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Preparation and Characterization of Aceclofenac-Loaded Nanosponges based on Eudragit L100 for Topical Application

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Abstract

Among the many NSAIDs generated from phenyl acetic acid is Aceclofenac (ACE). Its antipyretic, analgesic, and anti-inflammatory effects are noteworthy. However, the substance's delayed dissolution, limit permeability, and inadequate bioavailability are caused by its low solubility in water, which hinders its usefulness. Nanosponges (NS) are mesh-like tiny structures capable of encapsulating a diverse array of chemicals and pharmaceutical compounds. They augment the solubilisation ability of both hydrophilic and lipophilic medicines and exhibit a spherical porous colloidal structure. They enhance the bioavailability, the efficacy and safety of medication delivery of pharmaceuticals through extended drug release. They also serve as targeted delivery systems; avoid harm caused by medicine and as tactics for detoxification. This study aims to prepare and optimize an aceclofenac-Eudragit L100 NS to improve permeation through skin and release of ACE in controlled manner. The emulsion solvent diffusion procedure is used to prepare the NS that are based on Eudragit L100. The effect of the amounts of polyvinyl alcohol (PVA) and the drug-to-polymer ratio on NS properties are explored. Particle size, product yield, ACE trapped inside NS percentages, and drug release are the aspects that be considered during characterization. The results indicated that the product yield and drug entrapment % were significantly reduced as the concentration of Eudragit L100 was increased, while the particle size was significantly increased. A significant change was seen in product yield and entrapment percentage as the PVA content was increased. It also caused a notable increase in particle size. It could be concluded that Eudragit L100 can be used as a polymer matrix to incorporate ACE. In future investigations, ACE would be manufactured as a topical gel.

Key words. Aceclofenac , Eudragit L100,Gel ,PVA , Nanosponges and In vitro drug release .

Introduction

A novel class of colloidal structures made of solid nanoparticles with colloidal and nanosized holes is known as a nanosponge (NS). These structures are hyper-cross linked polymers. NS change the pharmacokinetic properties of active components, which increases drug bioavailability and makes poorly soluble drugs more soluble in water. On top of that, the medications are released in controllable manner [(1)].

When it comes to topical formulations, NS hold a lot of potential for accurate medication delivery. Their average diameter is less than 1 µm, and they can be filled with different materials. Their size is similar to that of a virus. Some of the methods used to prepare NS include solvent evaporation method, hyper cross-linked cyclodextrins, emulsion solvent diffusion, and ultrasound-assisted synthesis [(2)].

There was no skin irritation and improved bioavailability due to NS's spongy nature, which contains nanoscopic pores, and its ability to retain its integrity when mixed with the gel [(3)].

Aceclofenac is an excellent candidate for sustained-release formulations due to its short biological half-life of about 4 hours and the requirement for more frequent dose [(4)].

It has a very low solubility in water but good solubility in 96% ethanol. ACE recommended for the treatment of rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis symptoms, both acutely and chronically [(5)].

This work aimed to prepare and optimize an aceclofenac-Eudragit L100 as nanosponges for enhancing ACE release in controlled manner.

Materials and Methods

Materials

The following ingredients were obtained from China (Zhengzhou, Fushi Technology): aceclofenac powder, polyvinyl alcohol polymers, and eudragit L100. Dichloromethane was acquired from Germany: Merck KGaA. The remaining materials utilized in this investigation were all of analytical quality.

Method

Six different ACE NS formulations were prepared. Table (1) summarized the composition of the prepared NS. The synthesis of ACE NS is accomplished using the emulsion solvent diffusion method [6]. An ultrasonic shaker (Copley Scientific, UK), was used to dissolve ACE and Eudragit L100 in 5 ml of dichloromethane, creating the internal phase. The external phase was 50 ml of distilled water containing dissolved polyvinyl alcohol (PVA). With a hot plate magnetic stirrer (Joan lab, China) set at 1000 rpm, the internal phase was added dropwise to the external phase while the mixture was stirred for two hours at room temperature. The solid NS was able to precipitate from the organic solvent, which evaporated at this stirring rate. A vacuum pump and a Buchner funnel apparatus (Kennedy Manufacturing, USA) were used to collect the prepared NS. After that, it was rinsed three times with distilled water and then dried for twelve hours in a 40℃ oven (Memmert, Germany). The manufacturing processes used to create ACE NS are shown in Figure (1) [7,8].

Table 1. Composition of Various Eudragit L100 ACE Loaded NS.

