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NANOSTRUCTURED MATERIALS LOADED WITH IBUPROFEN AND COATED WITH A METHACRYLIC ACID COPOLYMER MATRIX

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Abstract. The synthesis of nanostructured materials (NMs) loaded with Ibuprofen (IBU) was carried out by a modified double emulsion (O/W/W) technique. An enteric coating which was made from the Eudragit L-100 (EL-100) and Eudragit L-30 D-55 (EL-30D-55) copolymers mixture was used as polymeric matrix. Nanostructured materials (NMs) were obtained varying the amounts of surfactant (20-40 mg) and the stirring rates (5600-15000 rpm). The average sizes of the NMs were around 300-800 nm. The best result of the nanostructured materials (NMs) process synthesis was reached with the combination of the copolymer EL-100 and EL-30 D-55. In this sample, the nanoparticles yield and encapsulation efficiency were higher than 40% and the release profiles presented a smaller burst. The release profiles indicated that the matrix used was suitable to prevent the contact of the active principle with the gastric medium and that this device could achieve an efficient release in the intestine.

1. INTRODUCTION

There is an increasing interest in optimizing efficacy of drug activity by using drug carrier materials. Since drug carrier plays a critical role on loading and releasing of the drug in a controlled way, many efforts have been devoted to design and prepare sustained and controlled drug-release systems [1-5]. Among the various drug carrier materials, organic polymer materials are widely used in scientific research and commercial field [6,7].

In the field of oral medicine, it is difficult to cure intestinal diseases completely because some ingested drugs would be adsorbed or digested in stomach before attain the intestine [8,9]. Specific Eudragit acrylic polymers were developed for oral dosage formulations with stepwise release of active ingredient in the gastrointestinal tract [10,11]. Eudragit L-100, (Fig. 1) a kind of anionic polymer with carboxyl groups, shows solubility dependence on the pH. The also called enteric polymers have been used to avoid any contact of drug with the enzymes at the pH stomach where it is 2-3 [12-14].

Ibuprofen (Fig. 2), a non-steroidal anti-inflammatory drug, is widely used for the relief of

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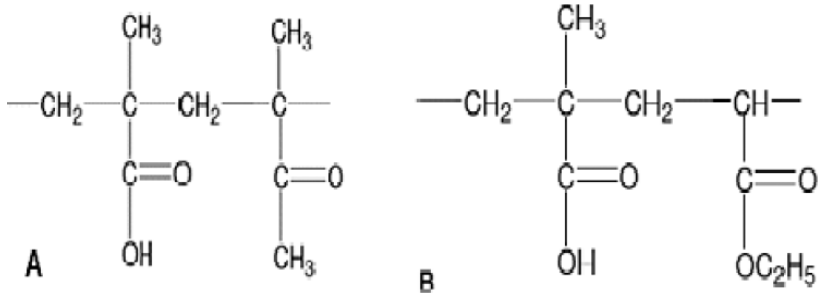

Fig. 1. Chemical formula of a) Eudragit L-100 b) Eudragit L30D-55.

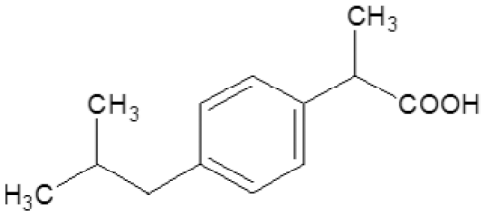

Fig. 2. Chemical formula of Ibuprofen.

mild-to-moderate pain in conditions such as migraine, rheumatoid arthritis and other musculoskeletal disorders [15]. It has a short plasma half-life of 1–3 h following oral dosing [16], which makes it necessary to be administered frequently in order to maintain a desired concentration. Therefore, it is an ideal candidate, which results in more reproducible drug absorption and reduces the risk of local irritations compared to single unit dosage forms, due to uniform spreading in the gastrointestinal tract [17].

