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Research Article

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Formulation, Development & Characterization of Floating Microspheres of Novel Calcium Channel Blockers

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Abstract The present study was aimed at the development of stomach specific drug delivery systems using various approaches like mucoadhesive. From the stock solution, a suitable concentration (10g/ml) was prepared with pH 1.2 Hydrochloric acid buffer solution and UV scan was taken between 200-400 nm. The absorption maxima of 278 nm was selected and utilized for further studies. From the stock solution, 10, 20, 30, 40, 50, and 60 g/ml solutions of Verapamil hydrochloride were prepared in pH 1.2 hydrochloric acid buffer solution. Weighed quantities of Verapamil Hydrochloride, ethyl cellulose, polyethylene oxide and hydroxy propylmethyl cellulose (HPMC K15M) were dissolved in a mixture of ethanol and dichloromethane (1:1 solvent ratio) at room temperature in a magnetic stirrer at 50 rpm for 50 min. For the DSC the samples were scanned from 400 to 4,000 cm^{-1} wave number. The dynamic scans were taken in nitrogen atmosphere at the heating rate of 10 °C min^{-1}. The energy was measured as Joules per kilocalorie. USP dissolution test apparatus II by spreading the microspheres (100 mg) on 900 ml of 0.1 N HCl containing 0.02 % v/v tween 80 as surfactant. The entrapment efficiency of F3, F7 & F9 was higher compared to other formulations. These formulations contained Ethyl cellulose (2%) and Polyethylene oxide (1%), HPMC K15M (1%) & Eudragit L100 (1%) respectively. The percentage yield of the microspheres was found to increase with increasing ethyl cellulose concentration, as was true in F3, F7 and F9. The encapsulation efficiency ranged between 53 ± 2.2 to 89 ± 1.9%, and was observed that the encapsulation efficiency increased with increasing amount of polymers in the hollow microspheres. The sphericity factors for all formulations were in the range of 1.01 ± 0.2 to 1.29 ± 0.6 and the sphericity values of best formulations F3, F7 and F9 were 1.05±0.2, 1.07 ± 0.1 and 1.16 ± 0.1 respectively. Systems that are designed as prolonged release can also be considered as attempts at achieving sustained release delivery.

Keywords Formulation & Development, Floating Microspheres, Novel Calcium Channel Blockers, Improve therapy, Mucoadhesive microspheres

Introduction

Floating drug delivery systems (FDDS) or hydro dynamically controlled systems are low-density systems that have sufficient buoyancy to float over the gastric contents and remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents, the drug is released slowly at the desired rate from the system. After release of drug, the residual system is emptied from

the stomach [1].

The system composed of a drug reservoir encapsulated in a microporous compartment having pores on top and bottom surfaces. The peripheral walls of the reservoir compartment were completely sealed to prevent any physical contact of the un dissolved drug with walls of the stomach. Novel levodopa gastro retentive dosage form based on unfolding polymeric membranes which combines extended dimensions with high rigidity. It was folded into a large size gelatin capsules. In vitro studies showed that unfolded form reached within 15 minutes after administration and it was confirmed in vivo in beagle dogs. The unfolded form was maintained for at least 2 hours. It was concluded that this dosage form could improve therapy of different narrow absorption window drugs. However, there are possibilities of the polymeric films to get stuck in the esophagus causing extreme discomfort to the patient or drug related injuries and repeated administration of rigid dosage form may result in gastric obstruction [2-4].

The present study was aimed at the development of stomach specific drug delivery systems using various approaches like mucoadhesive or floating. Mucoadhesive microspheres of Verapamil hydrochloride, were prepared.

Material and Methods

Reagents [5]

0.1 N Hydrochloric acid (HCl)

8.5 ml of concentrated hydrochloric acid solution was diluted with distilled water upto 1000 ml to give 0.1 N HCl.

0.2 M Potassium chloride

14.911 g of potassium chloride was dissolved in 1000 ml of distilled water.

0.2 M Potassium dihydrogen phosphate

Accurately weighed 27.218 g of potassium dihydrogen orthophosphate was dissolved in 1000 ml of distilled water.