Formula*	Ratio of drug to polymer	PVA concentration (w/v %)	DW(ml)**	DCM (ml)**
F1	1:0.5	0.125	50	5
F2	1:0.5	0.25	50	5
F3	1:1	0.125	50	5
F4	1:1	0.25	50	5
F5	1:3	0.25	50	5
F6	1:0.5	0.75	50	5

***all formulations were developed with 100 mg of ACE and a stirring duration of 2 hours.**
****DW: distilled water, DCM: dichloromethane.**

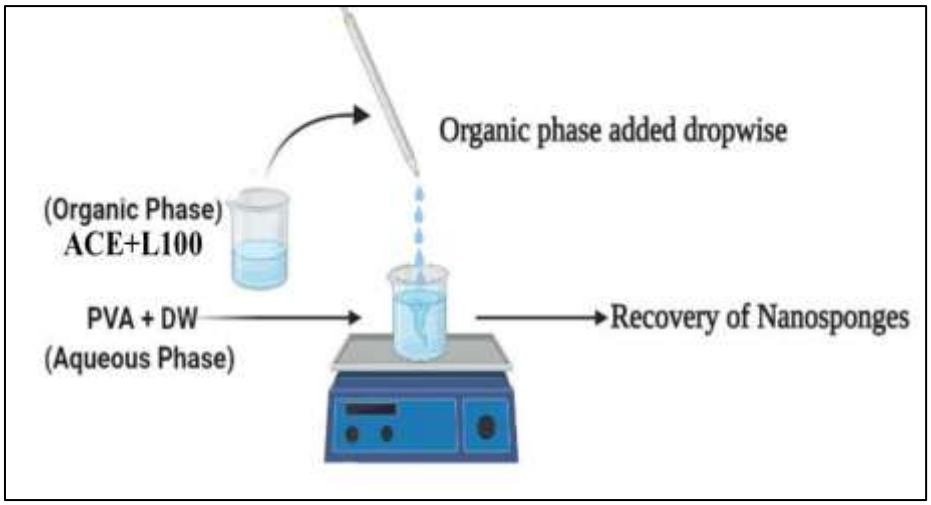

Figure 1. The emulsion-solvent diffusion process.

Characterization of ACE-NS formulations

Product yield determination

The product yield (PY%) calculated by dividing the dry formula weight by the total weight of the drug and polymer, as shown in equation (1) [9]:

$$PY\ (\%) = \frac{practical\ weight\ of\ nanosponge}{theoratical\ weight\ (polymer+drug)} \times 100 \quad \text{Eq 1}$$

Particles size evaluation

The vesicle size and dispersity were measured by dynamic light scattering (DLS) at 90ºϴ using an ultra-zeta sizer, all measurements were performed in triplicate and expressed as Mean ± Standard Deviation (M±SD, n=3).

The PDI is calculated by dividing the standard deviation by the mean droplet size and ranges from 0 to 1 [10].

Entrapment efficiency and drug loading of nanosponges

The entrapment effectiveness of a polymeric carrier is defined as the ratio of the drug mass actually trapped within the carrier to the initial drug loading. The following equation (2) is used to calculate it:

$$\text{Entrapment efficiency (\%)} = \frac{Actual\ loading}{Theoretical\ loading} \times 100 \quad \text{Eq. (2)}$$

The following equation (3) is the theoretical drug loading, which was determined by comparing the dosage of the medication with the total dosage and excipients utilized to make the NS:

$$\text{Theoretical loading (\%)} = \frac{Total\ drug}{Total\ drug + Total\ excipients} \times 100 \quad \text{..... Eq. (3)}$$

For actual drug loading to make the NS, 10 mg of the dry prepared NS loaded with drug was mixed with 2 mL of distilled water. The mixture was then centrifuged at 13000 rpm for 20 minutes. After eliminating any solid particles, the remaining transparent liquid was tested for unbound ACE linked to the NS. An ultraviolet-visible (UV) spectrophotometer (Carry100 UV, Varian, Australia), was used to measure the amount of light absorbed at ACE maximum absorbance wavelength (λ max.) of 273 nm. By mixing 10 mg of the dried powder with 10 ml of ethanol, the drug content in the NS could be measured. After that, a micro syringe filter with a pore size of 0.22 µm is used to pass the solution through after subjecting it to sonication. Analyzing the filtrate's absorbance with (UV) spectrophotometer allows one to determine whether ACE is present. To determine the real loading, the following equation (4) was used [11].

$$\text{Actual loading (\%)} = \frac{Total\ drug - Free\ drug}{mg\ of\ dried\ powder} \times 100 \quad \text{Eq. (4)}$$

Solid-State characterization of Aceclofenac Nanosponge

Differential scanning calorimetry (DSC) and Fourier-transform infrared spectroscopy (FTIR) were used to evaluate the physical properties of ACE-NS. The purpose of this analysis was to ensure that ACE-NS was free of impurities, its crystallinity and that the medicine and its excipients did not interact in any way. The drug, the drug and polymer in a physical mixture, and the optimum NS formula were all subjected to the analysis. To find out what the drug's crystallization state, the (DSC-600, Shimadzu, Japan) used to measure heat flow versus temperature [12].

After adding 5 mg of the powder to an aluminum pan, it was sealed. The sealed pan was contrasted with a reference sealed blank metal crucible. In a nitrogen atmosphere, the temperature was raised from 25°C to 300°C at a rate of 10°C/min. Fifty milliliters of nitrogen were being pumped out every minute. For the optimized solubilizing the drug and polymer and evaporated easily after diffusion. Because it is so easy to use, the emulsion solvent diffusion method was selected [16].

formulation using the KBr pellet technique, Fourier Transform Infrared (FTIR) spectra (FTIR600; Shimadzu, Japan). were recorded for the pure medication, physical mixture of drug and polymer, and the resulting pellets. The KBr hydraulic press was used to fabricate the pellets at a hydraulic pressure of 150 kg/cm^2. The spectra were acquired by utilizing an FT-IR 2500 instrument, which has a resolution of 4 cm^{-1}, to scan a range of 4000-400 cm^{-1} at ambient temperature [13].

