Original Article

The effect of formulation and process variables on prepared etoricoxib Nanosponges

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ABSTRACT

To assess the improvement in solubility and drug release using Nanosponges (NS) deliver system. ETX loaded NSs (ETX-NS) were prepared using the emulsion solvent diffusion method. The ratio of drug: polymer (1:0.25,1:1.5: 1:2 and 1:3) for formulas F1, F2, F3, and F4 respectively, the type and quantity of organic solvent used in the preparation, stirring rate, and sonication effects on the solubility, particle size, and entrapment efficiency and entrapment efficiency of prepared NS were investigated and evaluated.

The solubility of ETX was enhanced when formulated as EXT-Ns. The increase in EC proportion in EXT: EC ratio caused a decrease in solubility of prepared EXT-NS ($p<0.05$), while sonication increases the solubility of NS associated with a reduction in particle size at a constant ratio of EXT: EC (1:2). Increasing PVA content from 2gm (F3) to 3 gm (F3-B) caused a high increase in the solubility (2.704 mg/ml) of NS with a significant reduction in particle size (110 nm). Particle size results confirmed that prepared NS were in the nanosized range with a Polydispersity Index (PDI) of less than 1 (=0.005). The ratio of EXT: polymer, sonication, and PVA quantities variables have a significant effect on EXT-NS solubility, particle size, and %EE. Improved % EE (90%) and efficient reduction in particle size can be obtained by increasing the quantity of PVA in the formulation and using the suitable drug: polymer ratio and stirring speed during processing.

Keywords: Nanosponges, Nonsteroidal anti-inflammatory drugs, Solubility, Particle size

Introduction

Nanosponges (NS) are spherical structures with cavities of nanosized incapacity that can capture lipophilic drugs and enhance their poor solubility. These spherical Ns are non-toxic, non-irritant, and stable at high temperatures up to 300 °C in comparison with other drug delivery systems, act preferentially as a topical delivery system, and may extend an incorporated drug for up to 12 hours. These structures have a wide range of pH (1-11) and self-sterilizing because of the average diameter of the cavity or pore (250 nm -1µm). Furthermore, formulations of NS are low-cost with free-flowing properties as general and highly compatible with a wide range of ingredients. EXT is a class II drug with low solubility in water and high permeability, having a partition coefficient (LogP) of 3.9 [1-3].

It is a whitish to creamish colored powder with a molecular formula of C18H15ClN2O2S, the IUPAC nomenclature 5-chloro-6'methyl3- [4- (methylsulfonyl) phenyl] 2, 3 'bipyridine, a molecular weight of 358.84 g /mol [4].

ETX is a COX2 inhibitor with high selectivity, a member of the Nonsteroidal Anti-inflammatory Drug (NSAID), and available in film-coated tablets in the following doses: 30, 60, 90, and 120 mg (2,3). This study was subjected to investigate the effect of formulation and process variables include the ratio of drug: polymer, quantities of PVA, type of organic solvent, stirring speed, and sonication effects on enhancing solubility of ETX-NS, reduction of particle size, and entrapment efficiency [4, 5].

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Materials and Methods

Materials

Etoricoxib, as a generous gift from the Pioneer pharmaceutical industry, Iraq. Ethyl cellulose (EC) and polyvinyl alcohol (PVA)

polymer powders were obtained from Hangzhou Hyper Chemicals Limited, China.

Preparation of ETX-NS

ETX-NS were prepared using an emulsion solvent diffusion technique with various EC and PVP proportions **Table 1**. ETX and EC dissolved in 20 mL Dichloromethane (DMN) or acetone (internal or dispersed phase). Then, this organic phase was slowly added to the external or aqueous continuous phase (PVA dissolved water) using a specific syringe pump. Stirring was conducted by the magnetic to mix the two steps for two hours at various stirring speeds. Filtration was used to collect the resultants' NS and dried in a 40 °C oven for 24 hours [6].

