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Formulation and *in vitro* evaluation of floating microspheres of acyclovir

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ABSTRACT

The aim of the present work was to prepare floating microspheres of acyclovir to prolong residence time in stomach and to sustain the release of acyclovir. Acyclovir loaded floating microspheres were prepared by emulsion solvent diffusion method with combination of polymers (Ethyl cellulose and HPMC K4M). The resultant microspheres were evaluated for micromeritic properties, loss on drying, particle size, percentage entrapment efficiency, buoyancy, in vitro drug release and model fitting kinetics. Scanning electron microscope, Fourier transform infrared (FTIR) spectroscopy and differential scanning calorimetry were used to investigate the physical state of the drug in the microspheres. The particle size of the microspheres was in the range of 146.01-221.41µm. Percentage entrapment efficiency was between 63%-84% w/w. Microspheres remained buoyant for more than about 8 hours. The results of FTIR spectroscopy and differential scanning calorimetry indicated the stable character of acyclovir in microspheres and also revealed absence of drug polymer interaction. The formulation A3 showed results of *in vitro* drug released (91.24%) and acyclovir microspheres showed release from slow to sustained for more than 8 hours. Surface morphology by SEM analysis, and stability studies were carried out for the best formulation A3. All the stability studies for the formulation A3 showed no significant change in the percentage drug release studies and percentage buoyancy.

Keywords: Acyclovir, Floating microspheres, HPMC K4M, Ethyl cellulose.

INTRODUCTION

The most convenient method for controlled delivery is undoubtedly oral but oral controlled release formulations that exhibit greater absorption in stomach and upper small intestine have not been successfully prepared with conventional oral approach.

Acyclovir is a potent antiviral drug with low toxicity belonging to the deokiguanosin family. It is widely prescribed for the treatment of herpes simplex virus infections as well as in the treatment of varicella zoster infection. It has maximum absorption in stomach and upper part of small intestine. Due to low gastric retention, the bioavailability of drug is low (10-20%) as large portion of drug misses the absorption window when given orally owing to an important first pass metabolism. The recommended adult dosage of acyclovir is 200 mg twice daily or 400 mg once daily. The effective treatment of genital herpes simplex requires administration of 1000 mg of acyclovir in 5 divided doses a day. An alternative dose of 800 mg leads to plasma fluctuations, thus a sustained release dosage form of acyclovir is described. The short biological half life of drug (~2-3 hours) also favours development of a sustained release floating formulation.

MATERIALS & METHODS:

Acyclovir was supplied as gift sample by Wockhardt Pvt. Ltd, Aurangabad. And the assay value reported was 98.55 % as on basis. HPMC K4M and Ethyl cellulose was supplied as a gift sample by Axon Drugs Pvt Ltd, Chennai. Dichloromethane, Poly vinyl alcohol, Concentrated hydrochloric acid were purchased from Qualigens fine chemicals, Mumbai. and Triethyl citrate were purchased from Loba chemie Pvt, Ltd, Mumbai. The formulations were formulated using Mechanical Stirrer and Magnetic Stirrer (Remi equipments limited, Mumbai) and evaluated using USP tablet dissolution apparatus (Veego scientific VDA-8DR), UV Visible Spectrophotometer (ELICO SL159 / Shimadzu-1700 Pharmaspec), FTIR spectrophotometer (Shimadzu S4008), Digital pH meter (ELICO-LI120), Differential scanning calorimeter (Shimadzu DSC 60, Japan) and Particle size analyzer (Malvern Particle size analyzer (Master Seizer 2000)).

