DOI:https://doi.org/10.31351/vol27iss2pp66-76

Studying the Effect of Variables on Acyclovir Microsponge

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Abstract

The aim of the present investigation was to develop a microsponge delivery system of acyclovir to control its release when applied topically thereby reducing dosing frequency and enhancing patient compliance. The microsponge was produced by oil in oil emulsion solvent diffusion method. The effect of different formulation and process variables such as internal phase volume, polymer type, drug polymer ratio, stirring speed and stirring duration on microsponge production yield, loading efficiency, particle size, and *in vitro* drug release was evaluated. The results showed that the microsponge F2 prepared from Eudragit RS polymer had optimum physical properties regarding loading efficiency of ٩٩.71±0.7% and production yield which was 85%. Also, F2 showed 66% drug release through 8 hours. Accordingly, the oil in oil emulsion solvent diffusion method is an effective technique to formulate microsponge with maximum production yields and loading efficiency for acyclovir.

Keywords: Microsponge, Acyclovir, Controlled topical release, Oil in oil emulsion solvent diffusion method.

دراسة تاثير المتغيرات على اسفنجيات الاسايكلوفر المايكروية

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الخلاصة

الغرض من الدراسة المقدمة هو تصنيع وتقييم عقار الاسايكلوفير كاسفنجيات مايكرويه للتحكم بتحرر العقار عند وضعه على الجلد لغرض تقليل عدد الجرع اليومية وزيادة امتثال المريض للعلاج. حضرت الاسفنجيات المايكرويه بواسطة طريقة شبه المستحلب المتضمن انتشار المذيب إلى المكون الخارجي الزيتي ثم اختبر تأثير متغيرات طريقة التحضير كنسبة الدواء إلى البوليمر، سرعة الخلط ، مدة الخلط ، نوع البوليمر ، وحجم المذيب في الطور الداخلي للمستحلب . كما تم تقيم الاسفنجيات بالنسبة الى حجم الجسيمات ، الحصيلة الإنتاجية ، كفائة التحميل ، خصائص السطح وتحرر الدواء خارج الجسم. أثبتت النتائج ان التركيبة(٢) أبدت أفضل الخصائص الفيزيائية للاسفنجيات المايكروية ، كالقابلية الإنتاجية بمقدار ٧،٠ ± ٧١،٩٩ % ، وقابلية التحميل التي كانت٨٥ % . كما أن التركيبة (٢) أبدت تحرر للدواء خارج الجسم ٦٦ % خلال ٨ ساعات . من خلال نتائج الدراسة تبين نجاح طريقة شبه المستحلب المتضمن انتشار المذيب إلى المكون الخارجي الزيتي لتحضير أسفنجيات الاسايكلوفير المايكروية بقدرة إنتاجية و كفاءة تحميل عالية.

الكلمات المفتاحية :اسفنجيات ، الاسايكلوفير ، التحكم بتحرير العقار ، طريقة شبه المستحلب المتضمن انتشار المذيب.

Introduction

Microsponges are polymeric porous microspheres used for controlled delivery of medications [(1)] .This technology has been used for topical formulations and recently for oral administration. Microsponges are biologically safe and are believed to contribute towards reducing side effects, improved stability, increased elegance and enhanced formulation flexibility[(2)]. Even though microsponges are very small in size; they are not able to permeate the skin and are not absorbed systemically; therefore, they are considered drug delivery systems for topical use. Microsponges accumulate in the tiny nooks and crannies of the skin, and release slowly the entrapped drug as the skin requires[(3)].

Microsponges MS can be incorporated into a wide variable of pharmaceutical dosage form, like tablets[(4)], capsules[(5)], powders, creams[(6)], topical emulsions [(7)], gels [(8–10)] , etc. Acyclovir ACV was the first antiviral to be approved for the treatment of viral infections such as herpes simplex, varicella zoster virus and Epstein Barr infections[(11)]. ACV is hydrophilic in nature having water solubility of 2.5mg/ml at 37° C. The chemical structure of ACV is shown in Figure 1.

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Received: 8 / 8 / 2018
Accepted: 3 / 10 / 2018

Topical delivery of ACV is considered to be attractive choice for viral infection of the skin, as targeted therapy is enabled, circulating drug levels and risk of renal insufficiency will be reduced[12]. Poor penetration across the skin decreases the therapeutic value of topical ACV marketed creams [13]. High potency formulation and five applications per day are required to achieve the desired antiviral effect, which may cause skin irritation and decrease patient compliance. The aim of the present study was to develop ACV microsponges intended for controlled topical delivery of ACV.

The o/o emulsion solvent diffusion method was adopted to prepare ACV microsponge due to low solubility of ACV in the external oil phase; the drug loss was minimized leading to an increase production yield and drug loading efficiency. Different formulation variables (internal phase volume, polymer type, drug to polymer ratio) and processing variables (stirring speed and stirring duration) were evaluated to maximize the encapsulation efficiency and optimize release characteristics.

