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Der Pharmacia Lettre, 2010, 2(5): 83-94
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Formulation and evaluation of acyclovir microcapsules using biodegradable and non-biodegradable polymers

Krishnan P.N[1], Saraswathi R[1], Dilip C[1], Rama Rao N[2], Sambasiva Rao K.R.S[3] Raghvendra[4]

[1]AL Shifa College of Pharmacy, Keezhattur, Perinthalmanna, Kerala – 679325. India.
[2]Chalapathi Institute of Pharmaceutical Sciences, Lam, Guntur, Andhra Pradesh-522034. India.
[3]Department of Biotechnology, Acharya Nagarjuna University, Guntur, Andhra Pradesh-522510.India
[4]Aligarh college of Pharmacy, Aligarh, Uttar Pradesh. India.

ABSTRACT

To formulate and evaluate Acyclovir microcapsules using biodegradable and non-biodegradable polymers namely egg albumin, guar gum and ethyl cellulose and its invitro evaluation. The Acyclovir microcapsules were prepared using different concentrations of egg albumin, guar gum and ethyl cellulose. The microcapsules were prepared using solvent diffusion method and heat coagulation method. The microcapsules were then studied for entrapment efficiency at two different stirring speeds, drug polymer compatibility and surface morphology. The invitro release study was also done. Further kinetic modeling was employed to find out the release mechanisms. Acyclovir loaded microcapsules formulated with guar gum showed an entrapment efficiency of 92.97%, with ethyl cellulose 91.96% and the entrapment efficiency with egg albumin was 90.61%. The SEM showed that the microcapsules were free flowing, non aggregated and spherical between 700-1000 µm in diameter. The surface was wavy in microcapsule formulated with guar gum, porous in microcapsules using ethyl cellulose and smooth in the case of microcapsules formulated with egg albumin. The FTIR spectrum showed that there is no interaction between the polymer and the drug. The invitro release study was found to be the best in the case of acyclovir microcapsules formulated with guar gum. The rate of drug release follows a time dependent process based on fickian diffusion and korsemeyer-peppas model that the drug release is by diffusion and by erosion. The acyclovir microcapsules using various polymers can be used as oral controlled delivery of the antiviral drug acyclovir.

Key words. Polymers, Acyclovir, Microcapsules

INTRODUCTION

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body in order to promptly achieve and thereby to maintain the desired concentration. Recently, several technical advancements have been made. They have resulted in the development of new techniques for drug delivery. These techniques are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity, or targeting the delivery of drug to a tissue. These advancements have led to the development of several novel drug delivery systems that could revolutionalize the method of medication and provide a number of therapeutic benefits and the ultimate aim of these systems are to achieve the extended duration of drug levels but the methods of achieving this and the clinical performance of the products can vary considerably. Prolonged release or sustained release dosage forms have many advantages in safety and efficacy over immediate release drug product in that the frequency of dosing can be reduced, drug efficacy can be prolonged and intensity of adverse effects can be decreased. Many techniques are capable of controlling the rate of the drug delivery. Sustaining the duration of therapeutic activity and / or targeting the delivery.Sustained release[1,2,3,4.] systems are designed to achieve slow release of drug over an extended period of time after administration of single dose. If the system can provide control whether this be of a temporal or spatial nature or both of drug release in the body it is considered as a controlled release system. Control delivery attempts to sustain drug action at a predetermined rate by maintaining a relatively constant, effective drug level in the body. Microencapsulation is a process in which tiny particles or droplets are surrounded by a coating to give small beads with many useful properties. In its simplest form a microcapsule is a small sphere with a uniform wall around it. The material inside the microcapsule is referred to as the core, internal phase, or fill, whereas the wall is sometimes called a shell, coating or membrane. Most microcapsules have diameters between a few micrometers and a few millimeters. A range of materials are suited for use as the capsule material: lipids, wax, crystal starch, modified starch, cellulose, phospholipids and other polymers.[5,6**]** Many microcapsules however bear little resemblance to these simple spheres. The core may be a crystal a jagged adsorbent particle, an emulsion, a suspension of solids or a suspension of smaller microcapsules. The microcapsule even may have multiple walls. The uniqueness of microencapsulation is the smallness of the coated particles and their subsequent use and adaptation to a wide variety of dosage forms and product applications which otherwise might not have been technically feasible. Because of the smallness of the particles, drug moieties can be widely distributed throughout the gastrointestinal tract, thus potentially improving drug sorption. Converting liquids to solids, providing environmental protection, improved material handling properties, colloidal and surface properties can be altered, control the release characteristics and masking or protecting the core material as well as decreasing the volatility.Microencapsulation[7,8] holds great promise for increased product value and effectiveness, particularly within the pharmaceutical field. There are great benefits arising from the use of microencapsulation in Pharmaceutical products. For many treatments, microencapsulation will allow patients to take lower doses for the same pharmacological effect, lower the risk of side effects, allow patients to take fewer doses; e.g. one a day instead of five a day, enable taste – masking for children's medicines.[9,10].Microencapsulation will enable the production of taste masked chewable tablets, powders and suspensions, sustained or prolonged action medication, single layered tablets containing chemically incompatible ingredients, new formulation concepts for cream, ointments, dressing, aerosols, plasters, injectables and suppositories. Antiviral drugs are a class of medication used specifically for treating viral infections. Like antibiotics, specific antiviral are used for specific viruses. Antiviral drugs are one class of anti-microbial, a larger group which includes antibiotics, antifungal and anti-parasitic drugs. They are relatively harmless to the host and therefore can be used to treat