Eudragit L-100, can release drugs to the medium of pH 7.4 (intestinal), but hardly releases drug molecules in medium pH 1.2 (stomach) [18,19]. It should provide several advantages: preventing irritant drug leakage in the stomach, leading to intestinal drug release, and enhancing the stability of the tablets used. Therefore, if the designed pH-controlled release system could effectively delay the release of model drug in simulated gastric fluid and release it in simulated intestinal fluid without any restrictions, it might be an ideal pH-sensitive drug carrier [20-22].

The specific aim of this study is to develop a nanoencapsulation process of ibuprofen by the modified double emulsion technique using copolymers EL-100 and EL-30D-55 as an enteric coating polymer matrix. The chemical-physics properties of materials obtained and their behavior dissolution profiles were studied in order to apply them in the medical field. Therefore, oral administration in very small doses of the product, might be able to decrease side effects of the traditional oral and maintain therapeutic levels for an extended period of time.

2. EXPERIMENTAL

2.1. Materials

Eudragit L 100, EL30D-55, and Triethyl citrate (TEC) were obtained from Evonik (Darmstadt, Germany). Ibuprofen, a white crystalline powder with the purity of 99%, was purchased from Sigma–Aldrich (Poole, UK). All the other chemicals products were of analytical grade.

2.2. Preparation of nanostructured materials (NMs)

Nanostructured materials were obtained by a modified double emulsion (O/W/W) process, in the first step the IBU was dissolved in ethanol and emulsified on EL-100 and EL-30D-55, varing the stirring rates between 5860-15000 rpm (Table 1), for

Table 1. Experimental conditions.

Samples	IBU (mg)	Polymer 200 (mg)	TEC* (mg)	Stirring speed (rpm)	Stability of emulsion
Exp.1	20	EL 100	20	5860	high
Exp.2	20	EL 100	40	7500	high
Exp.3	20	EL 100/ EL 30D-55(1:0.05)	20	5860	high
Exp.4	20	EL 100/ EL 30D-55 (1:0.05)	40	15000	high

*TEC - (Triethyl citrate) surfactant

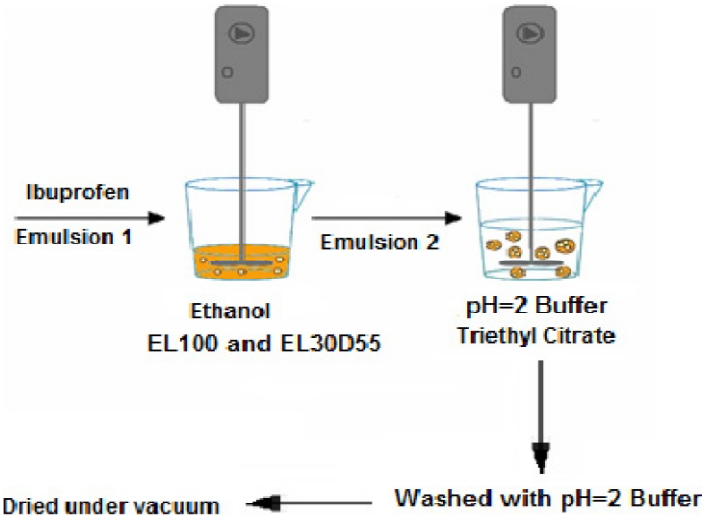

Fig. 3. Schematic representation of the process of Ibuprofen nanoencapsulation.

30 min. In the second step, the above obtained emulsion was dropped to a solution containing a pH 2 buffer (KCl 0.2 mol / HCl 0.2 mol) and triethy citrate as surfactant in different amounts (20-40 mg). Finally, the material was centrifuged at 1000 rpm for 10 minutes and washed with acidulated water of pH=2. The vacuum drying was conducted for 8 hours at 30 °C and samples stored at room temperature (Fig. 3). This process was performed under different conditions (see Table 1).

2.3. Yield (*Y*) and Encapsulation Efficiency (*EE*)

The yield (*Y*) was calculated as a percentage using Eq. 1:

$$Y(\%) = \left(m_{NMs} / m_{T}\right) \times 100, \quad (1)$$

where: m_{NMs} = mass of powder recovered after drying, m_{T} = initial mass of ibuprofen plus Eudragit L-100 and Eudragit L30D-55 in formulation.