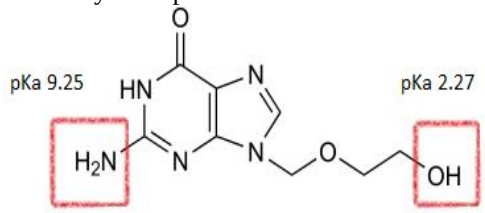


Figure 1**. The chemical structure of acyclovir**

Materials and Methods

Materials

Acyclovir ACV was purchased from Hangzhou Hyper Chemicals limited, China. Eudragit RS 100 and Eudragit RL 100 were from Evonik Röhm GmbH, Germany**.** Light liquid paraffin, acetone, n-hexane, magnesium stearate, potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium hydroxide, and cellulose nitrate filter with molecular weight cut off 8000-14000.

Methods

All ACV microsponge formulas were prepared by oil in oil quasi-emulsion solvent diffusion method. The internal phase was prepared by dissolving the polymer into acetone by sonication using SONOREX (Bandelin, Berlin, Germany) to obtain a clear polymeric solution. The drug and Mg-stearate were added to the prepared polymeric solution. The internal phase is added drop wise at a rate of 1 ml per min to 100ml of liquid paraffin with stirring at (500, 1000and 1500 rpm) by using mechanical overhead stirrer (RZR 2041 Heidolph TM, Schwalbach, Germany) for (30, 60 and 120 min) at which the organic solvent could evaporate, and the solidified MS precipitated. The MS were collected by filtration using of Büchner filtration device and was washed six times using n- hexane and left to dry over night at room temperature. The composition of various MS formulations is given in Table 1.

Table 1 .Composition of ACV loaded microsponges

Formula Code	Polymer type	Drug / polymer ratio	Solvent volume (ml)	Stirring rate (rpm)	Stirring time (min.)
F1	ERS	1:1	5	500	60
F2	ERS	1:1	7.5	500	60
F3	ERS	1:1	10	500	60
F4	ERS	1:1	7.5	500	30
F5	ERS	1:1	7.5	500	120
F6	ERS	1:1	7.5	1000	60
F7	ERS	1:1	7.5	1500	60
F8	ERS	2:1	7.5	500	60
F9	ERS	1:2	7.5	500	60
F10	ERL	1:1	7.5	500	60
F11	ERS: ERL	1:1	7.5	500	60

*All microsponges were prepared by using 0.5 g of polymer and 100 ml of liquid paraffin. *Magnesium stearate was added as a percentage weight relative to the acetone volume

Characterization of microsponges formulation

Determination of production yield

The production yield of the microsponge was obtained from the weight ratio of filtered and dried microsponge amount of each formulation to total solid material amount in the dispersed phase (drug, polymer and Mg stearate) as given in the following equation:

$$\mathbf{PY(\%)} = \frac{\textit{Practical weight of the MS}}{\text{Theoretical weight}\ (\text{drug,polymer and Mg stearate})} \times 100\%$$

Determination of loading efficiency LE

The drug content was determined in the prepared microsponge by crushing the MS using a porcelain mortar. Accurately weighed 100 mg of this powder was extracted in 100 ml of NaOH. The solution was sonicated for 15 min and left for 24 hours. The solution then was filtered and diluted (if necessary, a sample of 1ml was withdrawn from this solution, diluted to 100 ml with NaOH) and assayed spectrophotometrically at 264 nm (**Cecil spectrophotometer CE7200;UK**) to determine the ACV content. The loading efficiency was determined according to the following equation:

$$\textit{LE}(\%) = \frac{\textit{Actual drug content of MS}}{\textit{Theoretical drug content of MS}} \times 100\%$$

Determination of particles diameter

The optical microscope **(**Olympus, Japan**)** was equipped with graduated eye piece and was used to estimate the average diameter of ACV microsponges, under (10 X) magnification power, in which, 100 MS particles of each batch were examined. The average particle diameter was determined according to the following equation:

$$d_{av} = \frac{\sum nd}{\sum n}$$

Where: d_{av} is the average diameter of particles (μm), n is the number of particles per group, and d is the middle value (μm).

Scanning electron microscope (SEM) study

The surface topology and particle morphology of the prepared microsponge was studied using Carl Zeiss - SUPRA 55VP FE-SEM, Germany. The samples were first loaded on sample stub using a double side carbon tape and sprinkle the powder on it then tight all stubs on the specimen's holder after simple blowing to remove non-adherent particles. The prepared samples were loaded on SEM via air-lock door which depends on low voltage to avoid charging.

Solid state characterization of microsponge

DSC, XRD and FTIR were employed to characterize the physical state of the drug in the prepared microsponge and to confirm the absence of interaction within formulation. The pure drug (ACV), polymer (Eudragit RS), Mg-stearate, the physical mixture of all formula components and AVC MS, were investigated individually using each of these techniques.