Hydrochloric acid buffer (pH 1.2)

50.0 ml of 0.2 M potassium chloride was placed in a 200 ml volumetric flask, to this 85.0 ml of 0.2 M hydrochloric acid was added and then made up to the volume with water.

Analytical Methods

Verapamil hydrochloride:

The method described by Florey K was followed [6].

Stock solution:

Verapamil hydrochloride in pH 1.2 hydrochloric acid (HCl) buffer (100 g/ml).

Scanning:

From the stock solution, a suitable concentration (10 g/ml) was prepared with pH 1.2 Hydrochloric acid buffer solution and UV scan was taken between 200-400 nm. The absorption maxima of 278 nm was selected and utilized for further studies.

Standard Plot:

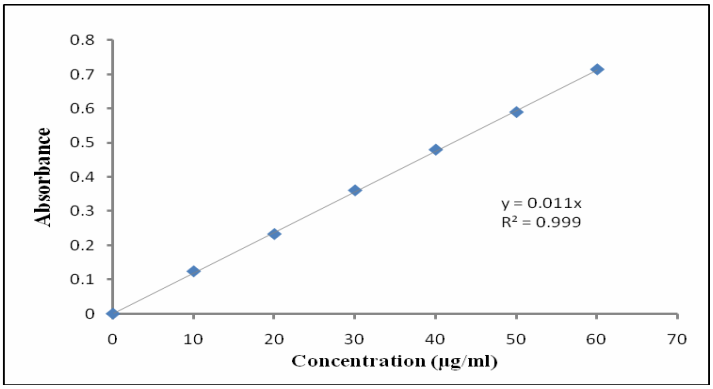

Figure 1: Standard plot for Verapamil hydrochloride in pH 1.2 hydrochloric acid buffer

From the stock solution, 10, 20, 30, 40, 50, and 60 g/ml solutions of Verapamil hydrochloride were prepared in pH 1.2 hydrochloric acid buffer solution. The absorbance was measured at 278 nm and a graph of concentration versus absorbance was plotted. Standard plot data of Verapamil hydrochloride in pH 1.2 hydrochloric acid buffer solution is reported in table and graph in figure 1.

Formulation of Hollow Microspheres [7-11]

Floating microspheres with a central hollow cavity were prepared by using a modified Quasi-emulsion diffusion technique. Weighed quantities of Verapamil Hydrochloride, ethyl cellulose, polyethylene oxide and hydroxy propylmethyl cellulose (HPMC K15M) were dissolved in a mixture of ethanol and dichloromethane (1:1 solvent ratio) at room temperature in a magnetic stirrer at 50 rpm for 50 min. This solvent was poured drop wise into 100 ml distilled water containing 2 ml of Tween 80 maintained at a temperature of 50 ± 2 °C. The resultant solution was stirred with a pitched-blade-type impeller type agitator at 1100 rpm for 3 h to allow the volatile solvent to evaporate. This resulted in the formation of microspheres. Different ratios of polymers were used to prepare the microspheres.

Evaluation Parameters for Floating Hollow microspheres

Fourier transform infra red spectroscopy (FT-IR)

FTIR spectra of pure drug and its formulations were obtained by a FT-IR Shimadzu 8400S (Japan) spectrophotometer using the KBr pellet method. The samples were scanned from 400 to 4,000 cm^{-1} wave number.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry was performed on pure sample of drug and its formulation. Calorimetric measurements were made with empty cell (high purity alpha alumina discs) as the reference. The dynamic scans were taken in nitrogen atmosphere at the heating rate of 10 °C min^{-1}. The energy was measured as Joules per kilocalorie.

Percentage drug entrapment efficiency [12]

Floating microspheres equivalent to 4 mg of drug was dissolved in 10 ml ethanol. The samples were assayed for drug content using UV spectrophotometer at 228 nm after suitable dilution. No interference was found due to the other components of floating microspheres at 228 nm. The percentage drug entrapment efficiency and yield were calculated as follows.

