Research Article

Formulation and Development of Hydrochlorothiazide Floating Microspheres

A Prashanth kumar*
Prasad institute of pharmaceutical sciences, Jangaon, Warangal, Andhra Pradesh, India.
***Corresponding author's E-mail:** Prashanthalay14@gmail.Com

ABSTRACT

The objective of the present study is to develop suitable gastro retentive floating microspheres of Hydrochlorothiazide and to study release kinetics of drug with a view to reduce the dose frequency and to achieve a controlled drug release via gastric retention with improved bioavailability. Formulations were evaluated for dimensional stability study, in vitro drug release profile, buoyancy properties. The optimized formulation also tested for stability studies as per ICH guidelines. The dimensional stability of the formulation and the drug release rate was prolonged with increasing the concentration and viscosity of the polymer. Floating microspheres loaded with hydrochlorothiazide were prepared by Emulsion solvent evaporation method. The prepared microspheres were evaluated by micromeritics properties, *in vitro* drug release, floating ability and drug entrapment efficiency. Kollidone SR, cellulose acetate, Acrycoat S 100, Methocel K4M, Methocel K15M, Methocel K100M, are used as polymers, Ethanol and Acetone are used as solvents and liquid paraffine is used as oil phase in the preparation of floating microspheres. SEM is used to determine the surface morphology of microspheres.

Keywords: Emulsion solvent evaporation, Floating ability and drug entrapment efficiency, Gastro retentive floating microspheres, Hydrochlorothiazide, *In vitro* drug release.

INTRODUCTION

Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 micrometer. The Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, which are biodegradable in nature. Solid biodegradable microspheres incorporating a drug dispersed or dissolved throughout particle matrix have the potential for controlled release of drugs.[1] Microspheres are small in size and therefore have large surface to volume ratios. The concept of incorporating microscopic quantities of materials within microspheres dates back to the 1930s and to the work of Bungerberg de joing and co-workers on the entrapment of substances within coacervates. The potential uses of microspheres in the pharmaceutical have been considered since the 1960's and have a number of applications.[2,3] The use of microspheres in pharmaceuticals have a number of advantages Viz., Taste and odor masking, conversion of oils and other liquids to solids for ease of handling, protection of drugs against environment (moisture, heat, light and oxidation), separation of incompatible materials, to improve flow of powders, production of sustained release, controlled release and targeted medications.[4] The most important physico-chemical characteristics that may be controlled in microspheres manufacture are; particle size and distribution, polymer molecular weight, ratio of drug to polymer, total mass of drug and polymer.[5,6,7] A number of different substances both biodegradable as well as non-biodegradable have been investigated for the preparation of microspheres; these materials include polymers of natural origin or synthetic origin and also

modified natural substances.[8] A range of microspheres prepared using both hydrophilic and hydrophobic polymers. Hydrophilic polymers includes gelatin, agar, egg albumin, starch, chitosan, cellulose derivatives; HPMC, DEAE cellulose.[9,10]

Advantages of Floating Microspheres

- Improves patient compliance by decreasing dosing frequency.
- Bioavailability enhances despite first pass effect because fluctuations in plasma drug concentration is avoided, a desirable plasma drug concentration is maintained by continuous drug release.
- Better therapeutic effect of short half-life drugs can be achieved.
- Gastric retention time is increased because of buoyancy.[11]
  Drug releases in controlled manner for prolonged period.
- Site-specific drug delivery to stomach can be achieved.
  Enhanced absorption of drugs which solubilizes only in stomach.
- Superior to single unit floating dosage forms as such microspheres releases drug uniformly and there is no risk of dose dumping.[12]
- Avoidance of gastric irritation, because of sustained release effect, floatability and uniform release of drug through multiparticulate system.[13]
- The flow characteristics and packability of the resultant microballoons are much improved when compared with the raw crystals of the drug.