infections. Most of the antiviral now available are designed to help deal with HIV, herpes virus, which is best known for causing cold sores but actually covers a wide range of diseases, and the hepatitis B and C viruses, which can cause liver cancer. Researchers are now working to extend the range of antiviral to other families of pathogens.[11,12**.]** The emergence of antiviral is the product of a greatly expanded knowledge of the genetic and molecular function of organisms, allowing biomedical researchers to understand the structure and function of viruses, major advances in the techniques for finding new drugs and the intense pressure placed on the medical profession to deal with the deadly virus. Eleven drugs approved by the Food and Drug Administration for the treatment of viral infections (other than these caused by human immunodeficiency virus). They are seven nucleoside analogues, two closely related 10- carbon ring amines, one pyrophosphate analogue, and a recombinant protein produced in bacteria. Acyclovir[13,14] is poorly water soluble and has poor oral bioavailability (10-20%) hence intravenous administration is necessary if high concentrations are required. When orally administered, peak plasma concentration occurs after 1-2 hours. Acyclovir has a high distribution rate; only 30% is protein –bound in plasma [15]. The elimination half life of acyclovir is approximately 3 hours It is renally excreted partly by glomerular filtration and partly by tubular secretion. The USP describes guar gum as a gum obtained from the ground endosperms of Cyamopsis tetragonolobus. It consists chiefly of a high molecular polysaccharide, **[**16,17] composed of galactan and mannan units combined through glycoside linkages, which may be described chemically as a galactomannan. Guar gum occurs as an odorless or nearly odorless, white to yellowish-white powder with a bland taste. Ethyl cellulose is a tasteless free flowing white to lighten colored powder. Egg albumin is obtained from the hen's eggs. In the solid state, albumin appears as yellow, brownish amorphous lumps, scales or powder. Denaturation can be induced by heating to 56°C by vigorously shaking with various acids. Albumin is a complex protein consisting of a single polypeptides chain of about 400 residues, a maximum of two phosphate residues per mole, and an oligosaccharide side chain of composed of mannose & glucosamine residues. Acyclovir being an antiviral drug was microencapsulated using egg albumin, Guar gum [18,19] and ethyl cellulose. Egg albumin and Guar gum being natural biomaterials was selected for micro encapsulation, Guar gum[20,21] has a good binding property so has got adhesive property towards drugs in a better manner when compared to that of egg albumin and ethyl cellulose. The release of the drug was done in the acidic pH and then in the alkaline pH and evaluation was done as per the plan of work. The work is evaluated against the marketed preparation of Acyclovir

