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Research Article

FORMULATION AND INVITRO EVALUATION OF FLOATING MICROSPHERES OF ACETAZOLAMIDE

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ABSTRACT

The aim of present work was to prepare floating microspheres of Acetazolamide to prolong residence time in stomach and to sustain the release of Acetazolamide. The floating microspheres were prepared by solvent evaporation method using different polymers like ethyl cellulose and hydroxypropyl methyl cellulose (different ratios of 1:1, 1:2.1:3, 1:4, 1:5 & 1:6), in the mixture of dichloro methane and ethanol at ratio of (1:1), with Tween 80 as the surfactant. The average diameter and surface morphology of the prepared microsphere were characterized by optical microscope and scanning electron microscopic methods respectively. The particle size of microspheres was in the range of 100-600 µm. The results of FT-IR spectroscopy indicated the stable character of Acetazolamide in microspheres and also revealed absence of drug polymer interaction. Percentage encapsulation efficiency was between 25.1% - 68.2 %. Microspheres remained buoyant for 12 hrs. The invitro drug release study showed that Acetazolamide released from the microspheres was slow and sustained for 12hrs. The invitro drug release revealed that batch F 2 was having 97.4 % cumulative release at the end of 12 hrs when compared with others batches, due to lower polymer to drug ratio there was a significant increase in drug release, seen at the 1:2 ratio. Formulation F 2 found to be best formulation among the other formulation.

Keywords: Gastro retentive dosage form, Floating microspheres, Acetazolamide, Invitro drug release.

INTRODUCTION

Controlled drug delivery systems have been developed which are capable of controlling the rate of drug delivery, sustaining the duration of therapeutic activity and/or targeting the delivery of drug to a tissue [1]. Floating microspheres are gastroretentive drug delivery systems based on non effervescent approach. Hollow microspheres are in strict sense, spherical empty particles without core. These microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, ideally having a size less than 200 micrometer. Solid biodegradable microspheres incorporating a drug dispersed or dissolved throughout particle matrix have the potential for controlled release of drugs. Gastro-retentive floating microspheres are low-density systems that have sufficient buoyancy to float over gastric contents and remain in stomach for prolonged period. As the system floats over gastric contents, the drug is released slowly at desired rate resulting in increased gastric retention with reduced fluctuations in plasma drug concentration.

When microspheres come in contact with gastric fluid the gel formers, polysaccharides, and polymers hydrate to form a colloidal gel barrier that controls the rate of fluid penetration into the device and consequent drug release. [2] As the exterior surface of the dosage form dissolves, the gel layer is maintained by the hydration of the adjacent hydrocolloid layer. The air trapped by the swollen polymer lowers the density and confers buoyancy to the microspheres. However a minimal gastric content needed to allow proper achievement of buoyancy. Hollow microspheres of acrylic resins, Eudragit, polyethylene oxide, and cellulose acetate; polystyrene floatable shells; polycarbonate floating balloons and gelucire floating granules are the recent developments. [3]

Acetazolamide is an Anti epileptic and Anti hypertensive agent [4], the conventional dosage form of the tablets results in poor bioavalablity [5], it can be efficiently and successfully formulated by non-aqueous solvent evaporation method by improving the solubility of the drug. Gastric retention will provide advantages such as delivery of drugs with narrow absorption windows in the stomach or the proximal part of the small intestine.

MATERIALS AND METHODS

Acetazolamide was received as a gift sample from Glen mark generic Ltd in Mumbai. HPMC K15M and Ethyl cellulose 25cps were purchased from Molychem. Mumbai. All other materials were used in the experiment are of analytical grade.

Preformulation studies

The term preformulation is self explanatory, i.e., the study conduct very beginning stage of the dosage form development. This is necessary since the physicochemical properties of the drug must be known to design a suitable dosage form therefore, before the formulation of floating microspheres of Acetazolamide; experiments were conducted to understand the physicochemical properties of the drug and excipient and their compatibility. [6]

Drug-excipient compatibility studies

The successful formulation of a suitable and effective solid dosage form depends upon the careful selection of the excipients. Excipients are added to facilitate administration, promote the consistent release and bioavailability of drug. It's necessary to study the compatibility of excipients with drug. Here IR spectroscopy was used to investigate and predict any physicochemical interaction between components in a formulation and to the selection of suitable compatible excipient. [7]

Infra red spectrophotometer (IR)

Infrared (IR) spectroscopy was conducted and the spectrum was recorded in the wavelength region of 4000 to 400 cm^{-1}. The procedure consisted of dispersing a sample (drug alone, polymers alone and mixture of drug and Polymers in KBr and compressing into discs by applying a pressure of 7 tons for 5 min in a KBr press. The pellet was placed in the light path and the spectrum was obtained.

