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Effect of SLS on ethyl cellulose containing cyclobenzaprine HCl floating microspheres

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ABSTRACT

The purpose of this investigation was to design and develop floating microspheres of Cyclobenzaprine HCl by emulsion solvent diffusion technique using Ethyl cellulose polymer to achieve an extended retention in upper GIT and there by improved bioavailability. The microspheres were evaluated for particle size analysis, drug entrapment efficiency, buoyancy studies, polymer compatibility study, *In-vitro* release studies and surface morphology characterized by Scanning electron microscopy (SEM) and X- ray diffractometry. The Microspheres with Ethyl Cellulose have an average size range of 199.86 ± 07.67 to 387.58 ± 05.99 µm. The entrapment efficiency was found to be in the range of 72.75 to 84.1%. The *In-vitro* release studies of the drug from the best formulation GC_b exhibited a sustained release of 72.60 % as studied over 24hrs. Release was best explained by zero-order kinetics model and it shows that the drug release follows diffusion mechanism. GC_b formulation has showed appropriate stability for 90 days by storing the formulation at room temperature. FT-IR data revealed that, compatible and there was no interaction between the drug and excipients added in the formulation. The X-Ray diffraction pattern of a formulation (GC_b) showed a combined pattern of those of the polymers and drug i.e. amorphous and crystalline respectively. SEM images of various SLS containing formulations revealed that the floating microspheres were spherical with no visible major surface irregularity, having smooth surface and also observed hollow microspheres.

Key words: Cyclobenzaprine HCl, Ethyl cellulose, Floating microspheres, Drug release.

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INTRODUCTION

One of the most feasible approaches for achieving a prolonged and predictable drug delivery in the GI tract is to control the gastric residence time by using gastro-retentive dosage forms (GRDF s). It remains in the gastric region for several hours and hence prolongs the gastric residence time of drug. It has several advantages over immediate release dosage form including the minimization of fluctuations in drug concentration in plasma and at the site of action over prolonged periods of time, resulting in optimized therapeutic efficiencies and reduce the side effect, reduction of total dose administered and reduction of administration frequency leading to improved patient compliances [1- 2].

Floating microspheres are gastro-retentive drug delivery systems based on non-effervescent approach. These microspheres are characteristically free flowing powders having a size less than 199 µm and remain buoyant over gastric contents and 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 [3- 4].

Cyclobenzaprine HCl, chemically it is 3-(5H-dibenzo [a, d] cyclohepten-5-ylidene)-N, N dimethyl-1-propanamine hydrochloride. Cyclobenzaprine HCL is a skeletal muscle relaxant and a central nervous system (CNS) depressant. Cyclobenzaprine HCL has a long half life up to 18 hrs and the absorption slowly but well absorbed after oral administration in the upper part of GIT hence it is suitable for gastro-retentive system. The present work consists of design and development of floating microspheres of Cyclobenzaprine HCl using ethyl cellulose in different proportions.

MATERIALS AND METHODS

Materials

Cyclobenzaprine HCl was received as a gift sample from Aurobindo pharma, Hyderabad, India. Ethyl cellulose and SLS was received as a gift samples from Glukem pharmaceuticals (P) Ltd, India. Dichloromethane, Ethanol, HCL and Tween 80 were purchased from Aasha Chemicals limited, Hyderabad. All other ingredients were of analytical grade.

For the present study instruments like Electronic balance (Essae, DS-852 series), pH meter (EI), Mechanical stirrer (Eltek motor, Mumbai), Double Beam UV Spectrophotometer (Analytical, Germany), USP dissolution test apparatus (TDT- 08L, Electro Labs, Mumbai), Optical microscopy (Khera Instruments Pvt Ltd, Delhi), Scanning electron microscopy (JSM-5610, JEOL Japan), Fourier transform-Infrared spectrophotometer(FT-IR) (Perkin Elmer Life sciences, USA), and X- ray diffractometer (XRD) were used.

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Method

Preparation of floating microspheres

The floating microspheres were prepared by emulsion solvent diffusion method [5] established by Kawashima et al with required modifications. Briefly the drug and Ethyl cellulose are shown in **Table 1** were mixed in ethanol at various ratios by using blending solvent dichloromethane. The slurry was introduced into 250ml beaker containing 0.2% SLS while being stirred at 750rpm by mechanical stirrer for 1hr at room temperature and allow the solvent to evaporate completely there by the obtained microspheres were collected by filtration. The collected microspheres are dried for 1hr at room temperature and subsequently stored in desiccators.

