Consequences of Formulation Variables on Physicochemical Properties of Indinavir Sulfate Microspheres.

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ABSTRACT

Indinavir sulfate with ethyl cellulose microspheres was successfully prepared by an oil-in-oil emulsion solvent evaporation technique using acetonitrile: dichloromethane (1:1) and light liquid paraffin as primary and secondary oil phases respectively with span 80 as a droplet stabilizer. In present study the effect of formulation variable e.g., polymer to drug ratio, viscosity of ethyl cellulose, volume of light liquid paraffin and effect of processing temperature on yield, encapsulation efficiency, particle size and in vitro drug release characteristics of the Indinavir microspheres were investigated. The prepared microspheres were spherical with stable nature of drug within the formulations confirmed by Fourier transform infrared spectra. The mean sphere diameter and encapsulation efficiencies depended strongly on the drug to polymer ratio, viscosity of polymer, volume of processing medium and processing temperature. The release of indinavir sulfate was diffusion controlled and influenced by the drug to polymer ratio, viscosity of polymeric phase, volume of light liquid paraffin used and processing temperature condition.

Keywords: Microspheres, Indinavir Sulfate, Oil-In-Oil Emulsion Solvent Evaporation, Mixed Solvent System.

INTRODUCTION

Microspheres were used for controlled drug delivery, targeting drug delivery thus decreasing the side effects and eliminating the inconvenience of repeated injections[1, 2]. A great number of microencapsulation techniques are available for the formation of sustained release microparticulate drug delivery systems. One of the popular methods for the encapsulation of drugs within water insoluble polymers is the emulsion solvent evaporation method. Ethyl cellulose (EC) was used as a model encapsulation material. It is a water insoluble polymer which is widely used in pharmaceuticals as a wall material for sustained release microcapsules[3]. This is due to its high safety, good stability, easy fabrication and cheapness. In the above study a mixed solvent system (MSS) has been used as a dispersed medium with a suitable non-aqueous processing medium to enable formation of oil1-in-oil emulsion. Components of the MSS can be selected from any of the commonly available organic solvents such as dichloromethane, ethyl acetate, acetone, acetonitrile, ethanol[4, 5, 6]. Having chosen oil as the processing medium, it is imperative that the solvent for polymer be immiscible with oil. Acetonitrile is a unique organic solvent, which is polar, water miscible and oil immiscible[7]. All other polar organic solvents like methanol, ethyl alcohol, ethyl acetate, acetone, dimethylsulphoxide and tetrahydrofuran are oil miscible and do not form emulsions of the polymer solution in oil[8]. Polyhydric alcohols (low molecular weight polyethylene glycol, glycerol etc.) or other non-aqueous liquids can also be used instead of oil as the processing medium. With oil as a processing medium, use of acetonitrile alone

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as a dispersed medium did not ensure formation of a stable emulsion, and a non-polar solvent such as dichloromethane was included to decrease polarity of the acetonitrile solution. In this study, the mixed solvent system comprising 1:1 proportions of acetonitrile and dichloromethane was used.

The model drug Indinavir sulphate (IS), an inhibitor of the human immunodeficiency virus is the FDA approved drug for the treatment of AIDS. It is typically administered orally as capsule and oral solution. The virustatic drug has a very short half life that is 1.80 h[9]. Therefore, the purpose of the present study is to use the oil-in-oil (o/o) emulsion solvent evaporation method in order to prepare microspheres using EC as wall former and to investigate the effect of polymer to drug ratio, viscosity of ethyl cellulose, volume of light liquid paraffin and effect of processing temperature on the characteristics of the microspheres in order to prepare stable and effective sustained release formulation of IS with high entrapment efficiency and yield for better treatment of AIDS.

EXPERIMENTAL SECTION

Materials

Indinavir sulfate was obtained as a gift sample from Macleod Pharmaceuticals Ltd., Mumbai, India. Ethyl cellulose (EC) and span 80 were purchased from Central Drug House, Mumbai and Rajesh Chemicals, Pune, India, respectively. All other reagents and solvents used were of analytical grade.

