Formulation and evaluation of Gastroretentive hydrochlorothiazide Floating Microspheres: Statistical Analysis

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ABSTRACT

The main objective of this work is to formulate gastroretentive floating microspheres of hydrochlorothiazide and to study the effect of formulation variables like drug to polymer ratio and concentration of polymer dispersion. The hydrochlorothiazide floating microspheres were formulated by using orifice-ionic gelation technique. These microspheres were formulated by using sodium alginate as sustained release polymer, sodium bicarbonate as gas generating agent and calcium chloride as a cross linking agent. The rheological properties of the polymer dispersion were studied. The microspheres were formulated by different drug to polymer ratio's at various concentrations of sodium alginate dispersion. Thus formulated microspheres were evaluated for floating behaviour, drug entrapment efficiency, drug content, micromeritic properties, particle size, swelling index, invitro drug release studies and release kinetics. The better formulation was subjected for stability studies and SEM analysis. The effect of formulation variables on the entrapment efficiency and release rate constant was studied by statistical analysis. The hydrochlorothiazide microspheres formulated with 1:3 drug to polymer ratio and 3.5% sodium alginate dispersion concentration was selected as optimized formulation based on the results.

Key words: Hydrochlorothiazide, Gastro retentive drug delivery systems, floating microspheres, orifice-ionic gelation, sodium alginate.

INTRODUCTION

Gastro retentive drug delivery systems (GRDDS) are suitable for drugs with an absorption window in the stomach or the upper small intestine [1], for drugs which act locally in the stomach[2] and for drugs that are poorly soluble or unstable in the stomach and for drugs that are poorly soluble or unstable in the intestinal fluid[3]. Various approaches have been made to promote the retention of an oral dosage form in the stomach, like floating drug delivery systems (FDDS), expanding and swelling systems, bioadhesive systems, high density systems, modified shape systems and other delayed gastric emptying devices. FDDS or hydrodynamically balanced systems have bulk density lower than gastric fluid and thus remain buoyant in the stomach without affecting the gastric emptying rate for prolonged period of time[4].

In multi particulate drug delivery systems, dosage form of drug is divided into several discrete delivery entities in contrast to single entity dosage forms. The microspheres are having high degree of dispersion in the digestive tract there by decreases absorption variability and avoid dose dumping. The use of microspheres in pharmaceuticals have a number of advantages like taste and odour masking, protection of drugs against environment, production of sustained release, controlled release and targeted medications[5].

Hydrochlorothiazide is one of the best thiazide diuretic. It acts orally and the dosage used for treatment of congestive heart failure and hypertension ranges from 25 to 50 g daily alone or combination with other antihypertensive drugs upto 100 mg if necessary. Hydrochlorothiazide has half-life approx. 2.5 hours and oral bioavailability 70%[6]. It is only absorbed from the upper part of the duodenum and once it passes this absorption site, little or no absorption takes place[7].

Thus The objective of the present study was to formulate the floating microspheres of hydrochlorothiazide in order to retain the formulation in the stomach for better absorption and study the effect of polymer, polymer concentration and viscosity of polymer dispersion on drug release behavior and the buoyancy properties and Micromeritic properties of prepared formulations.

MATERIALS AND METHODS

Materials

Hydrochlorothiazide is gift sample from NATCO pharmaceuticals Pvt. Ltd, Sodium alginate, sodium bicarbonate, Calcium chloride and glacial acetic acid collected from SD fine chemicals Pvt. Ltd, Mumbai.

Methods

Drug-excipient compatability studies by FT-IR:

The physico-chemical compatability between the selected drug (Hydrochlorothiazide) and the excipients used in the research was tested by IR spectroscopy. The samples were scanned under diffuse reflectance using KBr pellet technique. The spectra were recorded for pure drug, pure polymer and drug-polymer mixture using FT-IR. Samples were prepared in KBr desks (2mg sample in 200mg KBr). The scanning range was 400-4000 cm^{-1} and the resolution was 2 cm^{-1}.