Determination of absorption maximum (λ_{max}) of Acetazolamide

A spectrum of the working standards was obtained by scanning from 200-400nm against the reagent blank to fix absorption maxima. The λ_{max} was found to be 265 nm. Hence all further investigations were carried out at the same wavelength.

Solubility studies

The solubility of the drug in the gastric content is an important criteria for the dissolution and absorption. To understand the solubility profile of Acetazolamide, saturation solubility studies were

carried out in various media with varying P^H range. The solubility was determined in 0.1N HCl, phosphate buffer 6.8 and 7.8. [8]

Methods of formulation:

Selection of polymers for formulation of floating microspheres

Ethyl cellulose and HPMC K15M was used as an excipient for sustained drug release formulations. For the formulation of floating microspheres of Acetazolamide, chosen ethyl cellulose 25cps and HPMC K15M as the coating material. [9]

Selection of method of formulation of floating microspheres

a) Aqueous-/Non-aqueous solvent evaporation method [9]

The choice of the method of formulation is mainly governed by the nature of the drug to be encapsulated, i.e., whether the drug is hydrophilic or hydrophobic one. As the drug chosen for the formulation as the floating microspheres of Acetazolamide is a water soluble drug the choice of the dispersion medium was non-aqueous media. The selection of an appropriate dispersion medium helps to increase the encapsulation efficiency by minimizing the drug loss into the dispersion media during the formulation process.

Selection of the dispersion medium

a) Light liquid paraffin/Heavy liquid paraffin

The non-aqueous media available are various vegetable oils and mineral oils. The vegetable oils (arachis oil, sun flower oil) are more prone to microbial contamination and rancidity the selection of an inert media was added. Mineral oils such as heavy liquid paraffin and light liquid paraffin were the next candidates for the selection criteria. Among the mineral oils, heavy liquid paraffin posed a challenged of consuming a lot of time for filtration, light liquid paraffin was opted as the processing medium for the formulation of microspheres. Light liquid paraffin is considered safe by the FDA direct use in foods and it is GRAS (Generally Regarded As safe) listed excipient. [10]

Volume of dispersion medium [9]

The volume of dispersion medium was also an important parameter for the optimization of formulation variables. Larger volume of the dispersion medium more is the chance for the dispersion of the drug and polymer solution ultimately affecting the size of the microspheres. Microspheres were prepared using 30 ml of the dispersion medium.

Formulation of floating Microspheres

The present study was carried out to develop floating microspheres of Acetazolamide in order to improve patient compliance and also to prepare user-friendly formulation.

In this case, six formulations of floating microspheres were prepared using different polymers such as ethyl cellulose-25cps and HPMC K15M, dichloromethane and ethanol as solvent mixture in 1:1 ratio. The detailed composition of each formulation is given in the table 2. The prepared floating microspheres are shown in the Fig. 1.

Fig. 1: Floating behavior of Hollow Microspheres in 0.1 N HCl containing Tween 80 (0.02%) after stirring for 12 h.

Floating microspheres containing Acetazolamide as a core material were prepared by the non-aqueous solvent evaporation method.[11] Briefly, the polymer was dissolved in the 1:1 ratio of solvent mixture of dichloromethane and ethanol to this mixture drug was added. The solution of drug and polymer mixture was poured drop by drop into light liquid paraffin while being stirred at 1200 rpm by a mechanical stirrer equipped with a three bladed propeller at room temperature. The stirred was continued for two hours (2 hrs) to allow to solvents (Dichloromethane, ethanol) to evaporate completely and the formed microspheres were collected by filtration. The microspheres were washed repeatedly with petroleum ether until free from oil. The collected microspheres were dried at room temperature for 24 hours then store in desiccators.

The schematic representation of formulation of floating microspheres of Acetazolamide was given below; it gives the formulation specifications of the prepared floating microspheres.

