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Preparation and Characterization of Etodolac as a Topical Nanosponges Hydrogel

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Abstract

Nanosponges (NS) of etodolac (ETO) was prepared using the emulsion solvent diffusion method. Etodolac took as a model drug because of its short half-life, low water solubility; NS is non-irritating, non-mutagenic, non-allergenic and non-toxic suitable to bind to poorly- soluble drugs within the matrix and improve their bioavailability .The effects of drug: polymer ratio, the effect of level concentration of internal phase and stirring time and other variables that effect on the physical characteristics of NS were investigated and characterized.The selected formula was lyophilized then incorporated into hydrogel ; which also evaluated .The results show that the formulation that contain Drug:Polyvinyl alcohol: ethylcellulose in ratio 1:3:2 is the best with smallest particle size 40.2±0.098nm with polydispersibility0.005 and in vitro release 97.6%±0.11, , ETO NS Carbopol hydrogel produced a significant($p<0.05$) improvement of the in vitro release than pure ETO hydrogel.

Keywords: Etodolac, Nanosponges, Hydrogel.

تحضير وتقييم دواء الايتودولاك كإسفنجيات نانوية كمستحلب مائي موضعي

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الخلاصة

الهدف من الدراسة تقديم صياغة هلام من اسفنجيات نانوية من دواء الايتودولاك قليل الذوبان وذلك لتعزيز الذوبان و تحرير بأشكال موضعية و استخدامه عن طريق الجلد كوسيلة بديلة لتقليل الاثار الجانبية على الجهاز الهضمي حضرت الاسفنجيات بطريقة انتشار المذيبات من شبه مستحلب ثم اختبرت عليها تأثيرات الخصائص الفزيائية للإسفنجيات كفاءة التحميل و وبعدها اضفنا الاسفنجيات الي الهلام المائي و تم تقيم الهلام المائي مختبريا، و أظهرت النتائج تفوق تحرر الهلام المائي الاسفنجي مختبريا

الكلمات المفتاحية :الايتودولاك، الإسفنجيات النانوية، هلام مائي .

Introduction

The most significant challenge with topical drug delivery is the barrier nature of the skin that restricts the entry of most drugs, to increase the therapeutic effectiveness of the existing drug molecules is by formulating them using novel nanocarrier systems and incorporating them into topical preparations [(1)]. Nanosponges are a novel class of nanoparticles, exhibiting promising potential in controlled drug delivery, especially topical formulations. Bezawada et al., define Nanosponges(NS) as biocompatible porous nanoparticles formulate as nano-sized colloidal carriers having the shape of tiny sponges in nature; with a size of about a virus and the average diameter (250 nm -1μm) which will fill with a variety of materials. Different methods of preparation of NS; ultrasound assisted synthesis ,emulsion solvent diffusion method, solvent method and hyper cross-linked - cyclodextrins [(2)]; Pervious work on NS ; like using Lemongrass oil a volatile oil extracted from the leaves of *Cymbopogon citratus* incorporated in an EC NS revealed that the spongy structure with minute pores and the sustained integrity of the NS structure when incorporated in the hydrogel showed no skin irritation and increasing bioavailability [(2)].

Etodolac (ETO) is a pyranocarboxylic acid that falls in the (NSAIDs) category. ETO is a weakly acidic drug that belongs to the Biopharmaceutical Classification System (BCS) class II type (practically water insoluble). The chemical name is [(±) (1, 8 -diethyl-1, 3,4, 9-tetrahydropyrano- [3, 4-b] indole - 1-acetic acid] with a molecular formula of C17H21NO3, and molecular weight is 287.36g/mol, Fig. (1) demonstrated chemical structure of ETO. [(3)]

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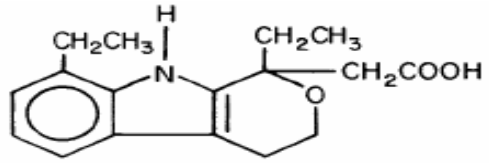


Figure (1) Chemical structure of etodolac[3]

Materials and Methods

Materials

Etodolac powder supplied by Hangzhou Hyper Chemicals Limited, China, Ethyl cellulose polymers powder N-type obtained from Hangzhou Hyper Chemicals Limited, China,, GCC Analytical reagent, UK supplied Dichloromethane. All other materials used in this study were of analytical grade.

Methods

Preparation of etodolac nanosponges

ETO NS was prepared by emulsion solvent diffusion method table (1). The internal organic phase consisted of different amounts of ETO and ethyl cellulose; all dissolved in dichloromethane (DCM) or acetone (internal or disperse phase); while PVA dissolved in 100 ml water (external or aqueous continuous phase). The disperse phase was added very slowly and continuously with the help of syringe into the aqueous phase then we put the formula under mechanical agitation for 2 hours at room temperature [4, 5].

Table (1) Composition of ETO NS.