- Drug targeting to stomach can be attractive for several other reasons. [14]

For the weakly basic drugs with poor solubility in the basic environment, the floating systems may avoid any chance of solubility to become the rate-limiting step in the release by restricting the drug to the stomach. Any solute released in the stomach will empty along with the fluids such that the whole surface of the small intestine is available for absorption. This is particularly useful when an absorption window exists in the proximal small intestine.[15] The positioned gastric release is useful for all substances intended to produce a lasting local action onto the gastroduodenal wall.

Limitations of floating Microspheres

- The major disadvantage of floating systems is requirement of sufficiently high levels of fluids in the stomach for the drug delivery.[16,17]
- The dosage form should be administered with a minimum of glass full of water.
- Floating system is not feasible for those drugs that have solubility or stability problems in gastric fluids.[18-19]
- Single unit floating capsules or tablets are associated with an "all or none concept," but this can be overcome by formulating multiple unit systems like floating microspheres or microballoons.[20]

- Drugs that irritate the mucosa, those that have multiple absorption sites in the gastrointestinal tract, and those that are not stable at gastric pH are not suitable candidates to be formulated as floating dosage forms.[21]

MATERIALS AND METHODS

Materials

Verapamil hydrochloride is gift sample from aurobindo chemicals pvt. Ltd, Kollidone SR, cellulose acetate, Acrycoat S 100, Methocel K4M, Methocel K15M, Methocel K100M, are procured from Hi Media labs Pvt Ltd, Ethanol, Acetone, liquid paraffine are collected from Micro labs.

Preparation method

Floating microspheres loaded with Verapamil hydrochloride were prepared by Emulsion solvent evaporation method as shown in figure 1.[22-23] Formulations were formulated using different polymers Kollidone SR, cellulose acetate, Acrycoat S 100, Methocel K4M, Methocel K15M, Methocel K100M.

Overall six formulations were formulated using different polymers, Methocel K4M (code F1), Methocel K15 (code F2) and Methocel K100M (code F3), Cellulose acetate (code F4), Acrycoat S100 (code F5), Kollidone SR (code F6) as shown in Table 1.

Table 1: Composition of floating microspheres of Hydrochlorothiazide (preliminary batches)

'F' code	Drug : Polymer	Organic solvent	Continuous Phase
F1	1:2	Ethanol	Liquid paraffin containing 0.01% tween 80
F2	1:2	Ethanol	Liquid paraffin containing 0.01% tween 80
F3	1:2	Ethanol	Liquid paraffin containing 0.01% tween 80
F4	1:2	Acetone	Liquid paraffin containing 0.01% tween 80
F5	1:2	Ethanol	Liquid paraffin containing 0.01% tween 80
F6	1:2	Acetone	Liquid paraffin containing 0.01% tween 80

Table 2: Micromeritics Properties and Characterization of prepared floating microspheres (preliminary batches)

'F'Code	Angle of repose	Carr's Index (%)	Hausner ratio	Mean Particle Size(µm)	% Yield	% Drug Entrapped	% Buoyancy at 12 hrs
F1	23.83 ±0.311	13.96 ±4.921	1.16 ±0.071	362.77 ±3.35	86.90 ± 3.70	85.6 ± 2.07	74.9 ±4.25
F2	22.63 ±0.601	15.88 ±0.566	1.20 ±0.007	255.81 ±2.29	77.18 ± 1.10	82.4 ± 2.75	61.8 ±2.35
F3	29.86 ±0.071	19.92 ±1.428	1.26 ±0.017	466.64 ±3.73	58.62 ± 4.09	76.4 ± 3.50	55.6 ±6.84
F4	31.42 ±0.035	29.43 ±0.239	1.27 ±0.042	642.66 ±5.86	36.86 ± 6.32	52.56 ± 1.67	46.20 ± 4.21
F5	29.89 ±0.559	29.24 ± 1.598	1.28 ±0.021	596.73 ±5.33	39.43 ± 5.85	54.47 ± 2.61	48.26 ± 3.56
F6	33.42 ± 0.672	32.30 ± 0.629	1.29 ±0.007	668.79 ±6.93	51.42 ± 7.63	58.56 ± 1.67	49.73 ± 2.46

