(Research Article)

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NANOENCAPSULATION OF NSAIDS NAPROXEN AND IBUPROFEN

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ABSTRACT: Naproxen and Ibuprofen, two non-steroidal anti-inflammatory drugs, are frequently prescribed in modern medicine. In this study, nanoencapsulation by solvent evaporation technique is used to formulate Naproxen and Ibuprofen loaded poly(DL-lactide-co-ε-caprolactone) nanospheres by means of Tween 80 and Span 80 as surfactant and co-surfactant respectively. Different formulations are prepared with different drug masses and same quantities of polymer and surfactants. This was done in order to evaluate the effect of the mass variation of the two drugs on the characteristics of the prepared nanospheres. The prepared nanospheres are studied for drug entrapment and drug loading efficiencies, particle size, morphology, FT-IR analysis and *in vitro* release. As a result, all nanospheres presented a spherical profile and homogeneous size distribution. Naproxen revealed the highest drug entrapment efficiency. FT-IR analysis showed no interaction for both drugs with the polymer. *In vitro* release of drugs from encapsulated PLC nanospheres showed that Ibuprofen release was faster than Naproxen release. Also the *in vitro* release was faster for formulations having the lowest amount of drug loaded.

INTRODUCTION: Recently, nanotechnology has been associated with many scientific uses. However, it remains under research and exploration in biomedical and pharmaceutical fields [1]. There are several factors that favor using polymeric nanospheres as carriers for pharmaceutical agents into the body. These reasons include [1, 2]:

- Control of the particle size and surface characteristics of nanospheres in order to reach a satisfactory drug targeting;
- Sustained and controlled release of the drug at target body organs, increasing the drug therapeutic efficiency and reducing its side effects;

- Easy modulation of the release profile and degradation of nanospheres depending on the choice of the matrix;
- Preservation of the drug activity due to the high drug loading in nanoparticles and the incorporation of the drug into the systems without any chemical reaction;
- Increasing the rate of dissolution and oral bioavailability of poorly absorbable drugs;
- Less amount of dose required;
- Use for different routes of administration.

Although nanospheres have many advantages, some inconveniences regarding their use are due to their small size and large surface area such as particle-particle aggregation, limited drug loading and burst release. Several main nanocarriers are used as drug delivery systems in the biomedical and pharmaceutical fields such as liposomes, polymeric micelles, dendrimers, carbon nanotubes, and polymeric nanoparticles [3 - 5]. One of the most common methods for the preparation of

nanoparticles based on the usage of biodegradable polymers, such as from Polylactic acid, Polylactic-co-glycolic acid and Poly caprolactone is the preformed polymer dispersion by the solvent evaporation technique [6]. The biodegradable polyester poly (DL-lactide-co-ε-caprolactone) (PLC) was used in this study for the preparation of nanospheres. PLC is biodegradable and biocompatible aliphatic polyester used as carriers in controlled delivery for a wide range of drugs [7].

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most frequently prescribed drugs in modern medicine. NSAIDs are very effective in the relief of pain or the reduction of fever and inflammation [8]. Two NSAIDs were chosen in this study, Naproxen (Nap) and Ibuprofen (Ibu) (**Fig. 1**). The use of Nap is limited by its short duration of action when administrated orally and has some side effects such as mucosal damage. As for Ibu, most frequent side effects are gastro-intestinal disturbance and bleeding, ulceration, and hypersensitivity. Advances in nanotechnology have reduced these side effects by the development of new polymeric nanospheres as drug carriers systems. Nanospheres polymeric coating is important in hindering any link of these drugs to the stomach membrane and maintaining a low and constant level of drug in the blood [9, 10].
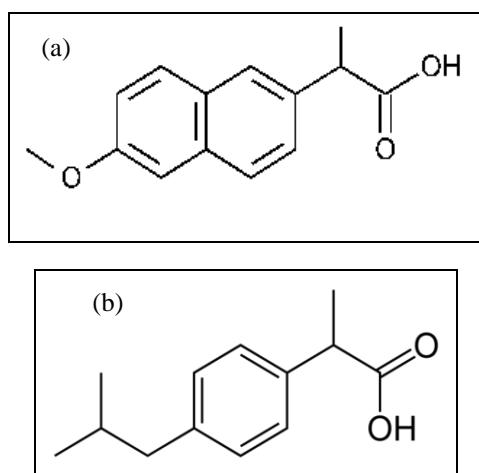
FIG. 1: CHEMICAL STRUCTURES OF NAP (a) AND IBU (b)

Two types of nanospheres using PLC will be synthesized by the polymer dispersion method for Nap and Ibu encapsulation. Tween 80 and Span 80 are used as surfactant and co-surfactant respectively. These two surfactants present many uses such as solubilisers, dispersants, and wetting

agents [11, 12]. The prepared nanospheres will then be and evaluated for several characteristics.