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

Yield of floating microspheres

The yield was determined by weighing the microspheres and then the percentage yield was calculated with respect to the weight of the input materials, i.e., weight of verapamil and polymers used. The formula for calculation of percentage yield is as follows.

$$\% \text{ Yield} = \frac{\text{Total weight of floating microspheres}}{\text{Total weight of drug and polymer}} \times 100$$

Scanning electron microscopy (SEM)

The surface morphology of the microspheres was examined by scanning electron microscopy (SEM; JSM-5200, Jeol, Tokyo, Japan) operated at 15 KV on samples, gold-sputtered for 120 s at 10 mA, under argon at low pressure.

Sphericity of the microsphere [12]

The sphericity of the prepared microspheres can be confirmed using a camera lucida by taking the tracings of the microspheres on a black paper. The tracings help to calculate the circulatory factor and confirm the sphericity

of microspheres if the obtained values are nearer to 1.

$S= P^2/12.5xA$

Where A is area (cm^2) and, P is the perimeter of the circular tracing.

Micromeritic properties of microsphere [13-14]

The microspheres were characterized by their micromeritic properties, such as particle size, bulk density, compressibility index and angle of repose (values useful in prediction of flowability).

Particle size

The particle size of the microspheres was measured using an optical microscopic method and the mean particle size was calculated by measuring 425 particles with the help of a calibrated ocular micrometer with stage micrometer.

Angle of repose

The flow characteristics of microspheres are measured by angle of repose. Improper flow of microspheres is due to frictional forces between the microspheres. The radius and height of the pile were then determined.

Tapped bulk density

The tapping method was used to determine the tapped density of the microspheres using tapped density testing apparatus (Electrolab tapped density tester ETD-1020) and percent compressibility index as follows.

Compressibility (Carr's) index

Carr‟s index is a dimensionless quantity, which proved to be useful to the same degree as the angle of repose values for predicting the flow behavior. Relationship between powder Flowabilty & % compressibility is shown in table 4.08. Carr‟s index is calculated using the formula

% Compressibility index = 1- V/Vo× 100

Where Vo and V are the volumes of the sample before and after the standard tapping.

Table 1: Relationship between powder flowability & % compressibility

% Compressibility range	Flow description
5-15	Excellent (free flowing granules)
12-16	Good (free flowing powder granules)
18-21	Fair (powdered granules)
23-28	Poor (very fluid powders**)**
28-35	Poor (fluid cohesive forces)
35-38	Very Poor
>40	Extremely poor

Floating Characteristics

***In vitro* buoyancy of microspheres**

USP dissolution test apparatus II by spreading the microspheres (100 mg) on 900 ml of 0.1 N HCl containing 0.02 % v/v tween 80 as surfactant. The medium was agitated with a paddle rotating at 100 rpm and maintained at 37° ± 0.5 °C for 12 h. Both the floating and the settled portions of microspheres were collected separately. The microspheres were dried and weighed. The percentage of floating microspheres was calculated using the following equation

$$\% \text{ Floating capability} = \frac{\text{Weight of floating hollow microspheres}}{\text{Initial weight of hollow microspheres}} \times 100$$

***In vitro* drug release study [13-14]**

The release rate of drug from formulations was determined using USP dissolution testing apparatus II (basket type). The dissolution test was performed using 900 ml of 0.1 N HCl, at 37± 0.5 °C and 50 to 100 rpm. Aliquots (5mL) were withdrawn at regular intervals for 12 h, sample was replaced by its equivalent volume of fresh dissolution medium to maintain the sink condition.

Results & Discussion

The strong solidified film produced at the surface of droplet, with further depletion of ethanol prevented rupture and shrinkage of microspheres during the evaporation of dichloromethane from the droplets.

Figure 2: Prepared Verapamil hollow microspheres

Selection of polymers

The polymers like ethyl cellulose, eudragit L 100, polyethylene oxide and HPMC were selected for hollow microspheres preparation as they are insoluble in aqueous media but are permeable and have the ability to produce pH-independent drug-release profiles and have release rate controlling ability, non-toxicity, non- irritancy, stability at gastric pH and compatibility with the drug [15]. Ethyl cellulose was selected as polymer for preparation of floating hollow microspheres for floating drug delivery and to improve the stability to the formulation. Eudragit L 100, delays the release of drugs in the gastrointestinal tract by virtue of its pH sensitive property, Polyethylene oxide and HPMC provide controlled release, hence the above polymers were selected for the study.