Field Emission Scanning Electron Microscopy

Field Emission Scanning Electron Microscopy (FESEM) used to examine the surface topography of the particles. Both the medication and the optimum NS formula were analyzed morphologically using FESEM (Zeiss-Gemini, Germany). The sample, which had been sputtered with gold to make it conductive, was attached using a double-sided adhesive tape [14].

In-vitro drug release study

The dialysis bag method was employed to investigate the medication release rate from ACE-NS. The dissolution medium comprised 250 ml of phosphate buffer at a pH of 7.4, with a specified quantity of ACE NS equivalent to 5 mg of pure medication suspended in 5 ml of phosphate buffer within a dialysis bag. The rotation speed was set at 50 rpm, and the temperature maintained at 32°C. At predetermined time intervals (5, 10, 15, 30 minutes and 1, 2, 3, 4, 5, 6, 7, 8 hours), five milliliters of the medium were withdrawn and substituted with an equivalent volume of dissolving medium to preserve a constant volume. The samples were examined photometrically at 272 nm [15].

Statistical analysis

The statistical analysis of the results was conducted using GraphPad Prism 8 program (version 8.0.1). The studies were performed three times and the results were presented as the mean value ± the standard deviation. The ANOVA test was employed to evaluate the level of statistical significance at level ($P < 0.05$) among different groups.

Result and Discussion

Preparation of Aceclofenac nanosponges

Eudragit L100 was chosen since it was more affordable and readily available than other alternatives, as well as to modify drug release in a controlled manner. To create a solid ACE-NS, dichloromethane was used as an organic solvent in the internal phase since it was effective in

Characterization of ACE-NS Formulations

The effects of various formulation parameters on NS characteristics, including drug entrapment efficiency %, particle size, and Product yield %, are detailed in Table (2).

Table 2. Characterization of nanosponges formulas

Formula code	Yield product percentage	Drug loading%	Entrapment efficiency (%)	Particle size (nm)	PDI
F1	66.37±2.2	61.6±2.3	92.4±3.5	142±4.5	0.17±0.01
F2	66.67±0.8	56.8±1.59	85.2±2.4	172.6±6.4	0.25±0.007
F3	56.89±1	25.65±1.87	51.3±3.7	438.9±13.3	0.46±0.03
F4	55.56±1	19.58±0.3	78.3±0.3	459±65.7	0.55±0.12
F5	22.67±0.6	20.88±1.2	41.7±2.4	519.6±8.9	0.58±0.16
F6	46.22±2.66	34.02±1.7	51±2.6	430.4±34.9	0.271±0.18

Impact of Drug-to-Polymer Ratio

Formulas F2, F4, and F5 were evaluated and the results were shown in Figure (2). According to the results, the particle size increased with increasing drug: polymer ratios. This is due to the fact that a higher drug-to-polymer ratio makes more polymer available for ACE-NS synthesis, which in turn causes the polymer to thicken and NS to grow in size [17].

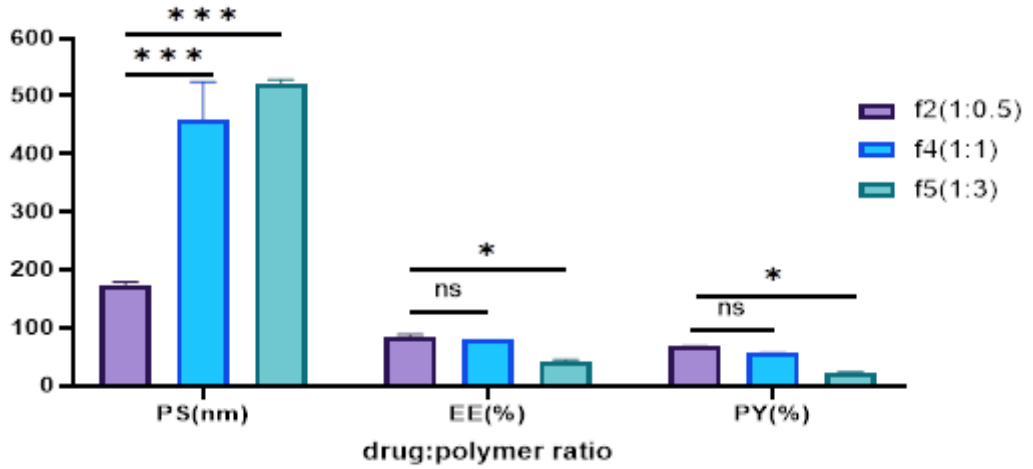

Figure 2. The effects of drug-to-polymer ratio on ACE-NS of the F2, F4 and F5 formulations.

