

Design of indomethacin-loaded nanoparticles: effect of polymer matrix and surfactant

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Abstract: Despite recent advances in nonsteroidal anti-inflammatory drug (NSAID) formulations, the design of targeted delivery systems to improve the efficacy and reduce side effects of NSAIDs continues to be a focus of much research. Enteric nanoparticles have been recognized as a potential system to reduce gastrointestinal irritations caused by NSAIDs. The aim of this study was to evaluate the effect of EUDRAGIT[®] L100, polyethylene glycol, and polysorbate 80 on encapsulation efficiency of indomethacin within enteric nanoparticles. Formulations were developed based on a multilevel factorial design (three factors, two levels). The amount of polyethylene glycol was shown to be the factor that had the greatest influence on the encapsulation efficiency (evaluated response) at 95% confidence level. Some properties of nanoparticles like process yield, drug-polymer interaction, particle morphology, and in vitro dissolution profile, which could affect biological performance, have also been evaluated.

Keywords: nonsteroidal anti-inflammatory, indomethacin, enteric polymer, polyethylene glycol, nanoparticles

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are popular and effective treatments for pain and inflammation. They can be delivered to patients by more than one route, but the oral route is the most desirable and preferred.¹ Nevertheless, when administered orally, they have a substantial toxicity profile, with several side effects.^{2,3} There are some differences in the propensity of single NSAIDs to cause gastrointestinal (GI) irritation, and recent studies have shown that more than 50% of patients taking NSAIDs have some damage associated with the upper GI tract.⁴ In this regard, the most common NSAID-induced adverse reactions include discomfort, ulcers, and bleeding.⁵

To understand both the desired and adverse effects of NSAIDs, it is important to understand the role of prostaglandins (PGs). PGs are potent mediators of inflammation that result in edema, pain, and vasodilation. The inhibition of these compounds is associated with analgesic and anti-inflammatory effects.⁵ NSAIDs block mucosal PG synthesis by inhibiting cyclooxygenase (COX) activity. Specifically, COX-1-derived PGs have been considered important in maintaining homeostasis of intestinal mucosa. Previously, COX-1 inhibition alone was thought to cause a reduction of blood flow in the intestinal mucosa and increased mucosal permeability, resulting in mucosal injury. Recently, a study using an animal model has shown that small-intestinal mucosal injuries occurred only after both COX-1 and COX-2 were inhibited.^{4,6}

Indomethacin is an NSAID used most commonly for the treatment of inflammation and pain resulting from rheumatic disease (arthritis), and less commonly in

postoperative pain management.⁷ Another study has shown that it can exhibit chemoprotective effects against tumors and reduce the risk of colon cancer.⁸ In spite of its benefits, indomethacin appears to have a high prevalence of gastric side effects.^{4,9}

Recently, special emphasis has been given to designing new oral delivery systems for existing drugs in order to improve therapeutic efficacy and/or reduce side effects. Some studies have reported attempts involving indomethacin derivatives, gastroprotective prophylaxis with proton pump inhibitors, gastric antisecretory drugs, or PG analogs (misoprostol) to protect the GI tract against mucosal damage caused by conventional NSAIDs.^{10–13} These alternatives have limitations in terms of efficacy and side effects; thus, there is no formulation yet that can completely avoid the side effects associated with NSAIDs.¹⁴ In this regard, enteric nanoparticles (NPs) have been recognized as potential carriers for reducing GI irritations caused by NSAIDs. Enteric NPs are solid colloidal particles, ranging in size from 1 to 1,000 nm, designed to pass through the stomach unaltered and then to dissolve in the intestine.¹⁵ They could be classified as target drug delivery systems that retain the efficacy and reliability of traditional NSAIDs, while reducing adverse effects.⁵ For the production of such enteric NPs, solvent extraction/evaporation methods have been widely applied since they can be used to encapsulate both hydrophilic and hydrophobic drugs.^{16–20} On the other hand, the selection of a suitable carrier and/or coating material is a critical parameter in designing targeted drug delivery systems.^{19,20}

In the present work, EUDRAGIT® L100 (Evonik Industries, Essen, Germany) and polyethylene glycol with average molecular weight (M_w) 2000 (PEG 2000) blends have been used in order to design a target delivery system while also enhancing drug bioavailability. The objective of this study was to evaluate the effect of EUDRAGIT® L100, PEG, and polysorbate 80 on encapsulation efficiency (EE) of indomethacin within enteric NPs. Some properties of NPs such as process yield (PY), drug–polymer interaction, particle morphology, and in vitro dissolution profile, which could affect biological performance, have also been evaluated.

Materials and methods

Materials

Indomethacin, PEG 2000, and polysorbate 80 (Tween® 80) were purchased from Sigma-Aldrich (St Louis, MO, USA). EUDRAGIT® L100, an enteric anionic copolymer based on methacrylic acid and methyl methacrylate (1:1), was generously supplied by Evonik. Analytical grade ethanol

(95.5%) was provided by Vetec (Vetec Química Fina Ltda, Rio de Janeiro, Brazil). Hydrochloric acid (32%) and sodium phosphate tribasic, used to prepare the dissolution media, were provided by Merck Millipore (Billerica, MA, USA). All chemicals were used as received, without further purification. Distilled water was used throughout this study.

Experimental design

A multilevel factorial design (2³) was used to study the effect of each factor, as well as the effects of interactions between factors, on the response variable. The studied factors were the amounts of Tween 80® (X1), PEG (X2), and EUDRAGIT® L100 (X3). The response variable was the EE (Y), expressed in percent (related to the amount of active ingredient encapsulated). Table 1 summarizes the independent and dependent variables. The resulted formulations are listed in Table 2. All experiments were carried out three times. The order of the experiments was fully randomized.