Methods

Preparation of Microspheres by o/o Emulsion Solvent Evaporation Method:

Microspheres were prepared by using o/o emulsion solvent evaporation method, using different ratios of EC to IS as given in Table 1. EC and IS were added (1:1/1:2/1:3/1:4) to the mixed solvent system consisting of acetonitrile and dichloromethane in a 1:1 ratio and mixed thoroughly by a magnetic stirrer. Then the polymeric phase was slowly added to 50/100/150 ml of light liquid paraffin 0.25 % v/v span 80 (HLB value of 4.3) as a surfactant while stirring at 500 rpm at 10ºC/25ºC/40ºC. After one h 10 ml of n-hexane was added, and the stirring was continued for a further 2 to 3 h. Obtained hardened microspheres were collected by filtration, washed with three portions of 50 ml of n-hexane and air dried for 12 h. The different experimental variables were given in Table 1. Batches were prepared in triplicate to obtain reproducible results.

Table 1: Formulation codes with quantities. Drug: Indinavir sulfate, EC: Ethyl cellulose VPM: Volume of processing medium, PT: Processing temperature.

Formulation Code	Drug : EC	Viscosity of EC (cps)	VPM (ml)	PT (ºC)
F1	1:1	22	50	25
F2	1:2	22	50	25
F3	1:3	22	50	25
F4	1:4	22	50	25
F5	1:4	46	50	25
F6	1:4	22	100	25
F7	1:4	22	150	25
F8	1:4	22	50	10
F9	1:4	22	50	40

Determination of Percentage Yield and Percentage Entrapment Efficiency:

Dried microspheres were weighed, and the percentage yield of microspheres was calculated using the following formula.

Percent Yield (%) = (Amount of microspheres obtained (g) / Theoretical amount (g)) x100

About 50 mg of accurately weighed drug loaded microspheres were triturated, added to 50 ml of triple distilled water (TDW), and kept shaking on mechanical shaker for 24 h. The resulting mixture was filtered (0.45 μm pore size) and analyzed spectophotometrically at 259 nm using Systronic 2101 UV – Visible spectphotometer after appropriate dilution. Entrapment efficiency of the microspheres was determined by using following formula.

% Entrapment efficiency = (Drug loading / Theoretical drug loading) x 100

Determination of particle size:

Microspheres were separated into different size fractions by sieving for 10 minutes using mechanical sieve shaker (Cuprit Electrical Co. India) containing standard sieves having apertures of 710, 500, 355 and 250 μm. The particle size distribution of the microspheres for all the formulations was determined and mean particle size of microspheres was calculated by using the following formula.

$$\text{Mean Particle size} = \frac{\Sigma \text{ (Mean particle size of the fraction X weight fraction)}}{\Sigma \text{ Weight fraction}}$$

Scanning Electron Microscopy (SEM)

The external morphology of the microspheres was analyzed by JEOL JSM – 5200, scanning electron microscope at 20 kV. Prior to examination the microspheres were fixed on a metallic support with a thin adhesive tape and microspheres were coated with gold under vacuum (fine coat, ion sputter JFC – 1110) to render them electron conductive.

Fourier Transform Infrared Spectroscopy (FTIR)

Drug polymer interactions were studied by FTIR spectroscopy. The spectra were recorded for pure drug, drug loaded microspheres and blank microspheres using FTIR JASIO (Model No. 410). Samples were prepared in KBr disks (2 mg sample in 200 mg KBr). The scanning range was 400 to 4000 cm^{-1}, and the resolution was 2 cm^{-1}.

***In vitro* Release Studies**

Drug release from microspheres was determined using an eight station USP I dissolution test apparatus (LABINDIA, Disso-2000, Mumbai, India) using 500 ml of TDW as a dissolution medium. Microspheres (100 mg) from size fraction 355 μm sizes were put in each basket. The dissolution medium was stirred at 100 rpm and maintained at 37 ± 0.5 ^{0}C. Samples of 10 ml were taken at aliquots were withdrawn and replaced by an equal volume of fresh dissolution medium. After suitable dilution, the samples were analyzed spectophotometrically at 259 nm. In order to determine the effect of processing temperature on drug release rate, mixed particle size microspheres were taken in to account for dissolution study as formulation F8 did not yielded comparable amount of microspheres of size 355 μm.