Differential scanning calorimetry (DSC)

Thermal analysis was carried out using DSC-600, Shimadzu, Japan by loading 5 mg of the powder into a sealed aluminum pan, which was compared with a sealed blank aluminum crucible as reference. The temperature was raised from 25°C to 300°C in a nitrogen atmosphere at the rate of 10°C per minute. The flow rate of nitrogen was 50 ml per minute.

X-ray diffractometry (XRD)

An XRD pattern was determined using X-Ray Diffractometer-6000, Shimadzu, Japan. The results were recorded over a range of 5–50° (2θ). The operating conditions were: voltage 40 kV, current 30 mA, scanning speed 8 deg /min.

Fourier transform infrared spectroscopy (FTIR)

The IR spectrum was recorded using FTIR 600 spectrometer, UK. The sample was crushed with potassium bromide with a porcelain mortar and pestle. The mixture was then compacted to form a translucent pellet with the aid of a mechanical die press to obtain KBr discs. These discs were scanned in frequency range of 4000-400 cm^{-1}.

In vitro release studies

Drug release rate from ACV microsponge was carried out through dialysis bag technique using USP dissolution test apparatus-II. The dissolution media consisted of 900 ml phosphate buffer having a pH of 7.4. Accurately, weighed samples of ACV microsponge equivalent to 50 mg of the drug was suspended in 5 ml phosphate buffer (pH 7.4) in a dialysis bag and the rotation speed was adjusted at 50 rpm at 37°C. At prescheduled time intervals (30min, 60min, 120min, 180min, 240min, 300min, 360min, 420min, and 480min) 10 ml of the media were withdrawn and replaced by an equal volume of dissolution medium to maintain a constant volume. The samples were filtered through a (0.45 μm, Millipore) filter, suitably diluted (if necessary ,1 ml of filtrate diluted to 10 ml with phosphate buffer solution) and analyzed at 252 nm using a double-beam Cecil spectrophotometer CE 7200; UK. These experiments were conducted in triplicate.

Kinetic modeling of ACV release

The data obtained from the *in vitro* release studies were fitted to different

mathematical expressions to describe the kinetic and mechanism of ACV release from the microsponge selected formula with the aid of DDSolver an Microsoft Excel add-in (14). The kinetic models used were zero order kinetic , first order kinetic , Higuchi model and Korsmeyer - Peppas [15]. The model with the highest correlation coefficient was the best fitted model.

Statistical analysis

Results of each experiment was reported as a mean ± standard deviation and were analyzed according to the one-way analysis of variance (ANOVA) test, using Microsoft Excel Program 2010. Differences were statistically significant at $p < 0.05$.

Results and Discussion

In this study. two types of Eudragit polymers (Eudragit RS and Eudragit RL) were used as a matrix forming agent for ACV microsponges. These polymers are known for their release retarding properties [16]. The internal phase solvent used in this experiment was acetone. The compatibility of acetone with liquid paraffin system is attributed to the partial polarity of acetone [17]. The oil in oil emulsion diffusion method successfully produced microsponge particle having a uniform spherical shape with high production yield and excellent loading efficiency owning to the low drug solubility in the external phase. The rationalization for Mg-stearate use, is the ability of metal stearate to form a protective shell surrounding the sponge particle and of great benefit in decreasing the coalescence and aggregation [18]. The microsponge production yield and loading efficiency is summarized in table 2

Table 2. Characterization of acyclovir microsponges

Formula code	Production yield (%)	Loading efficiency (%)	Particle diameter (um)
F1	67	98.77±0.3	47.23±0.75
F2	85	99.71±0.7	32.36±0.85
F3	88	88.59±0.36	26.57±0.51
F4	75.7	98.72±0.54	39.05±0.73
F5	87	99.28±0.2	30.64±0.5
F6	57	99.35±0.2	21.06±0.3
F7	45	97.31±0.5	16.84±0.4
F8	73	96.65±0.47	28.86±0.31
F9	85	94.05±0.23	51.13±0.32
F10	82	90.84±0.28	31.65±1.65
F11	83	98.12±0.35	30.23±1.45

Characterization of F1-F3 revealed that the MS were formed when the volume of inner phase ranged from 5 to 10 ml. The particle size significantly ($p < 0.05$) decreased with increasing the inner phase volume, because the lower inner phase viscosity led to breaking of the emulsion into smaller droplets forming microsponges with small particle size [19]. The loading efficiency was found to decrease at larger internal phase volumes because the reduction in the viscosity of the internal phase enhance drug escape to the periphery[20]. Accordingly; 7.5 ml of acetone were found to produce microsponge of acceptable properties in term of loading , yield and particle size.

Stirring duration was found to have significant effect on microsponge formation and 60 minutes was found to be most effective, while 30 minutes of stirring was insufficient for complete solvent diffusion and resulted in a lower yield. Stirring for 120 minutes did not significantly affect the microsponge particle size or loading efficiency ($p > 0.05$).