Table 1. Composition of etoricoxib Nanosponge Using Different Concentrations and Process Variables

Formula No.	EXT: EC (gm)	PVA (gm)	Water (ml)	DMN	Acetone	Time (hr)	Ultrasonic Time (min.)	Stirring Speed (rpm)
F1	1:0.25	2	100	20		2	3	1000
F2	1:1.5	2	100	20		2	3	1000
F2-A	1:1.5	2	100	20		2	0	1000
F3	1:2	2	100	20		2	3	1000
F3-A	1: 2	1	100	20		2	3	1000
F3-B	1: 2	3	100	20		2	3	1000
F3-C	1: 2	3	100		20	2	3	1000
F4	1:3	2	100	20		2	3	1000
F4-A	1: 3	2	100	20		2	3	750
F4-B	1: 3	2	100	20		2	3	1250

Etoricoxib content of prepared NS

Using a 50 ml volumetric flask, the ETX-NS equivalents to 20 mg of etoricoxib are dissolved in 40 ml dichloromethane and sonicated for 15 minutes. Dichloromethane was used to increase the volume to 50 mL. After that, the resulting drug solution was diluted with Dichloromethane, and the drug solution was tested for drug content with a UV-spectrophotometer at the specific λmax [7]. The percentage of drug content in the obtained nanosponge was determined using the following equation [8]:

$$\%\ ETX\ content = \frac{actual\ ETX\ weight \times 100}{theoretical\ ETX\ weight} \quad (1)$$

Saturated solubility

In aqueous media, the solubility of the pure ETX powder and prepared ETX-NS was determined. Excess amounts of pure ETX powder or dried ETX-NS were dispersed in 10 ml screw-capped vials of water; the vials were securely sealed. Then saturated solutions were kept for 48 hours, vials were held in a water bath shaker at 25 °C± 0.5 °C; after that, a UV-spectrophotometer was used to analyze the samples [9].

Variables affecting formulation

Nanosponges was prepared using different concentration of polymers and process variables (probe ultrasonication and stirring speeds). These variables were:

Effect of the drug to EC ratio on formulations

The effect of a different drug to polymer ratio 1:0.25, 1: 1.5, 1:2, and 1:3 was investigated in the formulas F1, F2, and F4, respectively. In each of these formulations, the quantity of PVA (2 gm) and the amount of Dichloromethane (20 ml) and stirring speed 1000 rpm were kept constant.

Effect of PVA concentration on prepared nanosponges

The effect of the concentration of PVA on the formulations of ETX-NS was evaluated in formulas F3, F3-A, and F3-B. In each of these formulas, the concentration of drug (1 gm), the concentration of EC (2 gm), the amount of Dichloromethane (20 ml), and the stirring rate 1000 rpm all kept constant while the amounts of PVA were varied as follow: 2 gm, 1 gm, and 3 gm in the formulas F3, F3-A and F3-B, respectively.

Effect of internal phase on the prepared nanosponges

Two types of organic solvents (DMN and acetone) were used to study the effect of organic solvent types on nanosponges formulation. Nanosponges formulas F3 and F3-C were prepared using DMN and acetone, respectively, while keeping other compositions and variables constant.

Effect of sonication on the prepared nanosponges

After achieving the EXT-loaded nanosponge formulation, sonication was applied for three minutes; amplitude 80% plus rate 5 seconds every 30 seconds, formulas of the same composition were used to investigate this influence; formula F2 and F2-A were used to study this effect.

The effect of stirring speed on the prepared nanosponges

Three different rates were used to demonstrate the impact of speed on particle size. To illustrate this influence, 750, 1000, and 1250 rpm were used in the formulas F3-A, F3, and F3-B while keeping other compositions and variables constant.

Characterization of prepared nanosponges

Particle size and size distribution of particles

The NanoBrook 90 Plus particle size scanner was used to determine particle size and Polydispersity Index (PDI); a dynamic light scattering technique that involves calculating the strength of light scattered by components in a mixture concerning the time at a scattering angle of 90o and a constant temperature of 25 °C [10].

Production yield percentage of prepared nanosponges

The Production Yield Percentage (PY%) of the prepared nanosponges determined the preparation method's efficiency. The (PY %) was determined by calculating the % of the actual weight of the obtained NS on the theoretical mass of drug and polymers [11]:

$$PY\% = \frac{\text{Obtained weight} \times 100}{\text{theoretical weight (Etoricoxib} + \text{polymers)}} \quad (2)$$