Experimental: Poly (DL-lactide-co-caprolactone) (86 mol% DL-lactide), Naproxen, Ibuprofen, Span 80, Tween 80, and acetone were purchased from Sigma-Aldrich Chemie Germany. All materials were of analytical grade and used as received.

Method Description: Preformed Polymer dispersion by the solvent evaporation method was used for the preparation of nanospheres containing Nap and Ibu. Different formulations of the two drugs were prepared by changing drug mass between 0.6 to 3.5 mg. As for the polymer and the surfactants, their masses were fixed respectively to 200 mg and 300 mg.

The organic phase consisted of PLC dissolved in 40 ml of acetone with Span 80 and the relevant drug mass. This was poured into 80 ml of an aqueous phase containing Tween 80. The solution which turns immediately milky as a result of the formation of nanoparticles was stirred for 3 hours. Acetone was finally removed using a rotary-evaporator (RE 200, Bibby Sterlin Ltd., UK) and the colloidal suspension concentrated to a final volume of 20-30 ml. This final suspension was centrifuged for 20 min with a high speed centrifuge (HERMLE Laboratory, Germany) at 16000 rpm to collect the nanospheres. Finally, the collected nanospheres were dried in an oven for 48 hours at 40 ℃ and the clear supernatant was reserved for the characterization tests [13].

Characterization:
a. Drug Encapsulation and Drug Loading: Encapsulated drug was calculated by subtracting the free drug in supernatant from the total drug introduced. The total drug introduced was calculated according to the used mass of drug. Free drug was determined from the clear supernatant, reserved from the centrifugation after separating the nanospheres from the aqueous medium. 2 ml of the supernatant were diluted in 20 ml of deionized water and analyzed with a UV/Vis spectrophotometer (Microplate Spectrophotometer, Epoch, Biotek, USA) at 332 nm for Nap and 264 nm for Ibu. % DE and %DL were calculated according to the following equations:

$$\% \ DE = \frac{\text{Encapsulated drug content}}{\text{Introduced drug content}} *100$$

$$\% \ DL = \frac{\text{Encapsulated drug mass}}{\text{Nanospheres mass}} *100$$

b. Particle Size: To determine the particle size of the prepared nanospheres, a small quantity of each sample is suspended in distilled water. Laser diffraction granulometer (LA950V2, Horiba instrument Ltd., France) was used for this study. The size distribution of nanospheres was determined, and the average particle size was calculated and expressed in nm.

c. Morphology: Nap and Ibu nanospheres were analyzed using Scanning Electron Microscopy (SEM) (LYRA 3 XMU, TESCAN, USA) to determine their morphological characteristics. The samples were set on a carbon conductive tape and covered with a platinum layer (10 nm thickness) using a sputter coater for testing.

d. FT-IR Analysis: To verify the absence of any possible chemical interaction between the drug and the polymer, FT-IR analysis (Frontier, Perkin Elmer, USA) was carried out for the prepared formulations. KBr pellets method was used. It is based on grinding and mixing each sample to be tested with KBr to form a very fine powder. The powder is then compressed using a manual hydraulic press into a thin pellet reading for testing.

***e. In-vitro* Study:** 10 mg nanospheres were incorporated in a dialysis bag having a molecular weight cut off 12000-14000. The dialysis bag was immersed in 5 mL PBS (0.01 M, pH=7.4) used as release medium and incubated at 37 ℃ in thermostat water bath. At specific time intervals, 1 mL of the release medium were withdrawn and replaced with 1 mL of fresh solution. Drug content was evaluated at 332 nm and 264 nm for Nap Ibu respectively.

RESULTS:

1. Nanospheres Preparation: The polymer dispersion technique was used to formulate Nap- and Ibu-loaded PLC nanospheres. 200 mg of PLC and 300 mg of both surfactants were used; the quantity of drug was varied between 0.6 and 3.5 mg. The drug formulations were referenced N1 to N5 for Nap and I1 to I5 for Ibu. Acetone was used as the organic solvent dissolve PLC and the drug forming an organic phase.