The effect of different polymers and polymer combination on ACV microsponge was studied in F2, F10 and F11 prepared from Eudragit RS, Eudragit RL, and Eudragit RS - Eudragit RL at 1:1 drug to polymer.

Particle size was found to be insignificantly affected with changing polymer type ($p > 0.05$) as the viscosity of the employed Eudragit polymers is similar resulting in comparable internal phase viscosity and similar viscosity difference between the internal and external phase [21] . The loading efficiency was significantly lower ($p<0.05$) with Eudragit RL microsponges because higher content of the ammonium group facilities the diffusion of some of the entrapped drug to the surrounding medium during formation of the microsponges[22].

Increasing the ratio of the drug to the polymer resulted in particle size significant reduction ($p<0.05$), since the amount of the polymer available per each microsponge was relatively lower than microsponge prepared at lower drug to polymer ratio [23]. Also, the Loading

Efficiency was significantly reduced ($p<0.05$) as lower amount of polymer to the drug produced lower viscosity enhancing drug escape to external media thereby reducing loading efficiency and drug entrapment in the MS[24].

Increasing the stirring speed reduced the production yield, due to adherence of the polymer to the walls of the glass container as a result of the vigorous turbulence created in the external phase [25]. Also, the particle size was significantly decreased ($p<0.05$) as the stirring speed increased, since high mechanical shear applied, resulting in a rapid splitting of the formed droplets, allowing less chance of coalescing into bigger droplets[26]. The most suitable stirring speed was found to be 500 rpm.

Scanning electron microscope (SEM)

SEM analysis of the selected microsponge formula is shown in Figure (2). MS were spherical in shape, having uniform size distribution with rough surface contains pores and crakes resulted from diffusion of the solvent.

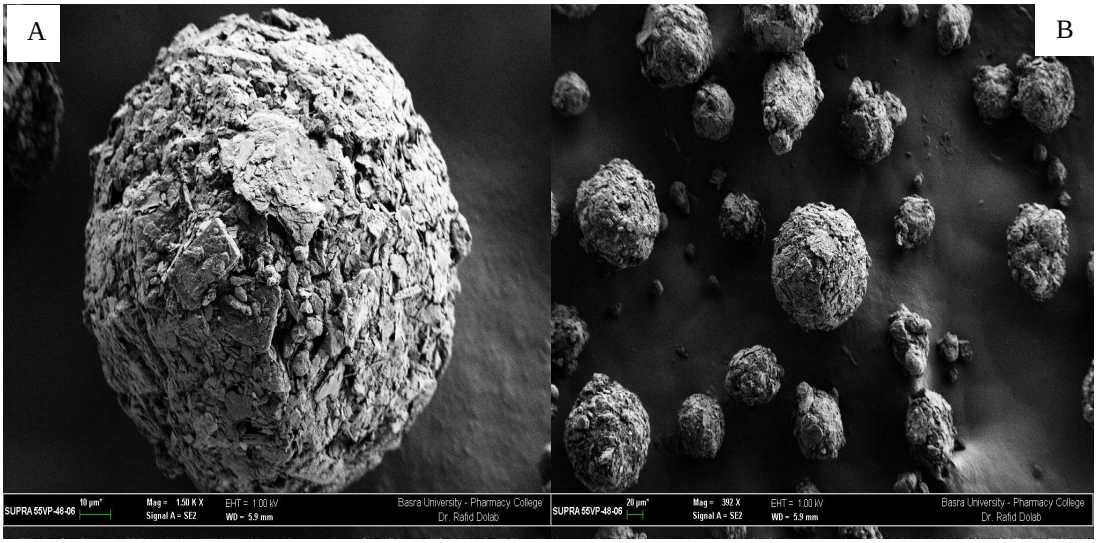


Figure 2. SEM of microsponge F2 [A] 1.5KX magnification [B] 1.0KX magnification

Solid state characterization of microsponge

According to DSC analysis illustrated in Figure 3, ACV displayed a sharp characteristic endothermic peak at 259°C corresponding to the melting point of the drug in the crystalline form, While Eudragit RS thermogram showed one transition only at 65°C which represents the glass transition temperature of the polymer in the amorphous state. The drug peak was obvious in the physical mixture which excludes in-compatibility. The disappearance of the melting point peak of acyclovir from the selected microsponge formula thermogram is explained by the uniform dispersion of the drug into the polymeric matrices [27] .