The mechanism of drug release (for formulation F1 – F7) was studied by Korsmeyer Peppas model. As given in the following formula[10].

$Mt / M\infty = Kt^n$

Where $Mt / M\infty$ is the fraction of drug released at time t, and k is the release rate constant and 'n' is the release exponent. The 'n' value is used to characterize different release mechanisms and is calculated from the slope of the plot of log of fraction of drug released vs. log of time.

Aging Studies

Aging studies were conducted on a formulation F5 in order to assess its stability after storage at 40 ^{o}C and 75 %RH for 6 months. Samples were withdrawn at 1, 3 and 6 months and evaluated for entrapment efficiency and *In vitro* drug release.

Statistical Analysis

Results are expressed as mean ± S.D for triplicate samples. The results were statistically analyzed significant differences among formulation parameters were determined by using UNISTAT® statistic version 3, Meglon, USA. Statistical significant was considered at $p<0.05$.

RESULTS AND DISCUSSION

Preparation of Microspheres by o/o Emulsion Solvent Evaporation Method

During optimization of the process various parameters was tried. It was found that for less than 500 rpm speed drug has got the tendency to settle down on the bottom, and at more than 500 rpm speed high turbulence was observed with adhesion of particles to surface of container. The minimum concentration of span 80 required to form the stable emulsion was found to be 0.25 %. The preparations were stirred for optimized time (3-4 h) which gave microspheres with proper strength.

Effect of Processing Variables on Percentage Yield and Percentage Entrapment Efficiency

The percentage (%) yield and percentage (%) entrapment efficiency of microspheres were given in Table 2. The yield was in the range of 76-91 %, and the entrapment efficiency was in the range of 69 to 90 %. When concentration and viscosity of EC increased, % entrapment efficiency increased. Higher the polymer to drug ratio, the higher probability of drug surrounded by polymer which acted as a barrier to prevent from diffusion of drug into the external medium. As the volume of the processing medium increased the entrapment efficiency significantly decreased. In higher volume of processing medium, the emulsion droplets can freely be moved in the medium, and they had very less chance to collide with each other, thereby yielding relatively small sized microspheres, which may have provided more surface area for drug to escape. Likewise when processing temperature was increased from 10ºC to 40 ºC the entrapment efficiency increased. It may be due to the fact that low processing temperature favored formation of smaller particle size, which in term provided more surface area for drug to escape to external medium.

Particle Size:

The morphology of microspheres was determined by SEM as shown in figure 1 which has shown that the microspheres were spherical in nature with rough surface. Microphotograph at higher resolution showed the presence of pores over the surface of microspheres. The mean particle size of microspheres was in the range of 271.34 to 501.32 μm as given in table 2. It was observed that as EC concentration, EC viscosity, and processing temperature of formulation was increased mean particle size increased. Volume of the processing medium had a significant role. As the volume was increased from 50 to 100 and to 150 ml, the mean particle size of microspheres decreased.

Table 2: Yield, Entrapment Efficiency (EE), Mean Particle Size (MPS) and parameters of Korsemeyer -Peppas model (r^2: Correlation coefficient, n: Release exponent) of the prepared microspheres.

Batch	Yield (%)	EE (%)	MPS (μm)	r^2	n
F1	91.86 ±1.68**	69.94 ± 2.0**	324.5 ± 1.93***	0.998	0.410
F2	94.35 ± 2.40**	82.80 ± 0.73**	367.1 ± 4.94***	0.992	0.445
F3	84.88 ± 7.40**	83.75 ± 1.15**	383.55 ± 4.26***	0.996	0.476
F4	82.93 ± 2.52	90.08 ± 0.81	434.95 ± 0.36	0.991	0.410
F5	83.00 ± 1.95**	94.03 ±1.30**	501.32 ± 3.38***	0.994	0.428
F6	84.50 ± 1.75**	83.66 ± 1.70**	384.47±1.97***	0.996	0.491
F7	81.12 ± 1.24**	81.61 ± 1.65**	307.45 ± 1.54***	0.997	0.480
F8	81.00 ± 3.50**	47.18 ±2.50**	271.34 ±5.29***	-	-
F9	64.00 ± 2.14**	93.77 ± 2.06***	578.54± 6.14***	-	-