MATERIALS AND METHODS

The microcapsules using guar gum [22.23,] was prepared by water-in-oil-oil(w/o/o) solvent diffusion method. A weighed amount of acyclovir (0.2 to 0.8gm) and guar gum were dissolved in 30ml of a mixture of acitonitrile and dichloromethane (1:1 v/v). The initial 50ml water-in-oil emulsion was formed by adding 2ml of deionized water to the drug polymer solution with constant stirring at 500rpm for 10 minutes. The w/o primary emulsion was then slowly added to 50ml light liquid paraffin containing span 80 (0.1 ml) as a surfactant with constant stirring for 2 hours. The glutaraldehyde (1 ml) was added and the stirring was further continued for 1 hour. The resulting microcapsules were separated by filtration, freed from liquid paraffin by washing with petroleum ether and finally air dried over a period of 24 hours in desiccators. Microcapsules using egg albumin were prepared by the water-in-oil-in-oil (w/o/o) double emulsion (heat coagulation) method. A weighed amount of acyclovir and the egg albumin were dissolved in 30 ml water. The initial w/o emulsion was formed by adding 0.1ml of span 80. Added albumin solution drop wise to liquid paraffin 50 ml and kept stirring for 20 minutes. 50 ml of liquid paraffin was heated in a heating mantle at a temp of 80^{O}C and to this albumin emulsion was

added drop wise and stirred for one hour. Precipitation of albumin formed which was separated by centrifugal process. The upper layer was discarded. The lower layer was washed four times with petroleum ether to remove traces of reactants and dried in an oven at 40^{o}C.The microcapsules using ethyl cellulose was prepared by water-in-oil-oil (w/o/o) solvent diffusion method. A weighed amount of acyclovir (0.2 to 0.8gm) and Ethyl cellulose were dissolved in 30ml of a mixture of acitonitrile and dichloromethane (1:1 v/v). The initial 50ml water-in-oil emulsion was formed by adding 2ml of deionised water to the drug polymer solution with constant stirring at 500rpm for 10 minutes. The w/o primary emulsion was then slowly added to 50ml light liquid paraffin containing span 80 (0.1 ml) as a surfactant with constant stirring for 2 hours. The glutaraldehyde (1 ml) was added and the stirring was further continued for 1 hour. The resulting microcapsules were separated by filtration, freed from liquid paraffin by washing with petroleum ether and finally air dried over a period of 24 hours in a desiccator.

Table No: 1 Formulation details of acyclovir microcapsules using Guar gum

Ingredients	FG-I	FG-II	FG-III	FG-IV	FG-V	FG-VI
Acyclovir (mg)	200	400	800	200	400	800
Guargum (mg)	2000	2000	2000	2000	2000	2000
Water (ml)	2	2	2	2	2	2
Solvent (ml)	30	30	30	30	30	30
Liquid paraffin (ml)	100	100	100	100	100	100
Span 80 (ml)	0.1	0.1	0.1	0.1	0.1	0.1
Glutardehyde (ml)	1	1	1	1	1	1
Speed (rpm)	1000	1000	1000	1500	1500	1500
Yield (%)	95.45	93.75	96.42	93.18	91.66	94.64
Entrapment Efficiency (%)	89.88	76.27	92.97	84.25	66.72	92.55

Table No: II Formulation details of acyclovir microcapsules using Egg albumin

Ingredients	FA-I	FA-II	FA-III	FA-IV	FA-V	FA-VI
Acyclovir (mg)	200	400	800	200	400	800
Egg albumin (mg)	2000	2000	2000	2000	2000	2000
Water (ml)	30	30	30	30	30	30
Solvent (ml)	Nil	Nil	Nil	Nil	Nil	Nil
Liquid paraffin (ml)	100	100	100	100	100	100
Span 80 (ml)	0.1	0.1	0.1	0.1	0.1	0.1
Glutardehyde (ml)	Nil	Nil	Nil	Nil	Nil	Nil
Speed (rpm)	1000	1000	1000	1500	1500	1500
Yield (%)	86.36	87.50	89.28	88.63	89.58	92.85
Entrapment Efficiency (%)	76.40	49.75	90.61	70.23	48.37	89.28

Table No: III Formulation details of acyclovir microcapsules using Ethyl cellulose