10 mg of each sample were dissolved in 10 mL of pH 6.8 intestinal medium. The UV absorbance of the solution was measured using an Optizen UV spectrophotometer at 264 nm. Encapsulation Efficiency (*EE*) was determined in duplicate for all batches using Eq. (2) [23]. Values were expressed as percentage:

$$Y(\%) = \left(m_{exp} / m_{teo}\right) \times 100, \quad (2)$$

where m_{exp} = mass of ibuprofen experimentally determined, m_{teo} = initial mass of ibuprofen.

2.4. Statistical studies

A multilevel factorial design (2^3) was used to study the effect of each factor, as well as the effects by the interactions between factors, on the response variable. The studied factors were the amounts of Surfactant (TEC) (*X*1), combination of polymers (EL-100 and EL-100+EL30D-55) (*X*2), and stirring speed (*X*3). The response variable were the *EE* (%) and *Y*(%). Table 2 summarizes the independent and dependent variables. The results of formulations are listed in Table 3. All the experiments were carried out three times. The order of the experiments was fully randomized. The factorial design consisting of 8 runs was analyzed using Statgraphics (Version 5 Plus; StatPoint Technologies, Inc., Warrenton, VA, USA). Statistical analysis was performed using an analysis of variance (one- way ANOVA). Variance analysis results for *EE* (%) were used to test the statistical significance of the estimated effects. *P*-

Table 2. Experimental design: factors and response.

Factors(independent variables)	Levels		Response(dependent variable)
	-1	+1	
X1: surfactant(TEC)*	20	40	*Y*(%) Yield
X2:Ratio (EL-100/EL30D-55)	0	1:0.5	*EE* (%) - Encapsulation Efficiency
X3: Stirring speed	5860	15000	

Table 3. Experimental design matrix, encapsulation efficiency and process yield values.

X1 (mg)	*X2* (mg)	*X3* (rpm)	*Y*(%)±SD	*EE* (%)±SD
+1.0	+1.0	-1.0	90±1	44±5
+1.0	-1.0	-1.0	92±3	43±2
-1.0	+1.0	-1.0	89±2	41±1
-1.0	-1.0	+1.0	89±1	44±1
-1.0	-1.0	-1.0	80±4	40±3
-1.0	+1.0	+1.0	95±1	53±5
+1.0	-1.0	+1.0	95±2	50±4
+1.0	+1.0	+1.0	97±5	60±2

values lower than 0.05 (*P*, 0.05) were considered statistically significant.

2.5. Scanning electron microscopy (SEM)

The morphology was analyzed by scanning electron microscopy (SEM JEOL-5600 LV) at 20 kV. Samples were coated with a layer of gold of approximately 20 nm using an EMS 550 sputter coating.

2.6. Dynamic light scattering (DLS)

The measurements were taken in a Zetasizer Nano instrument (Zetasizer Nano-ZS®; Malvern Instruments, Malvern, UK) at 25 °C. Samples were dispersed in hydrochloric acid 0.2 M by means of ultrasound and the resulting solution was filtered. Aliquots of 900 μL were measured in a small volume cuvette (DTS 1060 C). 20 runs were made for each sample (measurement angle was 90° backscatter detection). The average diameter (Z-average) and the poly-dispersity index (PDI) were measured for all formulations.

2.7. Fourier transform infrared spectroscopy (FTIR)

The sample (~5 mg) and KBr (~ 95 mg) were ground together in an agate mortar until the sample was well dispersed. The resultant powder was pressed until form a translucent wafer. Spectra were collected in a FT-IR-THERMO NICOLET Nexus-670 spectrophotometer. FTIR spectra were obtained in the wavenumber region between 500 and 4000 cm^{-1}.

2.8. X-Ray diffraction (XRD)

The analysis was carried out in an X-ray diffractomer (Rigaku, model Miniflex), with a Bragg–Brentano geometry and with a CuK_{α} radiation of 1.5408 Å wavelength, monochromator operated at 35 KV and 25 mA. The samples were scanned in the 2θ = 2-70° range, with a step time of 2 s and a step size of 0.05°.