Enteric NP preparation

In the present work, different formulations using indomethacin/EUDRAGIT® L100 (1:7, 1:10 w:w) have been developed (as shown in Table 2). A modified oil/water (O/W) nanoencapsulation method was used to encapsulate indomethacin into NPs. The general procedure represented in Figure 1 is as follows: the enteric NPs were formed by dropping 20 mL of an alcoholic polymer solution (concentrations listed in Table 2), with or without drug (indomethacin/placebo) and with or without hydrophilic polymer (40 mg) into 120 mL of acidic aqueous phase containing the surfactant (concentrations listed in Table 2), under stirring (Ultra Turrax Homogenizer, Model T-25; IKA, Wilmington, DE, USA) at 9,600 rpm for 5 minutes. The formed NPs were washed three times (Professional Ultrasonic Cleaner, USC 3300, Unique, Brazil) and collected by centrifugation (3,000 rpm, 10 minutes) (Refrigerated Centrifuge Sigma 4K 15; DJB Labcare Ltd, Buckinghamshire, England). Finally, samples frozen for 24 hours at –40°C were lyophilized for 4 hours (Freeze Bench Dryer, Enterprise IA, Terroni, Brazil) and

Table 1 Experimental design: factors and response

Factors (independent variables)	Levels		Response (dependent variable)
	–I	I	
X1: Surfactant (Tween® 80)	30	40	Y: Encapsulation Efficiency
X2: Hydrophilic polymer (PEG 2000)	0	40	
X3: Enteric polymer (EUDRAGIT® L100)	140	200	

Abbreviation: PEG 2000, polyethylene glycol with average molecular weight 2000.

Table 2 Experimental design matrix, encapsulation efficiency and process yield values

Formulation	X1	X2	X3	EE (%) ± SD	PY (%) ± SD
I 1	30	0	140	56 ± 1	91 ± 8
I 2	40	0	140	88 ± 4	92 ± 13
I 3	30	40	140	96 ± 5	71 ± 15
I 4	40	40	140	100 ± 0	75 ± 15
I 5	30	0	200	90 ± 4	94 ± 3
I 6	40	0	200	79 ± 5	94 ± 6
I 7	30	40	200	96 ± 2	87 ± 12
I 8	40	40	200	100 ± 0	89 ± 10

Notes: Three factors, X1, X2, and X3.

Abbreviations: PY, process yield; EE, encapsulation efficiency; SD, standard deviation.

stored at room temperature. All experiments were done at room temperature (25°C). Throughout this work, the term nanopowders will be used to refer to solid powders of NPs, often containing micron-sized agglomerates of NPs.

Nanopowder characterization

PY (%)

First, the designed formulations were evaluated by considering the PYs; according to previous work, it can be defined as the weight ratio of the amount of NPs to the total amount of polymer and active ingredient used.²¹ This parameter was calculated by using Equation 1:

$$\% \text{ PY(w/w)} = \frac{m_{\text{NP}}}{m_{\text{T}}} \times 100 \quad (1)$$

where PY (%) is the process yield in percentage, m_{NP} is the mass of powder recovered after freeze-drying, and m_{T} is the initial mass of indomethacin plus EUDRAGIT® L100

and PEG in formulation (weighted by using an analytical balance [AF-210AN; Bioprecisa, Curitiba, PR, Brazil]). The encapsulation method was accomplished in triplicate ($n = 3$). PY values were expressed as mean ± standard deviations.

EE (%)

The EE was determined by a spectrophotometric method (ultraviolet [UV] spectrophotometer [Mini UV-1204; Shimadzu Corp, Kyoto, Japan]). First, the calibration curve was obtained for indomethacin at 318 nm ($R^2 = 0.99974$). The amount of indomethacin loaded into NPs was estimated directly by dissolution of 10 mg of NPs in 10 mL of ethanol at room temperature. After the NPs had dissolved, UV absorption measurements at 318 nm were measured. Then, indomethacin concentrations were calculated from the equation of standard curve ($y = 17.75x$). All EE were calculated in triplicate ($n = 3$) by using Equation 2.²¹ EE values were expressed as mean ± standard deviations.

$$\text{EE (\%)} = \frac{m_{\text{exp}}}{m_{\text{teo}}} \times 100 \quad (2)$$

where m_{exp} is the mass of indomethacin experimentally determined and m_{teo} is the initial mass of indomethacin.

Fourier transform infrared spectroscopy analysis

For fourier transform infrared spectroscopy (FTIR) analysis we used a Universal attenuated total reflectance (ATR) accessory (infrared spectrometer; PerkinElmer Frontier, Waltham, MA, USA). The UATR module produces high quality spectra through the use of a pressure arm allowing good contact of the sample with the diamond crystal. The pressure arm

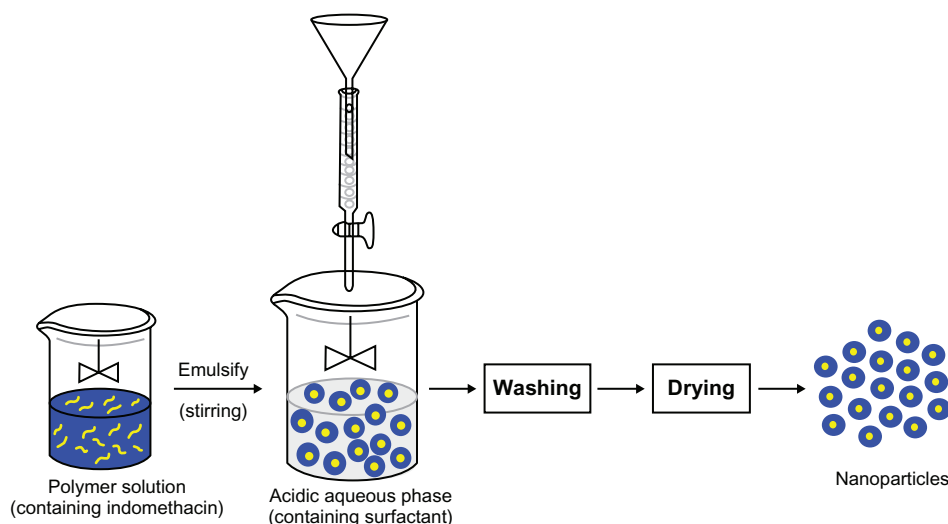


Figure 1 Schematic representation of nanoencapsulation procedure.

force indicator ensures first-class sample-to-sample and operator-to-operator reproducibility. All samples, including indomethacin, pure polymers, and nanopowders were analyzed under the same conditions (pressure 95%). FTIR spectra were obtained in the 4,000 and 650 cm^{-1} range, with a resolution of 1 cm^{-1} and accumulation of 100 scans (scan rate 0.5 cm^{-1}/s). A “background” scan was run before every analysis.