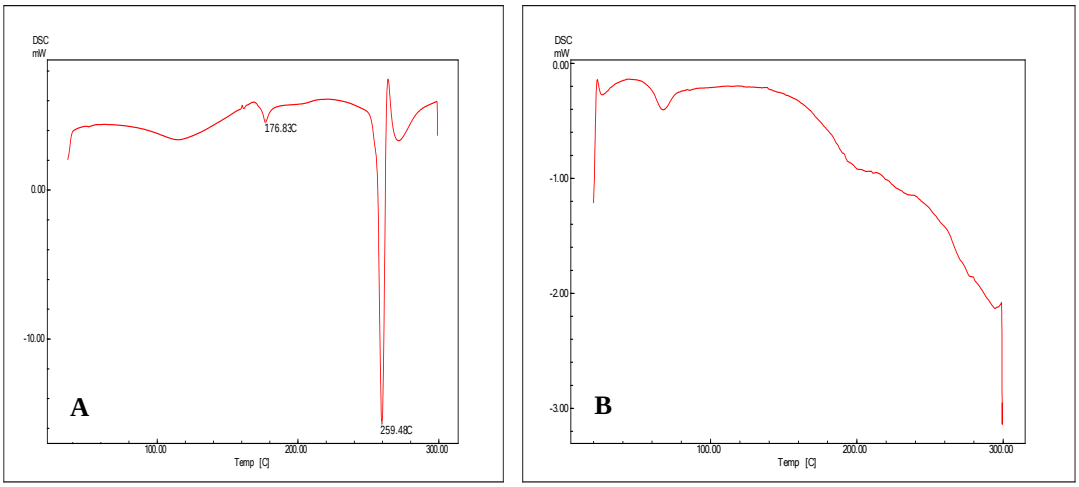
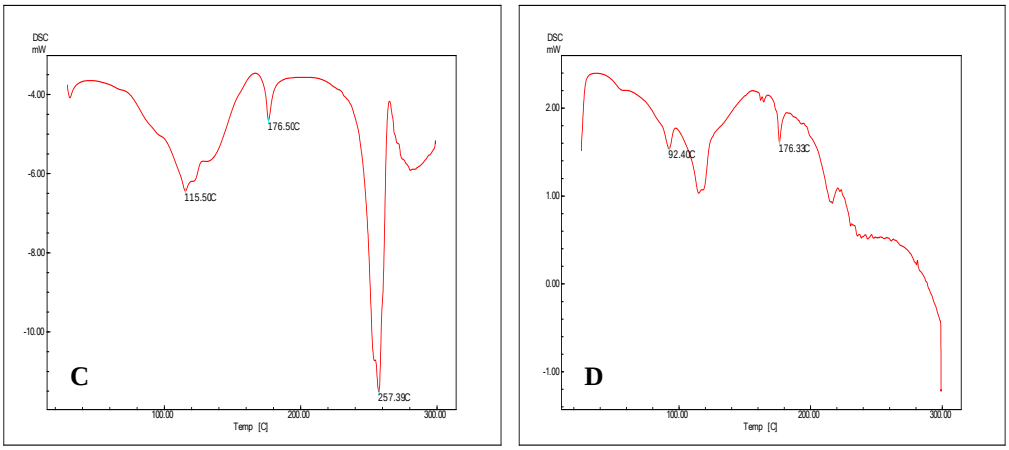


Figure 3. DSC thermograms of [A]pure ACV [B] Eudragit RS100



Continued Figure 3. Eudragit RS100 [C] physical mixture [D] microsponge F2

XRD diagrams are shown in Figure 4a,4b Eudragit RS100 showed typical diffraction form of amorphous materials, whereas the pure drug showed the diffractogram pattern of a crystalline material at 2θ of 7.0154 °, 29.1349° and 26.0690°. The physical mixture and the selected microsponge formula showed a diffraction pattern in which ACV characteristic crystalline diffraction peaks present at 2θ are in the same position of the pure sample but with reduced intensity due to the presence of the polymer[28] .