Evaluation of Floating Microspheres

The microspheres are characterized by their micromeritic properties, such as Particle size, Bulk density, Tapped density, percentage Compressibility index, Hausener's ratio and Angle of repose. The results are expressed as mean ± S.D (n=3). The shape and size distribution [7] of Floating microspheres in terms of average diameter (d_{avg}) was determined by using optical microscopic method. The moisture content [8] of formulated microspheres was calculated by the procedure proposed by (Leon Lachmann *et al*.). The moisture in a wet solid is that calculated on dry wet basis: this value is referred to as moisture content. An *In-vitro* floating ability [9] study was carried out using simulated gastric fluid USP containing 1% Tween 80 as a dispersing medium. Microspheres were spread over the surface of 500ml of dispersing medium at 37 ± 0.5^{o} C. A paddle rotating at 50 rpm agitated the medium. Each fraction of microspheres floating on the surface and those settled down were collected at a predetermined time point. The collected samples were weighed after drying. Floating ability was calculated by considering final weight of the microspheres to that of initial weight. Drug entrapment efficiency was determined by crushing 50mg of floating microspheres which were dissolved in 50ml of 0.1N HCl. The solution was filtered through a 0.45µ membrane filter, diluted suitably and the absorbance of resultant solution was measured spectrophotometrically at 290 nm using 0.1 N Hydrochloric acid as blank.

The release rate of Cyclobenzaprine HCl from floating microspheres was determined using United States Pharmacopeia (USP) Dissolution Testing Apparatus II (paddle method; Veego Scientific, Mumbai, India). The dissolution test was performed using 900 ml of 0.1N hydrochloric acid, at 37 ± 0.5°C and 50 rpm. A sample (10 ml) of the solution was withdrawn from the dissolution apparatus hourly over 24hrs and the samples were replaced with fresh dissolution medium [10]. The samples were filtered through a 0.45µ membrane filter and diluted to a suitable concentration with 0.1N hydrochloric acid. Absorbance of these solutions was measured at 290nm using a Shimadzu 1800UV/Vis double-beam spectrophotometer. Cumulative percentage drug release was calculated using an equation obtained from a standard curve.

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Characterization of Cyclobenzaprine HCl floating microspheres

FT-IR Studies: Fourier Transform Infrared Analysis (FT-IR) [11] measurements of pure drug, carrier and drug-loaded floating microspheres formulations were obtained using a Perkin-Elmer system 200 FT-IR spectrophotometer. The pellets were prepared on KBr-press under hydraulic pressure of 150kg/cm^2; the spectra were scanned over the wave number range of 4000 to 400 Cm-1 at the ambient temperature.

Scanning electron microscopy (SEM): The surface topography, morphology, particle size, etc., were determined by Scanning Electron Microscopy (SEM) [12] (Jeol, JSM-6360, Japan) (V. S. Masthiholimath *et al*.,). Dry micro particles were placed on an electron microscope brass stub and coated with gold in an ion sputter. Picture of micro particles were taken by random scanning of the stub.

X-ray Diffraction: Different samples were evaluated by X-ray powder diffraction [13]. Diffraction patterns were obtained by using X-ray diffractometer with a radius of 240mm. A system of diverging and receiving slits of 1^o and 0.1mm respectively was used. The pattern was collected with 40 Kv of tube voltage and 30 mA of tube and scanned over the range of 5-60.

Stability studies: The encapsulated floating microsphere formulations were subjected for stability study [13]. The stability study was carried out according to ICH guidelines at 40^0 C and relative humidity at 75 % for three weeks. For stability study, the formulation was selected as an optimum formulation depending upon release rate and floating properties. The microspheres were sealed in aluminum packing coated inside with polyethylene. These sample containers were placed in desiccators maintained at 75% RH. The product was evaluated for *In-vitro* drug release and drug content. The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under influence of a variety of environmental factors such as temperature, humidity and light, and enables recommended storage conditions.

Mathematical Modeling: The success of ethyl cellulose with SLS in controlling the release of the drug was studied under the following heads to understand the order and probable underlying mechanism involved in the release pattern. The various mathematical models studied [14] include zero order, first order, Higuchi model, Korsmeyer model and Hixson model.