Preparation of microspheres:

The floating microspheres containing Hydrochlorothiazide were prepared by orifice ionic gelation technique. Sodium alginate and gas forming agent sodium bi carbonate was dispersed in distilled water to form a homogenous polymer mixture. The drug hydrochlorothiazide was added to the polymer dispersion and mixed thoroughly on a magnetic stirrer to form a homogenous dispersion. The gelation medium was prepared by dissolving calcium chloride in 1.5% glacial acetic acid solution. The homogenous alginate solution was extruded using 24G syringe needle into the gelation medium. The distance between the edge of the needle and surface of gelation medium was about 10 centimetres. The gel microspheres formed were left in the solution for 30 minutes with gentle stirring room temperature to improve mechanical strength. After that the microspheres was collected and washed with distilled water twice, dried at 60^0c until get constant weight of microspheres. [8] The composition and conditions maintained during the preparation of microspheres were showed in table-1.

Table-1: Composition of Hydrochlorothiazide floating microspheres

S. No.	Formulation code	Drug (mg)	Polymer (mg)	Concentration of sodium alginate (%)
1	F1	250	250	3.5
2	F2	250	500	3.5
3	F3	250	750	3.5
4	F4	250	1000	3.5
5	F5	250	1250	3.5
6	F6	250	750	3.0
7	F7	250	750	4.0
8	F8	250	750	4.5

Studies on influence of sodium alginate dispersion viscosity:

Accurately weighed quantity of Sodiumalginate and sodium bicarbonate was dissolved in distilled water to get 3, 3.5, 4.0, 4.5 and 5% w/v solutions. The viscosity of the prepared polymer solutions were measured by using Brook field viscometer at shear rate of 0.13 to 0.66 and torque 10-50%. The viscosity of the polymer solution was noted as per Newtonian, Bingham and power law equations.

Newtonian equation:

$$\sigma = \eta\gamma$$

Where η = Viscosity in cps & γ = Shear rate in $sec^{-1.}$

Bingham equation:

$$\sigma = \sigma_\gamma + \eta_p\gamma$$

Where η_p = Plastic viscosity & σ_γ = Bingham yeild stress.

Power law:

$$\sigma^n = \eta\gamma$$

Where η = Viscosity coefficient and Exponent n= Index of pseudo plasticity.

Micromeritic properties of hydrochlorothiazide floating microspheres[9]:

The flow properties of prepared microspheres were investigated by measuring the bulk density, tapped density, Carr's index Hausner's ratio. The bulk and tapped densities were measured in a 10 ml graduated measuring cylinder. The sample contained measuring cylinder was tapped mechanically by means of constant velocity rotating cam. The initial bulk volume and final tapped volume were noted from which, their respective densities were calculated.

Drug content and entrapment efficacy of floating microspheres[10]:

10mg of drug equivalent formulations was dissolved in 100 ml of 0.1N HCl. The samples were assayed for drug content by UV- spectrophotometer by making suitable dilutions at 272 nm and the drug content was calculated. The percentage entrapment efficiency was calculated by using following formula.

% Drug entrapment = (Calculated drug content/ Theoretical drug content) x 100.

In vitro drug release studies[11]:

The drug release studies were carried out using dissolution apparatus USP type II. A weight of floating microspheres corresponding to 25mg of drug was placed in basket. The dissolution medium used was 900 ml of 0.1N hydrochloric acid at 37°C and 100 rpm. At specific time intervals, 5 ml aliquots were withdrawn and analysed by UV spectrophotometer at the respective λmax value 272 nm after suitable dilution against suitable blank. The withdrawn volume was replaced with an equal volume of fresh 0.1 N hydrochloric acid.

In vitro drug release kinetic studies:

In order to study the exact mechanism of drug release from the alginate microspheres, drug release data was analysed according to zero order, first order, Higuchi and Korsmeyer – Peppas equations. The order of drug release from alginate microspheres was described by using zero order kinetics or first order kinetics. The mechanism of drug release from matrix systems was studied by using Higuchi equation, and Korsmeyer drug release.