Significantly different from values of formulation F4 as $p < 0.05$ (**), $p < 0.005$ (***)

Increasing polymer concentration as well as viscosity of polymer produced a significant increase in the viscosity, thus leading to an increase of the emulsion droplet size and finally a higher microsphere size[11]. This is in agreement with the findings of Jeffery *et al.* 1991[12], who suggested that the higher concentration of polymer in the sample had led to increased frequency of collisions, resulting in fusion of semi formed particles and producing an overall increase in the size of the microspheres. The mean particle size of the microspheres was found to decrease with increase in volume of processing medium. When volume of processing medium was increased from 50 to 100 and to 150 ml, the mean particle size of microspheres decreased for the reason that when the volume of processing medium was increased, the emulsion droplets can freely be moved in the medium and they had less chance to collide with each other there by yielding small and uniform microspheres.

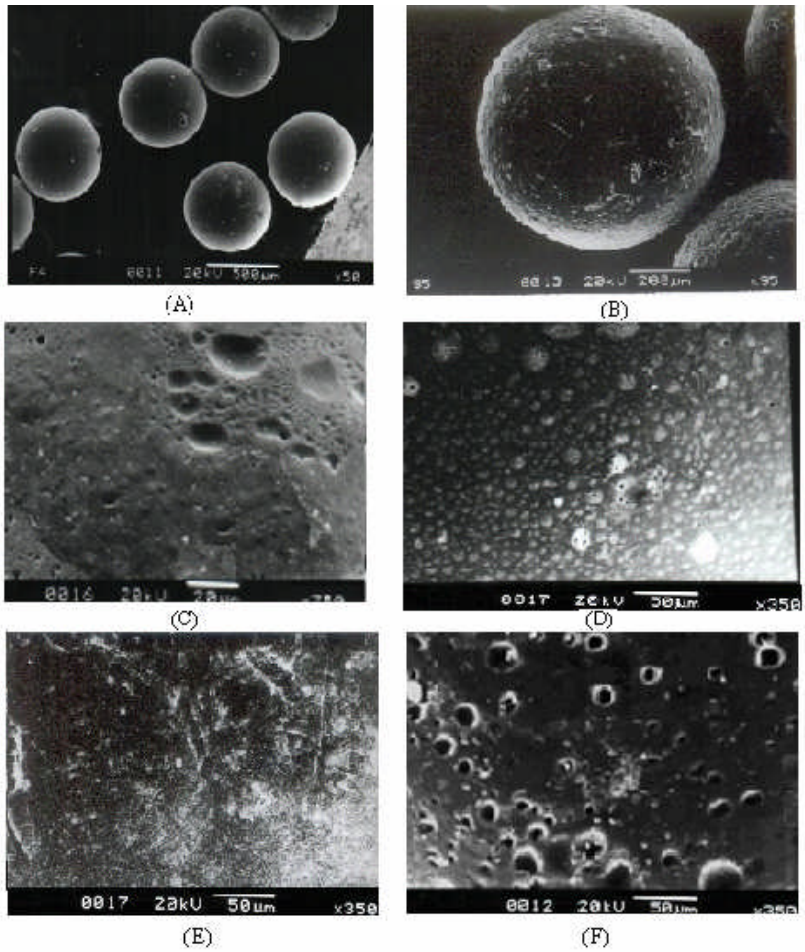

Figure 1: Scanning electron micrographs of drug-loaded microsphere of formulations: A: F4 at 50X, B: F4 at 90X, C: F4 at 350X, D: F7 at 350X, E: F8 at 350X and F: F9 at 350X.

Conversely, when the volume is only 50 ml, the emulsion droplets had more opportunities to collide with each other and fuse together to form larger microspheres.

