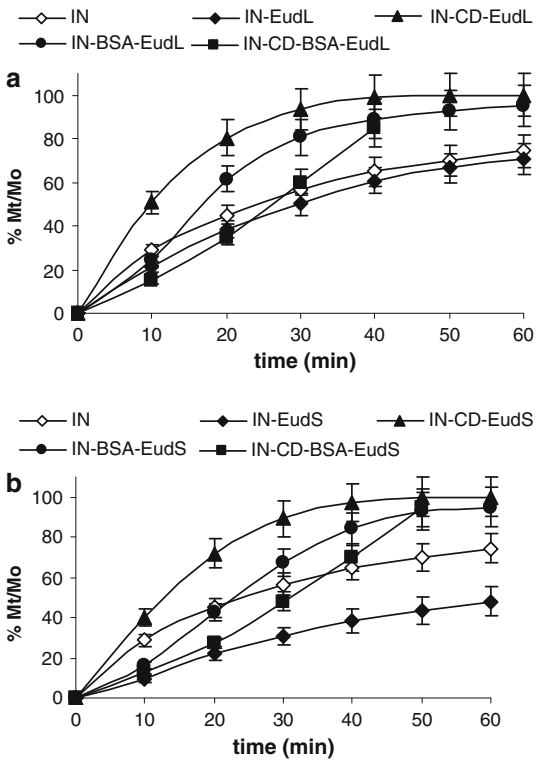

Fig. 8 Indomethacin release (% fractional amount ± SD, $n = 3$) at pH 7.4 from EudL (**a**) and EudS (**b**) coated samples

Moreover, the presence of CD, BSA or CD–BSA in the samples provided an higher drug availability with respect to the pure coated drug, confirming that complex and nanospheres are able to improve drug solubility (Fig. 8).

Conclusions

The solubility of IN can be increased by inclusion complexation with CD or protein/drug interaction with albumin nanospheres. The IN–CD complex and albumin nanospheres were characterized by enhanced release rate with respect to the commercial drug. Moreover, the inclusion complex IN–CD carried into BSA nanospheres provided a zero order drug release kinetic. The coating process with EudL and EudS allowed to obtain a negligible release at acidic pH without limit drug availability at pH 7.4.

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