Floating behavior[12]:

Three hundred milligrams of the microspheres were placed in 900 ml of 0.1 N hydrochloric acid. The mixture was stirred at 100 rpm in a dissolution apparatus for 12 h. After 12 h, the layer of buoyant microspheres was pipetted and separated by filtration. Particles in the sinking particulate layer were separated by filtration. Particles of both types were dried in a desiccator until constant weight was obtained. Both the fractions of microspheres were weighed and buoyancy was determined by the weight ratio of floating particles to the sum of floating and sinking particles.

% Buoyancy = [Wf / Wf + Ws)] x 100

where Wf and Ws are the weights of the floating and settled microspheres.

Swelling index[13]:

50 mg of microspheres were weighed and transferred to a petri plate containing 10ml of 0.1N Hydrochloric acid maintained at 37^0c. The microspheres were withdrawn at 1hr intervals upto 4 hours. The swelling index was calculated by using the Formula.

Swelling index = $(W_t – W_0 / W_t) \times 100$

Where W_0 = Initial weight & W_t = weight of microspheres at time t.

Particle size analysis[14]:

The particle sizes of the drug loaded formulations were measured by an optical microscope fitted with an ocular and stage micrometer and particle size distribution was calculated. The Olympus model (SZX-12) having resolution of 10 xs was used for this purpose. The instrument was calibrated at 1unit of eyepiece micrometer was equal to 1/10mm (10 µm). In all measurements at least 100 particles in five different fields were examined . Each experiment was carried out in triplicate.

Scanning electron microscopy analysis (SEM)[15]:

The shape and surface characteristics were determined by scanning electron microscopy. A working distance of 20nm, a tilt of zero-degree and accelerating voltage of 15kv were the operating parameters. Photographs were taken within a range of 50-100 magnifications.

Stability studies[16]:

The optimized formulation was kept at room temperature for 3 months. Then the microspheres are evaluated for percent drug entrapment and dissolution study.

Statistical analysis[17]:

All the means are presented with their standard deviation (mean± standard deviation). One way Anova and un paired student's t-test was used to compare the effect of drug to polymer ratio and concentration of sodium alginate dispersion on the entrapment efficiency and release rate constant. A p value <0.05 was considered significant.

Table-2: Rheological properties of polymer dispersions

S.No.	Concentration of polymer dispersion (%)	Viscosity of polymer dispersion (cps)	Correlation coefficient		
			Newtonian	Bingham	power law
1	3	436.15	0.9965	0.9462	0.9521
2	3.5	567.32	0.9979	0.9512	0.9310
3	4	652.40	0.9976	0.9126	0.9462
4	4.5	736.54	0.9943	0.9360	0.9517
5	5	861.30	0.9916	0.9398	0.9623