The prepared nanospheres were characterized for drug content, morphology, particle size, polymer-drug interaction and drug release.

Drug Encapsulation and Loading Efficiencies: The drug content was evaluated by the UV/Vis Spectrophotometer.

The percentage of encapsulation (% DE) and loading (% DL) of the different drug formulations are represented in **Table 1**.

TABLE 1: % DE AND % DL OF NAP AND IBU NANOSPHERES

Formulation Code	Quantity of Drug Introduced (mg)	%DE	%DL
N_1	0.6	50	0.15
N_2	1	79	0.4
N_3	2	86	0.8
N_4	3	91	1.4
N_5	3.5	65	1.1
I_1	0.6	52	0.2
I_2	1	63	0.3
I_3	2	67	0.7
I_4	3	85	1.3
I_5	3.5	66	1.2

The variation of % DE and % DL among the prepared nanospheres formulations of the two drugs are illustrated in **Fig. 2** and **3** respectively.
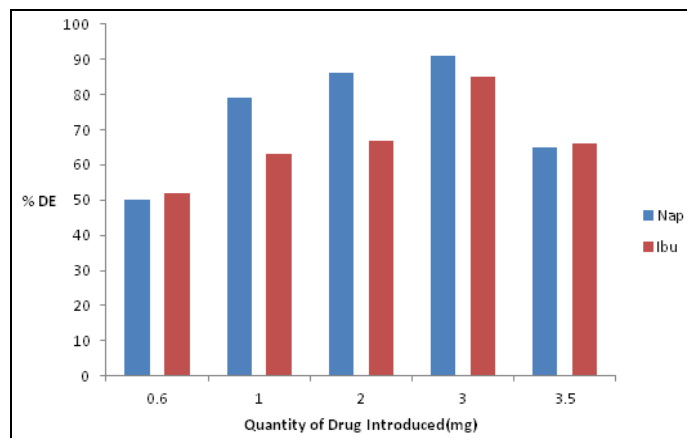
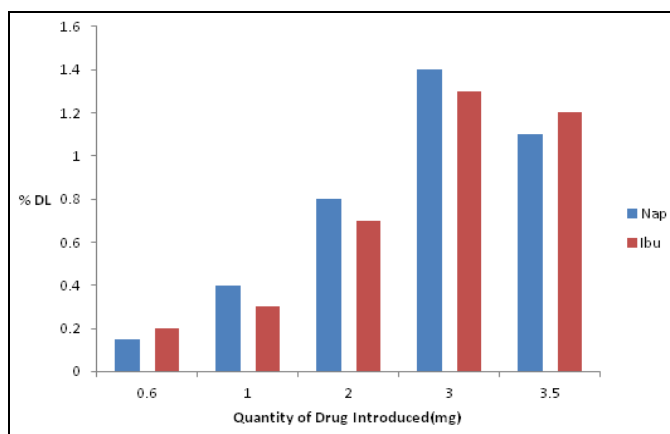**FIG. 2: % DE FOR NAP AND IBU FORMULATIONS**

FIG. 3: % DL FOR NAP AND IBU FORMULATIONS

3. Particle Size: Table 2 represents the average size of Nap and Ibu nanospheres formulations. For both drugs formulations, the size range is between 60 and 95 nm.

TABLE 2: AVERAGE PARTICLE SIZE FOR NAP AND IBU FORMULATIONS

Formulation Code	Average Particle Size (nm)
N_1	63
N_2	65
N_3	79
N_4	84
N_5	62
I_1	88
I_2	83
I_3	63
I_4	75
I_5	95

4. Morphology: SEM examination showed that the prepared nanospheres exhibited a spherical profile as shows **Fig. 4**.
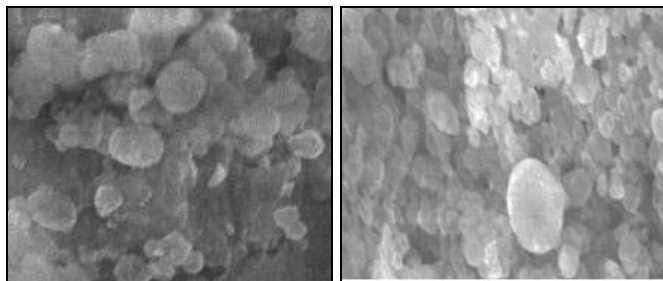

FIG. 4: PHOTOGRAPHS OF PLC LOADED NANOSPHERES BY SEM

5. Drug-polymer Interaction: The absence of drug-polymer interaction in the prepared nanospheres was studied by FT-IR spectroscopy. Spectra were recorded for the polymer PLC, the two drugs Nap and Ibu and drug-loaded nanospheres (**Fig. 5** to **9**).