The entrapment efficiency of prepared nanosponge

The entrapment efficiency was determined by dissolving 10 mg of dried NS in 10 ml of PBS of pH 7.4; in a volumetric flask, then put it in the bath sonicator for 1 minute. After centrifuging, the free drug remained in supernatant while entrapped drug retained in the NS; 1 ml from the clear solution was taken and filtered, diluted with PBS of pH 7.4 then the drug was detected by a UV spectrophotometric by using PBS of pH 7.4 as a blank, the measurement of drug EE% was carried out in triplicate, and average values used (8,9). The highest EE%, the greater the amount of drug entrapped inside each NS, drug entrapment efficiency can be determined by the following equation:

$$EE\% = \frac{\text{(W initial drug} - \text{W free drug) x 100}}{\text{W initial drug in the formula}} \quad (3)$$

Results and Discussion

The solubility of pure ETX powder was investigated in three different media (distilled water, phosphate buffer pH 7.4 with % w/v SLS, and phosphate buffer pH 7.4). According to the findings, ETX has a minimal solubility in phosphate buffer pH (7.4), as shown in **Table 2**; however, the solubility of EXT increased when SLS is added as a surfactant. The solubility analysis for ETX revealed that pure powder has low solubility in phosphate buffer (pH 7.4), while ETX NS showed comparatively higher solubility, as seen in **Table 2**. The prepared structure of NS increased surface area and improved hydrophilicity by reducing the particle size of ETX to the nanoscale using EC and PVA as well as hydrogen bonding between drug and nanosponges structure [12, 13].

Table 2. The Solubility of Pure Etoricoxib and Prepared Nanosponge Formulas

Formula No.	Ratio EXT: EC	Solubility in water (mg/ml)
Pure EXT #	-	0.084
F1	1:0.25	1.133
F2	1:1.5	0.930
F2-A	1:1.5	0.530
F3	1:2	0.715
F3-A	1: 2	0.642
F3-B	1:2	2.704
F3-C	1:2	0.886
F4	1:3	0.296
F4-A	1:3	0.271
F4-B	1:3	0.383

Solubility in Buffer (7.4 mg/ml) = 0.041

Solubility in Buffer 7.4 + 1% SLS (mg/ml) = 1.47

EXT: etoricoxib, EC: ethyl cellulose, SLS: sodium lauryl sulfate

The solubility of prepared NS was significantly affected by increasing quantities of EC with constant amounts of ETX, as seen in **Figure 1a**. An increase in the EC ratio caused a significant decrease in the solubility of ETX-NS. The best solubility was obtained with 1:0.25 and 1:1.5 of ETX: EC and the solubility were significantly increased by sonication, as seen in **Figure 1b**. F2-A was prepared without sonication had less solubility than F2, which was designed with sonication. This effect of sonication on solubility is attributed to an increase in the surface area of NS exposed to an aqueous vehicle [13].

As the PVA content of the nanosponge rises, the mean particle size of the nanosponge reduces at first , as shown in **Figure 2a** and this will lead to decrease the solubility by decrease the surface area ,a particular amount of PVA molecules was needed to reach a small size of the Nanoparticle, the majority of the

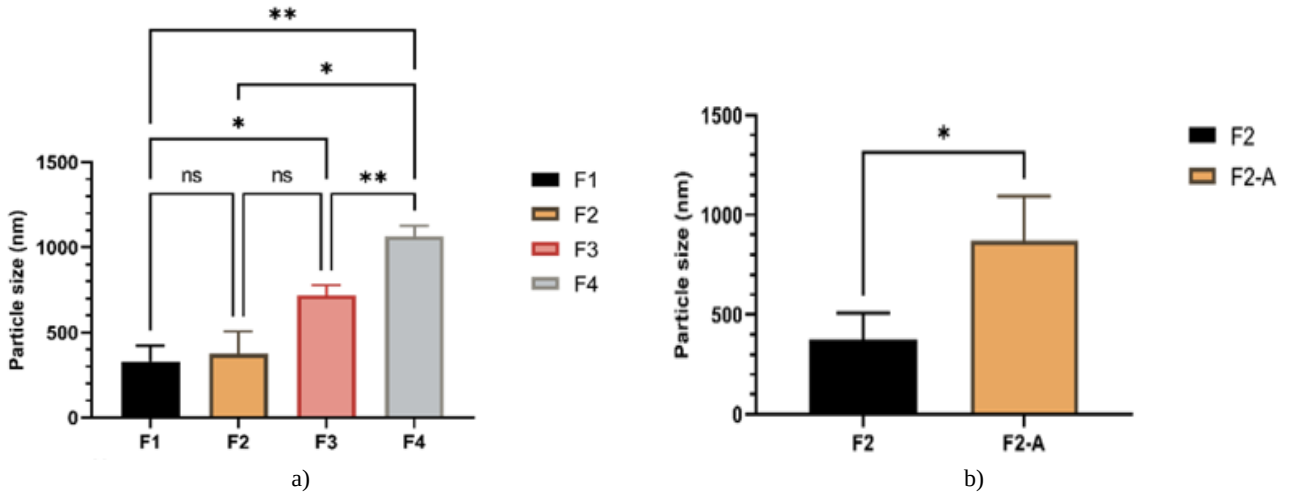
PVA concentration will remain in the continuous phase and play a significant role in the formation or stabilization of the droplets; as a result, the amount of PVA used in nanosponges technique is critical [14].