Schematic representation of formulation of floating microspheres of Acetazolamide

Step I-(A) Drug +Polymer dissolved in solvent mixture

Step II-(B) (A) - was added to light liquid paraffin

Step III- While stirred at 1200 rpm/ 2hrs

Step IV- Filtered and washed with petroleum ether

Step V- Drying at room temperature

Table 2: Formulation development of Acetazolamide floating microspheres

Formulation code	Drug: EC : HPMC	Dichloromethane: Ethanol	Dispersion medium(ml)
F1	1:1:1	1:1	30
F2	1:1:2	1:1	30
F3	1:1:3	1:1	30
F4	1:1:4	1:1	30
F5	1:1:5	1:1	30
F6	1:1:6	1:1	30

***** Each formulation contains 250mg of Acetazolamide

RESULTS AND DISCUSSION

Standard graph of Acetazolamide in 0.1 N HCl

The concentration of Acetazolamide and the corresponding absorbance were given in the table.3 and the plot of concentration verses absorbance was shown in Fig.2. The solution obeyed Beer-Lambert's law over a concentration range of 2 µg -18 µg /ml with a regression co-efficient of 0.9976. This standard curve was used further to estimate Acetazolamide in the in vitro studies.

Table 3: Standard graph values of Acetazolamide

S. No.	Concentration (µg/ ml)	Absorbance (265nm)
1	2	0.132
2	4	0.224
3	6	0.326
4	8	0.426
5	10	0.524
6	12	0.627
7	14	0.731
8	16	0.796
9	18	0.892
Slope		0.0257
Regression value		0.9976

Pre-Formulation Studies

Before formulation of drug substances into a dosage form, it is essential that drug polymer should be chemically and physically characterized. Preformulation studies give the information needed to define the nature of the drug substance and provide a framework for the drug combination with pharmaceutical excipients in the fabrication of a dosage form.

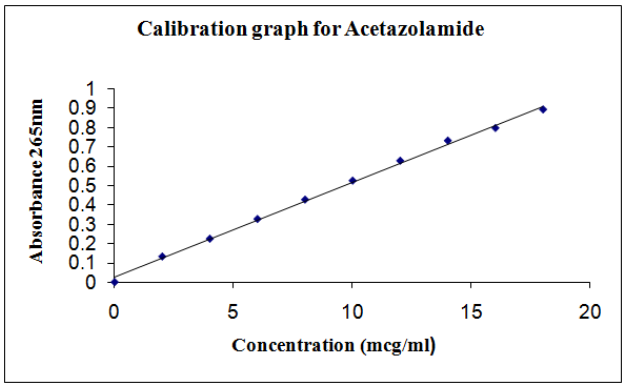

Fig. 2: Standard graph of acetazolamide in 0.1 N HCl

Compatibility studies

One of the requirements for the selection of suitable excipients or carrier for pharmaceutical formulation is its compatibility. Therefore in the present work a study was carried out by using IR spectrophotometer to find out if there is any possible chemical interaction of Drug with Ethyl cellulose-25cps and HPMC K15M.