RESULTS AND DISCUSSION

From the results of evaluation of the floating microspheres for preliminary screening it was found above in the table 2 that the microspheres prepared with cellulose acetate, Acrycoat and Eudragit S 100 were not showing the satisfactory results. The micromeritics properties of the prepared floating microspheres with

cellulose acetate, Acrycoat and Eudragit S 100 shown that the flow properties of the microspheres were poor. The value of angle of repose of formulation F1, F2 and F3 were found to be 33.42 ± 0.672, 31.42 ±0.035 and 29.89 ±0.559 respectively which indicates poor flow properties. % yield of the formulation F1, F2 and F3 were found to be 36.86 ± 6.32, 39.43 ± 5.85 and 51.42 ± 7.63 respectively. Drug entrapment efficiency of the formulation F1, F2 and

F3 were found to be 52.56 ± 1.67, 54.47 ± 2.61 and 58.56 ± 1.67 respectively. % Buoyancy at 12 h of the formulation F1, F2 and F3 were found to be 46.20 ± 4.21, 48.26 ± 3.56 and 49.73 ± 2.46 respectively. % yield, Drug entrapment efficiency and % Buoyancy at 12 hrs of the formulation F1, F2 and F3 were found to be comparatively less than formulation F4, F5 and F6. So formulation F4, F5 and F6 were selected for further studies with modifications.

Selection of the Formulations for Further Studies

The screening of microspheres formulations were based on different physicochemical and evaluation parameters. The optimistic formulations from above all are F4, F5 and F6. These formulations shows satisfactory results of different physicochemical parameters and evaluation parameters therefore modifications in these three formulations were made by varying the drug: polymer ratio, solvent systems and change in aqueous phase also investigated.

Optimization of the Selected Formulations

Optimization of the formulations F4, F5 and F6

Modification of all above formulations were affected by preparing the microspheres using different ratio of drug and polymer, combine effect of solvent system and change in aqueous phase also investigated. The floating microspheres were prepared according to the method given in the figure 1. They are designated as $K_1, K_2, K_3, A_1, A_2, A_3, C_1, C_2,$ and C_3. The detailed composition was given in the Table 3.

Table 3: Compositions of optimized formulations of floating microspheres

'F' code	Drug : Polymer Ratio	Organic solvent system [1:1]	Continuous Phase
K_1	1:1	Ethyl acetate: acetone	Liquid paraffin containing 0.01% tween 80
K_2	1:2	Ethyl acetate: acetone	Liquid paraffin containing 0.01% tween 80
K_3	1:1	Ethyl acetate: acetone	Liquid paraffin containing 0.01% tween 80
A_1	1:1	Dichloromethane: ethanol	Liquid paraffin containing 0.01% tween 80
A_2	1:2	Dichloromethane: ethanol	Liquid paraffin containing 0.01% tween 80
A_3	1:1	Dichloromethane: ethanol	Liquid paraffin containing 0.01% tween 80
C_1	1:1	Ethyl acetate: acetone	Liquid paraffin containing 0.01% tween 80
C_2	1:2	Ethyl acetate: acetone	Liquid paraffin containing 0.01% tween 80
C_3	1:1	Dichloromethane: ethanol	Liquid paraffin containing 0.01% tween 80

NOTE: Formulations K_1, K_2 and K_3 containing Kollidone SR. Formulations A_1, A_2 and A_3 containing Acrycoat S 100. Formulations C_1, C_2 and C_3 containing Cellulose acetate.