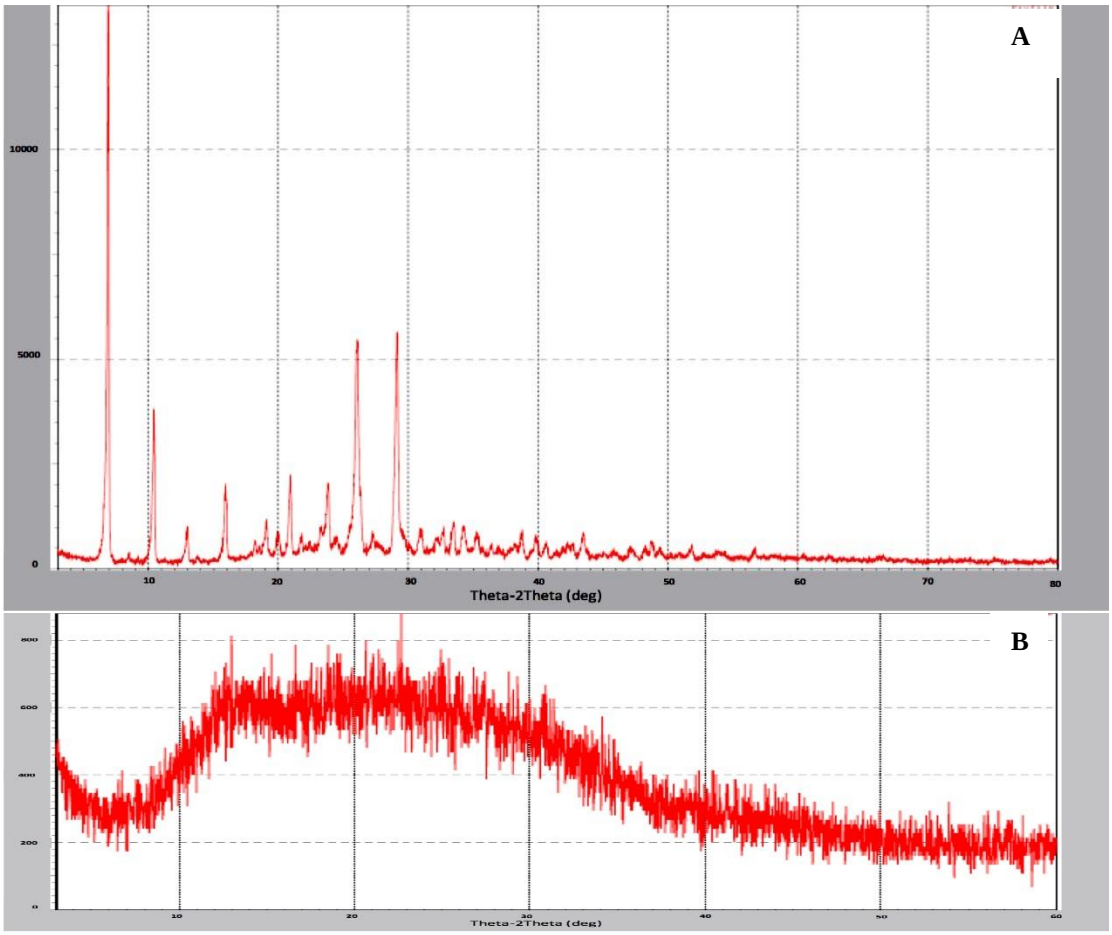


Figure 4. a. XRD of pure ACV [A]eudragit RS100 [B]