The effect of drug: polymer ratio

The entrapment efficiency of F3, F7 & F9 was higher compared to other formulations. These formulations contained Ethyl cellulose (2%) and Polyethylene oxide (1%), HPMC K15M (1%) & Eudragit L100 (1%) respectively. Microspheres were prepared using increasing EC concentration in combination with other polymers to assess the effect of polymer concentration on the size of microspheres. The mean particle size of the microspheres significantly increased with increasing ethyl cellulose.

The effect of stirring speed and mixing time

Average diameter of hollow microspheres was controlled by stirring speed. The ultimate mean diameter of hollow microspheres was determined by the particle size, which decreased with increasing rotational speed. The particle size increased with increasing concentration of polymer and particle size decreased with increase of stirring speed [16].

The effect of surfactant

These are substances which are added to the formulation to disperse a water- insoluble drug to form a colloidal dispersion. In other words, Tween 80 enhanced the solubility of the drug. Tween 80 was selected as the solubilizing agent in the formulation as it was compatible with the Verapamil hydrochloride.

Percentage yield

During the process of microencapsulation, the mechanical variables cause loss of final product and hence process yield may not be 100%. Hollow microspheres were weighed after drying and the percentage yield was calculated.

The percentage yield of the microspheres was found to increase with increasing ethyl cellulose concentration, as was true in F3, F7 and F9 [16].

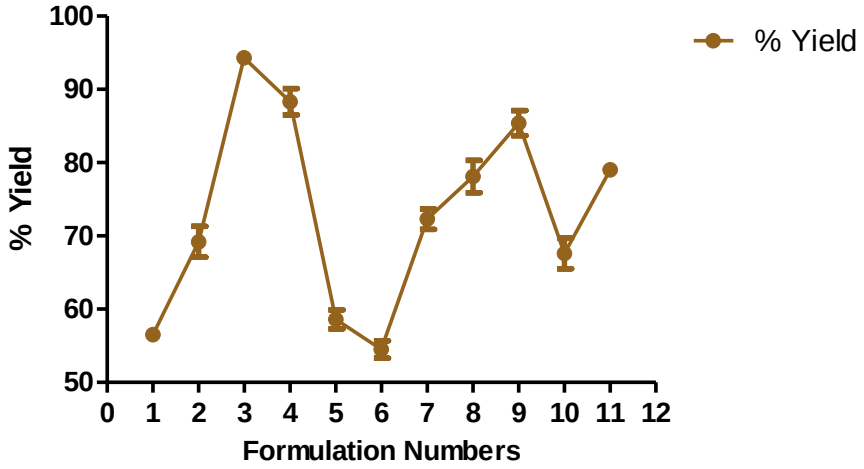

Figure 3: Effect on % yield of different formulation

Drug loading and encapsulation efficiency

The drug content test was carried out to ascertain that the drug is uniformly loaded in the formulation. Relatively high encapsulation efficiency was observed for all the formulations. The encapsulation efficiency ranged between 53 ± 2.2 to 89 ± 1.9%, and was observed that the encapsulation efficiency increased with increasing amount of polymers in the hollow microspheres. Formulations F3, F7, F9 showed relatively higher encapsulation efficiency as these formulations were composed of high concentration of polymer. When 1:10 & 1:20 (w/w) drug/polymer concentrations were used for both the ethyl cellulose, polyethylene oxide polymers combination ethyl cellulose, HPMC polymers combination & ethyl cellulose, eudragit L 100 polymers combination, the quality of hollow microspheres formed was poor. The extent of loading appears to influence the particle size of the microspheres. When loading is high proportion of larger particle formed is also high. Among all formulations, F3, F7 and F9 showed maximum percentage yield and drug loading [17].