Fig-1: Rheogram of 3.5% sodium alginate dispersion

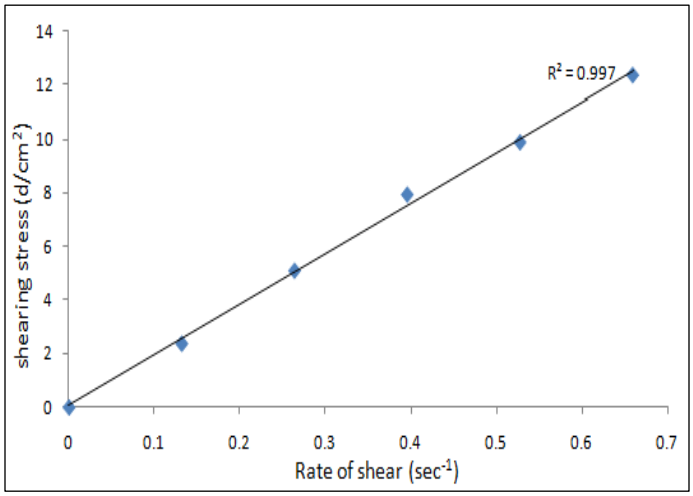


Fig-2: FT-IR graph of Hydrochlorothiazide

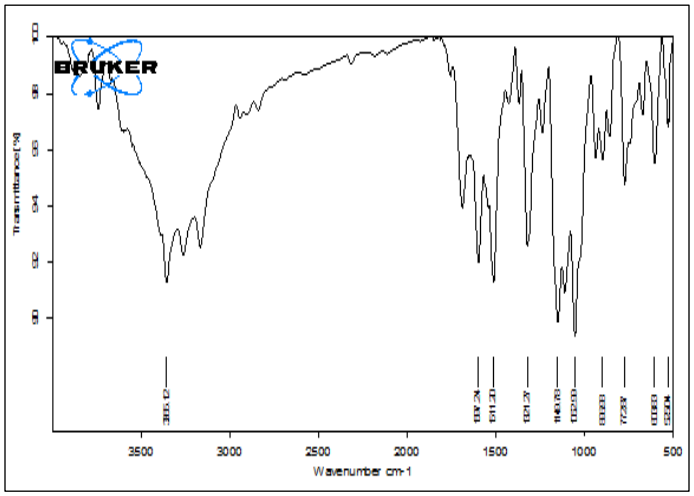


Fig-3: FT-IR graph of Sodium alginate

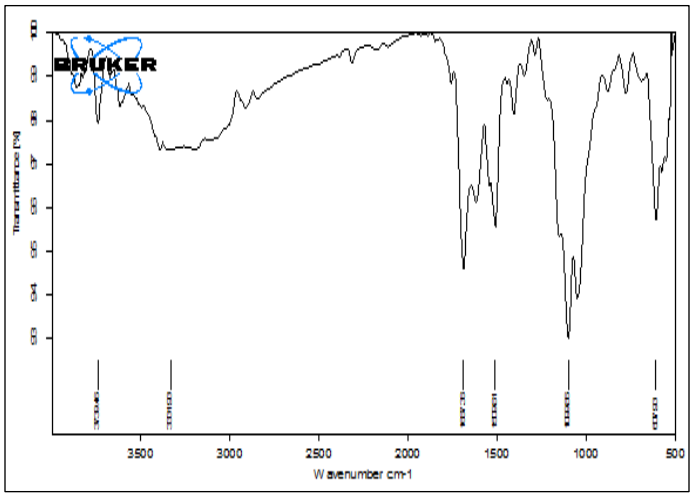


Fig-4: FT-IR graph of formulation containing Hydrochlorothiazide, sodium alginate and $NAHCO_3$

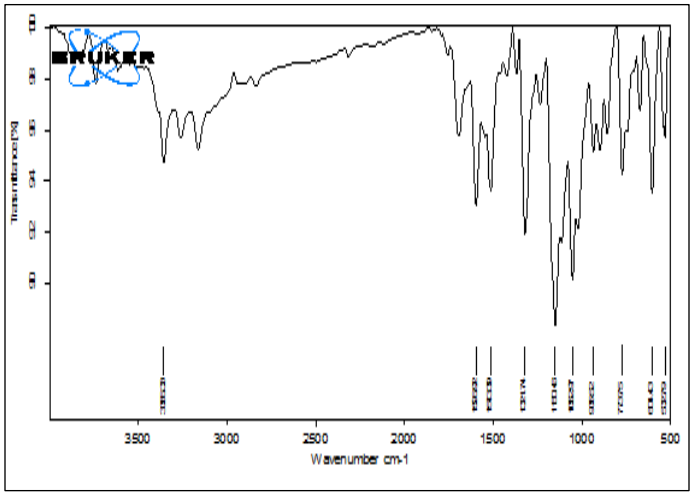


Table-3: Micromeritic properties of the Hydrochlorothiazide floating microspheres