Processing temperature also found to play a significant role in controlling the mean particle size of microspheres. In case of low temperature (10^0), when the polymeric phase was added to the oil phase and stirred, extensive emulsification of the polymeric phase takes

place, and then the solvent from polymeric phase diffuses in to the outer oil phase and evaporates from the system resulting in small size microparticles. But higher preparation temperature (40^0) increases the rate of solvent evaporation, and the emulsion droplets harden faster and stirrer shear force is not able to break the droplets and leads to formation of larger particle size[13].

In Vitro Release Studies

In vitro drug release of IS from microspheres of formulation F1 to F7 were shown in figure 2. It was found that the release rate decreased with increasing amount of the polymer. This can be explained by a decreased amount of drug present close to the surface (figure 1) and also by the fact that the amount of uncoated drug decreases with higher polymer concentration. Microspheres prepared using large volume of processing medium showed faster drug release. It may be due to the higher migration of drug to surface due with free movement of emulsion droplets with increasing volume of processing medium[12]. Formulation prepared at higher temperature showed a longer sustained release action as compared to formulation prepared at lower temperature as shown in figure 3. At lower processing temperature, the mean particle size of the microspheres was less than that at higher processing temperature. Therefore, the drug release from microspheres prepared at lower temperature was faster than that of microspheres prepared at higher temperature might be due to small size of the microspheres providing a large surface area for faster drug release[15]. This finding is consistent with the general rule *i.e.*, small size of microspheres provides large surface area for faster drug release.

In order to determine the mechanism of drug release the data obtained was fitted into Korsemeyer-Peppas model so as to find out 'n' value, which describes the drug release mechanism[10]. The 'n' value of formulation (F1 to F7) lies between 0 to 0.5 (table 2) indicating the mechanism of the drug release was diffusion controlled. For formulation F8 and F9 mixed particle sizes of microspheres were taken for dissolution study so the mechanism of drug release was not determined.

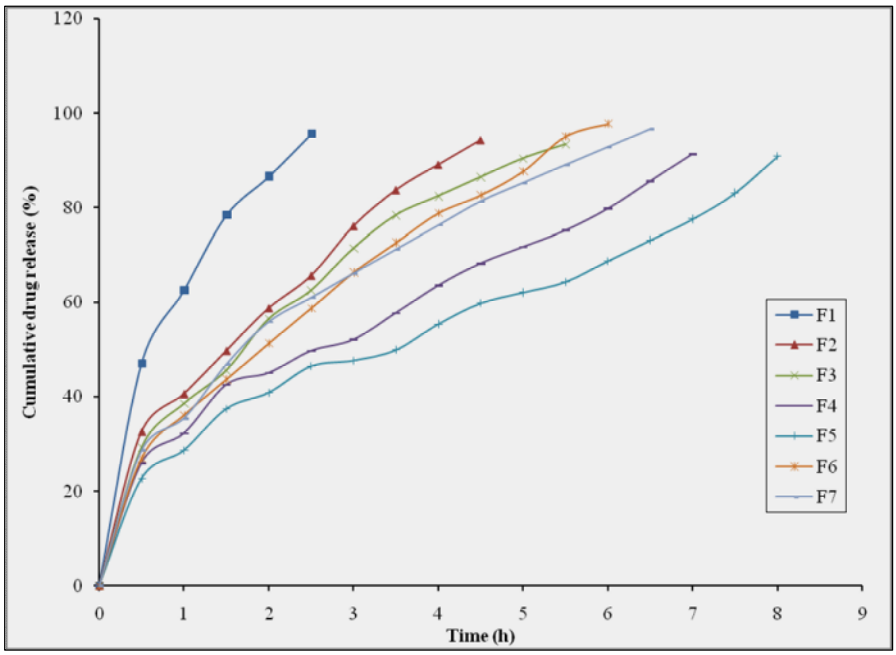

Figure 2: Cumulative percentage release of Indinavir sulfate from prepared microspheres containing sieved particles of size 355 μm.