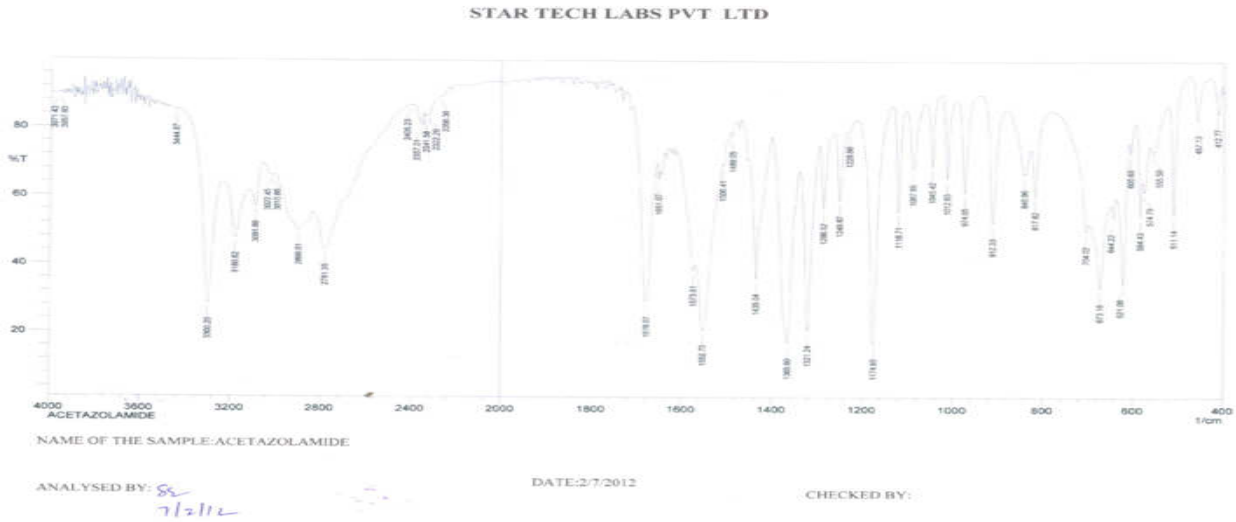

Fig. 3: FTIR spectra of Acetazolamide

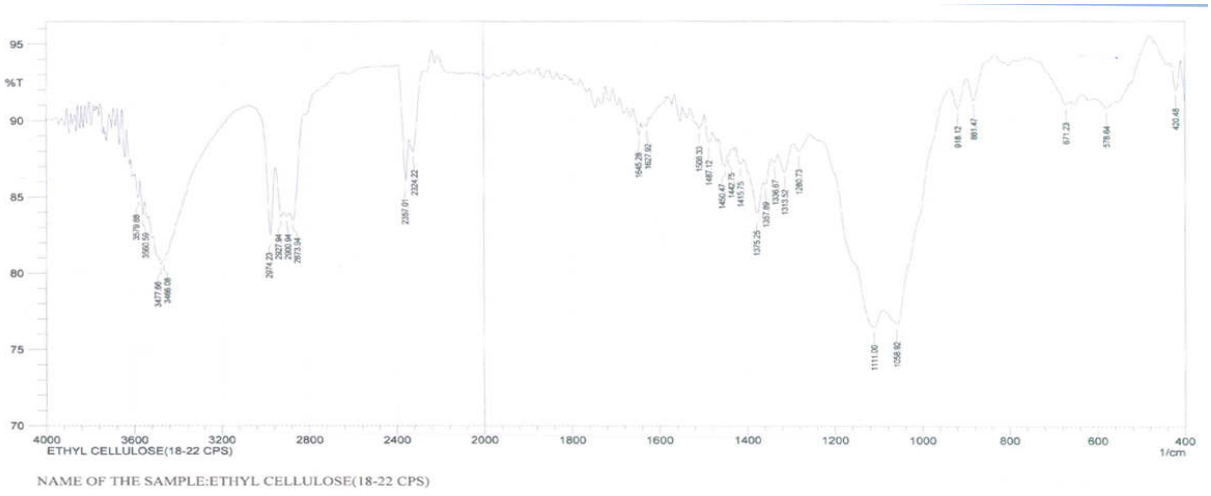

Fig. 4: FTIR spectra of ethyl cellulose-25cps

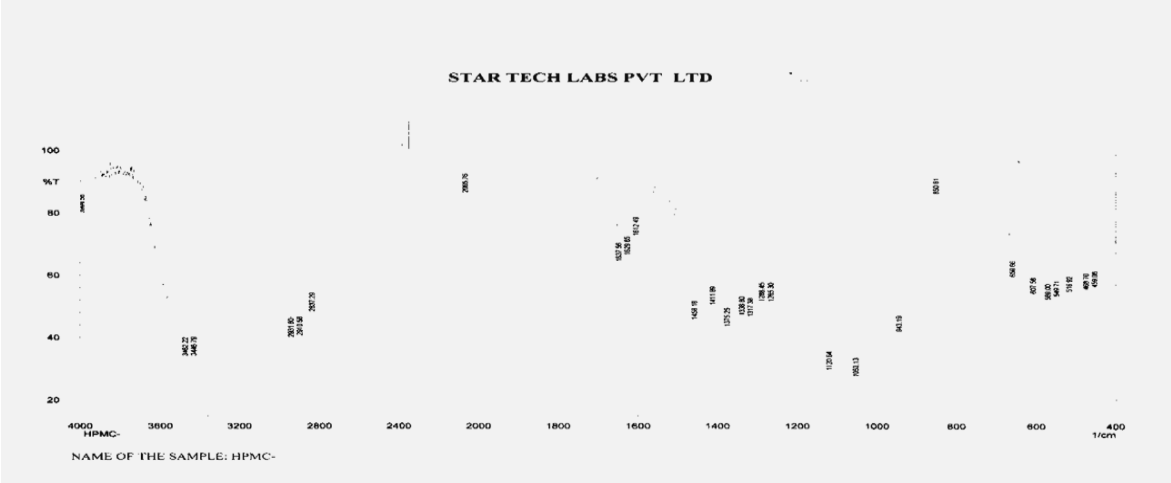

Fig. 5: FTIR spectra of HPMC K15M

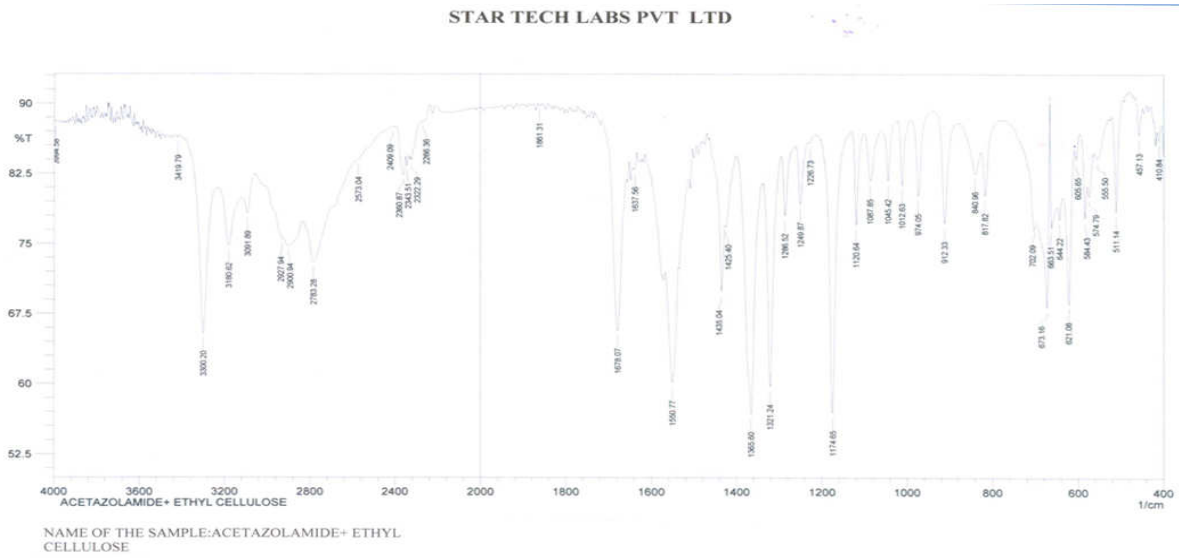

Fig. 6: FTIR spectra of Drug and ethyl cellulose-25cp

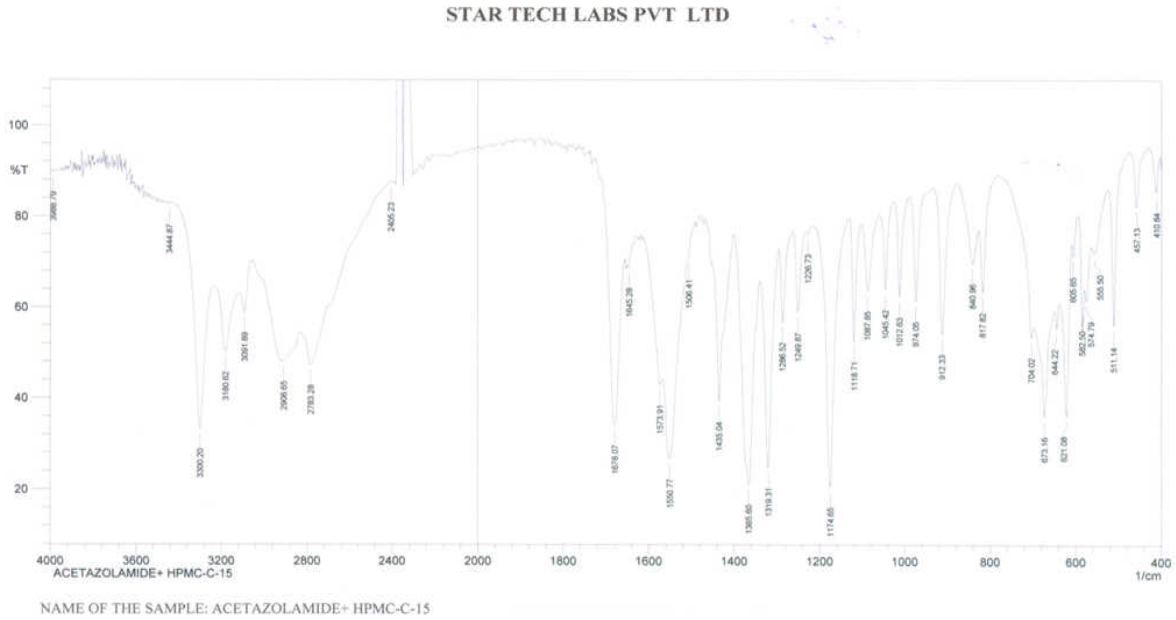

Fig. 7: FTIR spectra of drug and HPMC K15M

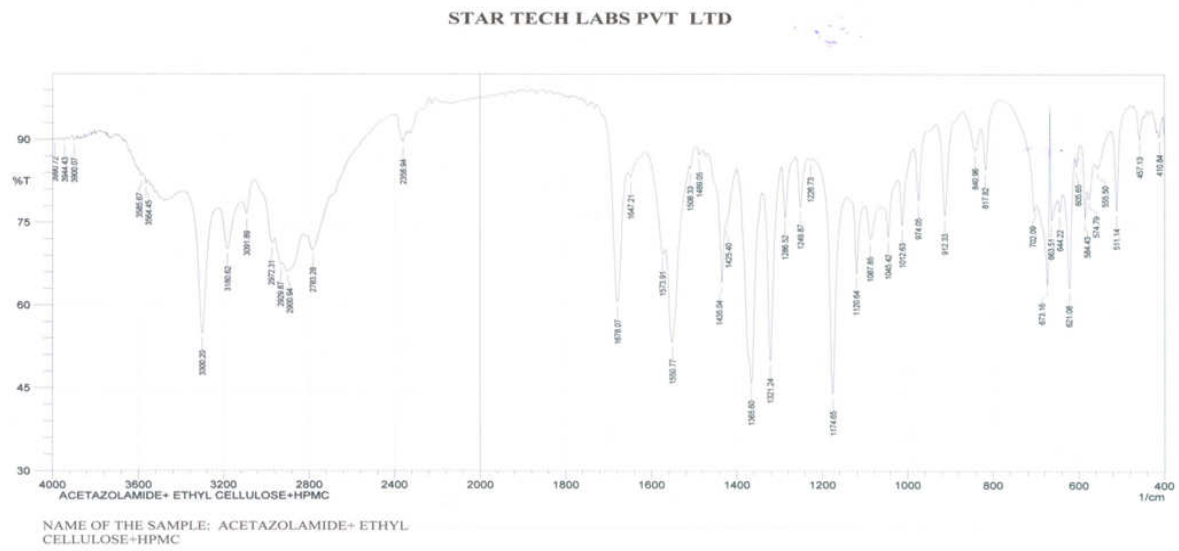

Fig. 8: FTIR spectra of drug, ethyl cellulose-25cps and HPMC K15M

Solubility studies

The solubility of Acetazolamide was determined in various pH ranges. The solubility of Acetazolamide was found to be 800 mg/ ml (pH 1.2). 340mg/ml in phosphate buffer pH 6.8 and 200 mg/ml in phosphate buffer pH 7.8. The results revealed that as the pH of the solution increased, the solubility of Acetazolamide decreased.