Formula no. / Ingredient	F1	F2	F3	F4	F5	F6	F7	F8
Etodolac (gm)	4	2	1	1	1	1	1	1
PVA (gm)	3	3	3	2	3	2	3	4
EC (gm)	2	2	2	2	2	2	3	2
Dichloromethane (ml)	20	20	20	20	_	20	20	20
Acetone(ml)	_	_	_	_	20	_	_	_
Distilled Water(ml)	100	100	100	100	100	100	100	100
Stirring speed (rpm)	1000	1000	1000	1000	1000	2500	1000	1000

Methods

Evaluation of the prepared nanosponges

Particle size and size distribution

Particle size and polydispersity index determination done by using the Nano Brook 90Plus particle size analyzer; which is a dynamic light scattering, works by measuring the intensity of light scattered by the molecules in the sample as a function of time [6].

The in-vitro dissolution profile of nanosponges

In-vitro drug release studies with a pretreated dialysis bag (Schuchardt dialysis membrane MWCO 12,000 Da) cut off. An aqueous dispersion of NS (10ml), is placed in dissolution test USP apparatus Type II. That fixed on 100 rpm and 37 ±0.5°C, ETO loaded NS evaluated by immersing it in 900 ml 7.4pH phosphate buffer [7,8] .

Zeta potential measurement (ZP)

ZP for selected formula determined by The Nano Brook 90Plus zeta sizer (BrookhavenInstruments USA) [9].

Freeze drying of the selected etodolac nanosponges

The formed NS sonicated for 10 minutes to avoid the presence of aggregates. Then centrifuged at 3000rpm for15min at room temperature to separate the uncomplexed drug as a residue below the colloidal supernatant. After that we filtrate by using filter paper to remove any undissolved particles [10]. The colloidal supernatants were freeze-dried to obtain ETO loaded NS [11].

Drug entrapment efficiency of nanosponges lyophilized powder

For the drug entrapment efficiency tests, 10mg of the NS powder was dissolved in 10ml phosphate buffer solution 7.4, in a volumetric flask then placed in bath sonicator to break the complex the free drug remained in supernatant while entrapped drug retained in the NS, the measurements was carried out in triplicate and standard deviation values was taken **[12].**

Saturation solubility of lyophilized powder

Performed for the selected formulation by dissolving the excess amount of lyophilized powder in 10 ml of phosphate buffer pH 7.4. Then the mixture was shaken for 48 hours at 25°C then filtered; and analyzed. (13)

The dissolution profile of lyophilized powder

In-vitro drug release studies were carried out using USP type II dissolution apparatus (50 rpm, 37 ±0.5ºC); 1 gm of ETO loaded Nanosponges lyophilized powder put in pretreated dialysis bag (cut off 12,000 Da). The dissolution media contain 900 ml of phosphate buffer pH7.4; At each fixed time intervals samples were taken and suitably diluted and analyzed; in triplicate (n = 3). (14)

Fourier transform infrared spectroscopic analysis (FTIR)

FTIR helps to check out the compatibility between the drug and the excipients.The FTIR spectra of pure ETO and lyophilized powder of the selected formula were obtained using (FTIR-8300 Shimadzu, Japan)[15].

X-ray powder diffraction (XRPD)

Powder X-ray diffraction can be used to emphasize the crystalline nature of the substances in the solid state, the diffractograms of ETO pure powders and lyophilized powders of the selected formulations analysis using Shimadzu XRD-6000 powder X-ray diffractometer [16].

Scanning electron microscope (SEM)

SEM can be used to study morphology, and surface topography of the prepared NS and the difference in morphology state of the pure drug and the product by using a Vega/TESCAN scanning electron microscope [17].

Preparation of nanosponges loaded hydrogel

The gel-forming polymer (Carbopol 934) 1 gm was soaked in 100 ml water for 2 hrs. Then dispersed by agitation using a magnetic stirrer to get a uniform dispersion. The dispersion was allowed to stand for 15 minutes so that all the entrained air expelled. Methylparaben (preservative); dissolved in a sufficient quantity of water pre-warmed to 40°C and incorporated. Then add triethanolamine (2% v/v) drop by drop with continuous mixing to neutralize the pH of polymer aqueous solution. Finally ethanolic solutions of lyophilized ETO NS and second formula of pure ETO powder incorporated in the polymer aqueous solution [18] . The Composition of ETO Nanosponge Loaded in Carbopol 934 Hydrogel in the table (2).

Table (2) Physical properties of the prepared hydrogel

Ingredients (% w/w)	F1	F2
Etodolac NS eq. to 2 gm Etodolac	12	-
Etodolac (gm)	-	2
Carbopol 934(gm)	1	1
Triethanolamine	2.0	2.0
Ethanol (95% v/v)	20	20
Deionized water q.s	100	100
Methyl Paraben(mg)	100	100

Determination of viscosity

Rheology of prepared NS loaded hydrogel formulation was studied by Myr Rotational (cup and bop) digital Viscometer with spindle no. R7, at 2-200 rpm, at room temperature; (n=3) (19).

Determination of etodolac content in the hydrogel formula

ETO content in the hydrogel was determined by taking specific quantity of the prepared gel which is equivalent to 10 mg of etodolac and transferred to 100 ml volumetric flask containing phosphate buffer (pH 7.4). The mixture then allowed to sonicate for 5 minutes followed by filtration then the filtrate was analyzed at λ max of ETO (20).