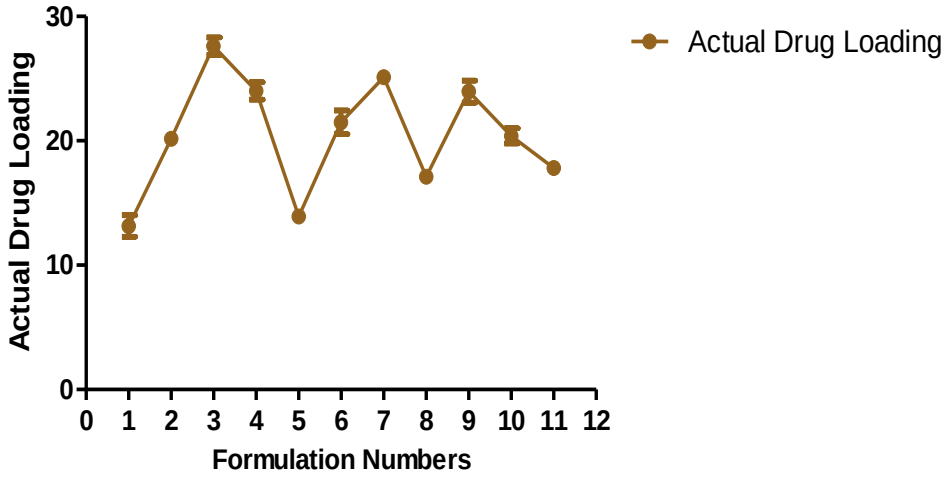

Figure 4: Effect on Actual Drug Loading of different formulations

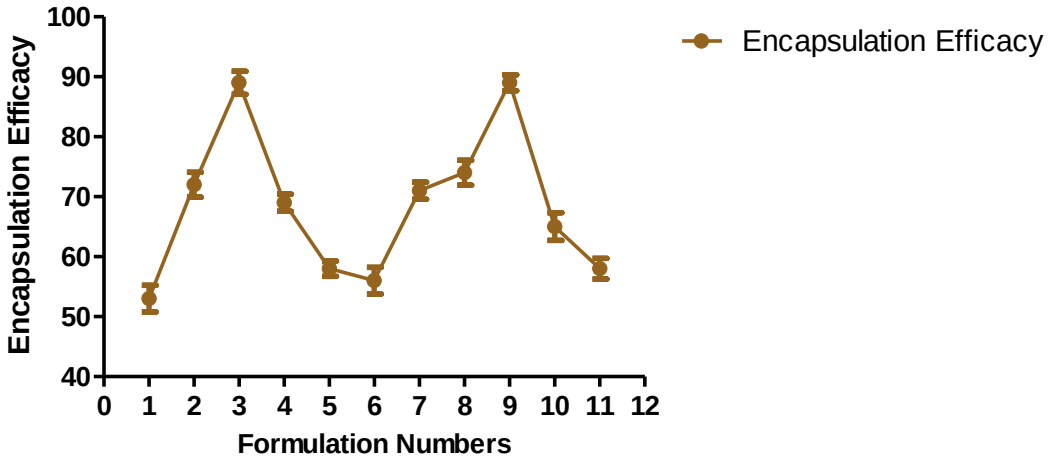

Figure 5: Effect on Encapsulation efficacy of different formulations

Fourier transform infrared spectroscopy (FT-IR)

The FT-IR spectra of the pure drug and formulation F3 indicated that the characteristics peaks of Verapamil Hydrochloride were not altered without any change in their position after successful entrapment in the hollow microspheres, thereby indicating no chemical interactions between the drug and carriers used. These results confirmed that the method used to prepare hollow microspheres did not affect the physicochemical properties of the systems [18].