2.9. Thermogravimetric analysis (TGA)

The samples were heated in a TA thermal analyzer Q50 at a heating rate of 20 °C/min with a nitrogen flux (15 cc/min). The TGA profiles were recorded in the temperature range of 30-800 °C. The weight of the sample used was about 9–11 mg.

2.10. In vitro dissolution testing

In vitro studies were performed on laboratory scale using a type II paddle apparatus according to USP XXXV specifications [24]. The rotation speed was 60 rpm and temperature circulation bath controlled at 37 ± 0.5 °C. The study was conducted under conditions that simulated gastric medium (pH 1.2 HCl 0.1 N). The samples were submitted to an intestinal medium (pH 6.8) (0.1 N HCl with Na_3PO_4 0.2 M (3: 1)) during two hours as is described for controlled release formulations [24]. Samples (3 ml) were evaluated at appropriate time intervals and the release of ibuprofen was followed spectrophotometrically at λ_{max} = 264 nm against a calibration curve. The calibration curve follows the (0.1511) $A + C = 0.1134$ behavior; where (*A*) is Absorbance and (*C*) is Concentration. Encapsulation efficiencies were also estimated spectroscopically. All samples were studied by duplicate.

3. RESULTS AND DISCUSSION

3.1. Yield (*Y*) and encapsulation efficiency (*EE*)

The encapsulation efficiency resulted quite low for all the experiments (Table 4); however, the most

Table 4. Results of yield and encapsulation efficiency.

Samples	*Y* (%)	*EE* (%)
Exp.1	80±4	34±3
Exp.2	95±2	39±4
Exp.3	89±2	36±1
Exp.4	97±5	48±2

acceptable experiment was Exp. 4. This result indicated the main role of the parameters under study (stirring rate, amount of plasticizer and amount of EL-30D-55) as it is shown in the statistical study.

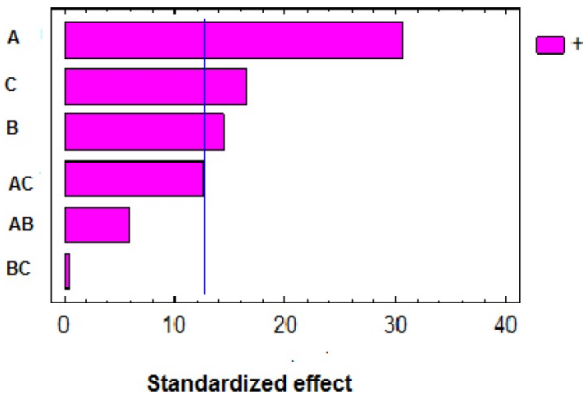

Fig. 4. Standardized Pareto chart for encapsulation efficiency.

3.2. Statistical studies

A variance analysis (ANOVA) was carried out in order to analyze the influence of the parameters such as stirring speed, combination of polymer and amount of surfactant over the encapsulation efficiency of Ibuprofen. It was also tested the interactions or relations between these variables.

From Table 5, ANOVA shows the effect of these variables over the encapsulation efficiency. It can be seen that factor *P* of the variable *A*, *B*, and *C* is lesser than *p* (0.05). This result confirms that there is a clear influence over the efficiency with a 95% of confidence. On the other hand interactions *AB*, *AC*, and *BC* have not any influence in the analysis.

It can be seen in the Pareto's graphic (Fig. 4), where the columns of *A*, *B*, and *C* variables exceed the vertical line indicating that they have influence in the response variable. While the vertical line is bigger the influence is higher. The stirring speed (*A*) is the independent variable that holds the higher influence on the response variable (*EE*). At higher stirring lower agglomerates formation are obtained and also smaller particles size (500-700 nm). In that way an homogeneous system can be obtained. The value of R^2 shows that the proposed model explains in a 99.0 % the efficiency of encapsulation variation.

3.3. Scanning electron microscopy (SEM)

Microphotographs recorded by electron scanning microscopy (Fig. 5) revealed an irregular surface and heterogeneous morphology with formation of agglomerates. The irregular shape presents some little spheres attached to the surface when the stirring rate is increased in the process of synthesis mainly with the use of polymer mixtures [25] (Exp.4).