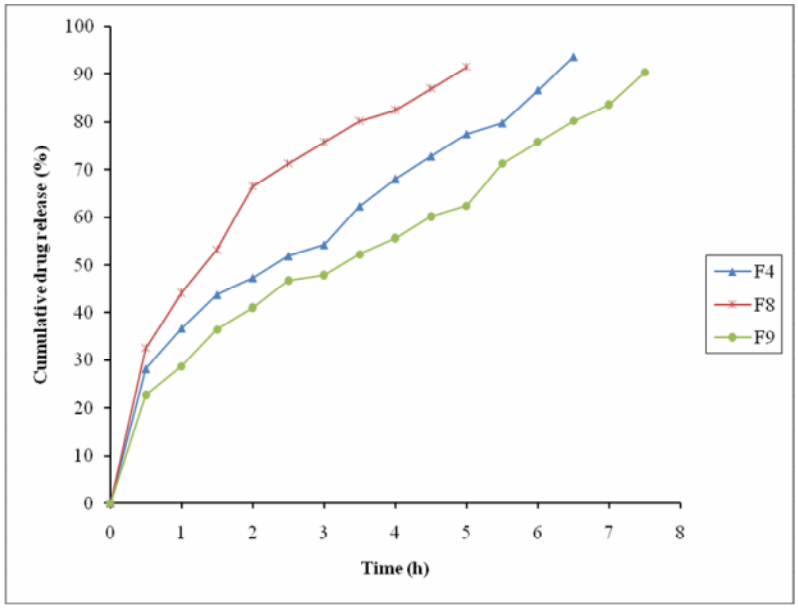

Figure 3: Effect of processing temperature on cumulative percentage release of Indinavir Sulfate from Microspheres Containing Unsieved Particles.

Drug-excipient compatibility study

Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of IS, EC loaded microspheres and blank EC microspheres were shown in figure 4. Drug spectrum showed prominent peaks at 3277 cm^{-1}, 3243cm^{-1}, 2974cm^{-1}, 2874 cm^{-1} 1681cm^{-1} and 1220 cm^{-1}corresponding to OH stretching, NH stretching of secondary amine, aromatic C–H stretching, (-CH_2-) stretching, C=O stretching and C-N stretching, respectively. IS loaded microsphere exhibited peaks within the same region confirming no interaction of drug with excipients during encapsulation process.

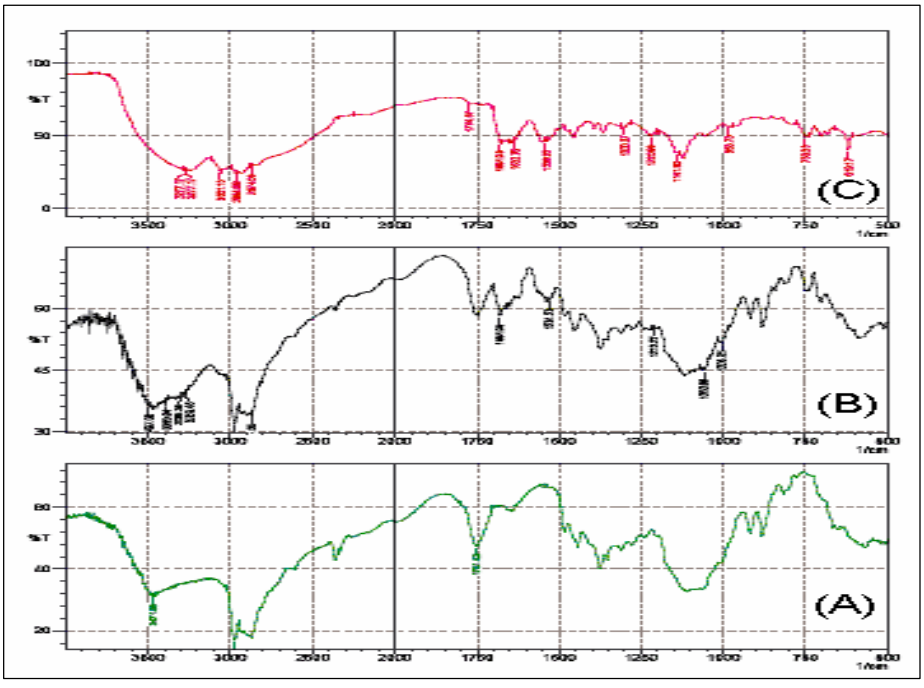

Figure 4: FTIR Spectra of A: blank microspheres, B: Formulation F4 and C: Indinavir Sulfate.