Differential scanning calorimetry (DSC)

There will be no detectable endotherm if the drug is present in a molecular dispersion or solid solution state in the polymeric hollow microspheres loaded with drug. In the present investigation, DSC thermograms of pure drug and drug loaded hollow microspheres were taken as shown in figure and data for the same is given in table. The thermal properties of the drug and the mixture of the drug and polymers are of importance as this ascertains the crystalline and amorphous status of the entrapped drug in the polymers thereby assessing the interaction among different components of the formulation during the fabrication process. [19]

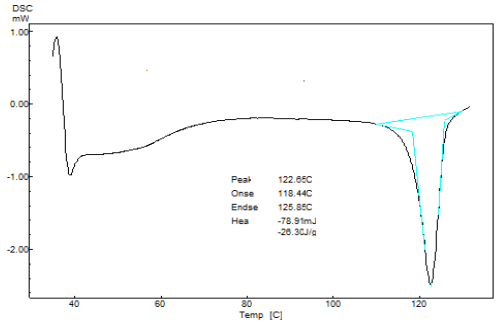

Figure 6: DSC thermograms of Verapamil Hydrochloride pure drug and hollow microspheres (F3)

Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) revealed discrete, spherical shaped microspheres with rough surface and presence of holes /hollow cavity due to the collapse of the wall of the microspheres during in situ drying process. Thus the rate of solvent removal from the embryonic microspheres exerted an influence on the morphology of the end product. Porous structure was observed on the surface of microspheres shell due to the rapid diffusion of the

solvent, there is also possibility of rupture of some microspheres. Microspheres floated more than 12 h because of presence of hollow cavity. SEM photographs are shown in figures.

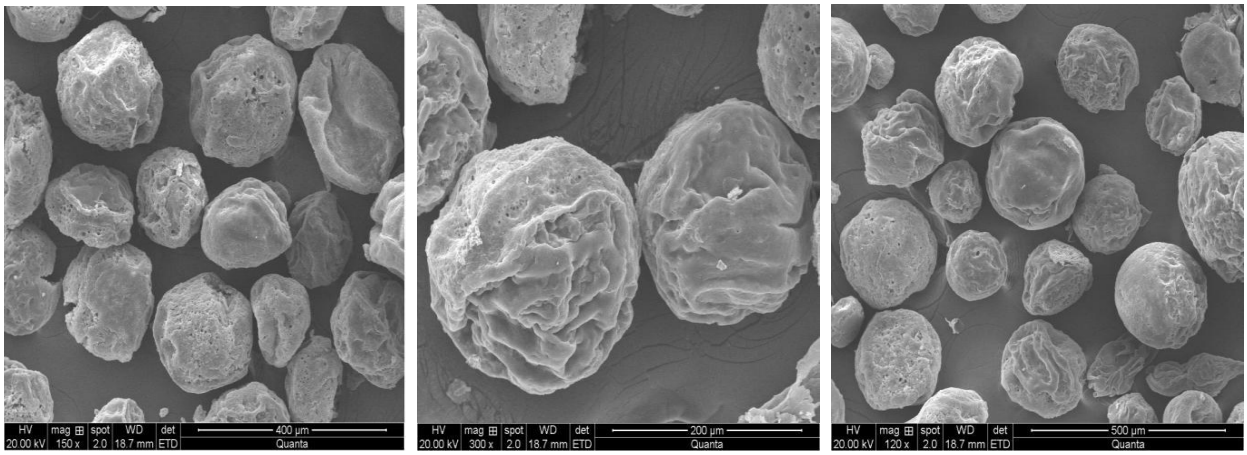

Figure 7: SEM photograph of microspheres at different magnifications

Sphericity of the microspheres

The sphericity of the prepared microspheres was confirmed and the calculated values were nearer to 1. The sphericity factors for all formulations were in the range of 1.01 ± 0.2 to 1.29 ± 0.6 and the sphericity values of best formulations F3, F7 and F9 were 1.05±0.2, 1.07 ± 0.1 and 1.16 ± 0.1 respectively. The sphericity value nearer to 1 indicated that the prepared formulations were spherical in nature.

Micromeritics properties

The obtained data along with related parameters are presented in table.

Angle of repose

The microsphere flow depends on the physical properties of the microspheres, bulk properties (size distribution, compaction) and processing environment (eg. storage and humidity). The values of θ ranged from 25° to 29° indicating that the obtained values were well within the limits for powder to have good flow properties. This result clearly showed that the prepared hollow microspheres have reasonably good flow property.

Compressibility index

The value of CI was found to be in the range of 13.7 to 26.1 %. The values of tapped density ranged between 0.138 to 0.281 g / cm^3. The values of compressibility index indicated fair to good flow properties.