Ingredients	FE-I	FE-II	FE-III	FE-IV	FE-V	FE-VI
Acyclovir (mg)	200	400	800	200	400	800
Ethyl cellulose (mg)	2000	2000	2000	2000	2000	2000
Water (ml)	2	2	2	2	2	2
Solvent (ml)	30	30	30	30	30	30
Liquid paraffin (ml)	100	100	100	100	100	100
Span 80 (ml)	0.1	0.1	0.1	0.1	0.1	0.1
Glutardehyde (ml)	1	1	1	1	1	1
Speed (rpm)	1000	1000	1000	1500	1500	1500
Yield (%)	90.90	95.83	94.64	93.18	95.83	96.42
Entrapment Efficiency (%)	82.32	79.72	91.76	90.03	71.05	90.21

RESULTS AND DISCUSSSION

1. Entrapment efficiency of Acyclovir microcapsules

Determination of entrapment efficiency of acyclovir loaded microcapsules
About 100 mg of the sample was taken and 60 ml of 0.1 M sodium hydroxide was added and dispersed well for 15 minutes. Then sufficient quantity of 0.1 M Sodium hydroxide was added to produce 100 ml. Mixed well and filtered. 15 ml of the filtrate was taken and 50 ml of water and 5.8 ml of 2 M Hydrochloric acid and sufficient water was added to produce 100 ml. To 5ml of this solution sufficient 0.1 M Hydrochloric acid was added to produce 50 ml and mixed well. The absorbance of the resulting solution was measured at 255nm, using 0.1 M hydrochloric acid in the reference cell. The same procedure was followed for all the samples.

The quantity of acyclovir present in 100 mg of the sample taken was calculated by the following formula

$$\frac{\text{Absorbance}}{\text{A (1\%, 1cm)}} \times \frac{100}{\text{Weight taken}} \times \text{dilution factor} \times 1000$$

A (1%, 1cm) of Acyclovir = 560

The entrapment efficiency of the 18 formulations was done and it was found that formulation with Guar gum the minimum % entrapment efficiency was 66.72% and maximum was 92.91%. Formulation with Ethyl cellulose the minimum to maximum % entrapment efficiency was 71.05% to 91.76 %. Formulation with Egg albumin % entrapment efficiency the minimum to maximum was 49.75% to 90.61%. The entrapment efficiency of the formulation with guar gum in FG-III at 1000 rpm was 92.97% and FG-VI at 1500 rpm was 92.55% consequently. The entrapment efficiency of the formulations with the ethyl cellulose in FE-III at 1000 rpm was 91.76% and FE-VI at 1500 rpm was 90.21% consequently. The entrapment efficiency of the formulations with Egg albumin FA-III at 1000 rpm was 90.61% and FA-VI at 1500 rpm was 89.28 % consequently

2 Compatibility study using FT-IR:

FT-IR was done for pure drug and 18 microcapsules formulation for drug identification. It indicates no chemical reaction between drug and polymers and also confirmed the stability of the drug during micro encapsulation process. The characteristic peaks were due to pure Acyclovir at 615cm $^{-1}$, 1215 cm $^{-1}$,1440 cm^{-1},1515 cm^{-1},1610 cm $^{-1}$, 1627 cm^{-1} for Aromatic ring - NH_2 Aromatic , ether, - OH binding secondary amine, primary amine, C=C ring aromatic stretching, C=O stretching have appeared in microcapsules spectra peaks , without any change in their position after successful encapsulation . The pure drug spectra peaks correlated with the microcapsules formulations peaks.

3. Particle size analysis of microcapsules:

The particle size analysis of formulations was carried out by optical microscopy and the size range of maximum particles in formulation was found. FG-I to FG-III (140 -160µm), FG-IV to FG-VI (80-100µm), FE-I to FE-III (160 -180µm), FE-Ⅳ to FE-VI (140 - 160µm), FA-I to FA-III (140 - 160µm) and FA-IV to FA-VI (60 - 100µm). The size range of particles were assessed between 80µm and 320µm.