The peak located at 1745 cm^{-1} of PLC is assigned to the stretching vibration of –C=O group. Peaks showing at 2900-300 cm^{-1} refer to aliphatic –CH stretching of the polymer. No peaks are located above 3000 cm^{-1} indicating the absence of –OH group. The broad bands appearing between 3000 and 3500 cm^{-1} in figures 6 and 7 are assigned to the stretching vibration of the –OH group in both pure drugs. **Fig. 8** and **9** show the spectra of Nap- and Ibu-loaded nanospheres respectively. The peak located at 1745 cm^{-1} is characteristic to the stretching vibration of the –C=O group of PLC. The broad band located between 3000 and 3500 cm^{-1} characterizes the stretching vibration of the –OH group of the drug. Peaks at 2900-3000 cm^{-1} is also present in the polymer.
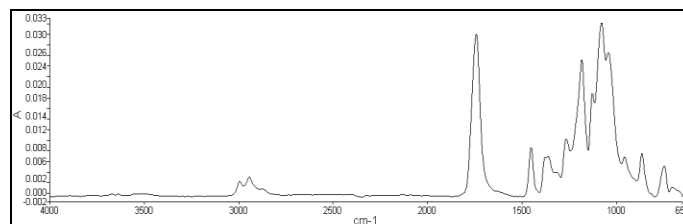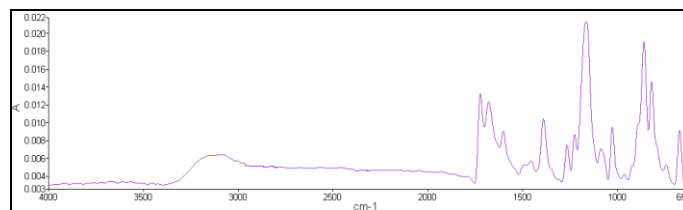

FIG. 5: FT-IR SPECTRUM FOR PLC
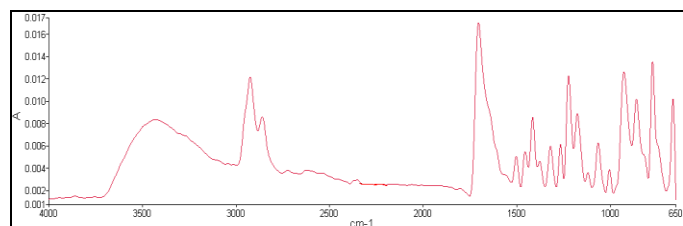

FIG. 6: FT-IR SPECTRUM FOR PURE NAP

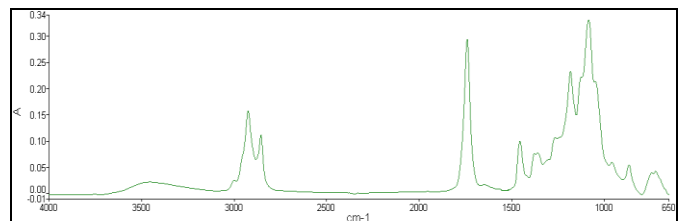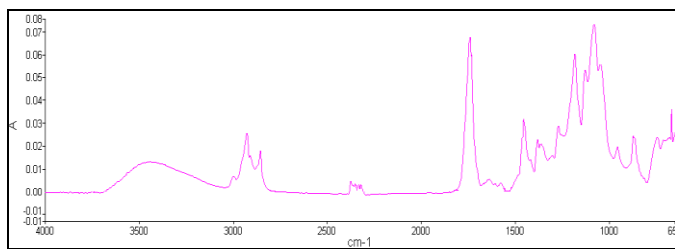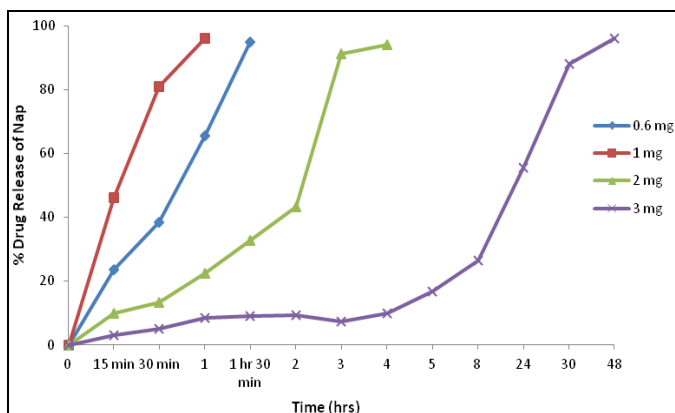
FIG. 7: FT-IR SPECTRUM FOR PURE IBU