Table 4: Micromeritics properties and Characteristics of Hydrochlorothiazide floating microspheres

F code	Mean Particle Size(μm)	% Compressibility	Hausner Ratio	Angle of Repose	% Yield	% Drug Entrapped	% Buoyancy at 12 hrs
K_1	344.70 ±3.81	13.86 ±0.26	1.17 ±0.041	25.42 ±0.67	97.40	83.8 %	72.2 ±2.687
K_2	360.75 ±3.30	14.30 ±0.62	1.19 ±0.007	24.42 ±0.03	84.85	84.7 %	73.8 ±3.253
K_3	382.50 ±3.09	16.43 ±0.23	1.24 ±0.017	23.89 ±0.55	87.16	82.6 %	68.6 ±2.121
A_1	252.45 ±4.63	16.25 ±1.59	1.24 ±0.028	22.83 ±0.31	77.14	82.9 %	62.7 ±0.849
A_2	253.80 ±2.27	15.86 ±2.92	1.21 ±0.028	22.63 ±0.60	75.15	81.3 %	61.8 ±1.273
A_3	279.00 ±1.27	17.78 ±0.56	1.26 ±0.07	29.88 ±0.07	73.59	80.6 %	63.6 ±0.636
C_1	418.95±8.81	17.92 ±1.42	1.26 ±0.016	29.46 ±0.58	44.93	75.6 %	47.0± 1.344
C_2	463.64 ±3.68	19.36 ±2.10	1.27 ±0.017	30.23 ±0.28	55.60	77.8 %	50.6 ±0.849
C_3	411.61 ±4.86	21.55 ±1.88	1.29 ±0.041	30.48 ±0.68	68.00	72.9 %	53.9 ±1.273

Characterization of optimized formulations

Micromeritics propertiesand evaluations like Yield of microspheres, Drug entrapment efficiency and in vitro floating ability of the optimized formulations were done and results are given in Table 4.

In vitro drug release studies[24-25]

The drug release studies were carried out using six basket dissolution apparatus USP type II. The microspheres were placed in a non reacting mesh that had a smaller mesh size than the microspheres. The mesh was tied with a nylon thread to avoid the escape of any microspheres. The dissolution medium used was 900 ml of 0.1N hydrochloric acid at 37°C. At specific time

intervals, 5 ml aliquots were withdrawn and analyzed by UV spectrophotometer at the respective lmax value 271 nm after suitable dilution against suitable blank. The withdrawn volume was replaced with an equal volume of fresh 0.1 N hydrochloric acid. The dissolution data of all nine formulations are given in Table 5.

Surface Topography (SEM) [26]

The surface morphology, shape and to confirm the hollow nature, microspheres were analyzed by scanning electron microscopy for selected batches as in figure 2. Photomicrographs were observed at required magnification operated with an acceleration voltage of 15 kV and working distance of 19 mm was maintained. Microspheres were mounted on the standard specimen-mounting stubs and were coated with a thin layer (20 nm) of gold by a sputter-coater unit to make the surface conductive.
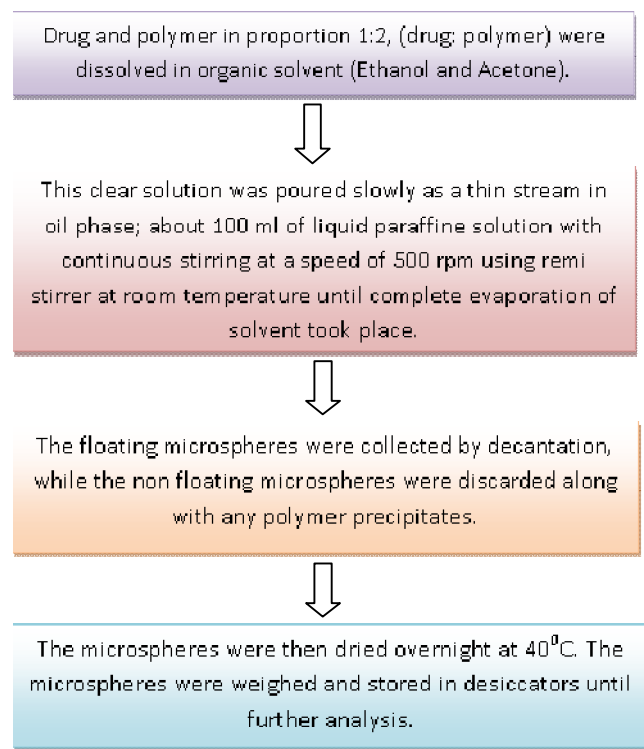