Micromeritic properties

Floating microspheres were found to be spherical in and having internal hollow, the flow properties of microspheres were listed in the table 4. Flow properties of batches were evaluated by measuring the angle of repose and compressibility index. In the evaluation of flowability of dry solid ,the substances shows excellent flowability of performance, when the angle of repose have the value less than 23^0 while when compressibility index has value below 13.35, no aid is needed for enhancing the flowability of power. Thus, angle of repose and compressibility index are indicates of good flowability of floating microspheres, showing no need for addition of glidants to enhance flowability.

SEM studies

Surface properties and internal structure of microspheres had been revealed by scanning electron microscopy (SEM). The microphotographs of cross section and surface view of microspheres of optimized batch F6 are shown in fig.3. The Fig. shows the smooth surface of the microspheres indicates that the microspheres produced by the non-aqueous solvent evaporation method are spherical with smooth surface and not aggregated. Their smooth surface indicated that Acetazolamide was embedded in the shell, as the drug particles were not present on the surface of the floating microspheres.

Table 4: Flow properties of microspheres

Formulation Code	Angle of repose	Bulk density (gm/cm^3)	Tapped bulk density(gm/cm^3)	Carr's Index
F1	18º96´	0.469	0.508	07.67
F2	19º73´	0.492	0.505	02.57
F3	20º21´	0.414	0.545	08.81
F4	20º14´	0.510	0.56	08.92
F5	21º61´	0.487	0.503	03.18
F6	23º74´	0.414	0.485	13.35