3.4. Dynamic light scattering (DLS)

The effect of ultrasonic waves on the agglomerates is observed in Fig. 6. Those aggregates were broken giving smaller entities in a range of 500-700 nm. In

Table 5. ANOVA for encapsulation efficiency.

Source	Sum of squares	*Df*	Mean square	*F*-Ratio	*P*-value
A	197.011	1	197.011	932.60	0.0208
B	43.7112	1	43.7112	206.92	0.0442
C	57.7812	1	57.7812	273.52	0.0384
AB	7.03125	1	7.03125	33.28	0.1093
AC	33.2113	1	33.2113	157.21	0.0507
BC	0.03125	1	0.03125	0.15	0.7662
Total error	0.21125	1	0.21125	-	-
Total (corr.)	338.989	7	-	-	-

Notes: *A* (*X3*): Stirring speed; *B* (*X1*): plasticizer (TEC); *C* (*X2*): combination of polymers EL-100 and EL-30D-55; *AB*, *AC*, and *BC* are interactions between the factors.
Abbreviations: ANOVA, analysis of variance: corr (corrected); *Df* (degrees of freedom).

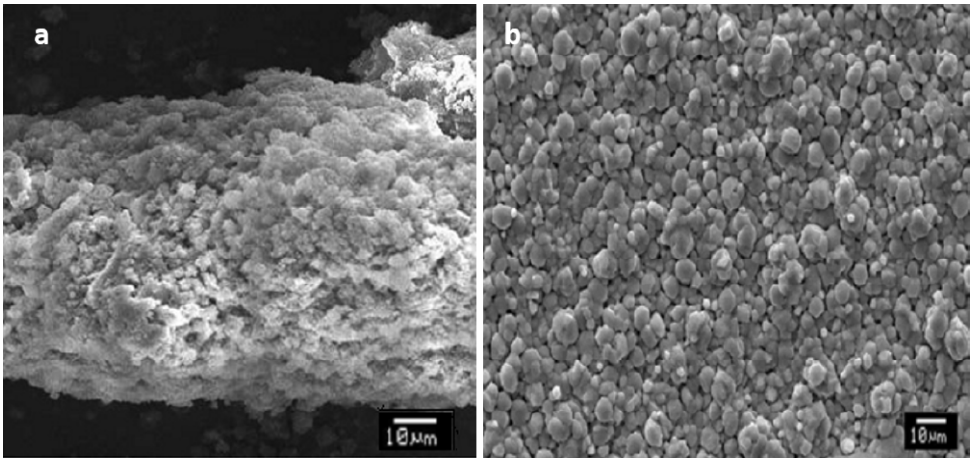

Fig. 5. Electron Scanning Microscopy (SEM) of obtained materials, a) Exp-1, b) Exp-4.