RESULTS AND DISCUSSION

The percentage yield of different batches was determined by weighing the floating microspheres after drying. The percentage yields of different formulation were in the range of 50.0 to 78.5%. The drug entrapment efficiency was decreased with increased polymer concentration in floating microspheres. The drug entrapment efficiency of different batches of floating microspheres was found in the range of 72.75 to 84.1%. The percentage moisture content decreased with increased polymer concentration in floating microspheres. The percentage moisture content of different batches of microspheres was found in the range of

6.88 to 22.60%. The Percentage yield, Drug entrapment efficiency and Percentage Moisture content results were shown in the **Table 2**. Results revealed that GC_a gives less percentage yield due to the rotation speed of the propeller / stirrer. High percentage moisture content of GC_a was due to the less polymer concentration. Drug Entrapment Efficiency of GC_a was higher when compared to other formulations. This can be attributed to the preparation characteristics of polymer used that could facilitate the diffusion of a part of entrapped drug to the surrounding medium during the preparation of floating microspheres.

The mean particle sizes of floating microspheres significantly increased with increasing polymer concentration were shown in **Table 3**. The particle size was found in the range of 199.86 ± 07.67 to 387.58 ± 05.99 µm for ethyl cellulose microspheres respectively. The tapped density of microspheres was found to range from 0.166 to 0.216 g/cc for ethyl cellulose. The bulk density ranged from 0.150 to 0.176 g/cc for ethyl cellulose microspheres. Angle of repose for Ethyl cellulose microspheres was between 18.71 to 23.09^0 thus, indicating good flow property for ethyl cellulose microspheres due to the polymer. The findings were supported by compressibility index, which were > 15 indicating good flow properties. Results concluded that all the prepared floating micro spherical formulations containing ethyl cellulose exhibits gave good flow properties.

The optical microscopic images of GC_b and GC_c Floating microspheres were shown in the **(Fig 1)**. Optical images showed that the prepared GC_b and GC_c exhibits spherical shape with an average size of 293.29µm.

The Scanning electron microscopy images of GC_b formulation **(Fig 2)** was observed to be mostly spherical and exhibits smooth surface. A SEM image of microspheres (GCb) shows the uniform encapsulation of Ethyl cellulose. Comparison of the various microsphere images revealed that the surface of the carrier particles at the medium level of polymer loading appeared to be more uniform and thinner than the rough and uneven encapsulation at high polymer coating. Some microspheres in the images are broken, which might be due to handling and processing.

FT-IR spectra of Cyclobenzeprine HCL and GC_b formulation were recorded. The Cyclobenzeprine HCL present in the formulation GC_b was confirmed by FT-IR as shown in **(Fig 3).** No predominant drug interaction was detected. Although there were some mild changes in 3600- 3200 cm^{-1} band width. This may be due to formation of band between drug and polymer but all other peaks and band shows in presence of drug in formulation. The region 3600- 3200 cm^{-1} was a stretching region of the functional group C-H of aromatic ring (3100- 3000 cm^{-1}), 2900–2880 (Methyne C-H stretch) and C-H of alkenes (3100- 3000 cm^{-1}) and C-H of alkane (3000 cm^{-1}). All these peaks have appeared in pure Cyclobenzeprine HCl and formulation GC_b indicating no chemical interaction between Cyclobenzeprine HCl and excipients. It also confirmed that the stability of drug during formulation.

The powder X-ray pattern of every from a compound is unique. The powder X-ray pattern of Cyclobenzaprine HCl , and formulation GC_b is shown in **(Fig 4)**. The X-ray pattern of

Cyclobenzaprine HCl indicated its existence in amorphous form. The X-ray pattern of formulation showed a combined pattern of those of the polymer and drug i.e. crystalline and amorphous. The drug is dispersed in the polymer matrix.

The floating test was carried out to investigate the floating ability of the prepared microspheres. Floating ability of different formulations was found to be differed according to the polymer ratio with SLS used as an aqueous phase (GC_a to GC_d formulations) showed floating ability over 24hrs. The results are given in **Table 4**.