In-vitro dissolution test of etodolac nanosponges loaded hydrogel

The *in-vitro* release of ETO from NS loaded hydrogel and ETO hydrogel formulas performed by using dissolution apparatus-II (paddle type). Hydrogel (2 gm) was performed using dialysis bag method with pretreated (dialysis membrane cut off 12,000 Da). The dissolution test performed ; with 900 ml dissolution media phosphate buffer pH 7.4, at 37 ± 0.5 °C with stirring speed of 100 rpm. Samples filtrated, diluted and analyzed; usually the drug release experiments were conducted in triplicate (n = 3); the statistic mean value of three readings was taken.

Result and Discussion

This study presents a new approach for the modification of polymeric nanoparticles as well as a new system for enhancing dissolution and release of poorly soluble drugs.

Particle size analysis and polydispersity index measurement

The effect of different parameters on the particle size and polydispersity index was studied using eight different formulations. The mean particle size (effective diameter) for formulations varied in the range from 46.9±0.358 nm to 1043.35±10.2 nm. The particle size and PDI for different formulations of different parameters is showing in the Table (3).

Table (3) The particle size, PDI of different formulations

Formula no.	Particle size ±SE*	PDI	% drug release (% ± SE)
F1	526.5± 0.125	0.352	59%± 8.767
F2	224.3 ± 90.7	0.005	66%± 8.022
F3	46.9± 6.5	0.005	97.6%± 12.201
F4	172.8± 0.169	0.005	89 %± 11.603
F5	354.68± 0.8	0.329	75%± 10.242
F6	111.5± 9.2	0.246	45 %± 5.998
F7	711.6± 90.7	0.005	50% ± 17.224
F8	1043.35± 10.2	0.352	45%± 8.870

***SE standard error, n=3**

Variables affecting formulations

Effect of the drug to polymer ratio

The formula 1 and 2 as seen in the Figure 2 and Figure 3; revealed that as the amount of drug increase; the particle size increase and *in- vitro* drug release reduced significantly ($p < 0.05$) ; Bohrey et al. observe the same result ; and explained it by the fact that a more considerable amount of drug results

in a more viscous organic phase (dispersed phase), making complex the mutual dispersion of the aspects and forming bigger nanoparticles [22].

While in formula 3 and 7 Figure 2 and 4 ; amount of ethyl cellulose polymer increased from 2 gm to 3 gm ;this cause an increase in particle size and significantly ($p< 0.05$) decrease in the amount of drug released; the same result was seen with Kılıçarslan et al., they prepared verapamil HCl loaded microspheres and discuss the effect of the drug/polymer ratio ; as the amount of polymer in the formulation increased, the drug release decreased; due to rising in thickness of polymer wall of NS particles will affect the diffusion path thus the drug not be quickly released in to the dissolution medium [23].

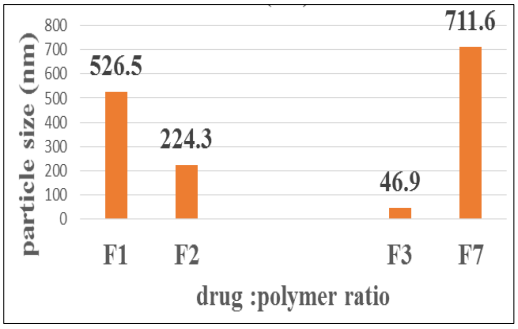


Figure (2) The effect of the drug to polymer ratio on the particle size of etodolac loaded nanosponge

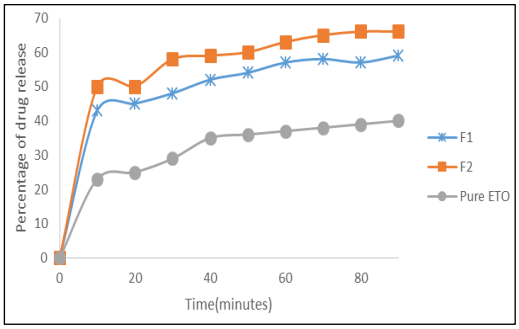


Figure (3) Dissolution profile of formulas F1, F2 in PBS of pH 7.4.

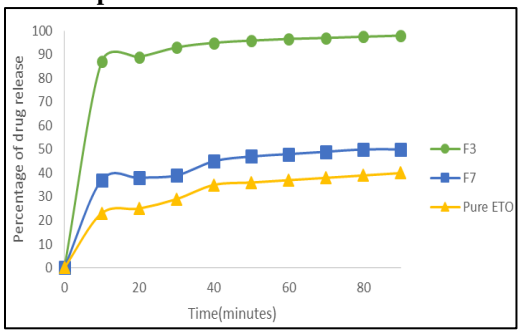


Figure (4) Dissolution profile of formulas F3, F7 in PBS of pH 7.4.