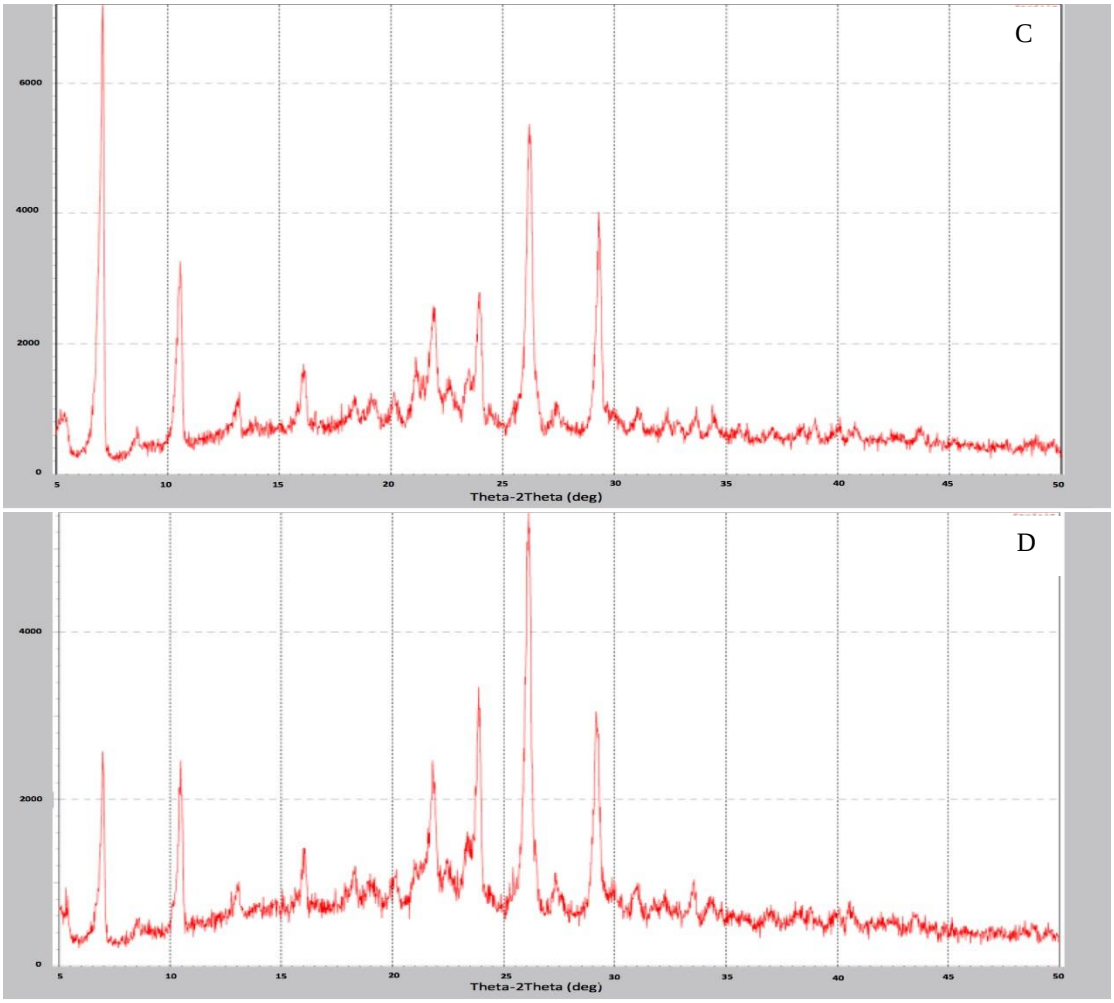


Figure 4b. XRD of Physical mixture [C] microsponge F2 [D].

The FTIR absorption bands of Pure ACV were noticed at 3519 cm^{-1} (O-H stretching), 3471 cm^{-1} and 3440 cm^{-1} (N-H stretching), (2962 cm^{-1} , 2927 cm^{-1}) (aliphatic C-H stretching a symmetric), 2854cm^{-1}, 2871 cm^{-1} (aliphatic C - H stretching symmetric), 1485cm^{-1} (aliphatic C -H deformation), 1716cm^{-1} (C = O stretching) and 1049. cm^{-1} (C-O stretching), while for Eudragit RS100, asymmetric C - H stretching bands appeared at 2993 and 2954 cm^{-1}, while the band at 2850 cm^{-1} represent symmetric C-H stretching, the carbonyl group C=O of an ester exhibited a stretching vibration at 1732cm^{-1}, as seen in Figure 5. The characteristic absorption bands of the drug were retained in the physical mixture and the selected microsponge formula with no new peaks appearance which confirms chemical computability between the formulation component and the absence of any chemical interaction.

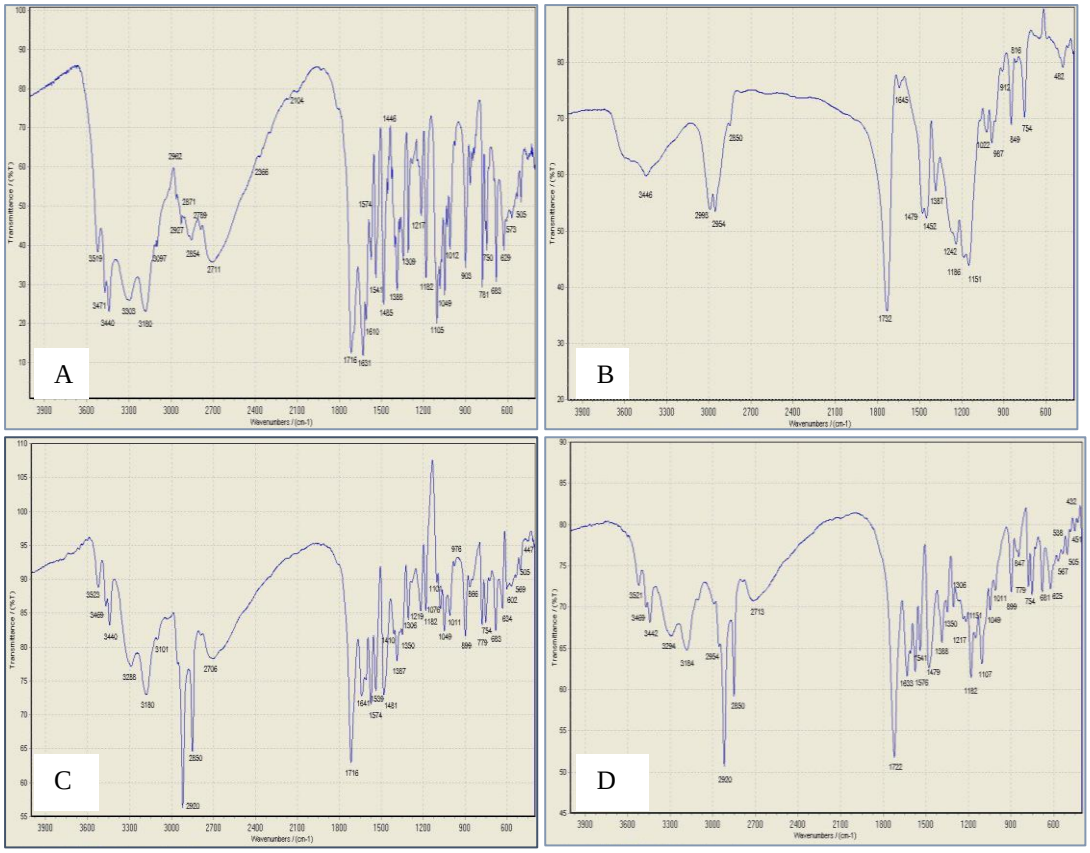


Figure (5): The FTIR spectrum of [A]pure ACV [B] eudragit RS100 [C] physical mixture [D] microsponge F2

Study of ACV release profiles from the microsponge

In vitro release study was performed to investigate the effect of different variable on drug release from the microsponges. The effect of changing drug polymer ratio on drug release was illustrated in figure (6). The best retardation is obtained as the amount of polymer to the drug is increased since increased particle size decreases the effective surface area and increasing the path length traveled by the drug molecule [29]. Besides , at higher drug ratio , more drug would present near to and on the surface of the microsponge which is in direct contact with the dissolution media accounting for the rapid release[30].