In-vitro Cyclobenzaprine HCl release studies were performed in 0.1N HCl for 24 hrs. Floating microspheres showed sustained release of the drug in acidic environment and the drug release was found to be approximately linear. The percentage drug release of Cyclobenzaprine HCl significantly decreased with increasing polymer concentrations **(Fig 5)**. The increased density of the polymer matrix at higher concentrations results in an increased diffusional path length. This may decrease the overall drug release from the polymer matrix. Furthermore, smaller microspheres are formed at a lower polymer concentration and have a larger surface area exposed to dissolution medium, giving rise to faster drug release.

The effect of SLS as an aqueous phase on the release rate was studied. In which formulation GC_b showed best appropriate balance between buoyancy and drug release rate. And the stability of GC_b after 90 days was studied and shown in **(Fig 6)**. The release data has been shown in **Table 5**.

The results for the mathematical modeling of the *in-vitro* drug release data for the floating microspheres have been compiled and the R^2 values shown in the **Table 6**. From the results it is apparent that the regression coefficient value closer to unity as in the case of the Zero order plots indicates that the drug release follows a Zero order mechanism. The data indicates a lesser amount of linearity when plotted by the First order equation. Hence it can be concluded that the major mechanism of drug release follows Zero order kinetics.

Further, the translation of the data from the dissolution studies suggested possibility of understanding the mechanism of drug release by configuring the data into various mathematical modeling such as Higuchi and Korsemeyer plots. The mass transfer with respect to square root of time has been plotted, revealed a linear graph with regression value close to one stating that the release from the matrix was through diffusion. The coefficient of determination (R^2) was found to be much closer to 1 for the Hixson- Crowell equation. Slope values suggest that the drug release from matrices followed diffusion and non-Fickian transport implicated.

CONCLUSION

From this study, it can be concluded that, the formulation retained for longer periods of time in the stomach (spatial control) and provides sustained release of the drug. Hence,

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cyclobezaprine HCL floating microspheres retained for longer periods of time in the stomach may leads to improve the therapeutic effect of the drug by increasing its bioavailability.

Table 1: Formulation of Cyclobenzaprine HCl floating microspheres.

S. No	Batch name	Drug : Ethyl cellulose	Ethanol: Dichloromethane	0.2% SLS (ml)
1.	GC_a	1:1	1:1	250
2.	GC_b	1:2	1:1	250
3.	GC_c	1:3	1:1	250
4.	GC_d	1:4	1:1	250

Table 2: Percentage yield, Drug entrapment efficiency and percentage Moisture content of floating microspheres

S.No	Batch name	Percentage yield	Percentage drug entrapment efficiency	Percentage moisture content
1.	GC_a	50.0 ± 0.816	84.1 ± 0.817	22.60 ± 0.816
2.	GC_b	74.33 ± 1.280	81.63 ± 0.012	11.06 ± 0.016
3.	GC_c	78.5 ± 1.637	73.08 ± 0.816	9.56 ± 0.130
4.	GC_d	74.0 ± 0.816	72.75 ± 0.984	6.88 ± 0.024

Table 3: Micromeritic studies of Cyclobenzaprine HCl Floating microspheres

Batch name	Particle size from optical microscopy (µm)	Bulk density (g/cm^3)	Tapped density (g/cm^3)	Hausner's ratio	Percentage Compressi -bility index	Angle of repose (θ)
GC_a	199.86 ± 07.67	0.150 ± 0.0016	0.166 ± 0.0008	1.107 ± 0.0016	09.64 ± 0.0124	23.09 ± 0.816
GC_b	260.92 ± 09.08	0.176 ± 0.0016	0.200 ± 0.0032	1.136 ± 0.0014	12.00 ± 0.1632	21.46 ± 0.849
GC_c	324.81 ± 08.73	0.168 ± 0.002	0.216 ± 0.0004	1.293 ± 0.0008	22.58 ± 0.0163	19.26 ± 0.024
GC_d	387.58 ± 05.99	0.166 ± 0.0008	0.214 ± 0.0004	1.289 ± 0.0021	22.43 ± 0.0124	18.71 ± 0.030

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Table 4: Percentage Floating ability at different time intervals of Cyclobenzaprine HCl Floating microspheres