X-ray diffraction analysis

Qualitative X-ray diffraction studies were performed using a PANalytical X-ray diffractometer (X'Pert PRO, equipped with auto X'Celerator; PANalytical, Almelo, the Netherlands). The samples (indomethacin, pure polymers, and nanopowders) were analyzed over a 2θ degree range of 10° – 100° at a scanning rate of $2^\circ/\text{minute}$.

Morphological analysis

Nanopowders were ultrasonically redispersed in hexane for approximately 10 minutes. Once the sample was dried it was coated by sputtering with a layer of gold of approximately 20 nm (Balzers, SCD 050). Samples were observed in a scanning electron microscope (SEM) (JSM-700 1F; JEOL, Tokyo, Japan), operated at 15 kV, and magnifications of between $300\times$ and $35,000\times$.

Dynamic light scattering

Dynamic light scattering (DLS) measurements were taken using a Zetasizer Nano instrument (Zetasizer Nano-ZS[®]; Malvern Instruments, Malvern, UK) at 25°C . Samples were dispersed in 0.1 M hydrochloric acid (HCl). Aliquots of 900 μL were measured in a small volume cuvette (DTS 1060C). A minimum of 20 runs were made for each sample (measurement angle was 173° backscatter detection). The intensity weighted average diameter (Z-average) and the polydispersity index (PDI) were measured for all formulations.

In vitro dissolution testing

Dissolution tests were carried out in a dissolution apparatus (dissolution tester Nova Ética, 299/6 ATTS; Nova Ética, São Paulo, Brazil). They were performed under simulated GI conditions (two stages, acid and buffer), as described for delayed-release dosage forms.²² Specifically, a solution of HCl 0.1 N (pH 1.2) and phosphate buffer (pH 6.8) prepared by mixing HCl 0.1 N with Na_3PO_4 0.2 M (3:1) were used for the acid and buffer stage, respectively. Accurately weighed NPs containing the equivalent of 20 mg of indomethacin were transferred to 200 mL of the dissolution medium at $37^\circ\text{C} \pm 0.5^\circ\text{C}$, at a rotation speed of 100 rpm (using paddle

stirring). At predetermined intervals, aliquots of the fluid were manually taken from the vessel and passed through the cell system on the UV spectrophotometer (Mini UV-1204; Shimadzu Corp) and returned to the vessel. After 2 hours of operation in 0.1 N HCl, the dissolution test was accomplished under buffer stage for 10 hours.

Statistical analysis of data

The multilevel factorial design, consisting of 24 runs (to be run in three blocks), was analyzed using Statgraphics (Version 5.1 Plus; StatPoint Technologies, Inc., Warrenton, VA, USA). Variance analysis results for EE were used to test the statistical significance of the estimated effects. *P*-values lower than 0.05 ($P < 0.05$) were considered statistically significant.

Results and discussion

Determination of PY

The encapsulation procedure was successfully developed since all PY values are higher than 70% (minimum 71%; maximum 94%). In the present study, PY values above 70% are a quite respectable result considering that PEG is highly soluble in water. Table 2 summarizes the mean PY values for all experiments. Individual PY values are shown in Figure 2.

From Figure 2, large individual differences for PY repeating experiments were observed. It is worth remembering that the PY is strongly influenced by the NPs' recovery and separation methods (including decantation, washing, and centrifugation processes). Thus, special care must be taken during NPs' recovery in order to achieve good reproducibility.

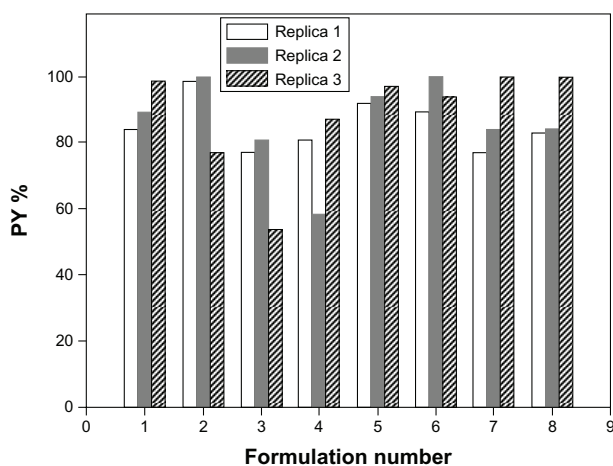


Figure 2 Process yield values of indomethacin encapsulation. Abbreviation: PY, process yield.

In general, PY values do not show great differences when EUDRAGIT® L100 is used in different concentrations. On the other hand, for the same EUDRAGIT® L100 concentration, a slight increase was observed in yield values as a function of surfactant concentration. Moreover, there was a negative effect of PEG concentration on PY, since lower PY values were obtained when higher PEG concentrations were used (Samples I3, I4, I7 and I8). These results can be explained due to the loss of the hydrophilic material in the outer aqueous phase during the encapsulation procedure. Therefore, low concentration of PEG reduces the final mass of NPs, as can be seen in equation 1. PY of placebo was around 77%.

