ejpmr, 2017,4(9), 723-730

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

FORMULATION AND CHARECTERIZATION OF ETHYLCELLULOSE MICROSPHERES OF AN ANTI VIRAL DRUG

M. Sreelatha*, R. Swapna, B. Vishnu Vardhanbabu, SK. Saira Banoo, Y. L. Swarupa, P. Anjaneyulu, P. Rajesh

Department of Pharmaceutics, Vagdevi College of Pharmacy, Gurazala, Andhra Pradesh.

***Corresponding Author: Prof. M. Sreelatha**

Department of Pharmaceutics, Vagdevi College of Pharmacy, Gurazala, Andhra Pradesh.

Article Received on 15/07/2017 **Article Revised on 05/08/2017** **Article Accepted on 25/08/2017**

ABSTRACT

Acyclovir (ACV), a synthetic analogue of 2'-deoxiguanosine, is one of the most effective and selective agents against viruses of the herpes group. Acyclovir microspheres were prepared by multiple emulsion solvent evaporation method, where DCM is used as a solvent for polymer ethylcellulose. PVA (emulsifying agent) is added to external aqueous phase, total of 8 batches are prepared by and evaluated for various parameters. Drug-excipients compatibility was performed by FTIR study. The average particle size of acyclovir loaded microspheres was found in range of 7.75-16.84 µm. Entrapment efficiency of drug loaded microspheres was found in range of 54.68-83.04%. The SEM of acyclovir loaded microspheres showed that the microspheres to be porous and spherical in shape, and also confirm the size of microspheres. Compatibility studies showed that acyclovir was compatible with ethylcellulose. The FTIR spectrum of physical mixture of drug and ethylcellulose, and drug loaded microspheres showed summation of peaks of drug and ethylcellulose. The in vitro release of optimized batch (F5) of drug loaded microspheres was found to be 86.1%. Higher correlation coefficient was observed in the Higuchi plot, indicating that the drug release from ethylcellulose microspheres was diffusion controlled.

KEYWORDS: Acyclovir, Microspheres, Ethyl cellulose.

INTRODUCTION

Previously people have been using conventional dosage form like tablet and capsule for the treatment of acute and chronic disease but these have to be taken several times to maintain the peak plasma concentration. In order to overcome this problem controlled drug delivery system was developed. The main objective of controlled drug delivery is to ensure optimum plasma drug concentration, thus enhancing efficacy and bio-availibity of drug with improved patient compliance. Controlled release refers to the use of a delivery system with the objective of releasing the drug into the patient body at a predetermined rate or controlled rate, at specific times or with specific release profiles.[1]

Several problems associated with use of conventional dosage forms for the controlled release includes: The drugs which are hygroscopic in nature are not suitable for filling into capsule because moisture will be absorbed from the shell, make them brittle in nature, leading it to crumble into pieces moreover the dried solution which requires previous dilution are unsuitable for capsule because if administrated as such leads to irritation into stomach. Bioavailability problems are associated with tablets because disintegration and dissolution is required before drug is available for absorption and sometimes it also causes gastrointestinal irritation. Various advancements have been made to overcome these drawbacks of conventional dosage form and microsphere is one of them.

Microsphere are free flowing solid particle made up of biodegradable and non-biodegradable material, ideally having a particle size less than 200µm and can be injected by an 18 or 20 number needle.[2] Microsphere eases sustained drug release and also reduces or eliminates gastrointestinal tract irritation. Microsphere is used to alter the drug release. Drug absorption and side effects due to irritating drugs against thegastrointestinal mucosa is improved because microsphere are made up of small particle size less than 200µm, which are widely distributed throughout the gastrointestinal tract.[3]

Advantages[4,5]

- Masking of odour or bitter taste.
- Improve physical stability and gastric enzymestability.
- Better process ability (improved flowability, dispersability).
- Reduced dose size.
- Reduced dosing frequency therefore improves patient compliance.

- Reduced toxicity.
- Absorption window.
- Gastric irritation problem can be overcome by microsphere.
- First pass metabolism is avoided.
- Biological half-life can be enhanced.
- Improve bioavailability.
- Microsphere provides increased therapeutic efficacy and prolonged duration of action.
- Microsphere provides controlled, sustained and targeted drug delivery.
- Microsphere can be injected into body because of small size and spherical shape.