S. No	Batch code	% Floating ability at different time intervals		
		8th hr	16th hr	24th hr
1.	GC_a	92.2 ± 0.124	86.7 ± 0.7760	81.23 ± 0.816
2.	GC_b	91.17 ± 0.124	87.52 ± 0.014	77.45 ± 0.069
3.	GC_c	91.0 ± 0.081	87.43 ± 0.042	74.89 ± 0.054
4.	GC_d	91.2 ± 0.081	85.19 ± 0.004	76.46 ± 0.004

Table 5: *In-vitro* drug release studies of Cyclobenzaprine HCl Floating microsphere

Batch code	% drug release at different time intervals						
	2nd hr	4th hr	8th hr	12th hr	16th hr	20th hr	24th hr
GC_a	78.55	82.71	85.08	86.79	89.46	92.16	95.68
GC_b	37.05	42.77	53.86	58.78	63.00	67.30	72.80
GC_c	28.65	29.35	36.70	42.02	47.31	52.55	57.08
GC_d	18.69	21.26	25.01	29.75	33.69	39.15	42.71

Table 6: Mathematical models for Cyclobenzaprine HCl Floating microspheres.

Batch code	Zero order	First order	Higuchi model	Korsmeyer model	Hixson model
GC_a	0.975	0.932	0.971	0.764	0.961
GC_b	0.958	0.988	0.994	0.938	0.981
GC_c	0.999	0.997	0.981	0.871	0.999
GC_d	0.998	0.995	0.973	0.868	0.996

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Fig. 1: Optical microscopic images of GC_b and GC_c formulations of Cyclobenzaprine HCl Floating microspheres.

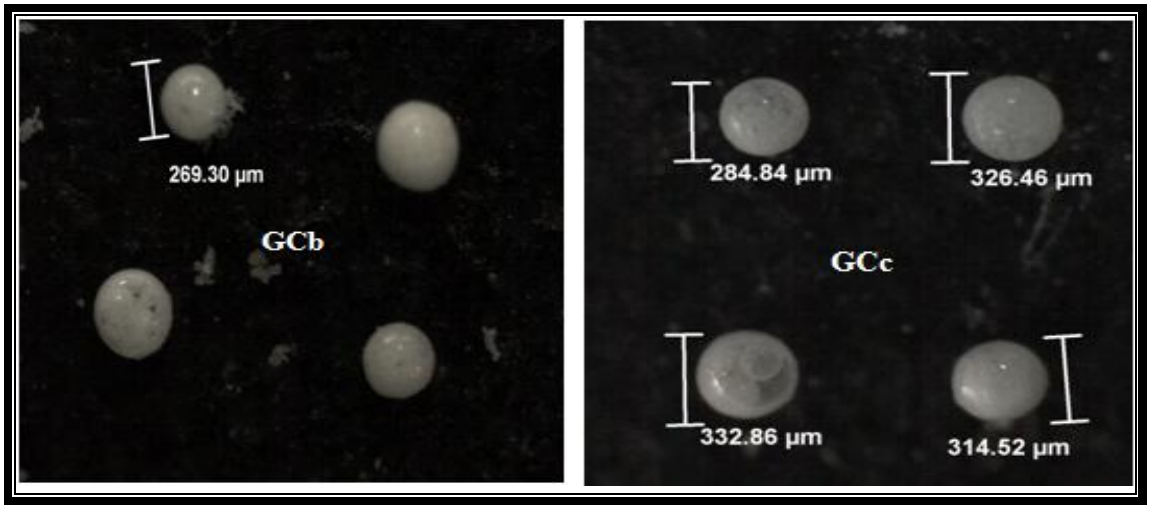

Fig. 2: Scanning electron microscopy image of GCb 1: Diameter of floating microsphere; GCb 2: Group of floating microspheres shows their diameter.