Particle size of hollow microspheres

The particle size range was between 223 ± 2.6 to 446 ± 5.2 and the mean particle size of the F3 microspheres was 312 ± 4.1. The microspheres prepared with ethyl cellulose, HPMC and eudragit showed higher particle size as compared with ethyl cellulose and polyethylene oxide combination ($p<0.05$). The mean particle size of the microspheres significantly increased with increase in polymer concentration which is in line with the report of Madan MK *et al*. Larger particles were produced due to the rapid polymer precipitation, leading to hardening and avoidance of further particle size reduction during solvent evaporation. Another possibility is that, by rapidly removing the solvent, the inward shrinking of the polymer gets avoided: this being achieved by slowly removing the solvent.

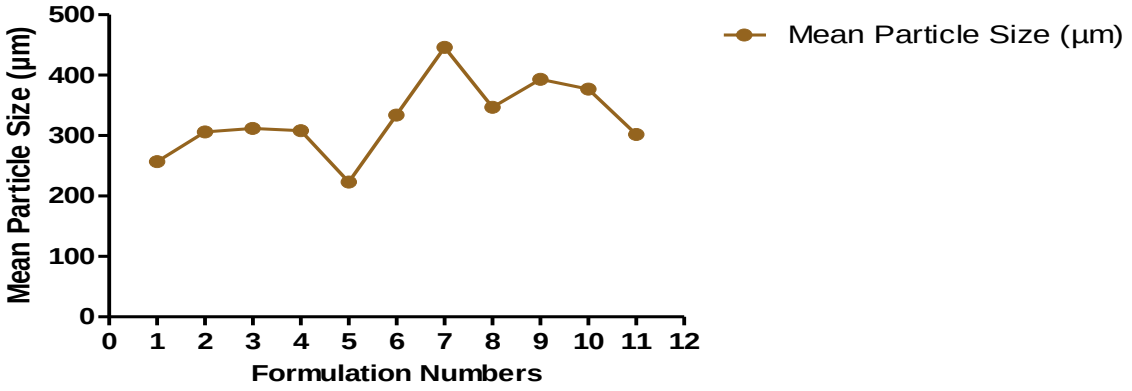

Figure 8: Effect on Mean particle size of different formulations

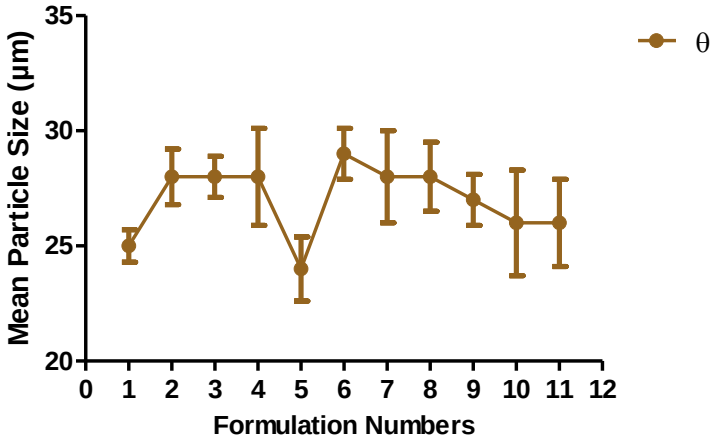

Figure 9: Effect on Mean particle size of different formulations

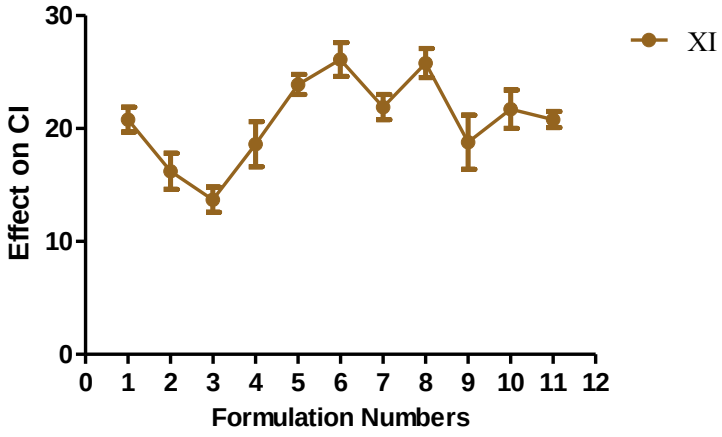

Figure 10: Effect on CI of different formulations

***In vitro* buoyancy of microspheres**

Despite the solution being stirred for more than 8 h, the hollow microspheres still floated indicating that microspheres exhibited excellent buoyancy effect. The density of values of hollow microspheres (<1.000

g/cm^3) was less than that of the gastric fluid (<1.004 g/cm^3) further supporting floating nature. The *in vitro* floating test was conducted on the microspheres which showed floating capability of about 70 ± 1.1 %. All the formulations showed buoyancy of more than 8 h. As concentration of ethyl cellulose increased buoyancy increased. The floating behaviour was controlled by varying the concentration of ethyl cellulose (7 cps) in different formulations. Among all formulations, F1, F3, F7 and F9 showed maximum percentage floating ability.