Acyclovir (ACV), a synthetic analogue of 2'-deoxiguanosine, is one of the most effective and selective agents against viruses of the herpes group. ACV is active against herpes simplex virus type 1 (HSV-1), herpes simplex virus type 2 (HSV-2), varicella zoster virus, and in a less extent against Epstein-Barr virus and cytomegalovirus. The mechanism of action of this drug has been extensively studied, and its antiviral activity has been shown to result from the inhibition of herpesvirus DNA replication.[6]

ACV was effective against cutaneous infections due to HSV-1, whose target site is the basal epidermis. However, it has been suggested that ACV topical therapy has a low efficacy, due to the lack of penetration of an enough amount of drug to the target site.[7] In this way, Parry et al.[8] found a good relationship between the free drug concentration at the basal epidermis and the *in vivo* antiviral efficacy for a variety of ACV topical formulations. Consequently, the quantification of ACV within the different strata of the skin will be essential to determinate its effectiveness.

MATERIALS

Acyclovir gift sample from Zydus Cadila, Ahmedabad**,** Ethylcellulose obtained from Reachem laboratory chemicals privated Ltd, Polyvinyl alcohol obtained from Himedia Laboratories Pvt. Ltd, Mumbai, Sodium chloride crystal obtained from s d fine – chem. Ltd, Mumbai, Carbopol 934P obtained from Research- Lab Fine Chem Industries, Mumbai**,** Triethanolamine obtained from Ranbaxychemicals Pvt. Ltd, Delhi**,** Methyl obtained from Chemicals private Ltd. Chennai**,** Chemicals and Reagents used as analytical grade.

FORMULATION DEVELOPMENT

For optimization of the drug: polymer ratio, PVA and NaCl concentration preliminary batches were made. The effect of various formulation and processing factors on microspheres characteristics were investigated by changing drug: polymer ratio, PVA concentration, NaCl concentration on external aqueous phase, stirring speed, time and the volume of external oil phase.

Experimental design

Experimentation is expensive in terms of time and resources. It is therefore necessary that the experimentation should be more efficient and systematic. Experimental design is the strategy of setting up experiments in such a manner that the information required is obtained as efficiently and precisely as possible.

The various formulation and process variables, involved in the development of pharmaceutical dosages can be divided into two groups. The independent variables, which are the formulation and process variables and the dependent variables which are the response or characteristics of the in-process material or resulting drug delivery system. In the formulation of the drug loaded microspheres three factors were varied. The formulation of microspheres was shown below table no-1.

Table 1: Composition of drug loaded microspheres

Ingredients	Batches							
	F1	F2	F3	F4	F5	F6	F7	F8
Acyclovir (mg)	500	500	500	500	500	500	500	500
Ethylcellulose (mg)	250	500	250	500	250	500	250	500
PVA (%)	0.2	0.2	0.5	0.5	0.2	0.2	0.5	0.5
NaOH 0.8M (ml)	5	5	5	5	5	5	5	5
Dichloromethane (ml)	15	15	15	15	15	15	15	15
Sodium chloride (%)	2.5	2.5	2.5	2.5	5	5	5	5
Water (ml)	60	60	60	60	60	60	60	60

Preparation of microspheres[9,10]

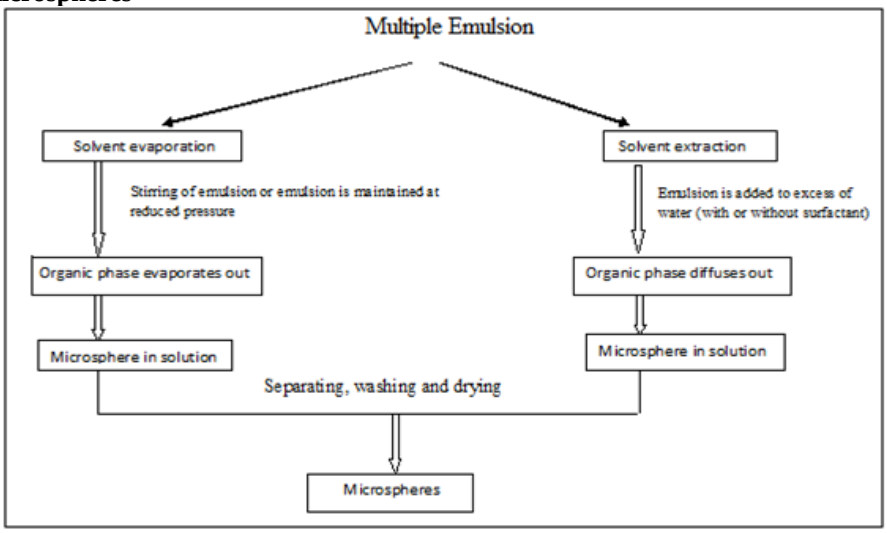

Fig. 1: Schematic representation of Microsphere preparation by Double emulsion method.