It was worthy to mention, when the drug to polymer ratio increases from F2 (1:0.5) to F5 (1:3) formula, a significant decrease ($P < 0.05$) in the percentage of drug entrapment, going from (85.2±2.4%) to (41.7±2.4%) due to the higher concentration of polymer. The rise in polymer concentration is responsible for the observed effect since it increases the viscosity. Low drug entrapment percentage was the end result of a tougher polymer covering that made drug transfer more difficult in a thicker medium [18].

The entrapment efficiency has a strong correlation with the product yield percentage. The fact that the proportion of drug entrapment follows the same pattern as the product yield proves this. For formula F2 and F5, the values dropped from (66.67±0.8%) to (22.67±0.6%) when the polymer to drug ratio increased, indicating a non-significant decrease ($p > 0.05$) in production yield. The reason behind this phenomenon is that the medium's viscosity is reduced when the amount of polymer is reduced and the amount of drug is increased. The drug component is able to diffuse more easily and a more malleable polymer coating is encouraged by this reduction in viscosity. The obtained results were inconsistent with that get by Yehia RM et al [19].

The effects of different concentrations of emulsifiers

To reduce surface interfacial tension, PVA is used as an emulsifying agent. The percentage of drug entrapment decreases as the PVA concentration is increased in formulas F2 and F6, with the polymer concentration held constant at a drug:polymer ratio of 1:0.5. This is because drug leaching is enhanced due to the nanoporous structure's enlargement brought about by the increased PVA concentration [20]. The non-ionic properties of PVA and the hydrophobic properties of ACE are responsible for the loss in product yield, as seen in Figure (3). Because of this, a bigger hydrophobic zone of PVA is formed, which leads to the dissolution of part of the drug and a decrease in product yield [21]. The effect on drug entrapment and product production was found to be statistically significant ($p < 0.05$). The viscosity of the emulsion increases with an increase in PVA percentage, which in turn causes larger droplet sizes. Similar finding was obtained by Nokhodchi A et al [22].

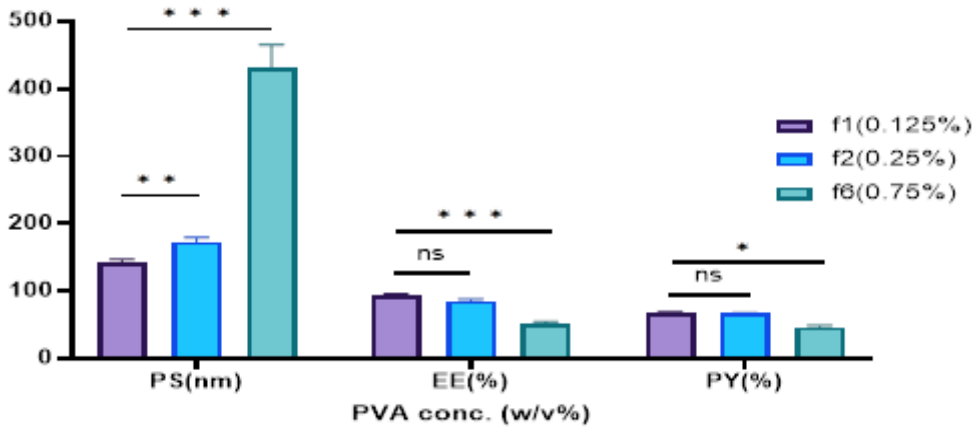

Figure 3.Effect of PVA on ACE-NS properties of F1, F2, F6 at constant 1:0.5 ratio of ACE to Eudragit L100.

On the other hand, when the concentration of PVA is increased in formulas F4 in a comparison with F3, while keeping the polymer concentration constant at a ratio of drug: polymer (1:1), there is a non-significant change in the results of the particle size, the percentage of drug entrapment and the product yield Figure (4).

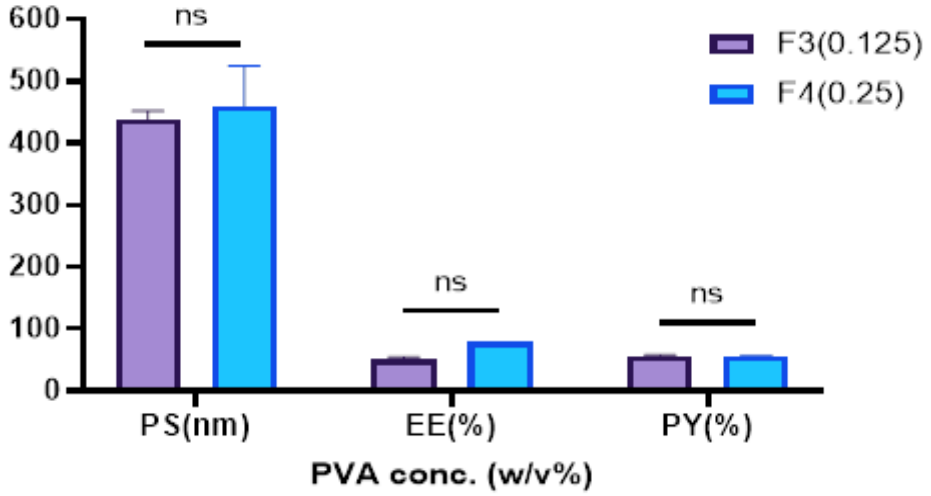

Figure 4. Effect of PVA on ACE-NS properties of F3, F4 at constant 1:1 ratio of ACE to Eudragit L100.