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PREPARATION OF FLOATING MICROSPHERES OF ACYCLOVIR BY EMULSION SOLVENT DIFFUSION TECHNIQUE

Floating microspheres containing acyclovir were prepared using emulsion solvent diffusion technique. For the preparation of floating microspheres, the rate controlling polymer used was ethyl cellulose and HPMC K4M in varying concentration (Drug: polymer, 1:0.5, 1:1.1, 1:1.5 and 1:2). Triethyl citrate (TEC) was added as a plasticizer in this formulation (10%). The method adopted for preparation is as follows:

The drug and polymer mixture (1:0.5, 1:1, 1:1.5 and 1:2) was dissolved in a dichloromethane (15ml) and plasticizer was added. The above mixture was dropped in a solution of polyvinyl alcohol (0.25%, 200 ml). The resultant solution was stirred with a mechanical stirrer for 1

hour at 500 rpm. The formed floating microspheres were filtered and washed with water and dried at room temperature and stored in a desiccator until further use.

Preformulation Studies:
Preformulation testing was an investigation of physical and chemical properties of a drug substance alone. Identification of drug was done by FTIR and melting point determination. Physiochemical properties and solubility studies were performed to know about the characteristic of drug. Determination of $?_{max}$ of drug and development of standard curve was performed using SGF (without enzyme). Drug polymer compatibility studies were performed by using FTIR & DSC, to evaluate the possible interactions between the active principle and the polymers.

Evaluation of non-effervescent floating microspheres:
Appearance, Percentage yield, Micromeritic properties (Bulk density, Tapped density, Angle of Repose, Carr's Index and Hausner's Ratio) and Loss on drying, were evaluated for formulated microspheres, Percentage Buoyancy, Drug Content Uniformity, Drug entrapment efficiency, *in-vitro* drug release studies, kinetics of *in-vitro* drug release were evaluated. Particle size determination, Surface morphology using SEM, and stability studies were carried out for selected best formulation.

Buoyancy Test:
Floating microspheres (50 mg) were placed in 0.1 N HCl (100 ml). The mixture was stirred at 100 rpm in a magnetic stirrer. The layer of buoyant microspheres was pipetted and separated by filtration at 1, 2, 4 and 8 hours. The collected microspheres were dried in a desiccator over night. The percentages of microspheres were calculated.

Entrapment Efficiency:
The various batches of the floating microspheres were subjected to estimation of drug content. The floating microspheres equivalent to 50 mg of acyclovir from all batches were accurately weighed and crushed. The powdered of microspheres were dissolved in ethanol (10 ml) in volumetric flask (100ml) and made the volume with 0.1 N HCl. This solution is then filtered through Whatmann filter paper No. 44. After filtration, from this solution accurate quantity (10 ml) was taken and diluted up to 100 ml with 0.1 N HCl. From this solution, accurate volume (2 ml) was pipette out and diluted up to 10 m1 with 0.1 N HCl and the absorbance was measured at 255 nm against 0.1 N HCl as a blank.

***In vitro* Drug release studies:**
In vitro release of acyclovir from floating microspheres was carried out using the USP dissolution test apparatus (Type-I). A weighed amount of floating microspheres equivalent to 200 mg of drug were filled into a capsule and placed in the basket. Dissolution media used was 900 ml of 0.1 N HCl (pH 1.2) maintained at 37 ± 0.5°C and stirred at 100 rpm. At predetermined time intervals, 10 ml of sample was withdrawn and replaced with equal amount of 0.1 N HCl (pH 1.2). The collected samples were filtered and suitably diluted with 0.1 N HCl and analyzed spectrophotometrically at 255 nm to determine the amount of drug released in the dissolution medium.

Kinetics of *in vitro* drug release:
To study the release kinetics of *in-vitro* drug release, data was applied to kinetic models such as Zero order, First order, Higuchi and Korsmeyer- Peppas.

Stability Studies:
The purpose of stability testing was to obtain a stable product which assures its safety. Floating microspheres (equivalent to 100 mg of active substance) was filled into capsule shell. Floating microspheres were tested for three months stability studies. *In vitro* drug released and buoyancy parameters were analyzed at the time interval of one month.

RESULTS AND DISCUSSION:
The identification of drug was carried out by FTIR spectroscopy and melting point. The physicochemical parameters such as color, odor, taste, solubility study and loss on drying were performed. The analytical profile of drug was evaluated for determination of absorption maximum, development of standard curve and percentage purity of drug.