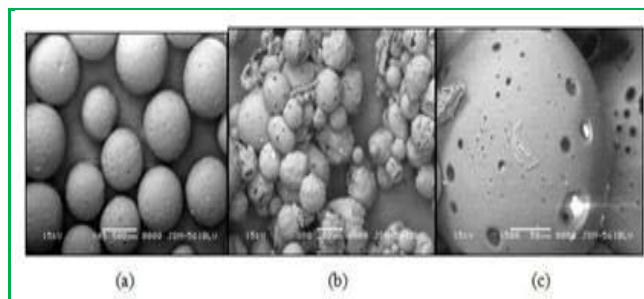**Figure 1:** Preparation method of floating microspheres

Figure 2: SEM of the kollidone SR microspheres (a), Acrycoat S100 microspheres (b) and cellulose acetate microspheres (c).

Kinetic Modeling of Drug Dissolution Profiles

The dissolution profile of all the batches was fitted to Zero order, First order[27-28] Higuchi[29-30] and Korsemeyer and Peppas[30-35] models to ascertain the kinetic modeling of drug release explained in table 6. The method of Bamba et al. was adopted for deciding the most appropriate model.[36]

Stability Study of the Optimized Batches

With the recent trend towards globalization of manufacturing operation, it is imperative that the final product be sufficiently rugged for marketing world wide under various climatic conditions including tropical, sub tropical and temperate. Stability studies were carried out as per ICH guidelines.[48] The floating microspheres were placed in a screw capped glass containers and stored at room temperature, (25 ± 2°C), Humidity chamber (40°C, 75 % RH), and in Refrigerator (2-8°C) for a period of 90 days. The samples were assayed for drug content at regular intervals. The graph of percent drug content versus time (in days) was plotted as in figure 3.
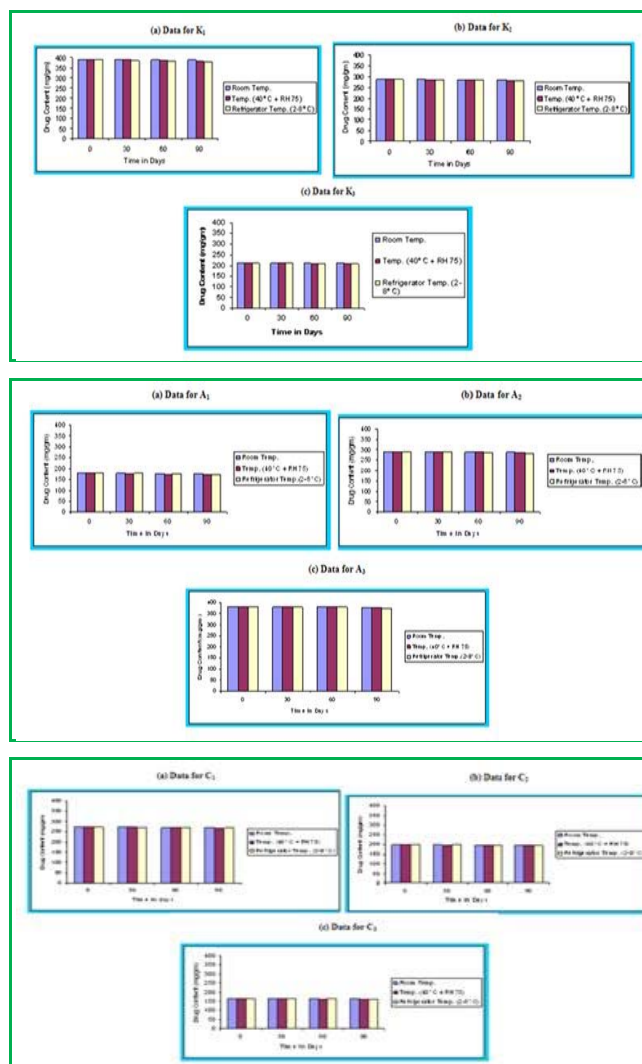