Formulation	Bulk density (gm/ml)	Tapped density (gm/ml)	Hausner's ratio	Carr's index	Angle of repose (^{0})
F1 (1:1)	0.2339±0.23	0.2843±0.19	1.21±0.13	17.72±17	29.25±0.13
F2 (1:2)	0.2613±0.42	0.3134±0.33	1.19±0.19	16.64±23	27.34±0.44
F3 (1:3)	0.3148±0.17	0.3675±0.19	1.16±0.24	14.34±26	24.77±0.24
F4 (1:4)	0.3793±0.33	0.4324±0.35	1.13±0.33	12.28±37	22.93±0.32
F5 (1:5)	0.4496±0.28	0.5017±0.24	1.11±0.32	10.38±29	20.32±0.27
F6 (3%)	0.2873±0.13	0.3439±0.17	1.19±0.14	16.45±0.19	27.79±0.23
F7 (4.0%)	0.3379±0.37	0.3852±0.33	1.14±0.12	12.27±0.15	22.78±0.12
F 8(4.5%)	0.3624±0.43	0.4012±0.45	1.10±0.09	9.67±0.11	21.02±0.33

Table-4: Physico parameters of the Hydrochlorothiazide floating microspheres

Formulation	Dug entrapment efficiency (%)	Drug content (%)	Total floating time (hours)	Swelling index (%)	Average particle size (µm)
F1 (1:1)	53.84±0.36	26.92±0.34	>12	69.15±0.46	435.20±1.78
F2 (1:2)	76.55±0.44	25.51±0.42	>12	72.36±0.38	560.98±1.57
F3 (1:3)	92.30±0.32	23.07±0.33	>12	75.18±0.43	750.30±1.33
F4 (1:4)	92.15±0.27	18.43±0.25	>12	82.13±0.45	930.15±1.69
F5 (1:5)	91.54±0.36	15.25±0.35	>12	84.27±0.35	1036.45±1.54
F6 (3%)	84.59±0.42	21.14±0.39	>12	71.83±0.53	679.35±1.92
F7(4.0%)	91.79±0.28	22.94±0.32	>12	78.35±0.45	840.28±1.75
F8 (4.5%)	90.50±0.27	22.62±0.31	>12	80.20±0.39	915.40±1.56