Choosing the optimum formulation

For the performance of further testing, the NS formulation that showed the best characteristic of the smallest particle size distribution, good product yield, highest drug entrapment and loading was selected. As a result, the formula F1 (table 2) was selected to evaluate the drug dissolution.

Characterization of ACE NS in a solid state

Differential scanning calorimetry

At 148.51℃, the DSC thermogram of pure ACE showed a clear endothermic peak, which is the same as the melting point of ACE in its crystalline form [23]. As shown in Figure (5), the DSC thermogram of the selected formula F1 show a depressed endotherm of drug at a lower melting point of 145.03 °C resulted in a relatively less crystalline nature [24].

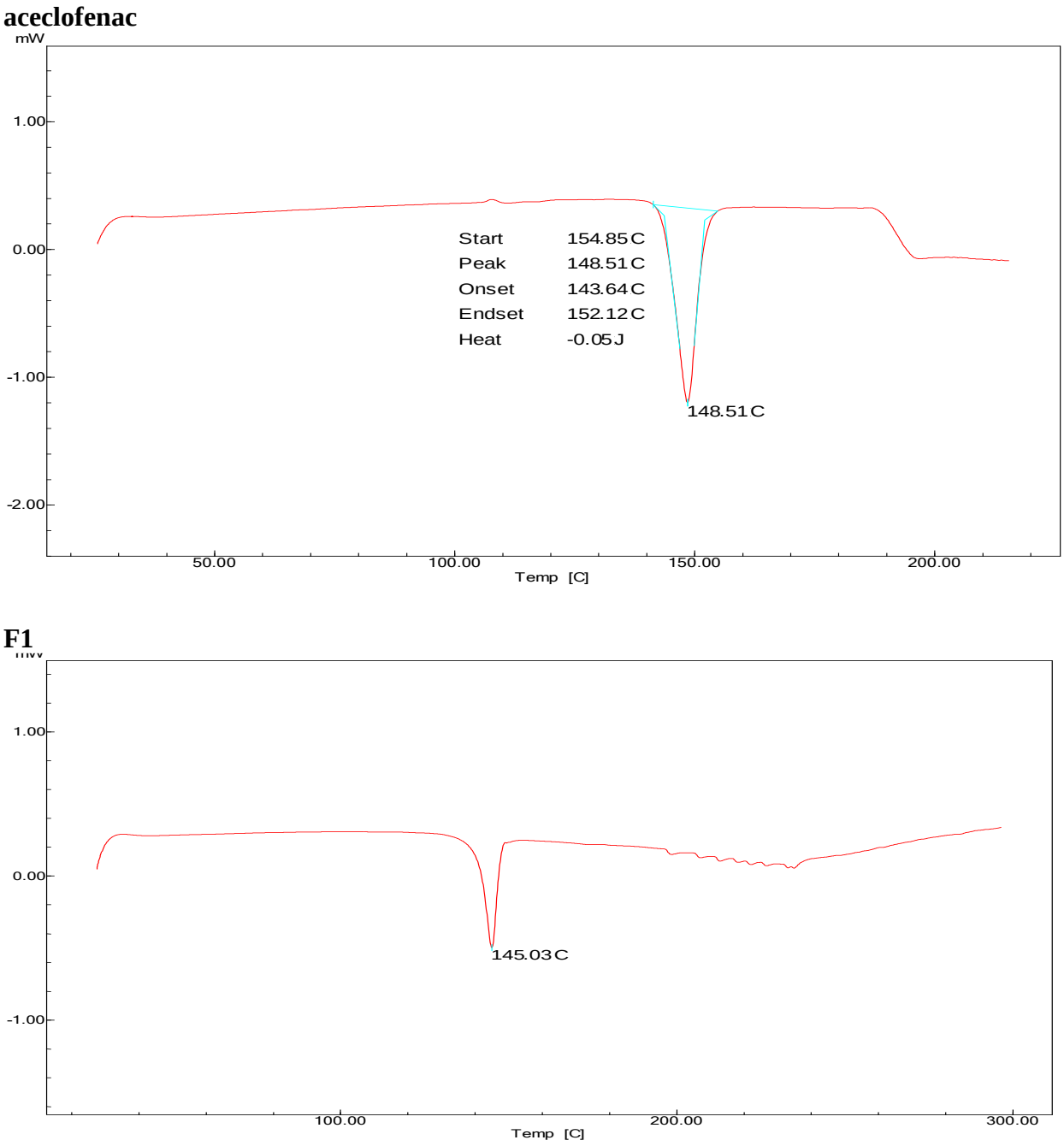

Figure 5. DSC Thermogram of pure ACE and formula (F1) nanosponge.