Aging Study:

Aging study data for formulation F5 is given in Table 3. The results indicated that formulation F5 did not show any physical changes after 1, 3 and 6 months. It was observed that entrapment efficiency, and drug release have shown no significant deference from the data of 0 month. Thus these results confirmed that the microspheres were stable at accelerated conditions.

Table 3: Aging study data for formulation F5. (EE: Entrapment Efficiency)

Time (Months)	EE (%)	Drug release for given time (%)				
		1 (h)	2 (h)	4 (h)	6 (h)	8 (h)
0	94.0 ± 1.3	28 ± 2	41 ± 1	55 ± 3	68 ± 2	91 ± 2
1	93.8 ± 1.0*	27 ± 1*	39 ± 2	56 ± 2*	69 ± 3*	90 ± 3*
3	93.2 ± 1.3*	28 ± 1*	43 ± 1	53 ± 4*	67 ± 2*	89 ± 1*
6	92.9 ± 1.1*	26 ± 3*	42 ± 1*	56 ± 1*	68 ± 1*	91 ± 1*

No significant difference as compared with the values for 0 month as $p > 0.05$ (*)

CONCLUSION

In terms of the above study, it could be concluded that a stable and sustained release formulation of IS with EC can successfully be prepared by utilizing mixed solvent system consisting of acetonitrile and dichloromethane in a 1:1 ratio. By optimizing processing parameters such as drug to polymer ration 1:4, viscosity of EC 46 cps, volume of processing medium 50 ml and processing temperature at 25°C, a stable and effective sustained release formulation of IS (F9) could be formulated with better entrapment efficiency, efficient release control, suitable particle sizes and desired drug release mechanism. This will certainly yield benefit for better management of AIDS.

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آثار متغيرات التركيب على الخصائص الفيزيوكيماوية للكريات المهجرية لسلفات الإندينافير

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ملخـص

تم بنجاح تحضير أجسام كروية مجهرية مايكرونية من الإيثيل سليلوز محملة بكبريتات إندينافير بتطبيق أسلوب تبخير المذيب العضوي في مستحلب زيت-في-زيت يتكون من خليط من الأسيتونيتريل و ثنائي كلورو ميثان (نسبة1:1) والزيت المعدني الخفيف كأطوار زيتية أساسية و ثانوية، على التوالي ، مع مادة سبان 80 كعامل مثبت للمستحلب. تتضمن الدراسة الحالية التحقيق في تأثير متغيرات التركيب مثل نسبة البوليمر للدواء، لزوجة الإيثيل سليلوز، حجم الزيت المعدني الخفيف وتأثير درجة الحرارة على ناتج الحبيبات ، كفاءة التغليف ، حجم الحبيبات و خصائص إطلاق كبريتات إندينافير في الظروف المخبرية. أظهرت الحبيبات المحضرة أشكالاً كروية كما أنها مستقرة كيماوياً كما أكدت أطياف الأشعة تحت الحمراء. أظهرت الدراسة إعتماد معدل قطر الحبيبات وكفاءة تغليف الدواء على نسبة و لزوجة البوليمر و حجم ودرجة حرارة وسط التحضير . وجد أن سرعة إطلاق كبريتات إندينافير تعتمد على خاصية الإنتشار و تتأثر بنسبة البوليمر ، لزوجة البوليمر ، وكمية الزيت المعدني الخفيف المستخدم و درجة حرارة وسط التحضير.

الكلمات الدالة: الحبيبات الكروية المجهرية، كبريتات إندينافير، تبخر المذيب لمستحلب زيت-في-زيت ، ونظام المذيبات المختلط .

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