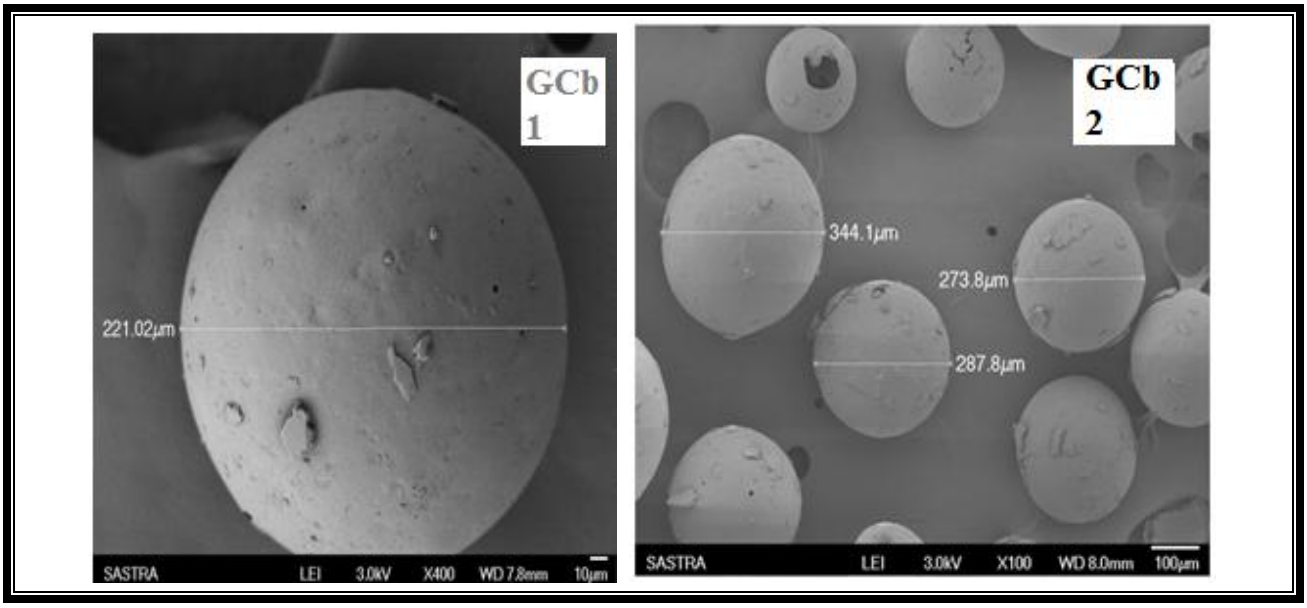

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Fig. 3: FT-IR Spectrum of Pure drug and GC_b

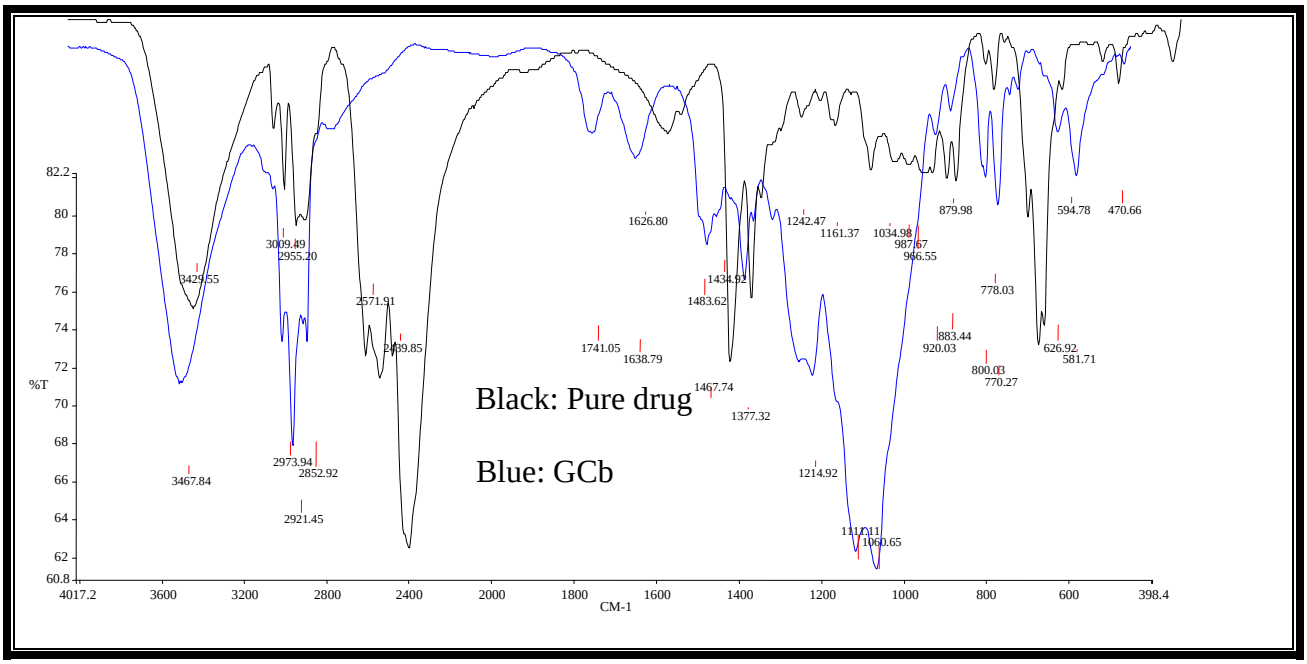

Fig. 4: X-ray diffraction pattern of pure drug and best formulation GC_b.