The effect of changing the type of organic solvent on particle size and solubility was observed in **Figure 2b**, It was discovered that decreasing in solubility resulted in a significant increase in mean particle size. Vaculikova *et al.* demonstrated this by observing the declining particle size of hydrochlorothiazide NP when comparing two organic solvents, dichloromethane and acetone; with dichloromethane, they obtained stable NP [15].

The impact of stirring speed on the formulated Etoricoxib loaded nanosponge characteristics was investigated; The intensity speed was found to affect the distribution of the drug and polymer into the aqueous process **Figure 3.**

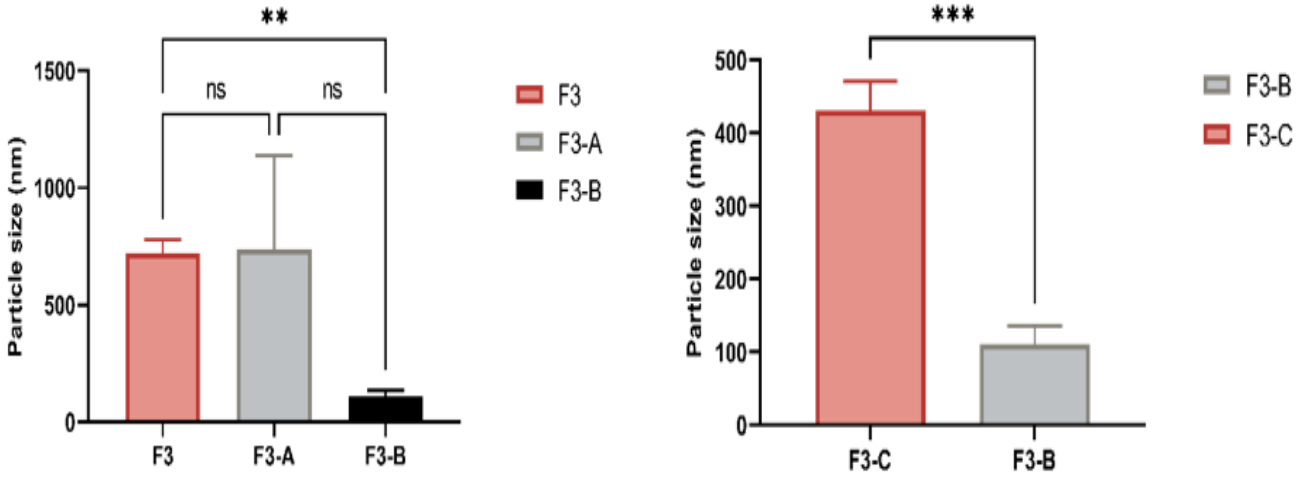
Because of the turbulence generated within the outer medium (external phase), the polymer adhered to the paddle at higher stirring speeds, and a substantial loss of the shaped Etoricoxib loaded nanosponge was also observed; The globules' likelihood can explain the increase in particle size at a low, stirring speed (**750 rpm**) to aggregate and fuse hence.