Fig. No. I Histogram of FG- III microcapsules

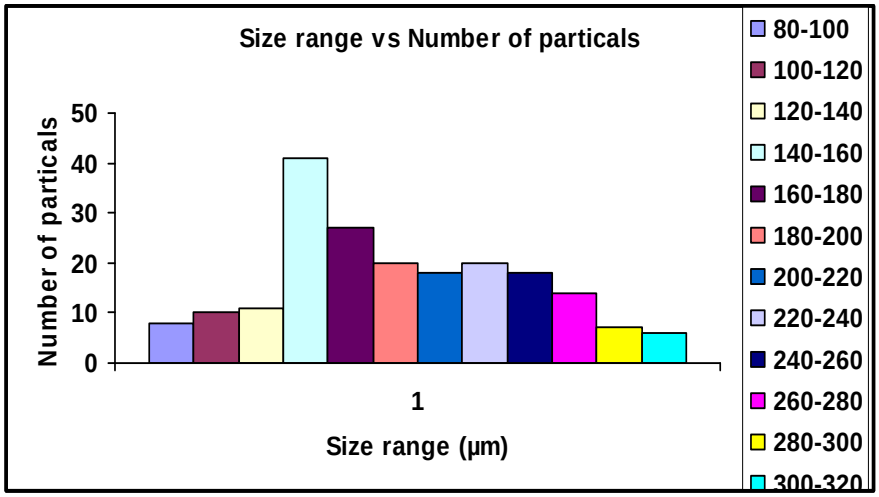


Fig. No : II Frequency distribution curve of FG- III microcapsules

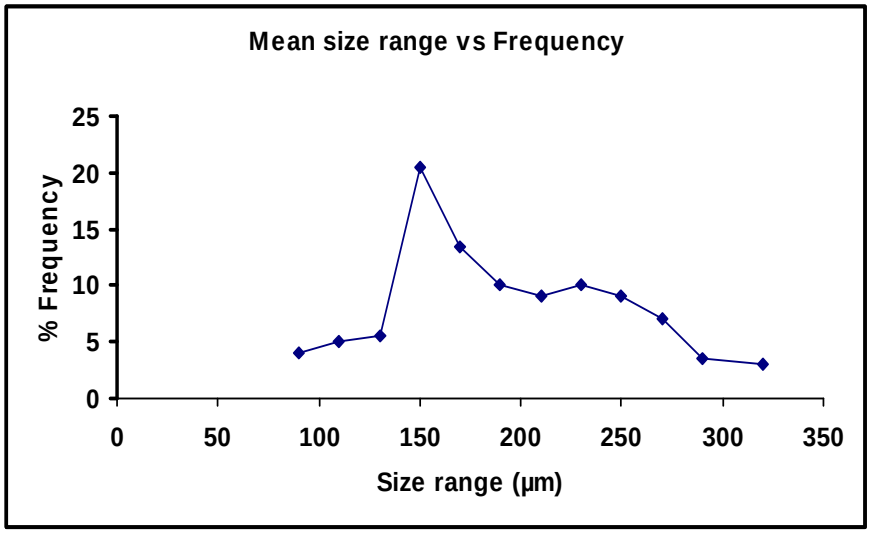


Fig. No. III Histogram of FG- VI microcapsules

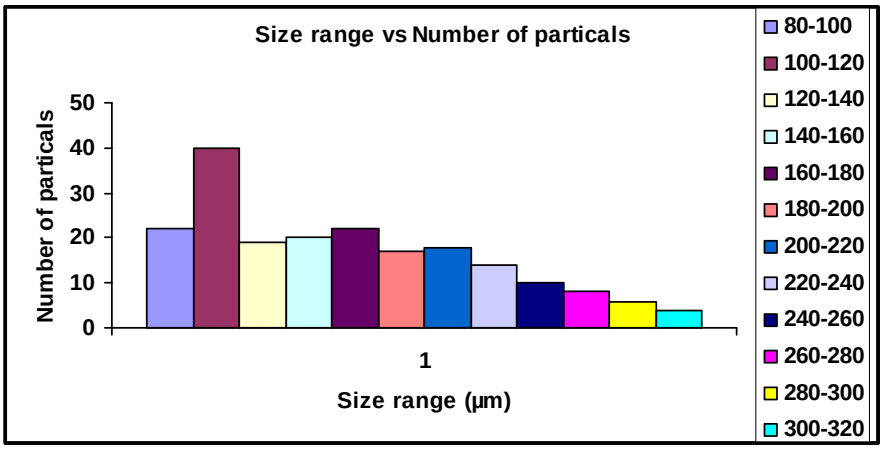
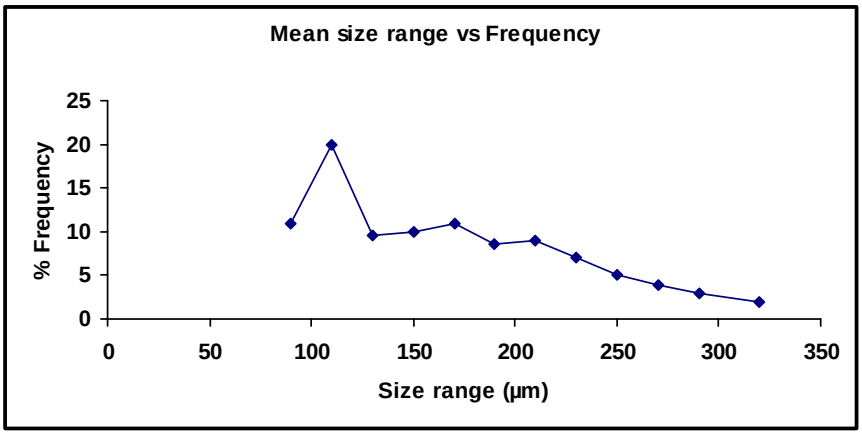