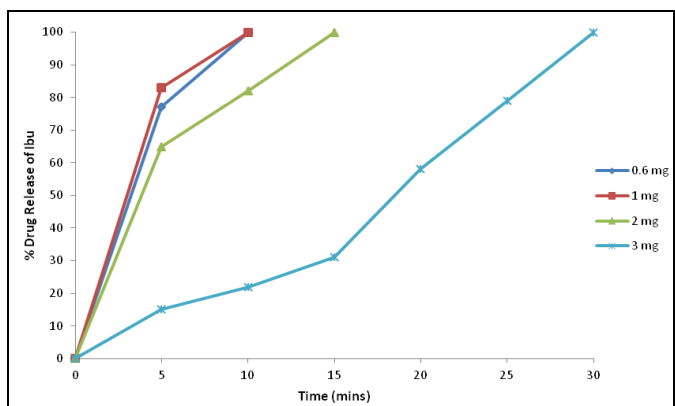**FIG. 8: FT-IR SPECTRUM FOR NAP-LOADED NANOSPHERES**

FIG. 9: FT-IR SPECTRUM FOR IBU-LOADED NANOSPHERES

6. *In vitro* Study: The *in vitro* release profile of Nap and Ibu with time is presented **Fig. 10** and **11** respectively.

FIG. 10: *IN VITRO* DRUG RELEASE OF NAP FORMULATIONS

FIG. 11: *IN VITRO* DRUG RELEASE OF IBU FORMULATIONS

DISCUSSION: In this study, the preformed polymer dispersion by the solvent evaporation technique was used to encapsulate the two NSAIDs Nap and Ibu into PLC nanospheres. This technique was chosen for different reasons [14]:

- Its rapidity in giving spherical particles with porous surface and controlled sizes in the nanoscale;
- Its simplicity, its cheapness and its reproducibility;

- Its application to a wide variety of polymers (PLC, PLGA and PLA) and drugs (hydrophobic and hydrophilic).

Incorporation of drug into PLC nanospheres required the use of Tween 80 as surfactant and Span 80 as co-surfactant. These two different surfactants have several roles [14, 15]:

- Disperse one phase in another immiscible phase;
- Stabilize the obtained emulsion;
- Prevent the coalescence and the aggregation of PLC nanoparticles;
- Synthetize nanospheres having a regular size and a small size distribution.

Many factors influence the properties of the nanospheres such as the nature of used materials, the quantity of drug in the dispersed phase, the concentration of surfactant and the operating conditions (agitation, temperature, pressure) [9, 14]. In our study, the only variable factor is the quantity of each drug in the dispersed phase which affects the nanospheres properties.

The comparison between the % DE and the % DL of nanospheres formulations relatively to the introduced mass of drug reveals that the formulations N_4 and I_4 show the highest % DE and % DL as shown in **Table 1** and **Fig. 2** and **3** respectively. Polymer properties, as precipitation is an important factor that affects drug encapsulation. Most attempts are based on a common idea that fast polymer precipitation on the surface of the organic phase can prevent drug loss into the aqueous phase increasing the encapsulation efficiency [16]. Nap-loaded nanospheres have more entrapment than Ibu-loaded nanospheres. In fact, the precipitation process of Nap is faster and its loss into the aqueous phase is prevented. However, this is not the case of Ibu. The latter diffuses more into the aqueous phase during the nanospheres formation, which explains the observed results.