Compatibility of drug and polymer mixtures were done by performing FTIR and DSC study. It was concluded that there were no interaction between drug and polymers.

Eight different formulations were prepared with different concentration and combination of polymers (Ethyl cellulose and HPMC K4M). Acyclovir microspheres were prepared by emulsion solvent diffusion method. All the formulations were evaluated for Appearance, Percentage yield, Micromeritic properties, Particle size, Loss on drying, Buoyancy test, Entrapment efficiency, *In vitro* drug release and Kinetics of *in vitro* drug release.

Percentage yield of microspheres were in the range of 68.44 % to 74.76 %. The observed bulk density was in the range of 0.689 to 0.836 g/cm^3, Tapped density was in the range of 0.735 to 0.912 g/ cm^3, Carr's index was in the range of 6.53 to 7.91 %, Hausner's ration was in the range of 1.04 to 1.11 and angle of repose was in the range of 19.41°C to 26.46 °C. All the formulations were having excellent flow properties.

Buoyancy percentage was found to be in the range of 56.30 to 62.56 % and the entrapment efficiency was in the range of 63.00 to 84.89 %. On comparing the major criteria in evaluation such as *in-vitro* drug release, Buoyancy percentage and entrapment efficiency, the Formulation A3 showed results of *in vitro* drug released (91.24%), Buoyancy percentage (62.56%) and entrapment efficiency (84.89%), were compared with all formulations A1 to A4 and B1 to B4. The buoyancy percentage, entrapment efficiency and *in vitro* drug release of

Table 1: Composition of acyclovir floating microspheres

S. No	Formulation code	Drug (Acyclovir) (gm)	Polymer (gm)		Plasticizer (TEC) (%)
			Ethyl cellulose	HPMC	
1	A1	1	0.5	-	10
2	A2	1	1	-	10
3	A3	1	1.5	-	10
4	A4	1	2	-	10
5	B1	1	-	0.5	10
6	B2	1	-	1	10
7	B3	1	-	1.5	10
8	B4	1	-	2	10

Table 2: Micromeritic properties

Formulation code	Bulk Denity gm/cm^3	Tapped Density gm/cm^3	Angle of Repose (°)	Hausner's Ratio	Percentage Yield(%)
A1	0.693± 0.026	0.742± 0.029	19.41 ± 0.41	1.07 ±0.22	69.7 ± 0.02
A2	0.689 ± 0.027	0.735 ± 0.00	20.18 ±0.57	1.04 ±0.34	68.6 ± 0.04
A3	0.774 ± 0.030	0.833 ± 0.035	22.53 ± 0.63	1.08 ±0.76	74.76 ± 0.03
A4	0.791 ± 0.033	0.857 ± 0.039	24.74 ± 0.62	1.08 ±0.12	74.54 ± 0.02
B1	0.806 ± 0.062	0.873 ± 0.045	20.09 ± 0.90	1.11 ±0.86	72.55 ± 0.04
B2	0.813 ± 0.063	0.881 ± 0.074	25.25 ± 0.67	1.08 ±0.45	69.9 ± 0.06
B3	0.836 ± 0.003	0.912 ± 0.046	26.46 ± 0.65	1.06 ±0.44	68.44 ± 0.08
B4	0.832 ± 0.062	0.902 ± 0.073	26.16 ± 0.35	1.08 ±0.87	69.16 ± 0.02

All the values are average of three determination ± S.D

Table 3: Buoyancy and entrapment efficiency

Formulation No	Buoyancy Test (%)	Entrapment Efficiency (%)
A1	57.87 ± 1.85	74.85 ± 0.19
A2	59.1 ± 1.37	69.83 ± 0.22
A3	60.31 ± 0.85	84.89 ± 0.22
A4	56.3 ± 1.69	81.55± 0.19
B1	62.56± 1.43	72.82± 0.43
B2	58.50± 1.23	63.00± 0.19
B3	56.81± 3.16	79.59± 0.19
B4	56.73 ± 1.35	73.01± 0.22