FESEM

Figure (6) shows the results of a FESEM morphological analysis of F1 compared to ACE row powder. The form of NS loaded with ACE was asymmetrical. As demonstrated in Figure (7), the rough and porous surface of NS was caused by the diffusion of DCM from the surface. PENJURI et. found an identical thing [25].

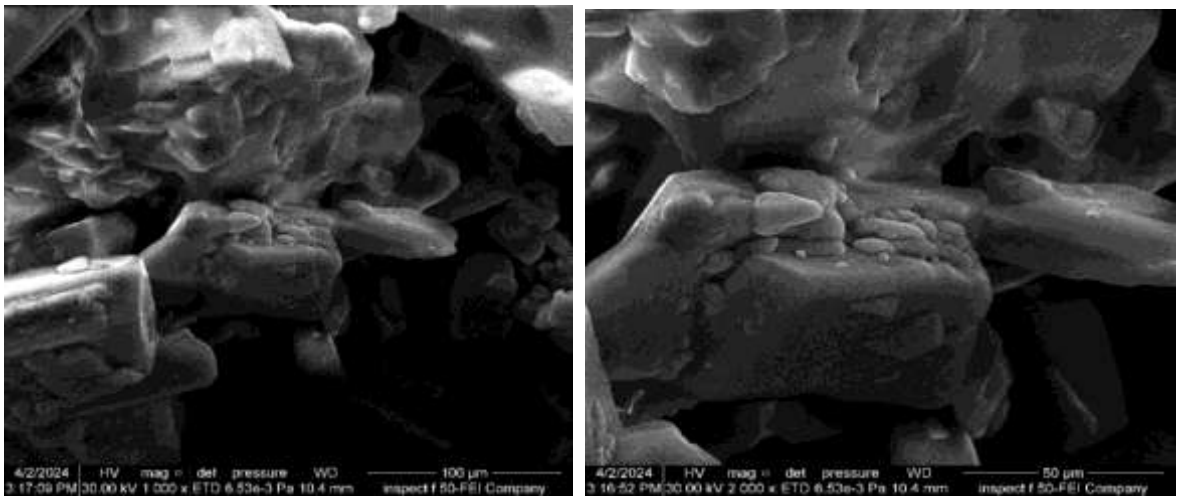

Figure 6. The FESEM of pure drug at 1000X and 2000x magnification.

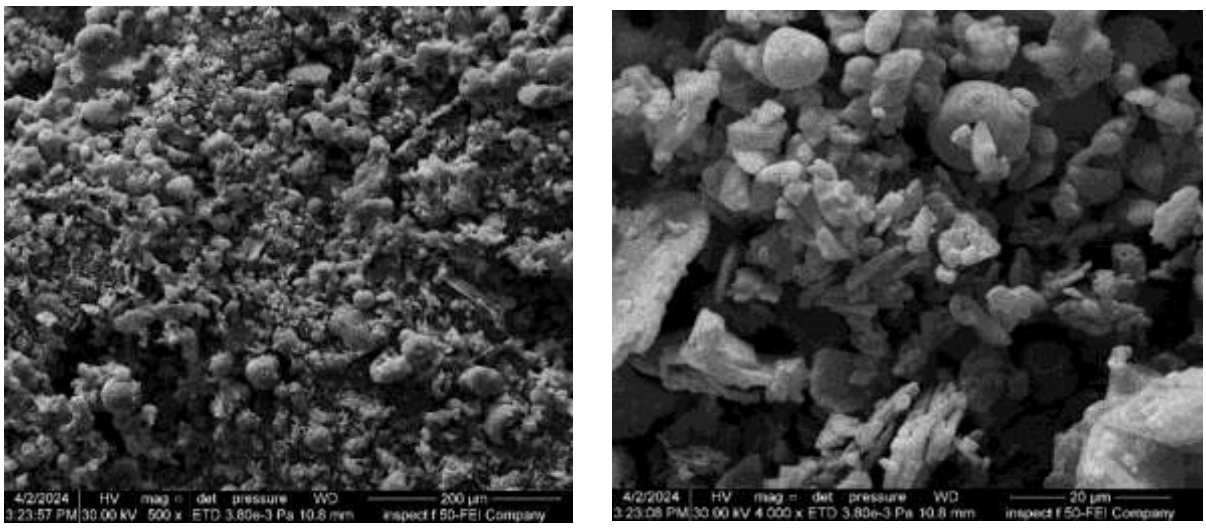

Figure 7. The FESEM of F1 at 500X, 2.00Kx and 4.00Kx magnification.

The Fourier-transform infrared spectroscopy

Here are the features of the absorption bands of ACE: for N-H or O-H stretching, 3318 cm-1; for C-H stretching (aromatic and aliphatic stretching vibrations, respectively), 3277 cm-1; for -C=O stretching, 1771 cm-1; and for -C=C stretching of aromatic compounds, 1585 cm-1 and 1506 cm-1. Figure (8) offers additional information on the noticeable bands. There was no evidence of drug-polymer interaction since the ACE peaks in the prepared NS did not differ significantly from those in the pure drug [26].