Effect of PVA concentration

The presence of PVA molecules stabilizes the emulsion nano droplets and prevents them from aggregation with one another. The PVA molecules must cover the organic/aqueous interfacial area of all the droplets. Hence a specific amount of PVA molecules is required to achieve small size of nanoparticles. We can notice from table (3) and Fig. 5 and Fig. 6 that the most uniform size distribution was obtained from 3% PVA in F3; as the amount of PVA increased F8 or decreased F4 from 3 % w/v; this cause insignificant effect ($p > 0.5$) on *in -vitro* drug release and particle size. As the concentration of PVA increased as in F8 this cause the micro size particle appearance instead of nanoparticles due to the increased viscosity of the aqueous phase causing reduction in the net shear stress available for droplet break down so the PVA concentration stays in the continuous phase. In this case PVA does not play a role, either in the emulsification or in the stabilization of the sponges. The same result was noticed with Zweers et al. and Feng et al. [24, 25] .

While in formula 4 as the amount of PVA decreased from specific level; the particle size decreased but cause the fragility of the formed NS during the process of dispersion. Which was noticed by a significant decrease in *in- vitro* drug release for F4. The particle size of NS seems to be dependent on the PVA concentration in continuous phase; the same result was reported by Kemala et al. [26].

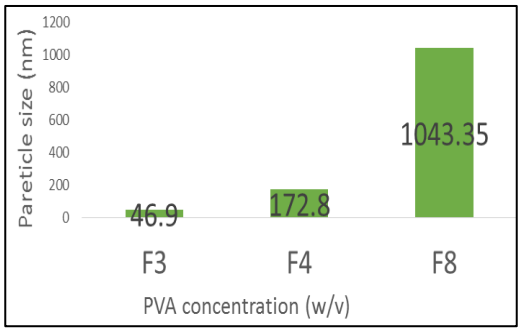


Figure (5) PVA concentration effect on ETO loaded nanosponge

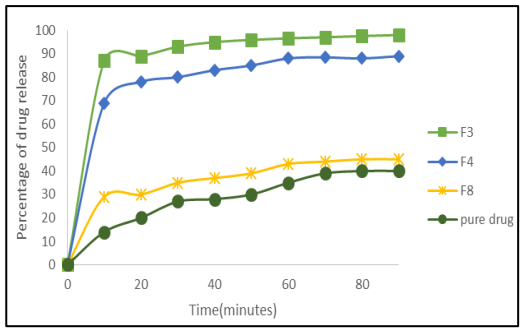


Figure (6) Dissolution profile of formulas F3, F4 and F8 in PBS of pH 7.4.

Effect of Type of internal phase solvent

Organic solvents have essential role in the preparation of NS. Beside dissolving the drug and polymer; organic solvents ensure the initial thermodynamic equilibrium with the aqueous phase. Also, during development the organic solvent diffused into the external phase and form NS; and this diffusion and consequently elimination of organic solvent mainly depend on its boiling point [27].

Formula 3 and 5 from table 3 and Fig.7; demonstrate this effect of change the type of organic phase causing increase in mean particle size with significantly ($p< 0.05$) decreasing *in- vitro* drug release. Because they depend on the physical properties of the organic solvent used; the solubility of DCM in water is low, but vapor pressure is very high. Therefore, DCM rapidly diffused into water and evaporated out resulting in fast precipitation of NS droplets (containing polymer with drug) without giving much time for drug molecules to partition into aqueous phase and aggregation. While acetone have higher boiling point (b.p. 56 °C) than DCM (b.p. 39 °C) so the evaporation is slower; therefore the in vitro release of acetone is low [28,29].

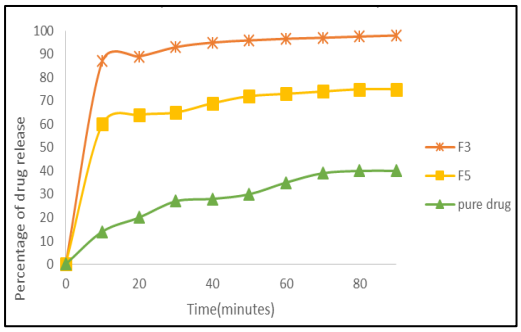


Figure (7) Dissolution profile of formulas F3, F5 pure drug in PBS of pH 7.4.

Effect of stirring speed

Formula 4 and 6 from table 3 and Fig.8; show the impact of mixing speed on NS formulations; increasing the stirring speed from 1000 to 2500 rpm cause insignificant ($p > 0.5$) decreasing of *in-vitro* drug release with decreasing of mean particle size; this explained by Srinivas et al.; at higher stirring rate decrease the mean particle size and the production yield was reduced because the polymer adhered to paddle due to high turbulence created within the external (1).

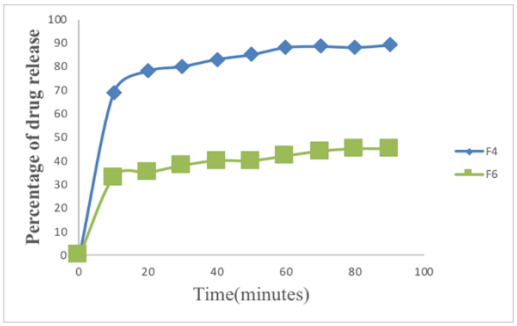


Figure (8) Dissolution profile of formulas F4, F6 in PBS of pH 7.4.