Determination of encapsulation efficiency

EE depends not only on the encapsulation method but also on polymer–drug affinity. Moreover, some formulation parameters, such as the weight ratio of polymer to drug or emulsifier type, will influence the amount of drug loading. Table 2 summarizes the mean EE values for all experiments (individual values are shown in Figure 3). As can be seen in Table 2, all EE values were greater than 50% (minimum 56%; maximum 100%). These values are understandable since indomethacin is a hydrophobic drug, showing higher affinity for polymer coating than for external aqueous medium (used during NP formation). Additionally, fast enteric polymer precipitation can prevent drug loss into the continuous phase, increasing EE. However, as shown in Table 2, formulation I1 had extremely low EE compared to other formulations, especially when compared to formulation I2. This experimental result seems to be a consequence of a negative synergistic

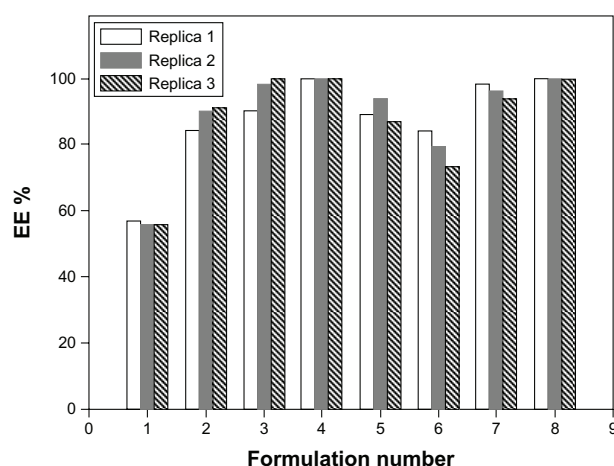


Figure 3 Encapsulation efficiency values of indomethacin.
Abbreviation: EE, encapsulation efficiency.

effect caused by the combination of low concentrations of Tween 80®, PEG, and EUDRAGIT® L100.

On the other hand, it is clear from Table 2 that higher PY values are not a necessary condition to obtain better EE values, and vice versa. Thus, higher EE was found for formulations containing PEG (I3, I4, I7, and I8). In contrast, the lowest PY values were observed for the same formulations. These results demonstrate that low PY values are due to the loss of the hydrophilic polymer. As expected, values that were quite consistent were observed for EE repeating the experiment from Figure 3. These results corroborate previous findings that EE depends on physical and chemical properties of encapsulating polymers, solvent systems, polymer–drug interactions, and properties of the continuous phase.²³

Statistical analysis

Table 3 shows each of the estimated effects and interactions for EE. The statistical significance of each effect was tested by comparing the mean square against an estimated experimental error (analysis of variance [ANOVA] shown in Table 4). In addition, the Pareto chart (Figure 4) shows each of the estimated effects in decreasing order of magnitude. The length of each bar is proportional to the standardized effect, which is the estimated effect divided by its standard error. The vertical line can be used to judge which effects are statistically significant. Any bar extending beyond the line corresponds to an effect which is statistically significant at the 95% confidence level. Then, in this study, there are seven effects that have *P*-values less than 0.05, indicating that they are significantly different from zero at the 95% confidence level.

Among the three studied factors, PEG was the most important for EE (see Figure 4). These results could be explained by the presence of polar groups in their chemical structure. Thus, such a structure makes it possible for the PEG to exhibit a nonionic cosurfactant behavior.²⁴ Consequently,

Table 3 Summary of estimated effects for encapsulation efficiency

Effects	Value	Standard errors
Average	88.1667	0.709033
A: Tween® 80	7.16667	1.41807
B: PEG 2000	19.6667	1.41807
C: EUDRAGIT® L100	6.0	1.41807
AB	-3.16667	1.41807
AC	-10.8333	1.41807
BC	-6.0	1.41807
ABC	10.8333	1.41807
Block	1.91667	2.00545
Block	-1.08333	2.00545

Abbreviation: PEG2000, polyethylene glycol with average molecular weight 2000.

Table 4 ANOVA for encapsulation efficiency

Source	Sum of squares	Df	Mean square	F-ratio	P-value
A: Tween® 80	308.167	1	308.167	25.54	0.0002
B: PEG 2000	2,320.67	1	2,320.67	192.34	0.0000
C: EUDRAGIT® L100	216.0	1	216.0	17.90	0.0008
AB	60.1667	1	60.1667	4.99	0.0424
AC	704.167	1	704.167	58.36	0.0000
BC	216.0	1	216.0	17.90	0.0008
ABC	704.167	1	704.167	58.36	0.0000
Blocks	11.0833	2	5.54167	0.46	0.6409
Total error	168.917	14	12.0655		
Total (corr)	4,709.33	23			

Notes: A, X1; B, X2; C, X3. AB, BC, AC, and ABC are the interactions between the factors.

Abbreviations: ANOVA, analysis of variance; corr, corrected; Df, degrees of freedom; PEG 2000, polyethylene glycol with average molecular weight 2000.

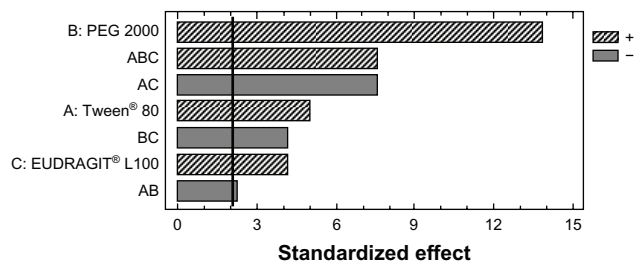
there is a greater stability in the system, resulting in higher EE values. The positive influence of this cosurfactant on the physicochemical properties of NPs can potentially be useful for other formulations. For this reason, special interest needs to be placed on polymers like PEG, which acts as a polymeric cosurfactant. In general, such polymers can improve EE and enhance the solubility of hydrophobic drugs.

In addition, R^2 (96.4%) and adjusted R^2 (94.8%) values indicate that the model as fitted is suitable to explain the variability in EE. The standard deviation of the residuals and the mean absolute error (average value of the residuals) were 3.47354 and 1.99306, respectively. On the other hand, the Durbin–Watson statistic (2.44467, $P = 0.0618$) shows that there is no indication of serial autocorrelation in the residuals.