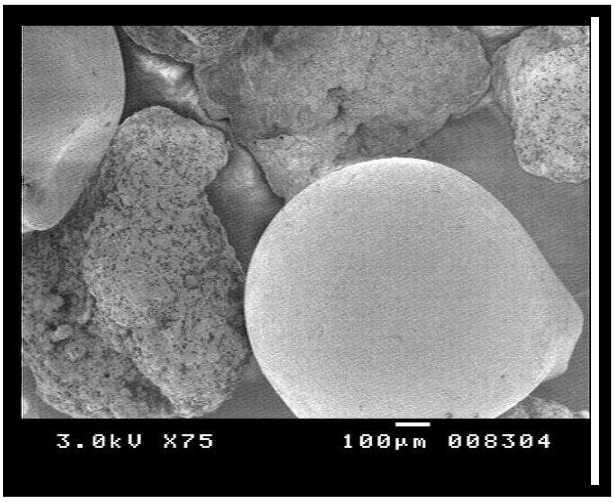

Fig. 9: Particle morphology of optimized floating microspheres shows the smooth surface

Post-Formulation Studies

Percentage yield of Acetazolamide floating microspheres

The yields of the individual formulations were calculated and a bar graph represents the yield of various formulations. The yield was high for the F6 amounting of 86.5% and the yield of F1 was low amount among all formulations to 61.6%.

Drug entrapment efficiency (DEE)

The drug entrapment efficiency of the prepared formulations were calculated and the results were given below. The entrapment efficiency was found to be 25.1% to 68.2% for formulation F1 to F6. The results obtained clearly indicated that the drug entrapment efficiency increased as the drug to polymer ratio increased. This may be attributed to the availability of more coat material per drug molecule. However, the entrapment efficiency was attributed to mixing technique used, physical compatibility with polymer used, and viscosity of media. Particle size may also play a part in the entrapment of drug.

Buoyancy Percentage

The buoyancy percentage for all batches was almost above 70%, which was studied for 12 h. The highest percentage was obtained with formulation F6. Average buoyancy in percentage was found to be 72.8% to 89.6%. In general with increase in the amount of polymers, there was an increase in the buoyancy percentage. The increase in the buoyancy percentage may be attributed to air and gel forming polymer HPMC K15M which caused swelling because of increased amount of the polymers present.

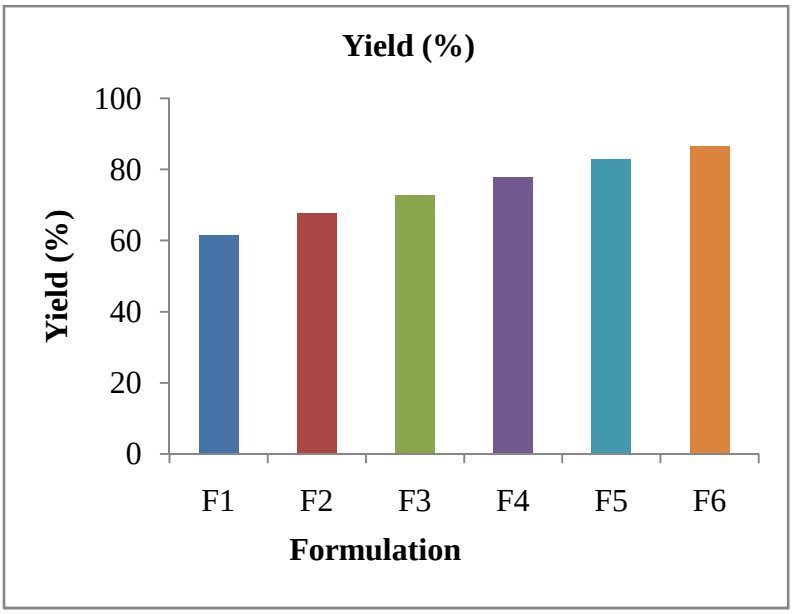

Fig. 10: Percentage yield of F1-F6 formulations

Table 5: Percentage of yield, Entrapment efficiency and Buoyancy Percentage data for F1-F6 formulations

Batch. No	yield (%)	DEE (%)	BP (%)
F1	61.6	25.1	72.8
F2	67.5	27.33	73.4
F3	72.8	36.4	79.5
F4	77.7	40.08	82.7
F5	82.7	53.6	85.6
F6	86.5	68.2	89.6