Zeta potential measurement (ZP)

High ZP value improve stability of the dispersion and will resist aggregation while colloids dispersion with low zeta potentials tends to coagulate or flocculate for the selected formulation of ETO loaded NS was-30.8 mV, the charge was negative due to the surface negative charge of PVA hydroxyl group that anchored on the surface of the NS ; the same result noticed with Salah et al., [30] the NS adequately stabilized by PVAnonionic surfactant that appeared to be the most suitable surfactant in reducing aggregation between nanoparticles the same result was reported by Lakshmi et al. [31].

Saturation solubility of freeze-drying nanosponges

The best formula for lyophilization; the batch F3 gave use the small particle size and lowest polydispersity index with best dissolution profile; table 1,3 and figure 3,4. The quantity F3 gives rise to fluffy mass powder showing highly porous structure; with a white cotton-like, these spongy materials was the same result that reported by Singireddy et al. [32]. The saturation solubility of the lyophilized powder was increased significantly ($p < 0.5$) from the pure drug solubility; It expands to 12± 0.7folds in pH 7.4; the same result reported by Rao et al. [33].

The dissolution profile of lyophilized powder

The lyophilized powder of NS Selected formula (F3) shown in Fig. 9. The release of ETO from the NS was higher than the release profile of pure drug within 90 min.

The percent of cumulative drug release of lyophilized F3 was more than 90% in less than 30 min as compared to less than 20% and 25% of the pure drug in the same in phosphate buffer pH 7.4 media.

Factors that are contributing to a fast release attributed to the reduction in particle size causing increase in the surface area and consequently enhanced the contact between particles and dissolution medium the same result seen with Torne et al. [34] .

Also, the plastic designs of the prepared NS structure and hydrophobic nature of EC, drug particles near the surface of the NS matrix could be initially diffused into the surrounding medium,

resulting in the increase in the pores and thus facilitating further drug release [21].

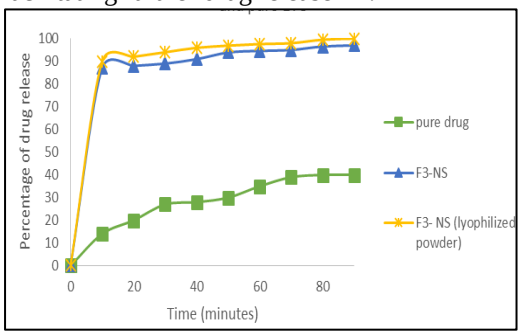


Figure (9) *in -vitro* drug release profile of ETO loaded NS lyophilized powder in PBS pH 7.4

Drug entrapment efficiency

The results show that each10 mg of lyophilized powder of NS of formulas F3 contain 97.45 % of ETO.

Fourier transform infrared spectroscopic analysis (FTIR)

The FTIR spectrum of pure etodolac and lyophilized powder given in figure (10) A, B. The results showed that the characteristic peak of ETO was C=O stretching vibration of COOH group in1743.33cm^{-1} is present in all the spectrums indicating that there is no chemical interaction between the drug other excipients [35].