Physico-chemical characterization

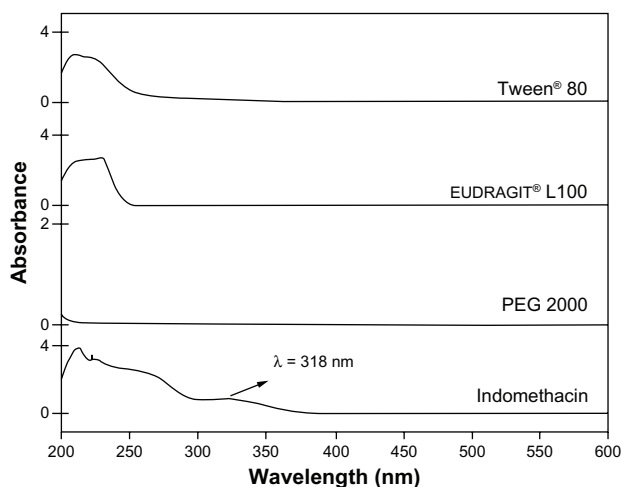
UV spectroscopy

UV spectra of all compounds used in the encapsulation procedure were measured in order to verify any interference in the quantification of indomethacin. As can be seen in

**Figure 4** Standardized Pareto chart for encapsulation efficiency.

Notes: A, X1; B, X2; C, X3. AB, BC, AC, and ABC are the interactions between the factors.

Abbreviations: PEG 2000, polyethylene glycol with average molecular weight 2000.

**Figure 5** UV spectra of indomethacin and pure polymers.

Abbreviations: UV, ultraviolet; PEG 2000, polyethylene glycol with average molecular weight 2000.

Figure 5, the PEG 2000 did not show absorption peak in the UV region. Also, EUDRAGIT® L100 and Tween 80® showed the UV absorption peaks at around 220 nm. Thus, the study of indomethacin can be accomplished at 318 nm since there is no interference of the excipients at this wavelength.

Infrared spectroscopy

Drug–polymer compatibility is of great importance in the design of pharmaceutical formulations, since formulation effectiveness (therapeutic activity and/or bioavailability) can be improved on the basis of their composition. From this perspective, mid-infrared absorption spectroscopy has been widely used to study drug–polymer interactions, since each molecule absorbs specific frequencies that are characteristic of its structure.^{25,26} In this regard, the two important regions for a preliminary examination of a spectrum are the ranges between 4,000–1,300 and 900–650 cm^{-1} . The high frequency section of the spectrum is called the functional group region. Moreover, the intermediate segment of the spectrum, 1,300–900 cm^{-1} is usually referred to as the “fingerprint” region.²⁵

Indomethacin, pure polymers, and each formulation were subjected to a qualitative FTIR spectroscopic analysis, to ascertain whether there is any interaction and/or incompatibility between the drug and the polymers used. As shown in Figure 6, the infrared polymer spectra are in agreement with previous works, showing characteristic bands for each molecule (see Table 5).²¹ For EUDRAGIT® L100, the absence of absorption bands in the “fingerprint” region is evidence that the polymers are free of impurities such as monomers, since there is no evidence of C=C bonds.

Pure indomethacin spectrum shows characteristic peaks for gamma-phases of indomethacin at 3,025 cm^{-1} (aromatic

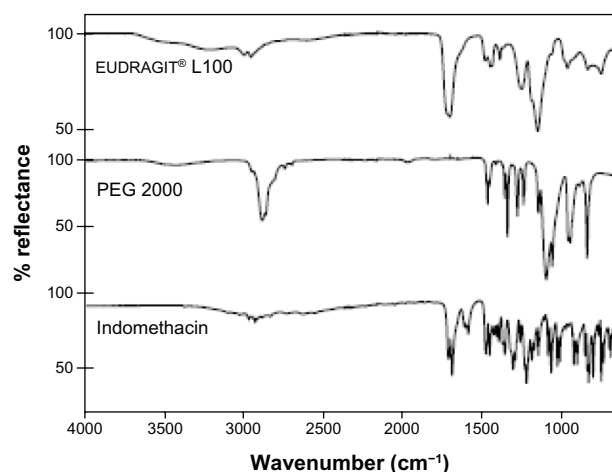


Figure 6 IR spectra of indomethacin and pure polymers.

Abbreviations: IR, infrared; PEG 2000, polyethylene glycol with average molecular weight 2000.

C–H stretching), 2,967 cm^{-1} (C–H stretching vibrations), 1,712 cm^{-1} (C=O stretching vibrations), 1,261 cm^{-1} (asymmetric aromatic O–C stretching), and 1,086 cm^{-1} (symmetric aromatic O–H stretching).^{27,28}

Infrared spectra of all NPs are shown to be identical (Figure 7). Therefore, only II formulation characteristic peaks are shown in Table 5. From the data obtained (Table 5), it was observed that characteristic peaks for NPs appeared with identical frequencies (or with minor differences) to those observed for the functional groups of the EUDRAGIT® L100. Thus, there was no evidence of strong chemical interaction between the drug and the polymers.

In spite of all formulations, NPs and pure EUDRAGIT® L100 showed similar spectra (see Figures 6 and 7) and a deeper fingerprint comparison revealed some subtle changes in the spectra of NPs (1,015 cm^{-1} , 929 cm^{-1} ,

800 cm^{-1} , and 793 cm^{-1}). These results suggest that the peaks are due to indomethacin molecules that are inside the NPs. However, indomethacin absorption patterns in the 1,300–650 cm^{-1} region are complex due to its aromatic structure. Consequently, this region may be less useful in structural characterization, since bands of strong or medium intensity could be more non-specific, while a weak band above 2,000 cm^{-1} may be characteristic of a specific group. In this regard, further studies will be necessary to have a better understanding of those results.