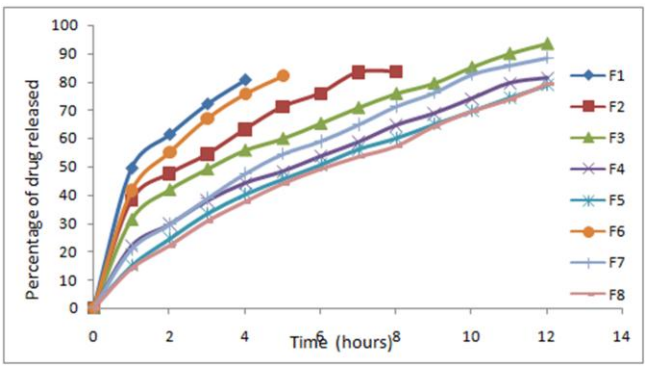


Fig-5: Drug release profile plot of Hydrochlorothiazide floating microspheres

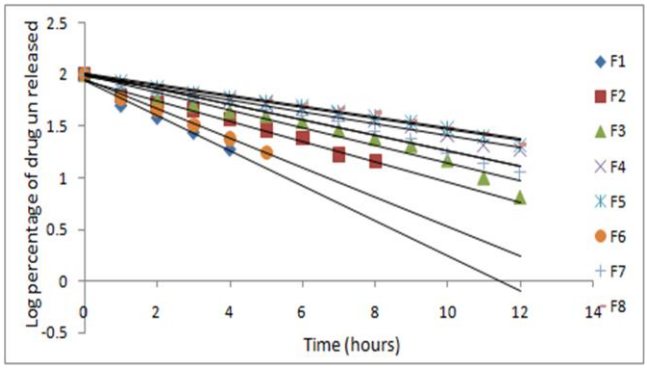


Fig-6: First order profile plot of Hydrochlorothiazide floating microspheres

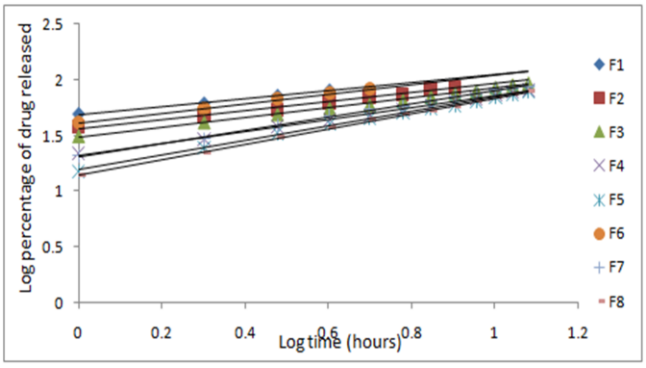


Fig-7: Peppas plot of Hydrochlorothiazide floating microspheres

Table-5: Kinetic profile of all formulations

Formulation	Zero order (r^2)	First order (r^2)	Higuchi (r^2)	Peppas (r^2)	n	Release rate constant K (hr^{-1})	$T_{50\%}$ (hr)	$T_{90\%}$ (hr)
F1 (1:1)	0.8586	0.9750	0.9913	0.9980	0.3532	0.3901	1.77	5.90
F2 (1:2)	0.8205	0.9861	0.9941	0.9946	0.4049	0.2279	3.04	10.10
F3 (1:3)	0.8231	0.9779	0.9873	0.9981	0.4384	0.1963	3.53	11.73
F4 (1:4)	0.9146	0.9924	0.9963	0.9980	0.4402	0.1312	5.28	17.55
F5 (1:5)	0.9483	0.9960	0.9896	0.9989	0.4667	0.1208	5.73	19.06
F6 (3%)	0.8748	0.9880	0.9973	0.9994	0.4307	0.3291	2.10	6.99
F7 (4.0%)	0.9320	0.9916	0.9936	0.9990	0.4501	0.1734	3.99	13.28
F8 (4.5%)	0.9658	0.9925	0.9822	0.9993	0.4809	0.1208	5.73	19.06

Stability studies:

Table-6: Drug release profile of optimized formulation

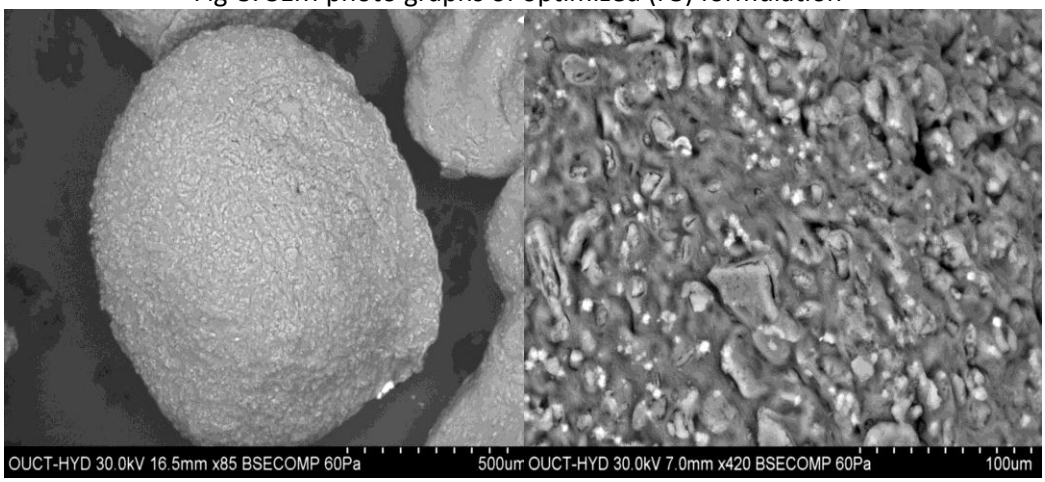
Time (hours)	Cumulative percentage drug release	
	Before storage	After storage
0	0.000	0.000
1	31.298±0.324	30.413±0.213
2	41.836±0.316	41.354±0.314
4	55.777±0.410	54.146±0.465
6	65.295±0.416	63.792±0.432
10	85.144±0.372	83.152±0.298
12	93.501±0.407	92.189±0.426