Fig. No : IV Frequency distribution curve of FG - VI microcapsules



4. Morphological evaluation of microcapsules:

The surface morphology of Acyclovir microcapsules was seen using Scanning Electron Microscope. The surface morphology was done with magnifications 50 x, and 2500x as shown .

SEM of Acyclovir Microcapsules

Fig No: V, FG- III (50x , 500 µm)

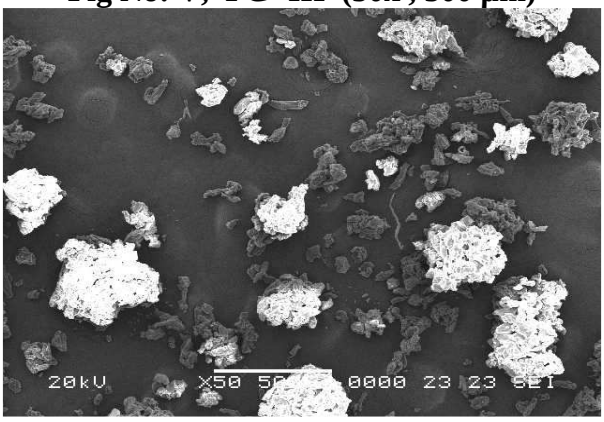


Fig No :VI, FG- III (2500x , 10 µm)

Fig No: VII, FG- VI (50x, 500 µm)

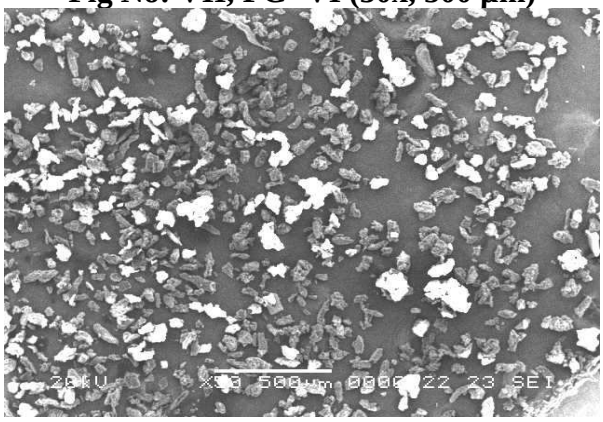
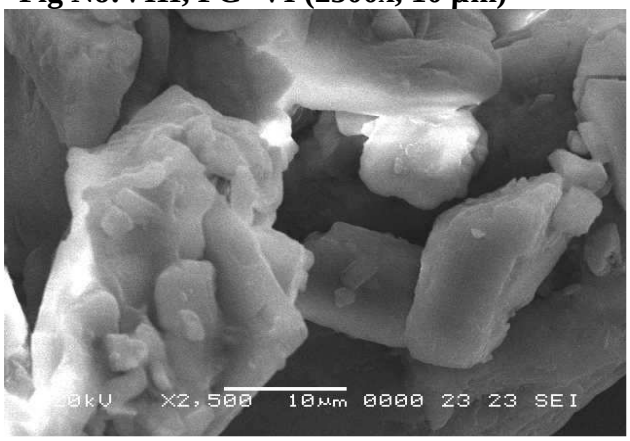


Fig No:VIII, FG- VI (2500x, 10 µm)