Figure 3: Graphical representation of stability studies of prepared floating microspheres (Formulation Code K1, K2, K3), (Formulation Code A1, A2, A3) and (Formulation Code C1, C2, C3)

Table 5: Dissolution data of formulation K1, K2, K3, C1, C2, C3, A1, A2, and A3

Time(h)	Cumu. % release k1	Cumu. % releasek2	Cumu. % release k3	Cumu. % release c1	Cumu. % release c2	Cumu. % release c3	Cumu. % release A1	Cumu. % release A2	Cumu. % release A3
1	30.39	29.14	31.02	34.993	35.373	28.088	3.006	4.184	3.222
2	36.95	35.82	34.22	43.498	40.576	34.27	10.008	12.906	12.88
3	41.96	40.69	39.56	52.359	46.242	38.434	16.736	16.072	15.226
4	48.15	47.98	48.12	61.038	55.354	47.923	21.824	19.656	19.98
5	53.13	54.16	54.6	69.143	63.183	54.062	26.646	26.738	28.95
6	56.99	56.26	55.36	78.613	68.243	57.192	30.264	32.821	31.284
7	60.16	59.94	59.68	84.290	77.51	67.656	35.664	37.944	34.56
8	68.03	68.12	68.32	89.130	85.49	73.578	41.842	43.218	40.986
9	75.49	75.14	75.13	-	90.35	78.142	47.366	47.662	48.632
10	83.31	81.2	80.86	-	-	83.45	54.224	56.818	56.016
11	87.18	85.58	82.38	-	-	87.47	59.424	62.548	63.525
12	94.75	89.13	84.29	-	-	87.89	63.922	67.128	69.962

Table 6: Kinetic data of drug release from various formulations

Code	Zero order	First order	Higuchi's kinetics	Peppas plots	n value
C_1	0.9438	0.8541	0.9753	0.9543	0.5614
C_2	0.9401	0.9399	0.9852	0.9695	0.5673
C_3	0.9257	0.9647	0.9842	0.9542	0.5475
K_1	0.9468	0.9518	0.9858	0.9790	0.5896
K_2	0.9240	0.9223	0.9734	0.9527	0.5276
K_3	0.9310	0.9691	0.9863	0.9693	0.5644
A_1	0.9977	0.9779	0.915	0.982	1.1545
A_2	0.9962	0.9657	0.9073	0.9873	1.0592
A_3	0.9899	0.9416	0.8916	0.9741	1.1317

Several Preformulation trials were undertaken for various proportions of drug and polymer by variation of the ethyl acetate-acetone ratio and dichloromethane-ethanol ratio. Kollidone SR, Acrycoat S 100 and Cellulose acetate were selected as matrixing agent considering its widespread applicability and excellent gelling activity in sustain release formulations and also having the pH-independent and reproducible drug release profile. It was found that Kollidone SR microspheres show desirable high drug content, yield, floatation and adequate release characteristics and hence was suitable for development of a controlled release system. The surface morphology and internal texture of floating microspheres were determined by scanning electron microscopy (SEM). Presence of pores were detected on the microspheres surface which increased in number and size after dissolution, it shows that the drug leach out through these channels.