Table-7: Drug entrapment efficiency of optimized formulation

Formulation	Drug entrapment efficiency (%)	
	Before storage	After storage
F3	92.30±0.32	91.82±0.41

Scanning electron microscopy analysis (SEM):

Fig-8: SEM photo graphs of optimized (F3) formulation



Statistical analysis: Table-8 Results of one way- analysis of variance

Parameter	Degrees of freedom	Sum of squares	Mean sum of squares	Fischer's ratio (F)	Significance
For % entrapment efficiency					
Regression	7	3868	552.6	552.6	<0.0001
Residual	16	16.00	1.000		
Total	23	3884			
For rate constant (k)					
Regression	7	0.2120	0.03029	25960	<0.0001
Residual	16	0.00001867	0.00000167		
Total	23	0.2120			

RESULTS AND DISCUSSION

FT-IR results: The FTIR spectrum of plain HCTZ (Fig-2) and optimized formulation (Fig-4) illustrates peaks at 3355.12, 3267, and 3170 cm-1 assigned to NH and NH2 stretching. It also shows peaks at 1597.24 and 1511.20 cm-1 corresponding to the heterocyclic ring system, and peaks at 2361 and 2339 cm-1 assigned to C-H stretching of the thiazide ring. In addition, it shows a peak at 1321 cm-1 corresponding to SO_2 asymmetric stretching and at 1150 and 1052 cm-1 corresponding to SO_2 symmetric stretching.

Effect of polymer ratio: To study the influence of polymer ratio on entrapment efficiency, drug release, and floating behavior different core: coat ratios (1:1, 1:2, 1:3, 1:4, 1:5) were used to prepare microspheres. The Micromeritic properties of the all the formulations were studied and the results are shown in the table-3. All the formulations showed good flow properties. The particle size of the all the formulations was studied by optical microscopy and the results were shown in table-3. The particle size of all the formulations was in between 435 to 1036µm. As the polymer ratio increases the particle size of the microspheres increases.

All the formulated microspheres were subjected to various physical parameters and results are shown in table-4. The drug entrapment efficiency for all the formulations was in between 53%-92%. The entrapment efficiency increases with the increased ratio of the polymer. As the ratio of the polymer increased more amount of the polymer is available to form a matrix around the drug. This may be attributed to the greater availability of active calcium binding sites in the polymeric chains and consequently, the greater degree of cross linking as the quantity of sodium alginate increased.

All the formulations were subjected to invitro dissolution studies in 0.1N HCl. comparative dissolution profile was in fig-5. The drug release rate was found to be reduced with increased polymer ratio. The slower release rate can be explained by the increase in the extent of swelling and the gel layer thickness that acted as barrier for the penetration medium, there by retarding the diffusion of drug from the swollen alginate beads.

Based on entrapment efficiency and drug release rate the core:coat ratio was optimized as 1:3 for preparing microspheres as it offers good entrapment efficiency, slow and complete release of drug.

Effect of concentration of sodium alginate dispersion: The size of the microspheres is decided by the droplet size of polymer dispersion contain drug delivered through the hypodermic needle. The droplet size of the dispersion is influenced by rheological properties of the polymer dispersion and the diameter of the hypodermic needle. change in diameter affects the surface area provided by the microspheres and hence affects the release rate of drug, so there is a need to study the influence of concentration of polymer dispersion on physico chemical properties of microspheres and release rate. The rheological properties of various concentrations (3% - 5%) the polymer dispersions were studied and the results are shown in the table-2. As the concentration of the polymer dispersion increases the viscosity of the polymer dispersion increases.