5. *In vitro* drug release study under simulated gastrointestinal conditions:

The in vitro dissolution apparatus used in the present study was specially designed. 100 mg of sample was weighed and placed in dialysis membrane. A 250 ml beaker with 187.5 ml of dissolution fluid was kept on a magnetic stirrer. A magnetic bead was placed and stirred at 100 rpm. The temperature of 250 ml beaker was maintained at 37±0.5°c. The dialysis membrane was tied at the bottom of two side open glass tube and it was immersed in the dissolution fluid so that it touches the surface of the dissolution fluid. Samples were withdrawn every half an hour for 2 hours in case of 0.1N HCl as dissolution medium and after two hours 62.5 ml of 0.2M tribasic sodium phosphate solution was added for change the pH 1.2 to 6.8 and samples were taken every 1 hour interval up to 6 hours. 5 ml of the sample was withdrawn and replaced with 5 ml of the dissolution medium. The samples were analyzed spectrophotometrically at 255 nm with suitable dilution. The dissolution as carried out for 18 samples using 0.1N HCl pH 1.2 and phosphate buffer pH 6.8 as dissolution medium. The release study was performed for eight hours.in simulated GI fluid. For FG-III at the end of the 2^{nd} hour the cumulative release was 38.76% and at the end of the 8^{th} hour it was 84.14%. For FG-VI at the end of the 2^{nd} hour the cumulative release was 40.11% and at the end of the 8^{th} hour it was 85.12%. For FE-III at the end of the 2^{nd} hour the cumulative release was 38.49% and at the end of the 8^{th} hour it was 81.93%. For FE-VI at the end of the 2^{nd} hour the cumulative release was 40% and at the end of the 8^{th} hour it was 86.72%. For FA-III at the end of the 2^{nd} hour the cumulative release was 38.76% and at the end of the 8^{th} hour it was 86.88%. For FA-VI at the end of the 2^{nd} hour the cumulative release was 39.70% and at the end of the 8^{th} hour it was 87.69%

Fig. No: IX, Comparative study of % Cumulative drug released vs Time FA – Formulations.

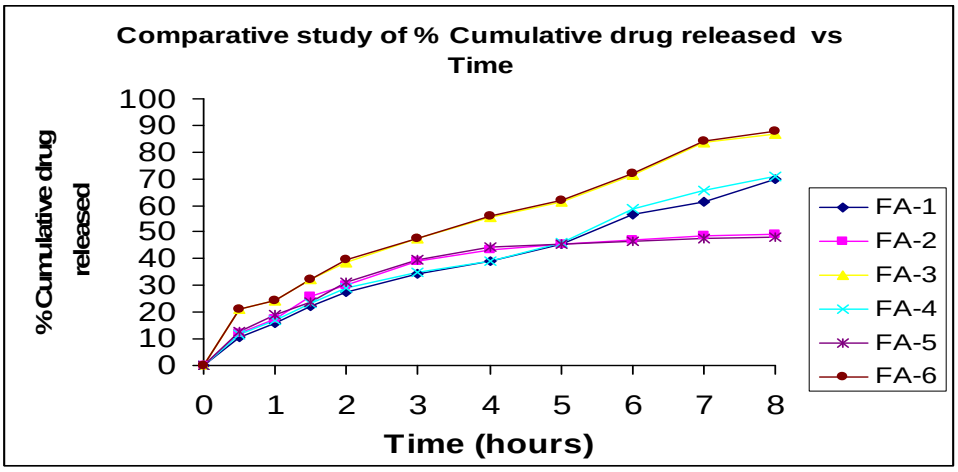


Fig. No: X, Comparative study of % Cumulative drug released vs Time FG – Formulations.