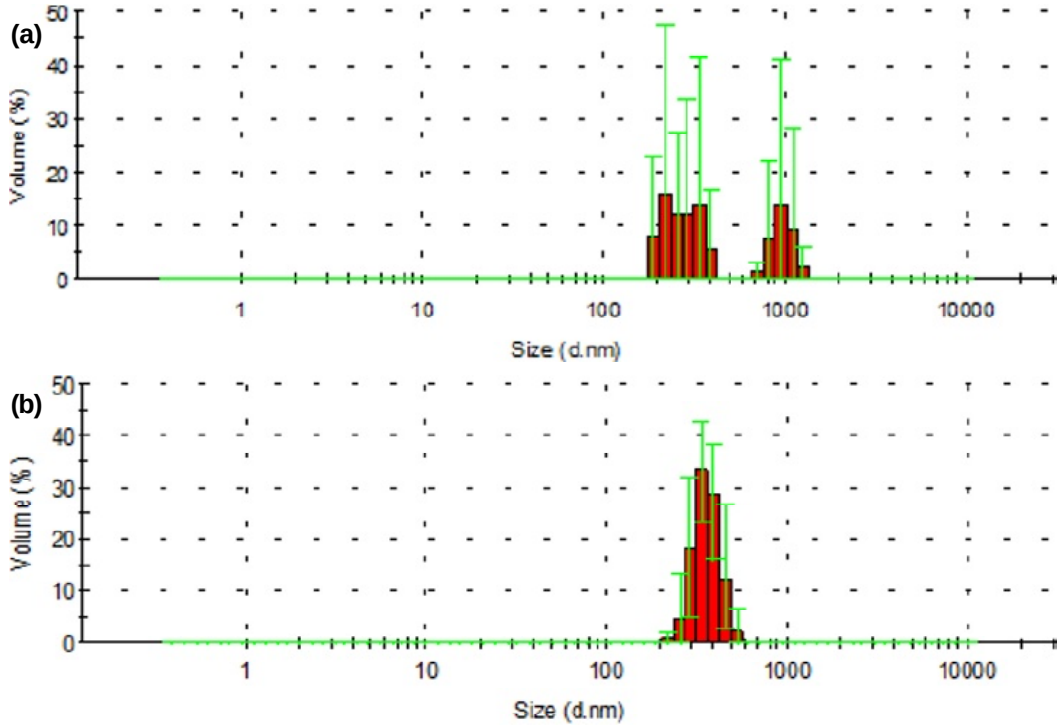

Fig. 6. Diameter distributions of the agglomerates treated with ultrasound by Dynamic Light Scattering (DLS), a) Exp.1, b) Exp.4.

one case, it was obtained two diameter distributions and, in the other case, a monodisperse distribution. This result suggests that agglomerates are loosely bounded and this feature can contribute as well as the irregular surface detected by SEM to appropriate release profiles.

3.5. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra were recorded for the polymers, the drug and the encapsulated materials as is shown in Fig. 7. The carbonyl vibration C = O around 1725 cm^{-1} correspond to carboxylic acids present both in the Ibuprofen and the polymer. The hydroxyl groups are associated to a broad band located between 3000 and 3500 cm^{-1}.

Other signals correspond to the CHx vibrations are seen between 2900-3000 cm^{-1} [26]. However, there is no evidence of possible interactions between Ibuprofen and the polymeric matrix due to the null displacement of the main bands of the functional groups and the complexity of the spectrum are not seen.

3.6. X-Ray diffraction (XRD)

The diffractograms correspond to the polymers indicate their amorphous nature (Fig. 8). The

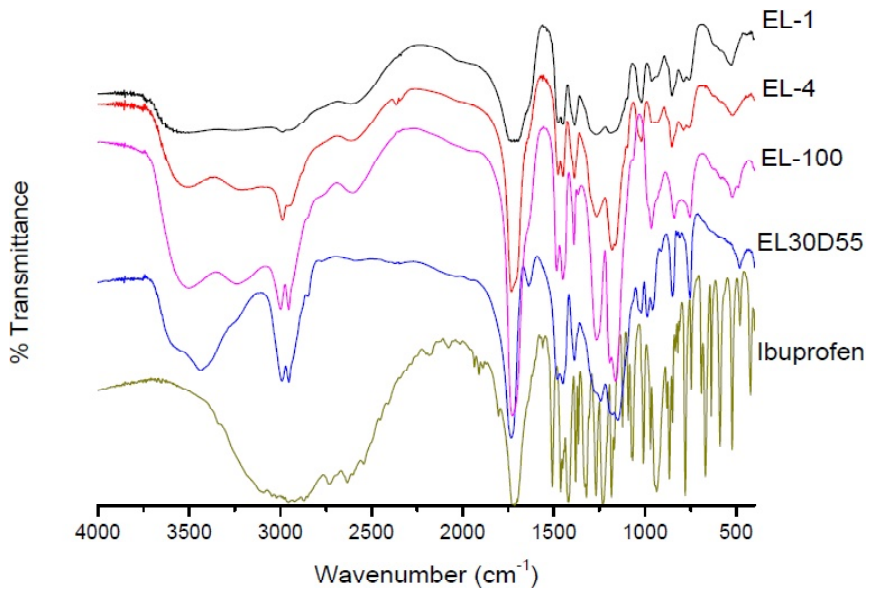

Fig. 7. Fourier transform infrared spectroscopy (FTIR) of the Exp.1, Exp.4, polymers and the Ibuprofen.