The hydrochlorothiazide microspheres formulated at low polymer dispersion concentrations less than 3% were not spherical and had a flatten base at the

points of contact with the drying vessels, however increase in the concentration of sodium alginate dispersion tend to make the particles more spherical. This indicates that at low alginate concentrations the particles were composed of loose network structure which collapsed during drying, other hand higher sodium alginate concentration formed dense matrix structure which prevented collapse of microspheres. As the concentration of sodium alginate in the aqueous dispersion increases above 4.5% the relative viscosity of dispersion was increased and it was difficult to transfer polymer dispersion through the hypodermic needle 24 gauze into the cross linking agent solution and increase in concentration moreover found a small tail one end of beads which significantly affects the flow properties and particle size distribution. Therefore the concentration of polymer dispersion was maintained in the range of 3-4.5% w/v to form microspheres.

The resulting microspheres were evaluated for various Micromeritic properties and the results are shown in table-3. All the formulations show good flow properties. The particle size was measured by using optical microscopy and the values are shown in the table-3. The average particle size was found to be in the range of 679 to 915 µm. The particle size increases with increase polymer concentration. The increase in the polymer concentration increases the swelling index of the microspheres. All the formulated microspheres were subjected to various physical parameters and results are shown in table-4. The drug entrapment efficiency for all the formulations was in between 84%-92%. There was no drastic change in the entrapment efficiency with changing in the polymer concentration.

All the formulations were subjected to invitro dissolution studies in 0.1N HCl. comparative dissolution profile was in fig-5. The percentage drug release from the formulations decreases as the viscosity of the polymer increased, it may be due to increase in diffusion path length for the dissolution medium into the microspheres. Based on the results the sodium alginate concentration was optimized as 3.5% for better entrapment, drug release and spherical microspheres.

Release kinetics: The drug release profile followed first order kinetics as the graph drawn in between log percentage drug un released vs time was found to be linear. The regression values from the first order equation and zero order equation showed. To ascertain the mechanism of drug release Korsmeyer-peppas equation was used, where Q is the percentage of drug release, t is the release time, k is a constant, incorporating structural and geometric characteristics of the release device and n is the release exponent indicative of mechanism of drug release. When n approximates to o.5, a Fickian or diffusion controlled release is implied, where 0.5<n<1 non Fickian transport and n=1 for zero order release. The drug release mechanism from microspheres was Fickian diffusion as n value is less than 0.5. The values of $T_{50\%}$ and $T_{90\%}$ and rate constant(K) for all the formulations are indicated in table-5.

Stability study: The optimized formulation was kept at room temperature and 75% relative humidity for 30 day. Then the microspheres were evaluated for percent drug entrapment and dissolution study. The results are shown in Table-6 and table-7. From the results, it can be concluded that there is no significant change in drug entrapment and dissolution release rate pattern of formulation after stability study.

SEM analysis: The SEM photographs (Fig-8) showed that the fabricated alginate microspheres were spherical in shape. It exhibited a particle size around 748 µm for F3 formulation.

Statistical analysis: The hydrochlorothiazide microspheres formulated with different drug to polymer ratio's and concentration of polymer dispersion were analysed by one-way ANOVA. The results shows (table-8) that the p value is less than 0.0001 it shows that there is more significant difference in the release rate constant and entrapment efficiency between the all formulations. These formulations are further analysed by t-test and the results shows that the p values is less than 0.005, it concludes that there is significant difference in the release rate constant between the optimized formulation and other formulations, but in case of entrapment efficiency there is no significant between F4, F5, F7, F8 and optimized (F3) formulation.

CONCLUSION

The entrapment efficiency and drug release rate was affected by polymer ratio. As the polymer ratio increases the particle size and the entrapment efficiency increases. The drug release rate was found to be reduced with increase in polymer ratio and drug:polymer ratio as 1:3 was selected to be optimum for better entrapment and maintaining drug release for 12 hours. The changes in the concentration of sodium alginate dispersion affect the drug release rate from the microspheres increase in the concentration of polymer dispersion increases the viscosity of the polymer dispersion leads to further increases the particle size of the microspheres. The percentage drug release from the formulation decreases as the viscosity of polymer dispersion was increased and 3.5% concentration of alginate dispersion was selected to be optimum for better entrapment and complete drug release within 12 hours.

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