X-ray diffraction analysis

Mechanical properties of polymers can be altered by the degree of crystallinity. For instance, because of the uniform arrangement of its chains within the lattice structure, a crystalline polymer will degrade more slowly than an amorphous one. However, crystalline polymers are brittle and usually less suited for drug delivery applications. Further, amorphous polymers possess poor mechanical toughness.²⁹ For that reason, polymers used in drug delivery are usually a mixture of crystalline and amorphous forms, as was the case in this study.

X-ray diffraction patterns of indomethacin and polymers are shown in Figure 8. While indomethacin and PEG show two diffractograms typical of crystalline material, rich in narrow and symmetric peaks, EUDRAGIT® L100 is predominantly amorphous in nature.

The observed powder pattern of indomethacin is in agreement with those reported in previous works.^{28,30} The diffractogram shows characteristic sharp intensity diffraction peaks at 2θ values of 11.7°, 17.1°, 19.7°, 20.9°, 21.9°, 24.1°, 26.7°, and 29.4°, which reflect the crystalline nature of the

Table 5 Assignment bands of indomethacin, PEG, EUDRAGIT® L100 and formulation II

Assignments	Indomethacin	PEG 2000	EUDRAGIT® L100	Formulation II
ν O–H	3,370 (w)	3,435 (w)	3,505, 3,224 (m)	3,478, 3,205 (m)
ν Csp ³ –H	2,967, 2,928 (w)	2,884 (s)	2,997, 2,952 (m)	2,995, 2,950 (m)
ν C=O	1,712, 1,690 (s)	–	1,704 (s)	1,701 (s)
ν C=C	1,613, 1,603, 1,588, 1,478, 1,454 (s)	–	–	–
δ C–H	1,428, 1,411, 1,396 (m-w)	1,467, 1,455 (s)	1,481, 1,448 (m)	1,480, 1,450 (m)
δ O–H	–	1,360, 1,342, 1,279, 1,241 (s)	1,389 (m-w)	1,387 (w)
ν C–N	1,372, 1,358 (m-w)	–	–	–
ν C–O	1,306, 1,291 (s)	1,149, 1,098, 1,060 (s)	1,253 (s)	1,320 (sh), 1,252 (s)
ν C–CO–O	1,233, 1,222 (s-m)	–	1,152 (s)	1,151 (s), 1,091, 1,074 (sh)
Aromatic ring	1,189, 1,148, 1,028, 1,012 (s)	–	–	1,015 (m-w)
γ O–H	1,086, 1,067 (s)	958, 947, 841 (s)	965 (m)	964 (m)
γ CH	926, 905 (s)	–	–	930 (m)
–	–	–	837, 752 (m)	838, 752 (m)
Aromatic ring	839, 832, 803, 752, 702 (s-m)	–	–	802, 794 (w)

Note: Intensities reported in semi-quantitative terms.

Abbreviations: PEG, polyethylene glycol; s, strong; m, medium; w, weak; sh, shoulder.

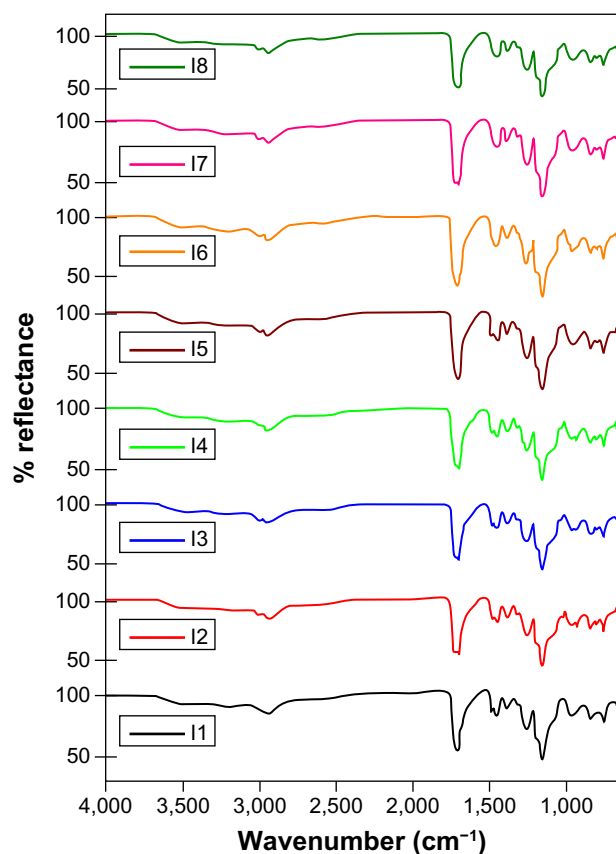


Figure 7 Comparison of IR spectra of indomethacin-loaded nanoparticles.
Abbreviation: IR, infrared.

drug (Figure 8). In the same way, PEG diffractogram shows characteristic diffraction peaks at $2\theta^\circ$ values of 19.3° and 23.4° . In contrast, EUDRAGIT® L100 diffractogram shows two amorphous halos between 10° and 37° (2θ).

As can be seen in Figure 9, X-ray diffraction patterns of all formulations show two amorphous halos between 10° and

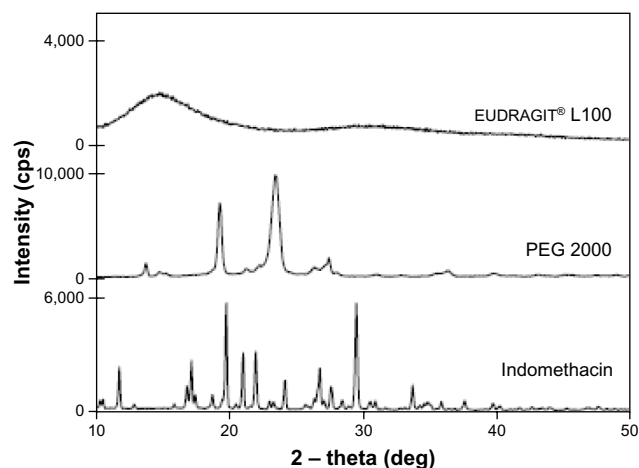


Figure 8 Qualitative X-ray diffractograms of indomethacin and pure polymers.
Abbreviations: cps, counts per second; PEG 2000, polyethylene glycol with average molecular weight 2000.