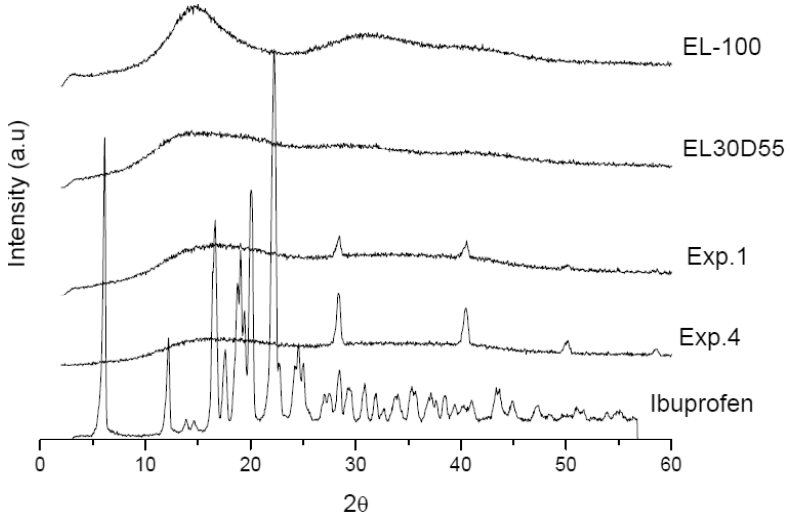

Fig. 8. X-ray diffraction patterns (XRD) of the Exp.1, Exp.4 samples, polymers and the Ibuprofen (IBU).

diffractogram of ibuprofen contains several signals and the most intense are seen between 2θ=5-25. These signals are not observed in the diffractograms of the Exp. 1 and Exp. 4 samples. It seems to indicate that ibuprofen is very disperses and/or that part of this could be remaining on the surface of the pores

3.7. Thermogravimetric analysis (TGA)

In thermogravimetric analysis (TGA) Fig. 9, the Ibuprofen (IBU) contained in the materials from Experiments 1 and 4 seem to be more stable in the presence of the polymers, suggesting some interaction. In these materials, the weight loss begins at lower temperature than the pristine polymers but it reaches a loss of about 40% amount higher than the IBU initially added. It suggests that successive washings cannot eliminate part of surfactant. Furthermore, a residual of about 10% is also observed but it can be due to Ibuprofen and also to the surfactant.

3.8. In vitro dissolution testing

Fig. 10 shows the release profiles Exp.1, Exp.4, polymers and the Ibuprofen, from there an IBU initial accumulation on the surface in both samples was observed (burst effect). Burst release can be attributed to fast drug diffusion through the polymer matrix near the surface or drug being present on the surface of the material. The localization of drug in the inner core or on the surface of the particle affects the diffusion path length and the release profile [27,28].

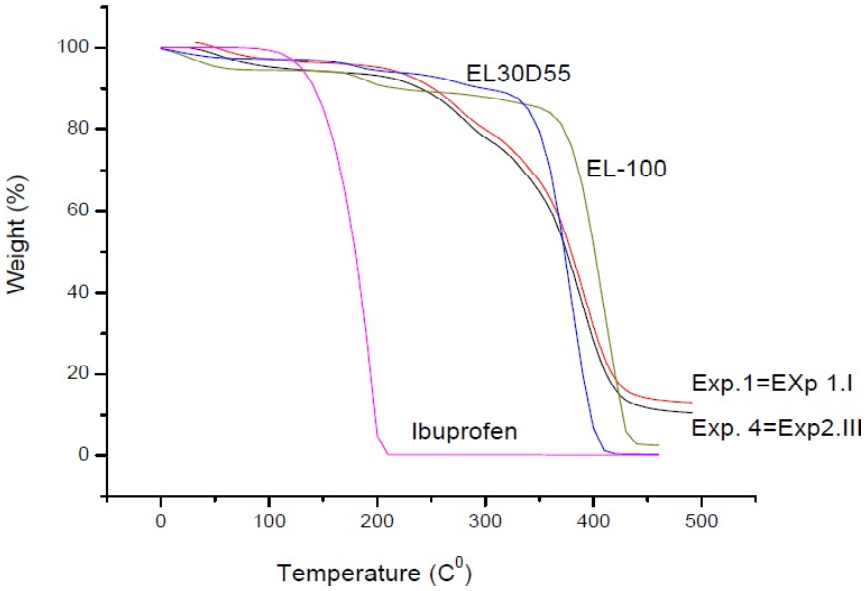

Fig. 9. Thermograms curves of the Exp.1, Exp.4, polymers and the Ibuprofen.