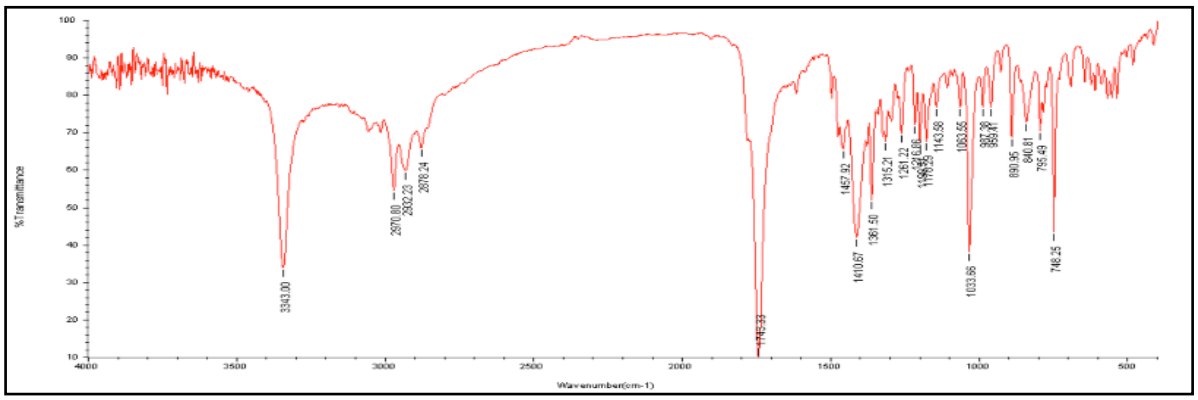


Figure (10) A.FTIR spectrum of pure etodolac

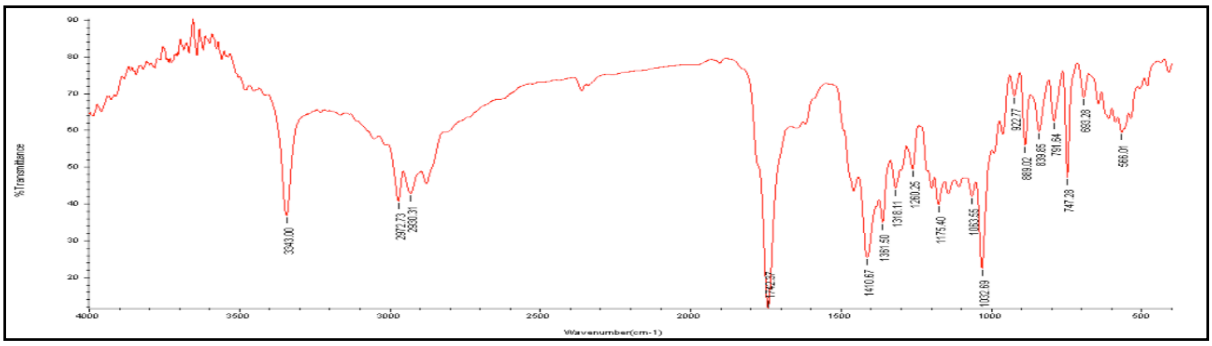


Figure (10) B . FTIR spectrum of ETO loaded NS lyophilized powder

Scanning electron microscope (SEM)

It can be seen that the raw drug particles have a rough surface with large particle size fig (11); while the SEM of F3 lyophilized powder fig (12) showed finely spherical, smooth, and porous due to diffusion of DCM from the surface PENJURI et. al. reported the same result [36].

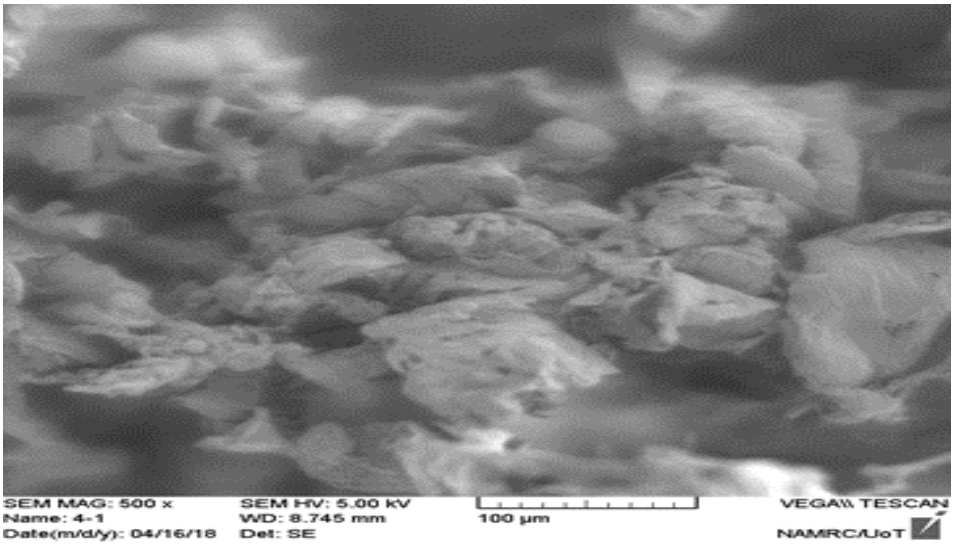
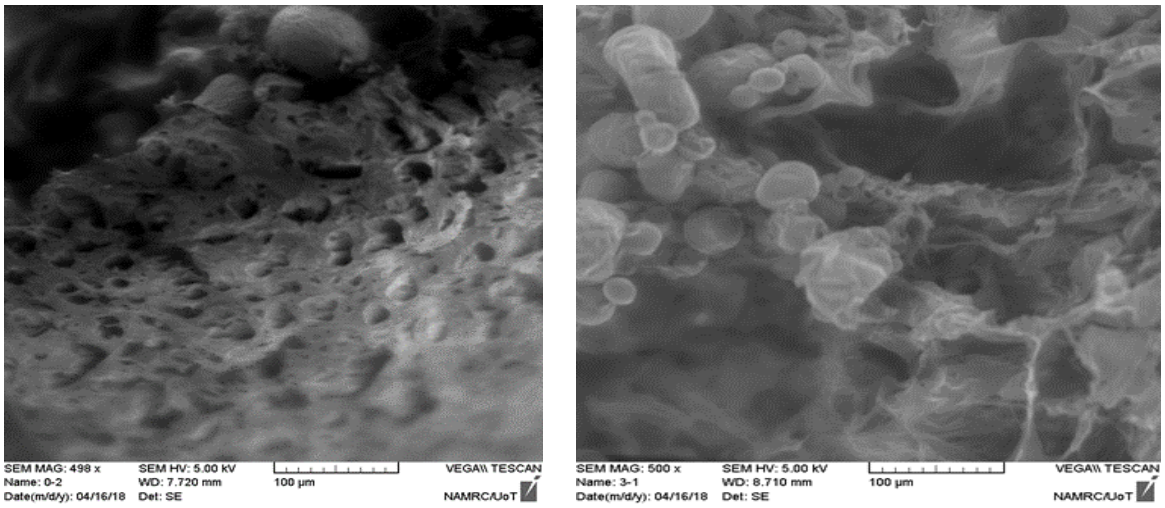