Another reason could be related to the drug molecular weight which influences its diffusivity through the polymer matrix. Drugs with low molecular weight can easily penetrate through the porous nanospheres, which leads to its loss by diffusion during or after preparation and thus to low drug encapsulation. Ibu has lower molecular

weight than Nap (206.29 g/mol and 252.24 g/mol respectively), so it will diffuse through the porous matrix inducing lower drug entrapment [17].

Nap- and Ibu-loaded nanospheres have the same particle size ranging from 60 to 95 nm (**Table 2**). This observation is due to the fact that the two kinds of nanoparticles were prepared under the same experimental conditions. In addition, the surface morphology of nanospheres analyzed using SEM shows that all prepared formulations have a spherical profile (**Fig. 4**). This proves the efficiency of the solvent evaporation technique in formulating homogeneous and reproducible nanoparticles.

The FT-IR spectrum of the Nap-loaded nanospheres (**Fig. 8**) shows a sharp peak at 1745 cm^{-1} present in the spectrum of PLC (**Fig. 5**). A broad band in the area between 3000 and 3500 cm^{-1} referring to the –OH group could be assigned to Nap (**Fig. 6**). The same results could be deduced for the FTIR spectrum of the Ibu-loaded nanospheres (**Fig. 9**) after comparison with the spectra of PCL (**Fig. 5**) and Ibu (**Fig. 7**). Consequently, the results indicate that there is no interaction between the drugs and the polymer: the distinctive peaks of PLC and the drugs appear in the FT-IR spectrum of nanospheres.

Moving to the *in vitro* study, the drug release rate in general depends on several factors [2]:

- Solubility of the drug;
- Desorption of the surface-adsorbed drug;
- Diffusion of the drug through the nanoparticle matrix;
- Degradation of nanoparticle matrix.

Concerning nanospheres, where the drug is evenly distributed, the release occurs by diffusion or degradation of the matrix. This release is related to the experimental conditions. The drug release mechanism is based on a diffusion process when it is faster than matrix degradation especially for drugs with low molecular weight. The rapid initial burst occurs mainly due to weakly bound or adsorbed drug to the surface of nanoparticles [2].

A comparison of drug release from formulations containing different drug loadings is done in this study. Formulations N$_5$ and I$_5$ were omitted from the study because their %DL are very close to those

of N$_4$ and I$_4$. *In vitro* release profiles of Nap and Ibu containing different drug loading are shown in **Fig. 10** and **11**. The release rate increases when the drug loading increases for the two types of drugs. The release or "burst" is found to be fast for formulations having the lowest amount of drug loaded (**Table 3**).

TABLE 3: *IN VITRO* COMPLETE DRUG RELEASE TIME

Formulation	% DL	*In vitro* Complete Drug Release Time
N$_1$	0.15	1 h 15 mins
N$_4$	1.4	48 h
I$_1$	0.2	10 mins
I$_4$	1.3	30 mins

On the other hand, the release is slow when higher quantity of drug is present in nanospheres, so the drug takes longer time to escape into the release medium. This result is confirmed for both drugs, where the formulations having the highest drug loading are characterized by the slowest release. Comparing the two drugs, the release of Ibu is faster than that of Nap. % DE of Ibu is lower than that of Nap which indicates that it is weakly adsorbed on the large surface of PLC nanoparticles, and explains the rapid initial burst of Ibu [17].

Another reason is that the matrix of PLC nanoparticles is hydrophobic inducing a low permeability of water across it. In the case of Nap, it has a strong interaction with the polymer due to its structure, which makes its dissolution in water difficult and retards its release [18].

CONCLUSION: Nanoparticles based on biodegradable polymers are useful carrier systems for the delivery of many drugs to specific body sites. They have many advantages including their extended release properties, size and biocompatibility. Naproxen and Ibuprofen were successfully encapsulated into PLC nanospheres using the solvent evaporation technique. Nanospheres were spherical in profile with a homogeneous size distribution. Naproxen gave higher % DE than Ibuprofen due to the excessive diffusion of Ibuprofen in the aqueous phase during nanoprecipitation and due to the lower molecular weight of Ibuprofen. As for the FT-IR analysis, no interaction between the drugs and PLC was shown. The *in vitro* release was fast for formulations

having the lowest %DL and was faster for Ibuprofen than for Naproxen.

CONFLICT OF INTEREST: The authors state that there is no conflict of interest in the present manuscript.

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