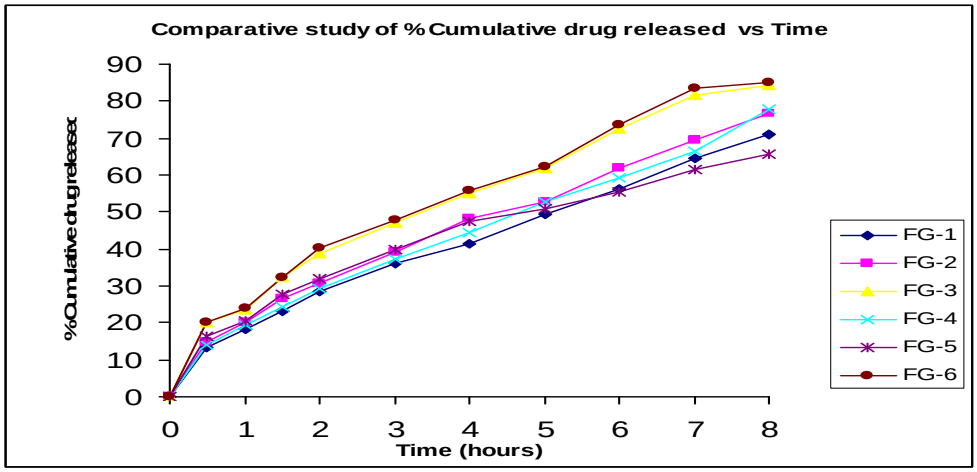
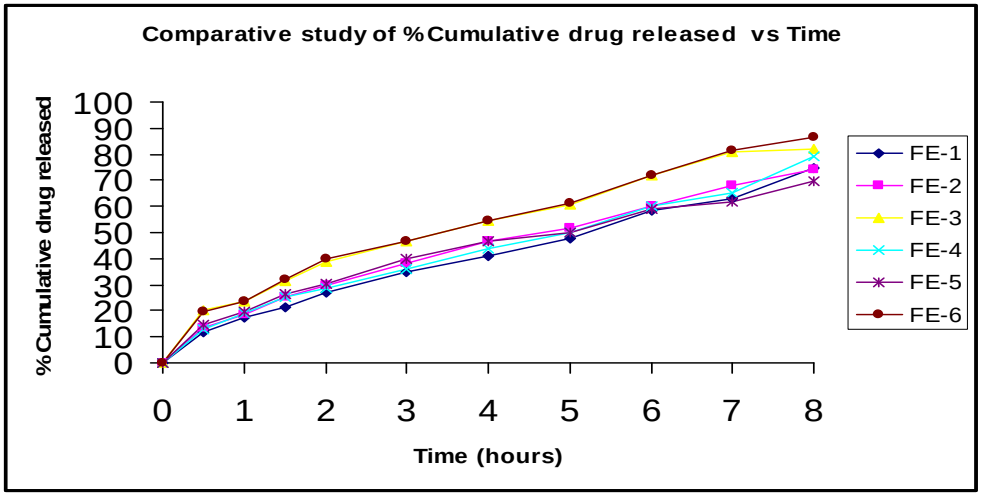


Fig. No. XI, Comparative study of % Cumulative drug released vs Time FE – Formulations



6. Mathematical modelling of Acyclovir microcapsules:

In order to investigate the mode of release of the microcapsules, the release data were analysed with the following mathematical models.

$Q_{1=} K_0^t$ (Zero Order kinetics)
Log $(Q_1/Q_0) = K_1^t/2.303$ (First Order Kinetics)
$Q_1 = K_{KP}^{tn}$ (Korsmeyer and Peppas equation)
$Q_t = K_H^{t1/2}$ (Higuchi's equation)

Where Q_t is the percent of drug released at time "t'', K_0 , K_1, K_{HC}, K_{KP}, and K_H are the coefficient of Zero order, First order, Korsmeyer-Peppas and Higuchi's equation.

CONCLUSION

From the entrapment efficiency done for the eighteen formulations it was seen that on increasing the rpm from 1000 to 1500 the entrapment efficiency decreased except in the case of the acyclovir microcapsules encapsulated with guar gum which was 92.97%. In the case of FT-IR after interpretation through the spectra it was confirmed that there were no major shifting of functional peaks between the spectra of the drug, polymer and the drug loaded microcapsules. It can be concluded from the IR spectroscopic studies that the drug acyclovir was entrapped in the polymer matrix and there was no chemical interaction because there was no shifting of the functional peaks From the SEM analysis it was seen that there was no burst in the encapsulated material. Surface was wavy in FG, porous in FE and smooth in FA formulations. The microcapsules were spherical, free flowing and non aggregated.

The rate of drug release follows a time dependent process based on fickian diffusion and korsemeyer- peppas model that the drug release is by diffusion and by erosion.

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