Figure (11) SEM of raw drug magnification 500X; size of particles (100µm)



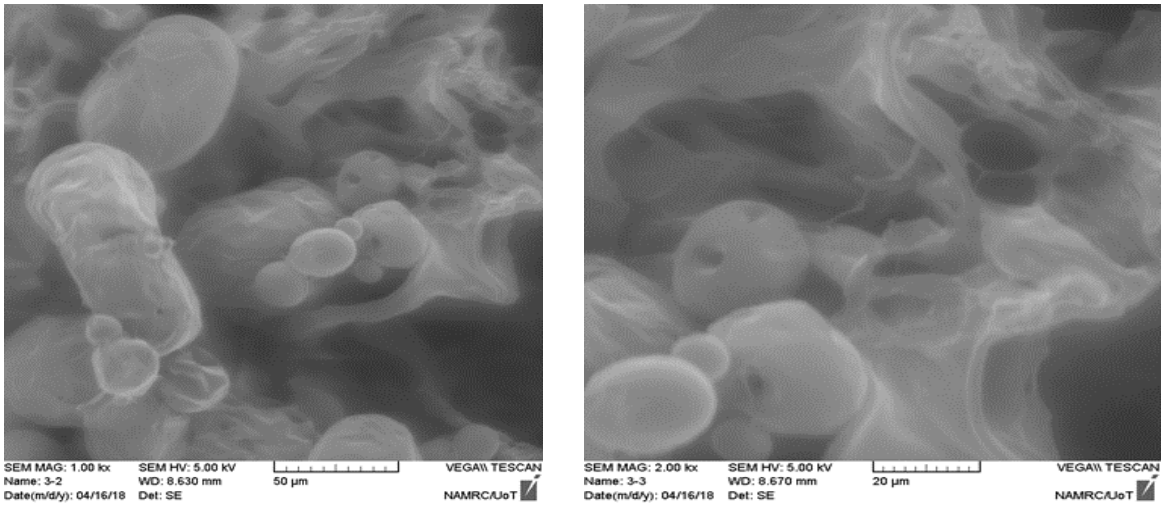


Figure (12) SEM of F3; at 498X,500X,1.00Kx and 2.00 Kx magnification

Powder X-ray diffraction analysis (PXRD)

PXRD patterns of ETO as a pure powder showed sharp diffraction peaks in the Fig. (13) indicates the crystalline nature of the pure etodolac. However, these characteristic peaks disappeared in the pattern of lyophilized powder as seen in Fig. (14) Producing a diffused pattern of very low- intensity peaks and shifting to a lower degree with complete absence of and diffraction peak, this mean ETO in the lyophilized powder is in an amorphous state; the same was reported by Rao et al [37] .

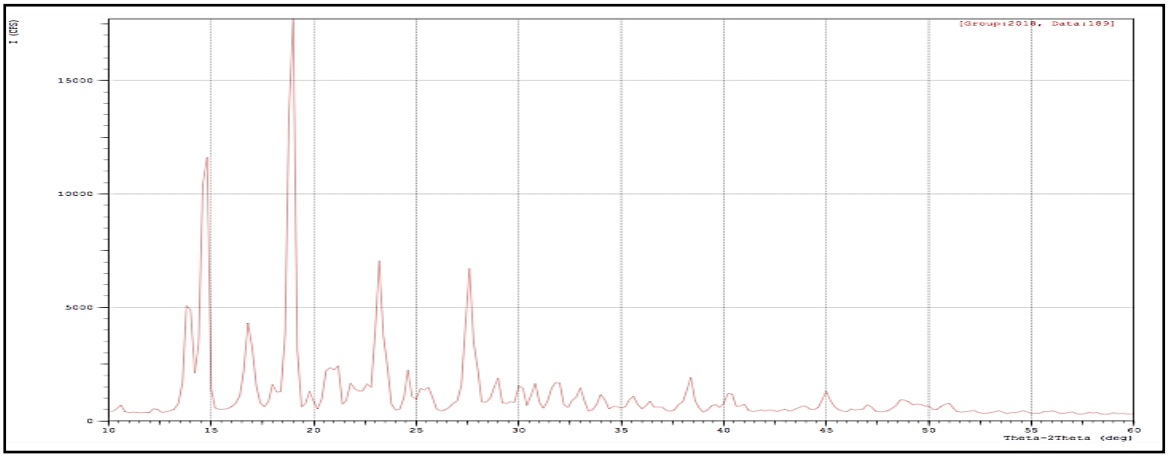


Figure (13) PXRD of pure drug.