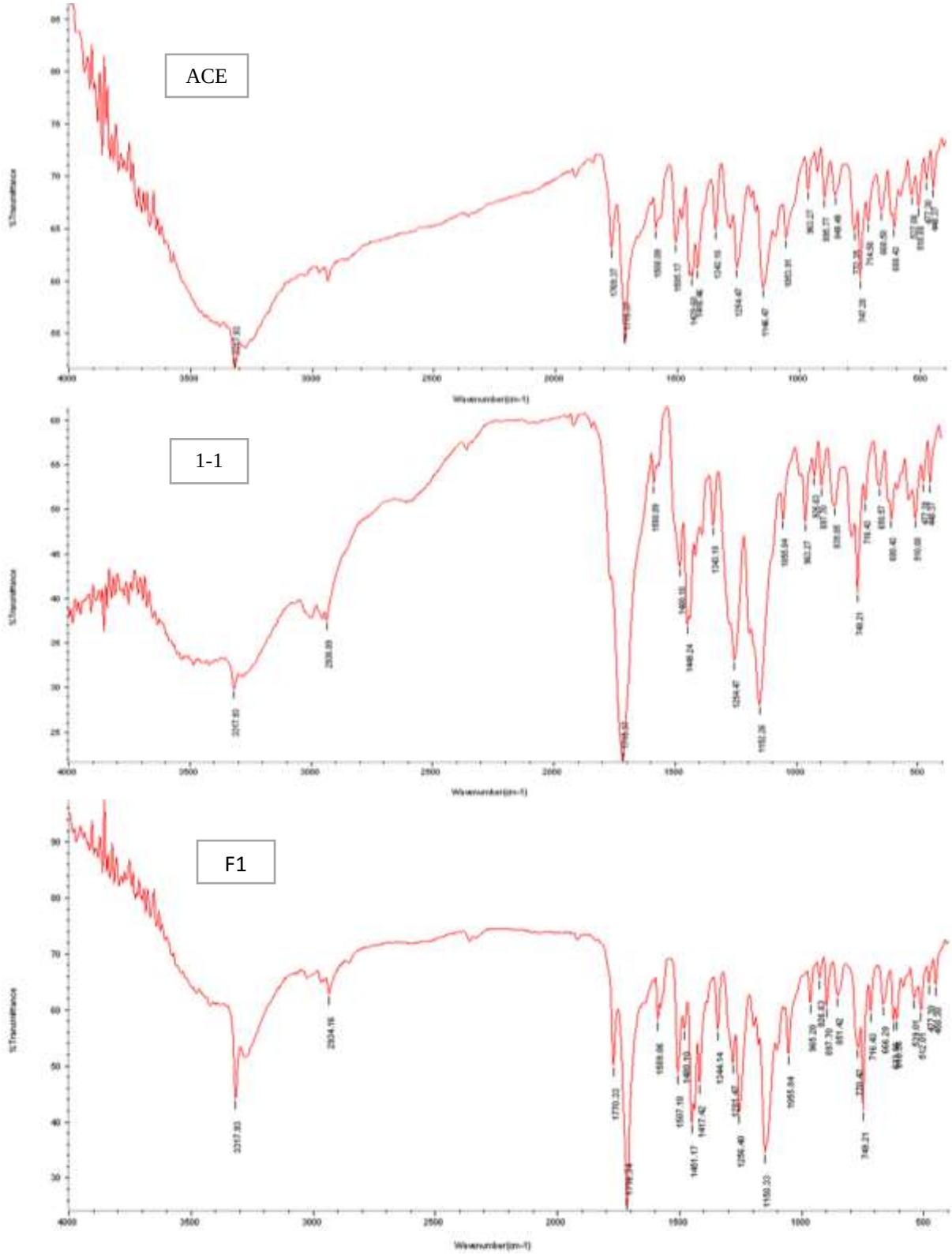

Figure 8. The FTIR spectra for pure ACE powder, physical mixture (1-1) and F1.

In-vitro drug release study

Figure (9) illustrated the percentage of ACE released from F1 in comparison to the release from a pure drug suspension. The results indicated that F1 showed (74.5±1.3%) of drug released in 8 h in comparison to the release of a pure drug (32.8±1.08%), this may be attributed to enhanced drug dissolution when integrated into NS, which is

likely due to the reduction in drug particle size that increases surface area and subsequently improves contact between particles and the dissolution medium. The results obtained align well with the Noyes–Whitney equation, which stated that a reduction in particle size results in an enhanced dissolving rate [27].