The microspheres were prepared by multiple (W/O/W) emulsion solvent evaporation method. In this method, acyclovir was initially dissolved in 5 ml of 0.8M NaOH (inner aqueous phase) and oil phase is prepared by dissolving different amount of ethylcellulose in 15 ml DCM. An aqueous drug solution was mixed with DCM containing ethylcellulose polymer and homogenized using an ultra turrax at 11000 rpm for 7 min. The resultant first emulsion (W/O) was slowly added into 60 ml of PVA solution (external aqueous phase) and homogenization continued for 1 min. to produce W/O/W emulsion.

In order to increase the diffusion rate of DCM from the emulsion drops into the external phase, 200 ml of external water phase (PVA solution containing the same concentration of PVA) was added to the W/O/W emulsion system under magnetic agitation. The system was stirred continuously for 8 h at room temperature to evaporate the DCM completely. After the microspheres had formed, they were vacuum filtered through whatmann filter, washed three times with distilled water and dried in vacuum oven at 35^{o}C.

FTIR SPECTROSCOPIC STUDIES:

The sample (5mg) was finely grounded and mixed with approximately 100 mg of dried potassium bromide (kept at 100^{o}C for 8 h). Fourier transform infrared spectroscopy (FTIR) of pure drug, ethylcellulose, physical mixture of drug and polymer (1:1 ratio) and acyclovir loaded microspheres were recorded between 4000-400 cm^{-1} using Jasco FTIR-4100. The major peaks were identified in the FTIR spectrum of the pure drug and infrared spectral assignment was done. This served as a tool for confirming identity of the drug. The physical mixture of drug and ethylcellulose and drug loaded microspheres were recorded to access the compatibility of drug with main excipients.

EVALUATION OF MICROSPHERES:

Products from various batches were evaluated for the following criteria.

Percent yield

The percent yield was calculated as the weight percentage of the final product after drying, with respect to the initial total amount of acyclovir and ethylcellulose.

Percent yield= (Weight of microspheres / Weight of drug + polymer) × 100

Particle size analysis[11]

The particle size was determined by dispersing 25 mg microspheres in 10 ml solution of 0.1% tween80 and their size was determined by laser light scattering using Malvern Hydro 2000 SM particle size analyzer or laser diffractometer (Malvern Instruments, Malvern, UK). The particle size was expressed as a volume mean diameter in micrometer. Each measurement was performed in triplicate and standard deviation was calculated.

Entrapment efficiency[12]

To calculate the entrapment efficiency of acyclovir into formulated particles. The microspheres equivalent to 25 mg of acyclovir was accurately weighed and crushed. The powder of microspheres suspended in 50 ml phosphate buffer pH 7.4 and shaken using a magnetic stirrer for 1 h. The mixture was filtered through 0.45 µm membrane filter and filtrate is subjected to spectroscopic analysis (Jasco V-530, Japan) at 252 nm for acyclovir content against phosphate buffer as a blank.

Entrapment efficiency= (Experimental drug content / Theoretical drug content) × 100

In vitro drug release[13]

The in vitro drug release study of drug loaded microspheres was carried out using USP (type-I) rotating basket dissolution test apparatus (Labindia DS-8000). A weighed quantity of microspheres equivalent to 100 mg of acyclovir was introduced into the basket. Dissolution

media used was 900 ml of phosphate buffer pH 7.4 and sodium lauryl sulphate (SLS) 0.5% (1:1) maintained at 37±0.5 °C and stirred at 75 rpm. At predetermined intervals (15, 30, 45, 60, 120, 180, 240, 300 and 360 min.), 5 ml of the sample was withdrawn and replaced with equal amount of fresh pre-warmed dissolution medium. The collected samples were filtered through 0.45 µm membrane filter and suitably diluted with phosphate buffer and SLS solution and assayed spectrophotometrically (Jasco V-530, Japan) for acyclovir at 252 nm.