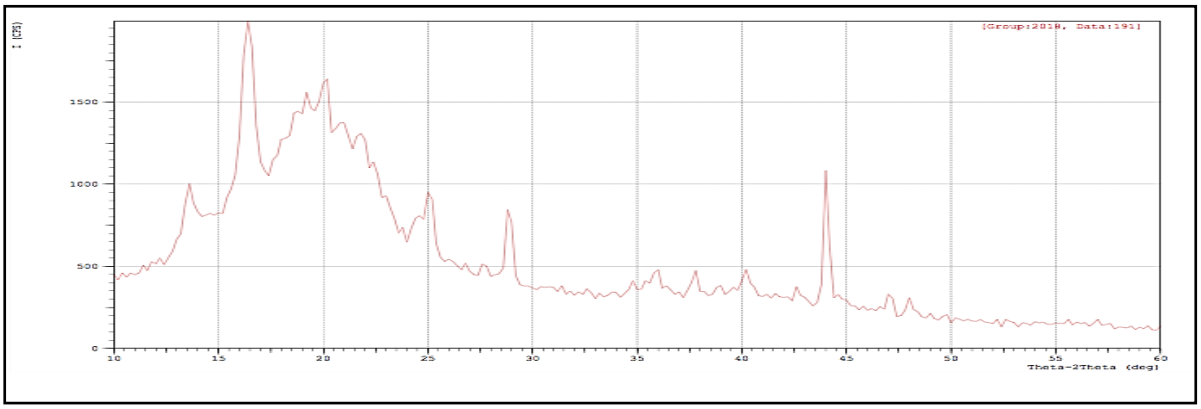


Figure (14) PXRD of selected formula F3

Physical properties of the prepared hydrogel

The physical appearance showed that systems gelled and pale to white non-transparent, with smooth, homogeneous constancy. This result agrees with that of research on miconazole nitrate loaded nanosponge gel [18]. The result of pH for ETO loaded NS hydrogel is 5.9±0.01; while the pH of ETO hydrogel was 6.7±0.01; which lies in the normal pH range of the surface; indicating the suitability of the formulations for application on the skin [38].

The viscosity of nanosponges hydrogel

Carbopol 934 hydrogel showed approximate viscosity between (32,000 -134,150) CP for ETO loaded NS hydrogel while ETO hydrogel showed viscosity between (45,000 - 140,546) CP; Figure [15]. The viscosity value of ETO loaded NS hydrogel is excellent because it provides easy removal of preparation from the container and smooth application of formulations on the infected surface; There is a significant difference (P<0.05) between formulations.[39]

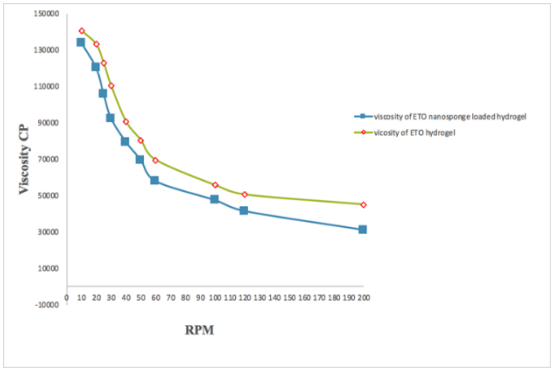


Figure (15) The rheogram of nanosponges hydrogel and pure etodolac hydrogel

Determination of etodolac loaded nanosponges content in the hydrogel formulas

The ETO NS contents in hydrogel are 97.5% ± 0. While in ETO hydrogel contents are 90.1% ± 0.44 from this we can conclude that ETO uniformly distributed in Both hydrogel formulations.

***In -vitro* drug release study**

From the release profile Fig. (16); ETO NS loaded hydrogel has produced a significant improvement in the dissolution rate which is significantly higher ($p<0.05$) than that of pure ETO hydrogel. *In -vitro* release of ETO nanosponge from hydrogel showed fast and complete statement in comparison to pure ETO hydrogel, the same result seen with; Aldawsari et al.; they prepared a topical hydrogel of lemongrass-loaded NS; the higher porosity of the larger particles would allow for the leakage of the ETO to the hydrogel during preparation, resulting in faster release rates [21] .

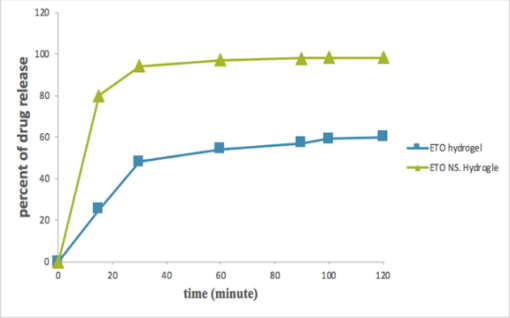


Figure (16) Dissolution profile of ETO from nanosponges hydrogel formula and pure ETO hydrogel in PBS (pH 7.4)

The ETO loaded NS lyophilized powder and in the liquid state showed the same cumulative % of drug release profile more than 97.67% release of ETO, within the same time; 90 minutes whereas the ETO NS loaded hydrogel released more than 86% of ETO in the same period Fig. (12).This delayed in the release of ETO NS loaded in hydrogel because of embedding of ETO NS into the hydrogel base which effectively controlled the release of ETO, so the release was a combination of the version of the drug from NS matrix carriers and subsequent diffusion through the microchannel structures of the Carbopol hydrogel base Song et al. reported the same result [40].

Conclusion

Emulsion solvent diffusion method was used because of its simplicity and reproducibility. Also, this method is seem to be promising for the preparation of ETO NS; it can achieve that NS carbopol hydrogel was greatly enhanced dissolution and release of etodolac when compared to pure etodolac hydrogel.

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