This will affect the solubility as large particles size have low solubility and changing in particle spherical shape will also decrease the solubility, a mixing speed of 1000 rpm was highly suggested to prepare the Etoricoxib loaded nanosponge [16, 17].



a) b)

Figure 1. a) The Effect of ETX: EC Ratio on Particle Size of Prepared NS. F1, F2, F3, and F4 Prepared with Various Ratios (1:0.25, 1:1.5, 1:2, and 1:3) ,b) The Effect of Sonication on the Particle Size of Prepared NS. Sonication Used in the Preparation of F2 and F2-A Prepared without Sonication



a) b)

Figure 2. (a) The Effect of PVA Quantities on the Particle Size of Prepared NS. F3, F3-A, and F3-B were Prepared with Various Amounts (2gm, 1gm, and 3 gm) ,(b) The Effect of Type of Organic Solvent on the Particle Size of Prepared NS. DMN was Used in the Preparation of F3-B and Acetone in F3-C

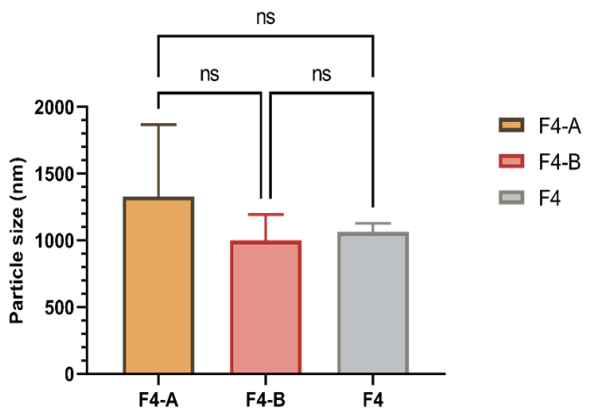


Figure 3. The Effect of Stirring Speed on Particle Size Prepared NS. Various Stirring Speed 1000,750 and 1250 rpm in Preparation of F4, F4-A, and F4-B

Particle size results confirmed that prepared NS were in the nanosized range with a Polydispersity Index (PDI) of less than 1 (=0.005). The particle size of nanosponges was inversely affected by the ETX: EC proportion. This may be attributed to the thickness of the polymer wall that is reduced at low ratios of EC, and NS will be smaller than those prepared with high portions of EC, and this can be explained by the viscous polymer caused by the polymer proportion increase from 1:0.25 to 1:3. Consequently, the high viscosity prevents the breaking of the emulsion formed during the processing of this method (emulsion solvent diffusion method) into smaller droplets. Therefore, nanosponges with larger particle sizes are formed [5, 18-20].

Moreover, the low concentration of the EC increases the diffusion of the internal phase into the external phase (water) lead to decrease time needed to form droplets and thus reducing the particle size. Further reduction in particle size was obtained with sonication. NS was prepared at lower stirring speeds 750 rpm; formula F4-A resulted in a larger particle size 1328 nm as compared to 1000 and 1250 rpm (formulation F4 and F4-B, respectively), for which the particle size was 998 and 1064 nm. With the higher speed of homogenization, more energy is released in the process, leading to a rapid dispersion of polymeric organic phase, and because of which NS was of small size [21].

% EE of 1: 0.25 ratio (F1) was significantly varied compared to the percentage of 1: 3 (F4). This indicates EXT's encapsulation is more efficient with lower content of EC in F1 (82%). In contrast to % PY, it is the highest value (81%) with a high ratio (1:3) of EC in EXT: EC ratio in F4. In addition, sonication increases % EE from 78% to 84% at a constant ratio of EXT: EC (1:2) while there is no significant effect of sonication on % PY. PVA quantities showed a substantial impact on % EE, which elevated to 90% using 3 grams of PVA compared with less quantity 1 gm and 2 gm both of them produced 72%, as illustrated in **Table 3**.

Table 3. The Effect of Stirring Speed on % PY and % EE Prepared NS Using Different Formulas

Formula	%PY	%EE
F1	75±8	69±8
F2	79±7	75±8
F2-A	83±11	75±6
F3	87±5	75±9
F3-A	85±12	72±6
F3-B	88±6	90±10
F3-C	83±7	76±6
F4	78±6	67±6
F4-A	79±5	70±4
F4-B	76±5	65±3

%PY: production yield percentage, %EE: entrapment efficiency

Conclusion

In the present study, ETX- NS improved the solubility of the poorly soluble drug ETX. The ratio of EXT: polymer, sonication, and PVA quantities variables have a significant effect on EXT-NS solubility, particle size, and %EE. Improved % EE (90%) and efficient reduction in particle size can be obtained by increasing the quantity of PVA in the formulation and using the suitable drug: polymer ratio and stirring speed during processing.

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