The λ_{max} noted in dissolution aliquots from different batches of microspheres did not show shift from 252 nm conforming absence of interference by other soluble being leached out. In order to investigate the mechanism of acyclovir release from microspheres of different batches, the release data were analyzed with different mathematical models such as zero order kinetic (cumulative amount of drug released vs. time), first order kinetic (log cumulative percentage of drug remaining vs. time), Higuchi model (cumulative percentage of drug released vs. square root of time) and Korsmeyer-Peppas model.

7.3.4. Scanning electron microscopy[15]

The shape and surface characteristics of the microspheres were observed by a scanning electron microscope (JEOL, JSM-6360A, Japan). The samples were placed on one side of an adhesive stub and the stub was then coated with conductive gold with sputter coater attached to the instrument. Once gold coating is complete, specimen are ready to be viewed on the SEM at 5 kV. Images may be scanned on a digital imaging system by computer enhancement.

RESULTS AND DISCUSSION

Percent yield

The percent yield was calculated as the weight percentage of the final product after drying, with respect to the initial total amount of acyclovir and ethylcellulose. Percent yield of all batches is tabulated in Table 2.

It was found that decrease in drug:polymer ratio increases the percent yield of microspheres. The percent yield of all the formulations was found to be satisfactory as all the batches showed higher production yield i.e. above 75%.

Table 2: Percent yield of microspheres.

Batch No.	Percent yield
1.	75.33
2.	80.5
3.	76.66
4.	88.5
5.	78
6.	83.8
7.	78.66
8.	87

Particle size analysis

The particle size of acyclovir loaded microspheres was analyzed by laser light diffraction using Malvern particle size analyzer. All the batches of microspheres show uniform size distribution. The average particle size (D_{90}) of drug loaded microspheres was found to be in range of 7.75-16.84 µm (Table 3). It was found that as the drug:polymer ratio was increased, the microspheres size was decreased.[76] With increasing amount of solvent, viscosity of internal phase decreased, resulting in decrease particle size. The increased amount of PVA an emulsifying agent, incorporated in microspheres also increases the particle size.

Table 3: Particle size of microspheres

Batch No.	Particle size (µm ±SD)
1.	7.75±0.86
2.	13.92±01.05
3.	14.35±1.13
4.	16.05±0.97
5.	8.50±0.66
6.	12.61±1.20
7.	13.22±1.16
8.	16.84±1.28

Entrapment efficiency

It was observed that the drug entrapment efficiency was decreased with the increased drug concentration and increased with increasing polymer concentration in microspheres. The data is tabulated in Table 4.

The increasing amount of solvent decreased the entrapment efficiency. Also the concentration of emulsifier employed increased, entrapment efficiency decreases. Since PVA is non-ionic emulsifier, a molecule associate away from the interface at higher concentration and dissolves some portion of drug resulting in reduction in entrapment efficiency. The increased amount of NaCl in external aqueous phase significantly increases the entrapment efficiency of acyclovir microspheres.

Table no: 4 % Entrapment efficiency of microspheres.

Batch No.	Entrapment efficiency (% ±SD)
1.	60.43±3.48
2.	72.04±2.04
3.	54.68±1.65
4.	64.46±1.49
5.	78.13±2.34
6.	83.04±1.18
7.	57.59±2.51
8.	66.1±1.80

In vitro drug release

Dissolution medium employed in the in vitro drug release studies from acyclovir microspheres was phosphate buffer pH 7.4 and 0.5% SLS (1:1). The in vitro release of the acyclovir from ethylcellulose microspheres is exhibited initial fast release which was

followed by nearly constant release of drugs. The initial fast release may be due to the presence of drug particles on the surface during encapsulation. Drug release rate was increased with increasing amount of acyclovir in the formulation. Higher level of drug corresponding to lower level of the polymer in the formulation resulted in an increase in the drug release rate. The in vitro drug release profiles for all batches of drug loaded microspheres were given in the table no-5 are graphically represented in Fig. 2.

Table no-5: Cumulative % drug release.