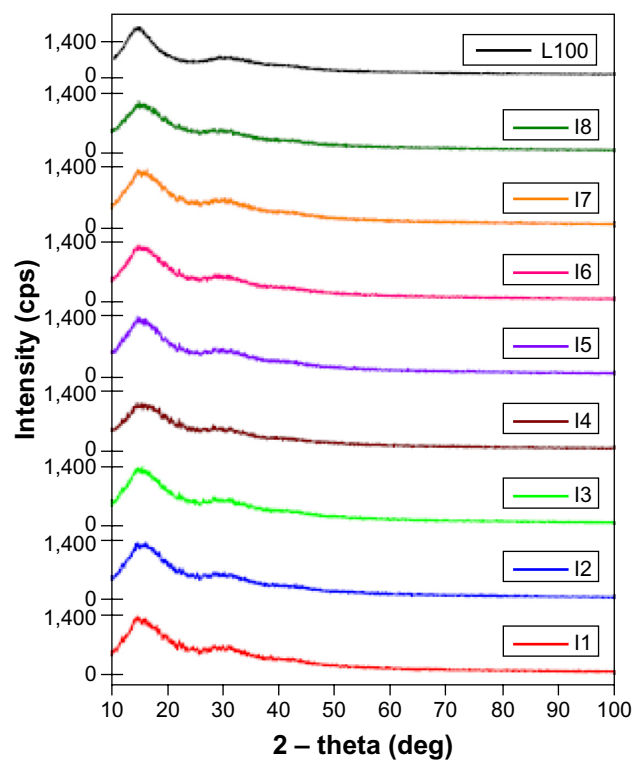


Figure 9 Comparison of the qualitative X-ray diffractograms of nanoparticles and EUDRAGIT® L100.

Abbreviation: cps, counts per second.

37° (2θ), as observed for the amorphous polymer. This result suggests a reduced degree of crystallinity of the drug in these formulations. Moreover, the absence of characteristic peaks of lower indomethacin crystallinity indicates a quite successful indomethacin encapsulation process, regardless of the amount of polymer matrix used.

Morphological analysis

In previous works, the effect of PEG in the morphology of NPs has been discussed.^{21,31} For this reason, in the current analysis, particular emphasis was placed on samples without PEG. Figure 10 shows the SEM images of the formulation 15 (without PEG) at different magnifications $\times 4,000$, $\times 8,500$, $\times 16,000$, $\times 25,000$, and $\times 33,000$. As can be seen, Figure 10A and B, obtained from different regions of the sample, are showing irregular ball and laminar structures. Figure 10C and D detail different regions observed in Figure 10B. Both laminar and irregular structures are a result of smaller particle aggregation. Finally, Figure 10E and F are showing three-dimensional aggregations of NPs (around 100 nm). This strong aggregation is due to the formation of intermolecular hydrogen bonds between hydroxyl groups (from methacrylic acid copolymer).³¹ It is worth mentioning that carboxylic acids act as both hydrogen bond acceptors (carbonyl) and hydrogen

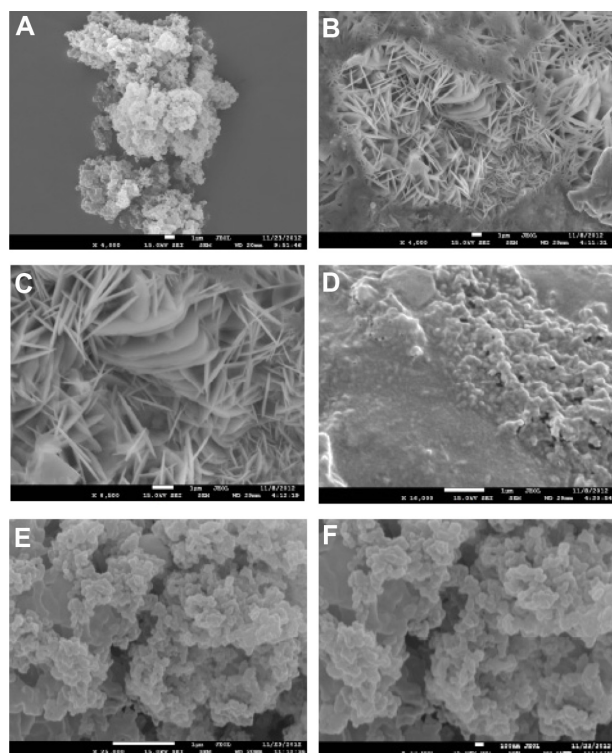


Figure 10 SEM micrographs of sample 15.
Notes: (A) $\times 4,000$; (B) $\times 4,000$; (C) $\times 8,500$; (D) $\times 16,000$; (E) $\times 25,000$; and (F) $\times 33,000$.
Abbreviation: SEM, scanning electron microscope.

bond donors (hydroxyl), participating often in hydrogen bonding. Hence, the interaction between the polyacid chains, and consequently the number of NPs aggregates, are governed by electrostatic effects.

Particle size and particle size distribution

Particle size and particle size distribution are important factors in the therapeutic performance of NPs. Batches with wide particle size distribution can show significant variations in drug loading and/or drug release. From DLS measurements, individual particle sizes and agglomerate sizes are around 100 nm and 1,000 nm, respectively. In general, very similar particle sizes were found for all formulations; it seems that no parameter, at the considered values, has great influence. On the other hand, all formulations show very high PDI in the range of 0.563–1.0. PDI measurements agree with the SEM images shown in Figure 10D–F, in which agglomerations might result from aggregation of smaller particles.