The prepared microspheres were evaluated for the micromeritics properties. The average of three readings was taken. The mean particle size, flow properties and standard deviation were calculated. The low standard deviation of the measured mean particle size, % Compressibility, Hausner's Ratio and Angle of Repose of all the 9 formulations ensures the uniformity of the microspheres prepared by emulsion solvent evaporation method. The mean particle size was found to be in the range of 252.45 ±4.63 μm to 463.64 ±3.68 μm. The variation in mean particle size could be due to variation in drug-polymer ratio. The % Compressibility of all the microspheres was found to be in the range of 13.86 ±0.26 to 21.55 ±1.88. The Hausner's Ratio of all the microspheres was found to be in the range of 1.17 ±0.041 to 1.29 ±0.041. The Angle of Repose of all the microspheres was found to be in the range of 22.63 ±0.60 to 30.48 ±0.68. For the all formulations, % drug entrapped was found to vary from 72.9 % to 84.7 % and it shows that the drug entrapment is higher in microspheres containing Kollidone SR and lower in microspheres containing cellulose acetate. For the all formulations, % yield was found to vary 44.93 % to 97.40 % and it shows that the yield is higher in microspheres containing Kollidone SR and lower in microspheres containing cellulose acetate.

All formulations floated for more than 8 hours on the simulated gastric fluid USP. But more than 60 % microspheres of Kollidone SR and Acrycoat S 100 were floated for 12 hours whether microspheres containing cellulose acetate did not show buoyancy up to 12 hours.

In the present study, *in vitro* release studies of the floating microspheres were carried out in 0.1 N hydrochloric acid at 37°C for a maximum period of 12 hours. At different time intervals, samples were withdrawn and cumulative % drug release was calculated. The percentage drug release of all the formulations is presented in Figure. Out of 9 formulations tried, the formulation K_1 was found to be satisfactory; since it showed prolonged and complete release with 94.75 % at end of 12 h.

The *in vitro* release data of all formulations were also subjected to model fitting analysis to know the mechanism of drug release from the formulations by treating the data according to zero order, first order, Higuchi and Korsmeyer-Peppas equation. The results are shown in Table. It can be interpreted from the result that the release of drug from the microspheres followed zero order kinetics. Further, the higuchi plot revealed that the drug release from the microspheres obeyed diffusion mechanism. It can be concluded that the formulation of microspheres (K$_1$) containing Hydrochlorothiazide and Kollidone SR (1:1) seems to be promising and further *in vivo* study must be carried out to check the efficacy of preparations. In *vivo* floating ability of microspheres was studied; X-ray photograph of dog stomach with barium sulphate containing floating microspheres is shown in figure. Stability studies for all formulations were performed for three months, at room temperature (25 ± 2°C), at refrigeration temperature (2 to 8°C), and at 40°C / RH 75%. The floating microspheres were stored at various above mentioned temperatures. The prepared microspheres were subjected for drug content analysis after every one month interval. Histogram was plotted between drug content (mg/gm) and time (In days), stability profile of different formulations at various temperatures is shown in figure. The data depicts that the floating microspheres stored at room temperature, refrigeration temperature, were found to be comparatively stable and at 40°C / RH 75 % there was less than 5% degradation at the end of three months.

CONCLUSION

Multi unit gastroretentive drug delivery system has additional advantage of absence of dose dumping as in single unit drug delivery. The present investigation described the influence of viscosity and drug: polymer ratio on Hydrochlorothiazide release. The release and drug entrapment efficiency of the microspheres were affected by the different grade of Methocel. It was found that the Kollidone SR had a dominant role in the drug release from microspheres rather than Acrycoat S 100 and Cellulose acetate. And it can be given in hard gelatin capsule form. Therefore, it may be concluded that drug loaded floating microspheres in combination with Kollidone SR are a suitable drug delivery system for Hydrochlorothiazide and may be used for effective treatment of several cardiovascular disorders.

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