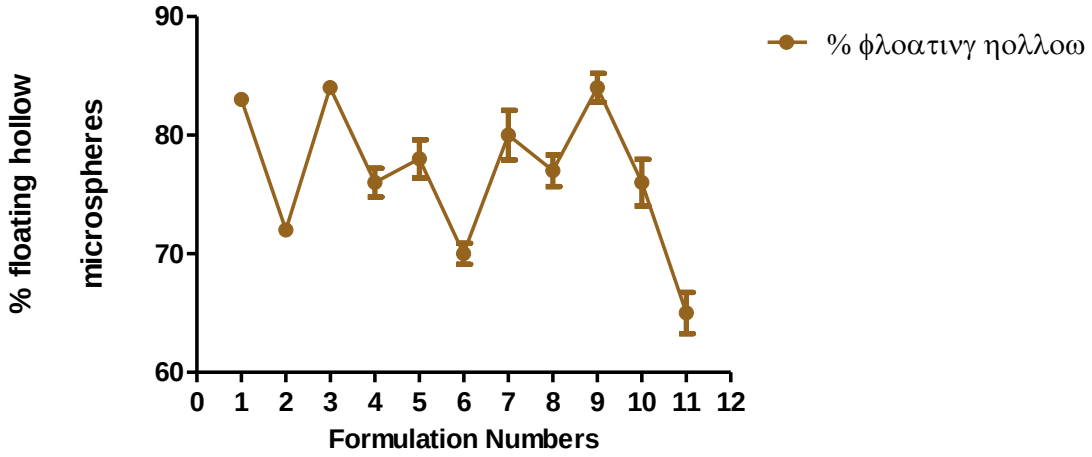

Figure 11: Effect on % Floating Hollow Microspheres of different formulations

***In vitro* drug release studies**

In vitro dissolution studies of Verapamil hydrochloride from floating hollow microspheres of Verapamil hydrochloride were carried out for all formulations in pH 1.2 hydrochloric acid buffer for 12 h using electrolab dissolution test apparatus II. It was found that formulations F1-F11 showed 43.0% - 80.76 % of drug release at 8 h and 75.3 % - 99 % of release at 12 h ($p<0.05$).

Microspheres prepared with ethyl cellulose and HPMC (F5, F6 and F7) showed less release compared to other combinations. This may be probably due to the gelation property of HPMC, which forms gel matrix after contact with the dissolution medium. F1 and F11 showed more than 99 % drug release at the end of 12 h, which may be due to low polymer concentration resulting in smaller particle size with larger surface area.

Conclusion

Systems that are designed as prolonged release can also be considered as attempts at achieving sustained release delivery. Repeat action tablets are alternative method of sustained release in which multiple doses of drugs are contained within a dosage form, and each dosage is related to periodic interval. Delayed release systems, in contrast may not be sustaining, since often function of these dosage forms is to maintain the drug within the dosage form for some time before release. Commonly the release rate of drug is no altered and does not result in sustained delivery once drug release has begun.

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