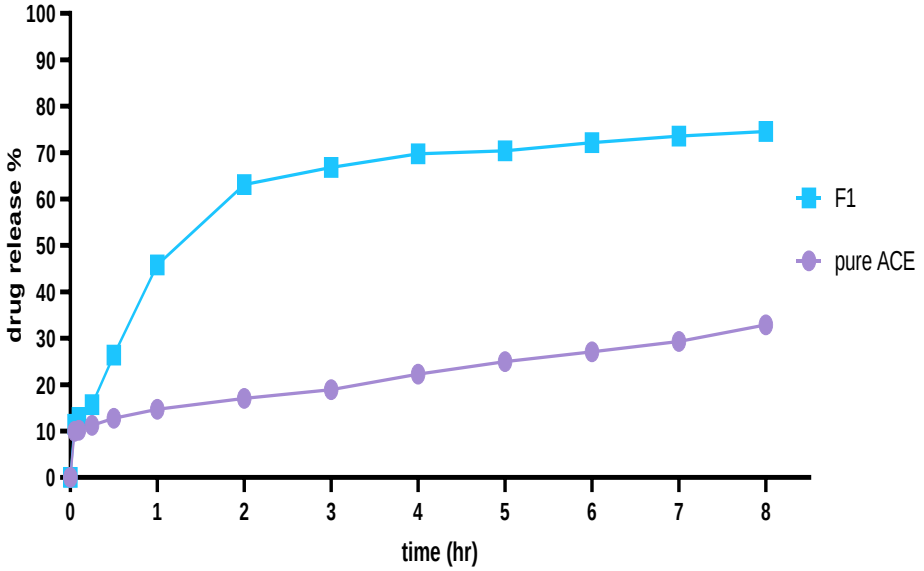

Figure 9. The % drug released from F1 Nanpsponge and a pure drug suspension.

Conclusion

This study concludes that Eudragit L100 can effectively serve as a NS polymer matrix. Additionally, Eudragit L100 was compatible with ACE. The study found that the drug: polymer ratio and PVA concentration are important factors that greatly affect the size of NS particles, the yield of the product, and the efficacy of drug entrapment. AC-NS (F1) showed enhanced release rate of drug as compared with pure drug suspension over 8 h.

Conflicts of Interest

There are no conflicts of interest, as we have declared.

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Ethics Statements

Neither animals nor humans were included in the investigation.

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: Lubna A .Sabri (L.A.S.); data collection: Mays Hassan (M.H); analysis and interpretation of results: L.A.S, M.H; draft manuscript preparation: L.A.S, M.H. All authors reviewed and approved the final version of the manuscript.

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تحضيروتقييم الاسفنج النانوي المحمل بأسيكلوفيناك على أساس يودراجيت L100 للاستخدام الموضعي

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[٢]فرع الصيدلانيات ،كلية الصيدلة ،جامعة بغداد، بغداد ،العراق .

الخلاصة

أسيكلوفيناك (ACE) هو دواء مضاد للالتهابات غير الستيرويدية (NSAID) مشتق من حمض فينيل أسيتيك. يمتلك خصائص ملحوظة مضادة للالتهابات، ومسكنة، وخافضة للحرارة. ومع ذلك، فإن فعالية المادة يعوقها انخفاض ذوبانها في الماء، مما يؤدي إلى ذوبان بطيء، ونفاذية محدودة، وتوافر حيوي غير كاف. الإسفنج النانوي (NS) عبارة عن جسيمات نانوية تمتلك بنية نانوية ثلاثية الأبعاد فريدة وتحتوي على تجاويف بمقياس النانومتر. تسمح هذه المساحات المجوفة باحتواء كل من المركبات القابلة للذوبان في الماء والمركبات القابلة للذوبان في الدهون، مما يساعد على تحسين فعالية وسلامة توصيل الأدوية. كما أنها تعمل كأنظمة توصيل مستهدفة، وتجنب الضرر الناجم عن الأدوية وطريقة لإزالة السموم. الهدف من العمل الحالي هو إعداد وتحسين إسفنجات نانوية ذات إطلاق متواصل من الأسيكلوفيناك-يودراجيت L100 من خلال دراسة تأثير عوامل الصياغة. يتم تحضير الإسفنجات النانوية المعتمدة على البوليمر يودراجيت L100باستخدام عملية نشر المذيبات المستحلبة. تبحث الدراسة في تأثير

العديد من متغيرات الصياغة على خصائص الإسفنج النانوي. يتضمن ذلك نسبة الدواء إلى البوليمر وكميات كحول البولي فينيل (PVA). يستلزم التوصيف تقييم حجم الحسيمات، ونسبة المنتج الذي تم الحصول عليه، والنسبة المئوية لانحباس الدواء داخل الجسيمات و تحرر الدواء في المختبر . تشير النتائج إلى أن زيادة تركيز يودراجيت L100 أدى إلى زيادة كبيرة في حجم الجسيمات وانخفاض كبير في إنتاجية المنتج ونسبة انحباس الدواء. أدت زيادة تركيز PVA إلى انخفاض كبير في إنتاجية المنتج ونسبة الانحباس. ومع ذلك، فقد أدى ذلك إلى زيادة كبيرة في حجم الجسيمات. ويستنتج من هذه الدراسة بامكانية استخدام يودراجيت L100 كبوليمر حامل لدواء الأسايكلوفيناك، والذي سيتم تصنيعه كهلام موضعي في الدراسات المستقبلية.

الكلمات المفتاحية: أسيكلوفيناك، ايودراجيت إل ١٠٠،كحول البولي فينيل , جل , الإسفنج النانوي وتحرر الدواء في المختبر.