Time in hrs	Cumulative % drug release							
	F1	F2	F3	F4	F5	F6	F7	F8
0.5	35	22.9	20.1	17.1	31.5	25.5	21.9	18.5
1	39.5	29.1	22.5	19.2	39.5	33.3	27.1	20.4
2	46	33.4	25.2	22.5	55.3	42.7	35.2	23.5
3	55	41.2	35.2	25.4	60.1	49.3	40.2	26.7
4	64.5	43.6	42	34.8	74.1	51.3	42.8	34.1
5	71.2	52.5	44.3	37.3	82	60.8	44.6	35.8
6	76.1	57.4	46.5	39.8	89.9	64.5	45.6	37.3

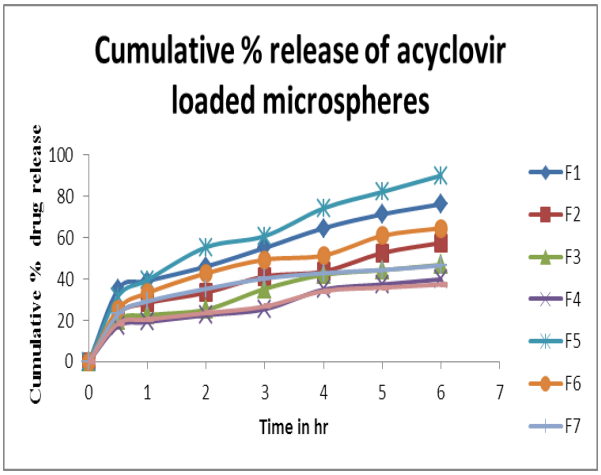

Fig. 2: Cumulative % release of acyclovir loaded microsphere.

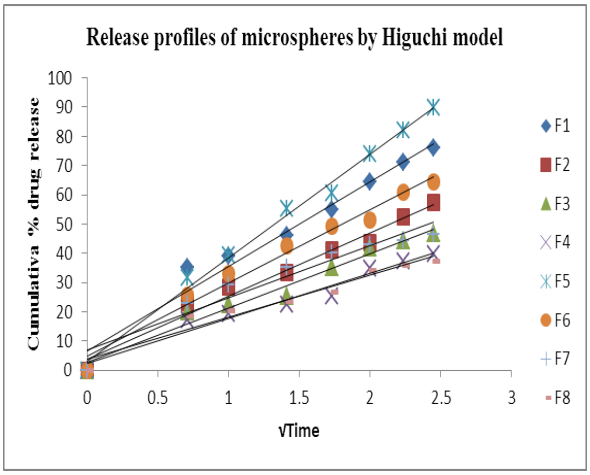

Fig. 4: Release profiles of microspheres by Higuchi model.

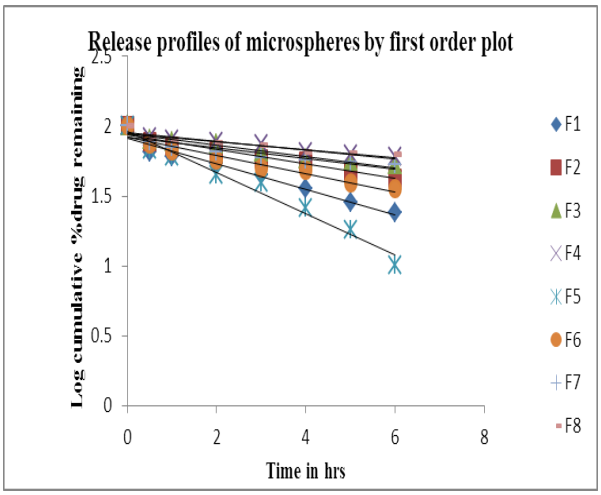

Fig. 3: Release profiles of microspheres by first order plot.

It was also showed that rate of drug release was higher for the batch having smaller particle size because of more surface area. To identify the kinetic of drug release from microspheres, release data was analyzed according to different kinetic models.

The in vitro release profile was applied on various kinetic models in order to find out the mechanism of drug release. The best fit with the highest correlation coefficient was shown in Higuchi, first order and followed by zero order equations, as given in Table 5. The rate constants were calculated from the slope of respective plots.

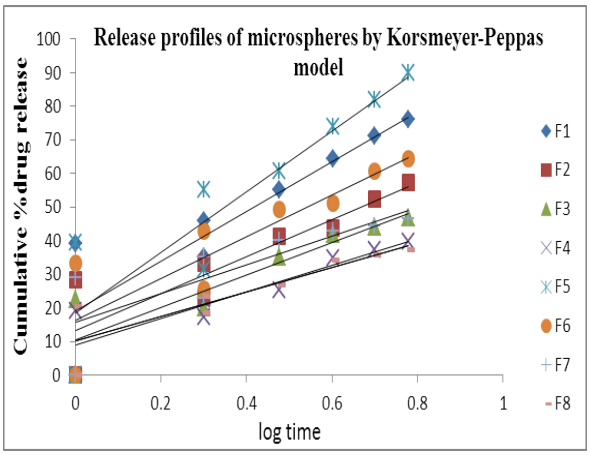

Fig. 5: Release profiles of microspheres by Korsmeyer-Peppas model.