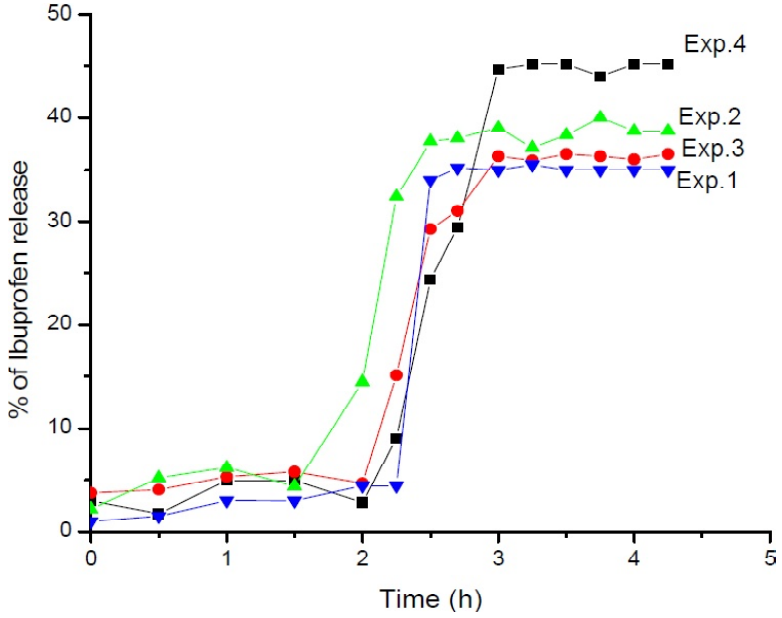

Fig. 10. Cumulative release profiles of Ibuprofen related to Encapsulation Efficiency (EE).

These profiles indicate a behavior typical of polymers pH dependent. All experiments reveal the nature of the enteric matrix used, which are not able to release the drug at acidic pH = 2 (stomach). The amount released at beginning is less than 5% it is quite acceptable. The release at basic pH = 6.8 (intestinal) reached up to 35 and 45%.

Comparing the four experiments, it is noted that Exp.4 sample has higher the amount released. That sharp differences seems not to be in the different nature or different amount of surfactant added but in the stirring rate. It was confirmed by the statistical analysis. Higher stirring rate stabilizes the emulsion giving a higher yield of material and higher *EE*, (see Tables 1 and 4). Experiments 1 and 3 are equivalent except on the polymers. In the case of Exp. 3 was carried out using a mixture of polymer being one of them an acrylate of known lower glass temperature [18,29].

However, the effect of plasticization did not produce any change in the profile confirming the above discussion. The lower glass temperature of the polymer used in Exp. 3 should produce a less stiff material that favorers the yield but not the *EE* as shown in Table 4. Interaction drug-polymer could be also operating in spite of FTIR gave no information. Mustafin and Kabanova [30] have published a similar result.

4. CONCLUSION

The modified double emulsion (O/W/W) process can be used to prepare nanostructured materials loaded with ibuprofen using an enteric coating copolymers by L-100 Eudragit and L30D-55 Eudragit mixture.

Statistical analysis of multilevel factorial design showed several significance effects on *EE* (95% confidence level). The stirring speed is the most important variable that has influence in the process of encapsulation.

The X-ray diffraction and FTIR spectra did not show evidence of strong interaction between the polymers and Ibuprofen. Morphological analysis indicated that NMs present an irregular surface with formation of agglomerates. The DLS showed a positive effect of ultrasonic waves on the agglomerates. It was posible to obtain smaller entities in nanometric range of 500-700 nm. Finally, all the formulations showed a delayed release. This will avoid gastric irritation caused for this type of drug and maintains therapeutic levels for a long period of time.

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