As an example, intensity, volume, and number distributions of sample 15 are shown in Figure 11. Three size distributions classes (around 100 nm, 1 μm and 6 μm) can be seen in Figure 11A and B, wherein the majority of the total particle volume comes from the 1 μm sizes. On the other hand, it is clear that agglomerations scatter much more light

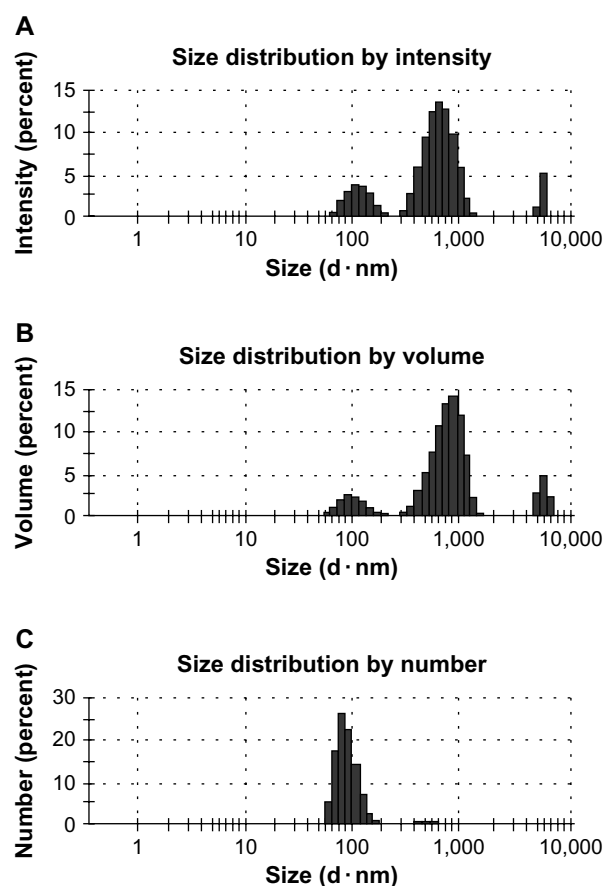


Figure 11 Particle size distributions.
Notes: Particle size distributions (A) intensity, (B) volume, and (C) number.

than small particles. Finally, on a number basis, it becomes more obvious that the 100 nm individual particles are the most significant size, as shown in Figure 11C.

In vitro dissolution studies

The therapeutic efficacy of a formulation intended to be administered by the oral route depends on its bioavailability.¹ It is well established that dissolution is recurrently the rate-limiting step in the GI absorption of a low soluble drug from a solid dosage form. Drug release of poorly water-soluble drugs like indomethacin has been shown to be unpredictable – it remains a problem for the pharmaceutical industry.²⁸ However, recent studies have shown that incorporation of small amounts of hydrophilic excipients, such as surfactants, alongside hydrophobic drugs could significantly improve its dissolution rate.^{28,32} Additionally, NPs coated with hydrophilic polymers such as PEG protect them from being engulfed by the macrophages or kupffer cells, thereby increasing their circulation time and enhancing drug bioavailability.²⁹

According to the release profile shown in Figure 12, it seems that there is no influence of PEG on in vitro

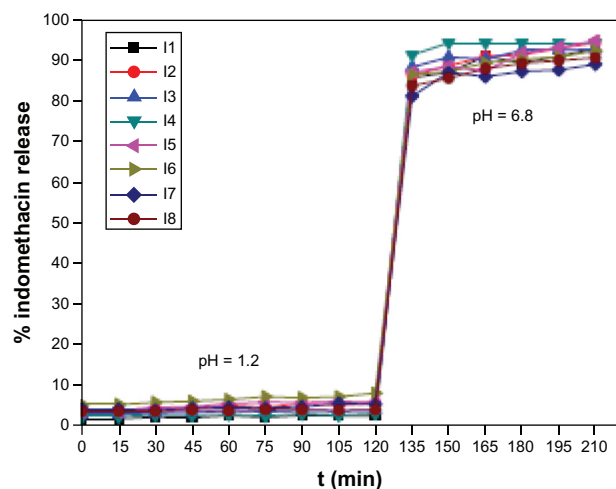


Figure 12 In vitro dissolution profiles of indomethacin loaded in nanoformulations.

indomethacin release from NPs. These results are in agreement with the low yield values, indicating low levels of PEG in NPs.

As can be seen in Figure 12, all dissolution profiles show the delayed release characteristics of an enteric coating. They show no release at acidic pH, while immediate releases were observed at pH 6.8. These results demonstrate that the anionic copolymer used has a pH dependent solubility (soluble at $\text{pH} > 6$) and is readily soluble in neutral to weakly alkaline solutions.¹⁵ Other terms such as “gastro resistant,” “enterosoluble,” and “pH sensitive” are used to refer to these dosage forms.¹ The results also reveal the success of the encapsulation process, since indomethacin seems to be inside the unswollen NPs.

Conclusion

This study clearly showed that the encapsulation procedure was successfully developed since all yield values and EEs were higher than 70% and 50%, respectively. Statistical analysis of multilevel factorial design showed seven significance effects on EE (95% confidence level), while PEG showed greater influence due to its cosurfactant behavior. On the other hand, X-ray diffraction and FTIR of NPs did not show evidence of strong interaction between the polymers and indomethacin. However, further theoretical studies will be considered to get a better understanding of the experimental changes in the $1,000\text{--}650\text{ cm}^{-1}$ region. Morphological analysis indicated that NP aggregates are governed by electrostatic effects. According to DLS measurements, the particle sizes and agglomerates sizes are around 100 nm and 1,000 nm, respectively. Finally, all formulations showed a delayed release, demonstrating great potential as indomethacin

carriers for oral administration. Toxicity studies and in vivo release of indomethacin remains to be investigated.

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Disclosure

The authors report no conflicts of interest in this work.

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