R^2 is the correlation coefficient and K_0, K_1 and K_H are the release rate constants of the zero order, first order and Higuchi models and n are the release exponent of Korsmeyer-Peppas model. Higher correlation coefficient was observed in the Higuchi plot (Fig.5). The drug release was proportional to square root of time, indicating that the drug release from ethylcellulose microspheres was diffusion controlled. The data obtained was also put in Korsmeyer-Peppas equation in order to find out n value, which describes the drug release mechanism.

Table 6: In vitro release kinetic parameters of acyclovir loaded microspheres.

Batch No.	% Release	Zero order		First order		Higuchi model		Korsmeyer-Peppas model	
		R^2	K_0	R^2	K_1	R^2	K_H	R^2	N
F1	73.05	0.903	12.17	0.987	0.548	0.991	29.82	0.996	0.408
F2	53.77	0.927	8.96	0.988	0.638	0.997	21.87	0.988	0.473
F3	44.92	0.929	7.48	0.992	0.668	0.996	18.34	0.984	0.458
F4	37.35	0.869	6.22	0.959	0.689	0.987	15.25	0.997	0.377
F5	86.1	0.871	14.35	0.988	0.438	0.982	35.15	0.996	0.351
F6	60.15	0.874	10.02	0.965	0.614	0.986	24.56	0.997	0.363
F7	49.65	0.874	8.27	0.901	0.653	0.982	20.27	0.998	0.364
F8	39.6	0.805	6.6	0.900	0.683	0.954	16.16	0.996	0.259

The n value of microspheres of different batches was between 0.26-0.48, indicating the mechanism of the drug release to be diffusion controlled. The release also showed high correlation with Korsmeyer-Peppas model, as shown in Table 6.

Drug release mechanism observed here was probably combination of two types of release: polymer matrix release and release through porosities. Since highest correlation was obtained with Higuchi treatment, it could be concluded that diffusion played a major role in control of drug release from the microspheres particle. Thus release mechanism was near to √t relationship. An increase in system size is expected to reduce release rates due to increased length of diffusion pathways and thus decreased drug concentration gradients[14].

Scanning electron microscopy

SEM image of pure drug acyclovir (Fig. 6) showed that drug is crystalline in nature, whereas optimized formulation (F5) of acyclovir loaded microspheres indicate that the microspheres to be porous and spherical in shape (Fig. 7). The pores were induced by the diffusion of solvent from the surface of the microspheres. When DCM diffuses out, nearly all of the dispersed phase is converted to the form of solid microspheres and separated particles appear. SEM analysis also revealed that the prepared microspheres found to be devoid of aggregation and hence might have possessed negligible surface charges. Thus the microspheres were found to possess physical stability.

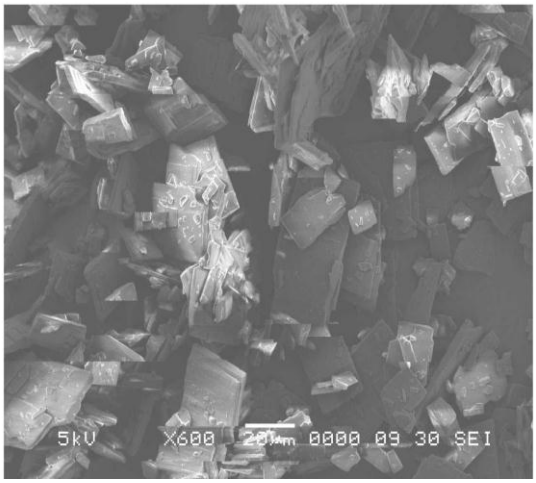

Fig. 6: SEM image of pure drug.