DEE- Drug entrapment efficiency, **BP**-. Buoyancy Percentage

Table 6: In vitro drug release data for F1-F5 formulations

Time(hrs)	F1	F2	F3	F4	F5	F6
0.5	41.33	30.57	27.78	12.62	11.83	10.04
1	44.09	36.2	32.23	30.37	24.04	22.69
2	45.4	40.1	38.6	37.6	34.36	31.62
3	47.2	44.50	42.5	40.7	39.02	38.53
4	50.2	49.8	47.9	45.05	42.32	41.1
5	55.6	54.12	51.2	50.24	47.6	44.4
6	66.8	65.06	62.8	60.8	50.7	46.6
7	74.5	73.2	72.2	63.6	53.8	48.7
8	77.5	76	75.9	64.1	56.12	50.6
9	84.3	80.3	78.4	69.8	59.08	55.4
10	88.1	85.2	80.5	70.9	63.9	57.8
11	96.6	96.2	86	73.2	65.51	59.3
12	99.6	97.4	87.9	75.6	66.8	61.04

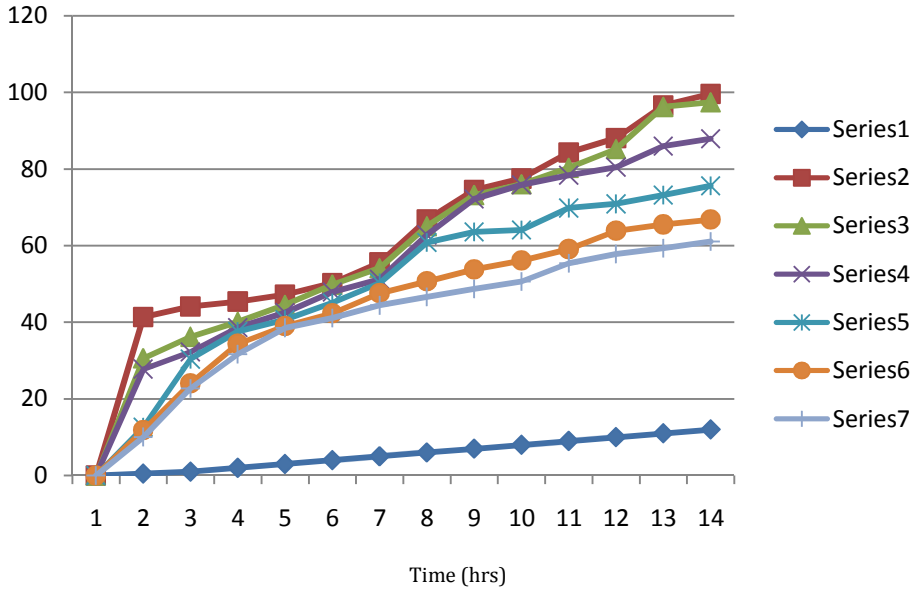

Fig. 11: *In vitro* drug release profile of Acetazolamide for batches F1-F6.

In vitro drug release studies

Microspheres were subjected to in vitro release using USP dissolution apparatus Type I in 900 ml of simulated gastric pH medium. The results of the in vitro drug release studies were given in the tables11 and figures 24. With all of the formulations, there was initial intermittent burst release. But the release seems to be somewhat sustained with increase in the amount of polymer. The release rate was found to be decreased in accordance with the increase in ratio of polymer used.

ACKNOWLEDGEMENT

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CONCLUSION

Gastro retentive floating microspheres of Acetazolamide, an Anti epileptic and Anti hypertensive agent can be efficiently and successfully formulated by non-aqueous solvent evaporation method. Different polymers such as ethyl cellulose (25cps) and HPMC K15M were used in the development of floating microspheres.

The results of Fourier transform infrared spectroscopy (FTIR) revealed that there was no drug excipient interaction. Formulated floating microspheres of Acetazolamide gave satisfactory results for various evaluated parameters like angle of repose, bulk density, tapped density, Carr's index, percentage of yield, drug entrapment efficiency, percentage of floating buoyancy, in vitro drug release and SEM studies. Results of In-vitro floating ability study indicated that the microspheres floated in the simulated fluid without enzyme (pH 1.2) more than 12 hrs. The SEM photographs revealed that the formulated floating microspheres were spherical in shape smooth textured. The yield of the formulated floating microspheres was in the range of 61.6% to 86.5%. The results of drug entrapment efficiency were in the range of 25.1% to 68.2%, as the core to coat ratio increased there was an increase in entrapment efficiency. The buoyancy percentage was found that in the range of 72.8% to 89.6% and the floating microspheres had no floating lag time. In-vitro release rate studies showed that the sustained drug release was observed in F2, at the lower polymer to drug ratio there was a significant increase in drug release, seen at the 1:2 ratio.

Further, the microspheres can also be compressed into tablets or filled into capsules. Further detailed investigation is required to establish bioavailability studies and efficacy including pre-clinical studies and clinical studies of these novel gastro retentive floating microspheres of Acetazolamide.

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