All the values are average of three determination ± S.D

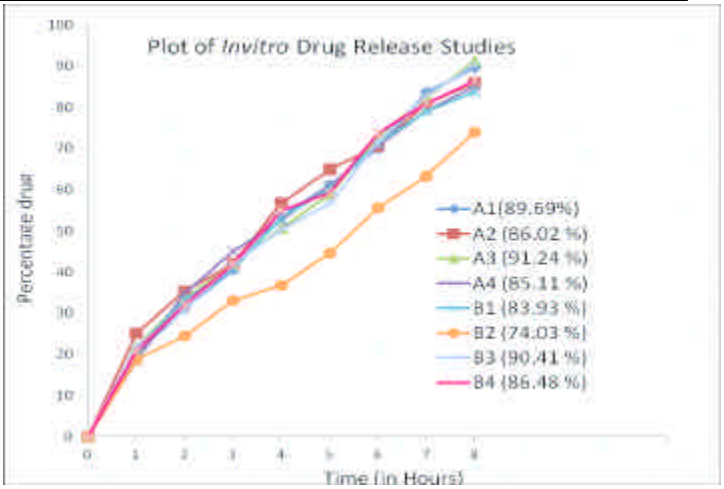

Figure 1: In vitro drug release profile of A1 to A4 and B1 to B4

Table 4: Different kinetic models for acyclovir floating microspheres

Formulation No.	Zero Order R^2	First Order R^2	Higuchi R^2	Peppas R^2	n	Best Fit Model
A1	0.9847	0.9605	0.9713	0.9972	0.7284	Peppas
A2	0.9560	0.9878	0.9981	0.9938	0.6136	Higuchi
A3	0.9814	0.9490	0.9725	0.9946	0.6864	Peppas
A4	0.9685	0.9882	0.9840	0.9966	0.7138	Peppas
B1	0.9701	0.9895	0.9831	0.9881	0.6743	Peppas
B2	0.9816	0.9726	0.9619	0.9809	0.6599	Zero order
B3	0.9835	0.9489	0.9679	0.9911	0.6928	Peppas
B4	0.9745	0.9800	0.9794	0.9978	0.6946	Peppas

Table 5: Stability studies of best formulation (A3)

Stability chamber	Time	Appearance	Drug content (%)	% drug release
40±2^{0}C with 75±5% RH	Initial	White	74.76±0.33	91.24±0.56
	1st month	No change	74.64±0.34	91.17 ± 0.82
	2nd month	No change	74.11±0.22	90.82 ± 0.75
	3rd month	No change	73.92±0.77	89.97 ± 0.68

All the values are average of three determination ± S.D

microspheres were increased in formulations with ethyl cellulose. The formulation A3 was selected as the best formulation among the eight formulations.

The kinetics of *in-vitro* drug release studies were determined by applying the drug released data to various kinetic models such as zero order, first order, Higuchi and Korsmeyer - Peppas. The formulation A3 was best fitted with Peppas model kinetics. All the stability studies for the formulation A3 showed no significant change in the percentage drug release studies and percentage buoyancy. The formulation A3 was concluded best formulation among the formulations were prepared.

CONCLUSION

The results obtained from this investigation are interesting and promising. The objective of the present investigation was to improve oral bioavailability of the poorly water soluble drug. For better absorption and enhanced bioavailability of some drug, prolongation of retention time of the dosage form in the stomach is essential. This problem can be solved by preparation of gastro-retentive drug delivery systems. An attempt was made to prepare floating microspheres of acyclovir using ethyl cellulose and HPMC. Ideal properties of floating microspheres include high buoyancy and sufficient release of drug in acidic condition. The prepared formulation (A3) showed best appropriate balance between buoyancy and drug release rate.

The developed formulation overcomes the drawbacks and limitations of sustained release preparations. Therefore multiple unit floating system, i.e., floating microsphere will be possibly beneficial with subject to sustain action. Major advantages of prepared formulation include, Easy of preparation, Good particle size, Good buoyancy and Sustained release over several hours . From the results obtained, it can be concluded that the objective of present study is achieved.

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