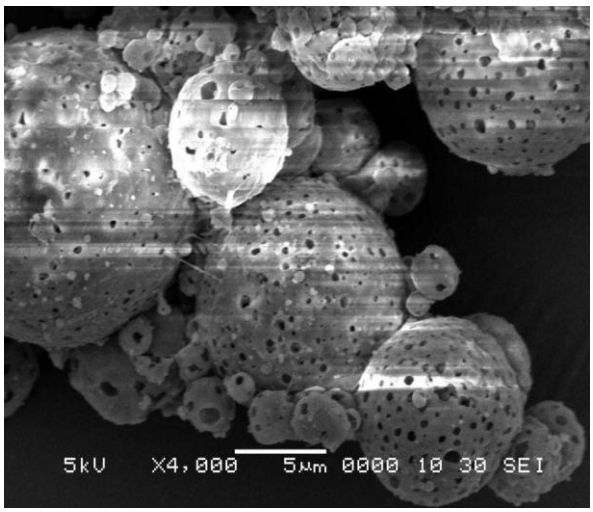

Fig. 7: SEM image of acyclovir loaded microsphere.

FTIR spectroscopic studies

The compatibility of acyclovir in ethylcellulose microspheres was evaluated through FTIR spectrophotometer. The FTIR spectra of acyclovir, ethylcellulose, physical mixture of drug and ethylcellulose (1:1) and optimized formulation (F5) of drug loaded microspheres were recorded. Fig. 8 shows FTIR spectrum which gives number of absorption peaks, some fine some broad with varying intensities in its complete region (4000-400 cm^{-1}). Acyclovir FTIR spectra shows (Fig. 8A) a peak at 3340.1 cm^{-1} is due to –OH stretching. The doublet peak at 3471.24, 3445.21 cm^{-1} is of 1^o amine, and a peak of 2^o amine observed at 3523.31 cm^{-1}. The peak at 2963.09, 1718.26, 1609.31, 1183.11 cm^{-1} is due to –CH, C=O, C=N and -O- stretching respectively. In ethylcellulose FTIR spectra (Fig. 8B) peak observed at 2980.45 cm^{-1} is of –OH group present on closed ring structure of the polymer repeating units. The peak observed at 2862.81 and 1270.86 cm^{-1} is due to –CH and -O- stretching.

The IR spectra of physical mixture of acyclovir and ethylcellulose (Fig. 8C) show characteristic peaks corresponding to drug, representing respective functional group were found to be present in the spectrum. There was no significant change in the spectra was observed. In acyclovir loaded microspheres (Fig. 8D) finger print region of formulation is slight different from both drug and polymer (Table 7).

In the final formulation the peaks intensity and nature of NH_2, NH, C=O, C=N and -OH group of drug have been slightly changed indicating physical association of drug and polymer in presence of solvent DCM, NaOH and PVA solution.

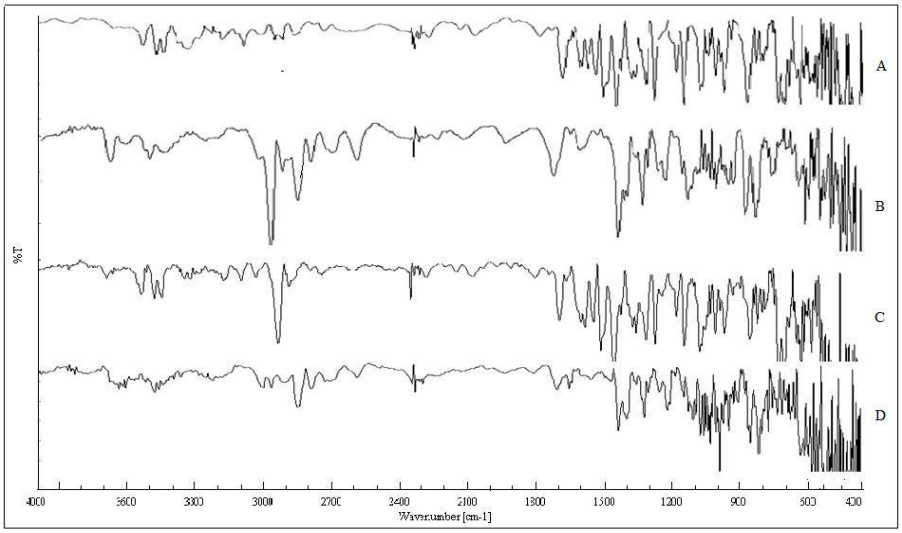

Fig. 8: FTIR spectra: A. Acyclovir; B. Ethylcellulose; C. Physical mixture of drug and polymer; D. Acyclovir loaded microspheres

Table 7: FTIR spectra of acyclovir and drug loaded microspheres.