The release profile is also affected by varying polymer type as shown in figure 7. ACV release rate increased from 66 % with F2 (ERS) to 80.22 % for F11(ERS: ERL) and 92.99 % for F10 (ERL) for a duration of 8 hours. Eudragit RS was the least permeable of the three formulas and the most capable of sustaining drug release. This is explained by different structural properties of the two polymers in term of percentage of the quaternary ammonium group present which is (10%) in Eudragit RL 100 while Eudragit RS 100 contains only (5%). Thus, Eudragit RL 100 is more permeable and release the drug at a faster rate than Eudragit RS[21].

The effect of stirring speed on drug release was evaluated by comparing the release behavior from F2, F6 and F7 prepared at stirring speeds of 500, 1000 and 1500 rpm as provided in Figure 8. Drug release was faster for F7 than F6 and F2 since it has lower particle size resulted from high stirring speed which led to an increase surface area exposed to the dissolution medium and subsequently release rates are faster.[31]

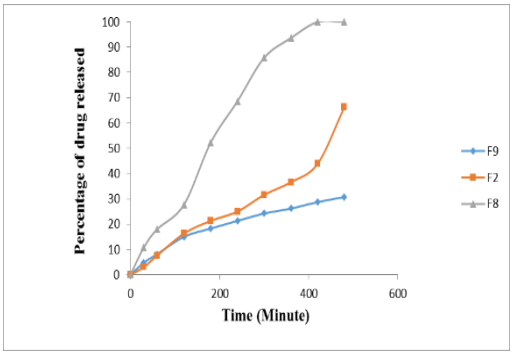


Figure 6. Effect of drug: polymer ratio on % drug release from F2, F8 and F 9.

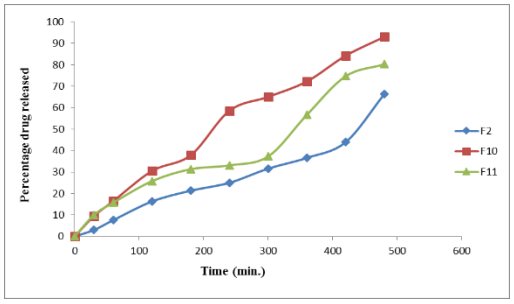


Figure 7. Effect of polymer type on % drug release from F2, F10 and F 11.

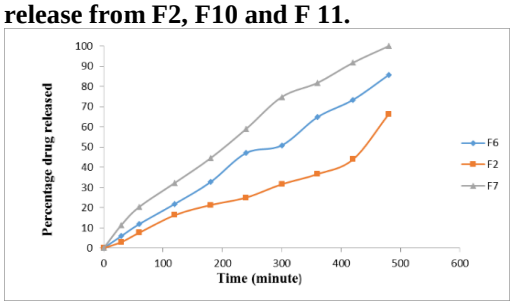


Figure 8. Effect of stirring speed on % drug release from F2, F6 and F7.

Kinetic Analysis of ACV Release Data from F2 Microsponge

The release of ACV from microsponge F2 obeys zero order release as their R^2 values gave the higher result, the release depends on the MS pore size and release of AVC from the pore rather than the concentration of AVC inside the MS. The mechanism of drug release is non - Fickian diffusion Supercase II as the release exponent " n " value of F2 microsponges is more than 1. Supercase II release behavior meaning that the release depends on polymer relaxation rather than water diffusion inside the microsponge as shown in table 3.

Table 3 . Kinetic analysis of ACV release data from F2 microsponge

Formula	Mathematical model for drug release kinetics										
	Zero order		First order		Higuchi		Hixson-Crowell		Korsmeyer-Peppas		
F2	K_0	R^2	K_1	R^2	K_h	R^2	k_{HC}	R^2	R^2	K_{KP}	n
	0.116	0.9386	**0.001**	0.8965	2.064	0.7570	0.000	0.9115	0.9452	0.047	1.153

Conclusion

It can be concluded from the results obtained in this study that the oil in oil emulsion solvent diffusion method is an effective technique to formulate microsponge based delivery system of acyclovir with maximum production yields and drug loading efficiency. The ACV microsponge formulated with the Eudragit RS at drug: polymer ratio of 1:1 is the best formulation among all the prepared batches in terms of capacity of extending drug release behind 8 hours thereby decreasing the number of applications and enhancing patient compliance.

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