S. No.	Vibration mode	Wave Number (cm^{-1}) range	Wave number (cm^{-1}) of acyclovir	Wave number (cm^{-1}) of drug loaded microsphere
1.	2^o amine (NH)	3500-3300	3523.31	3621.66
2.	1^o amine (NH_2)	3500-3300	3471.24,	3495.35,
3.	-OH stretching	3650-2500	3340.1	3248.5
4.	Alkane CH stretching	2962-2890	2963.09	2864.74
5.	ketone C=O stretching	1780-1660	1718.26	1749.12
6	Imine C=N stretching	1690-1640	1609.31	1605.45
7.	Ether - O – stretching	1300-1000	1183.11	1162.27

CONCLUSION

Microspheres based novel drug delivery system has been developed to provide sustained release medication for delivery of acyclovir. The acyclovir loaded microspheres prepared successfully by multiple emulsion solvent evaporation method, yields uniform size of microspheres and high entrapment efficiency. Thus as per study results it was concluded that our objective of formulation of acyclovir microspheres for sustained release is potentially beneficial use.

ACKNOWLEDGEMENTS

This work was supported by VAGDEVI COLLEGE OF PHARMACY, Gurazala for their continuous support and encouragement and for providing the necessary facilities.

REFERENCES

1. Vyas S.P and Khar R.K. Controlled Drug Delivery: Concepts and Advances, Vallabh Prakashan, 1 edition 2002, Reprint, 2010; 1-4.
2. Brahmankar, Biopharmaceutics and Pharmacokinetics, Vallabh Prakashan, 2 edition, 2009; 488.
3. V. Prasanth V, Moy Akash Chakraborthy, Mathew Sam T, Mathapan Rinku. Microspheres- An Overview. International Journal of Research in Pharmaceutical and Biomedical Sciences, 2011; 2(2): 332-338.
4. Sahil Kataria, Middha Akansha, Sandhu Premjeet et al. Microsphere: A Review. International Journal of Research in Pharmacy and Chemistry, 2011; 1(4): 1184-1197.
5. Meena Kedar Prasad, Dangi J.S., Samal P.K. et al. Recent Advances in Microspheres Manufacturing Technology. International Journal Of Pharmacy and Technology, March, 2011; 3: 854-893.
6. D.M. Richards, A.A. Carmine, R.N. Brogden, R.C. Heel, T.M. Speight, G.S. Avery, Acyclovir. A review of its pharmacodynamic properties and therapeutic efficacy. Drugs, 1983; 26: 378-438.
7. N.M. Volpato, P. Santi, P. Colombo, Iontophoresis enhances the transport of acyclovir through nude mouse skin by electrorepulsion and electroosmosis. Pharm. Res., 1995; 12: 1623-1627.
8. G.E. Parry, P. Dunn, V.P. Shah, L.K. Pershing, Acyclovir bioavailability in human skin. J. Invest. Dermatol, 1992; 98: 856-863.
9. Vyas S.P. and Khar R.K. "Targeted and Controlled Drug Delivery: Novel Carrier System, CBS Publishers & Distributors, New Delhi,1st ediotion 2002, Reprint, 2011; 417-451.
10. Okonogi SChaisri W, Henmnink WE, preparation of microspheres by double emulsion technique, Int Conf Bioencap, 3(1): 1-4
11. Perumal D, Dangor C.M, Alcock R.S, Hurbans N, Mooparnar K.R, Effects of formulation variables on in vitro release and micromeritic properties of modified release ibuprofen microspheres. J. Microencapsul, 1999; 16: 475-487.
12. Singh D, Saraf S, Dixit V.K, Saraf S, Formulation optimization of gentamicin loaded eudragit RS100 microspheres using factorial design study. Biol. Pharm. Bull, 2008; 31(4): 662-667.
13. Das M.K, Rao K. R, Evaluation of zidovudine encapsulated ethylcellulose microspheres prepared by water-in-oil-in-oil (w/o/o) double emulsion solvent diffusion technique. Acta Poloniae Pharmaceutica-Drug Research, 2006; 63(2): 141-148.
14. Sato T, Kanke M, Schroeder H, Deluca P.P, Porous biodegradable microspheres for controlled drug delivery. Pharmaceutical Research, 1988; 5(1): 21-30.
15. Crotts G, Park T.G, Preparation of porous and non-porous biodegradable polymeric hollow microspheres. J. Control. Release, 1995; 35: 95-105.