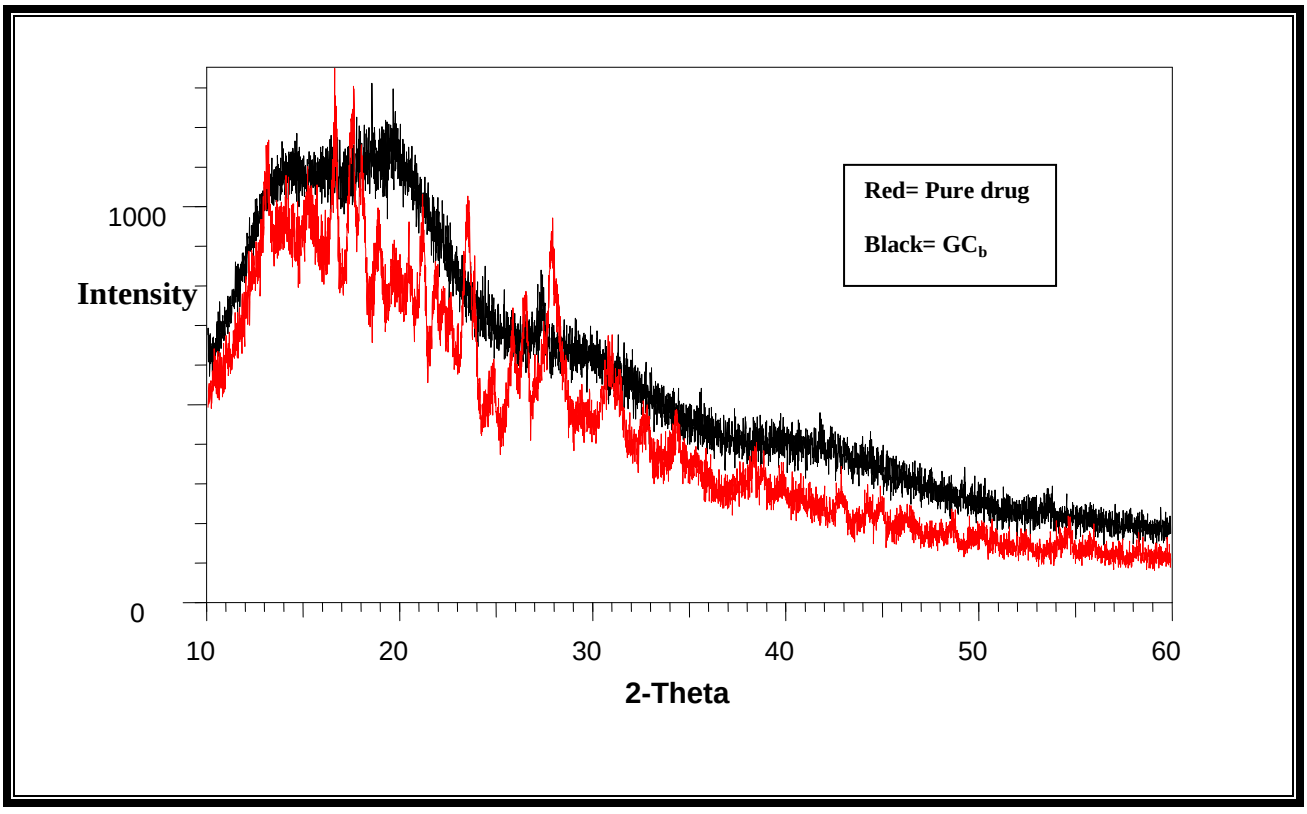

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Fig. 5: Effect of SLS on *In-vitro* drug release study of Cyclobenzaprine HCl floating microspheres.

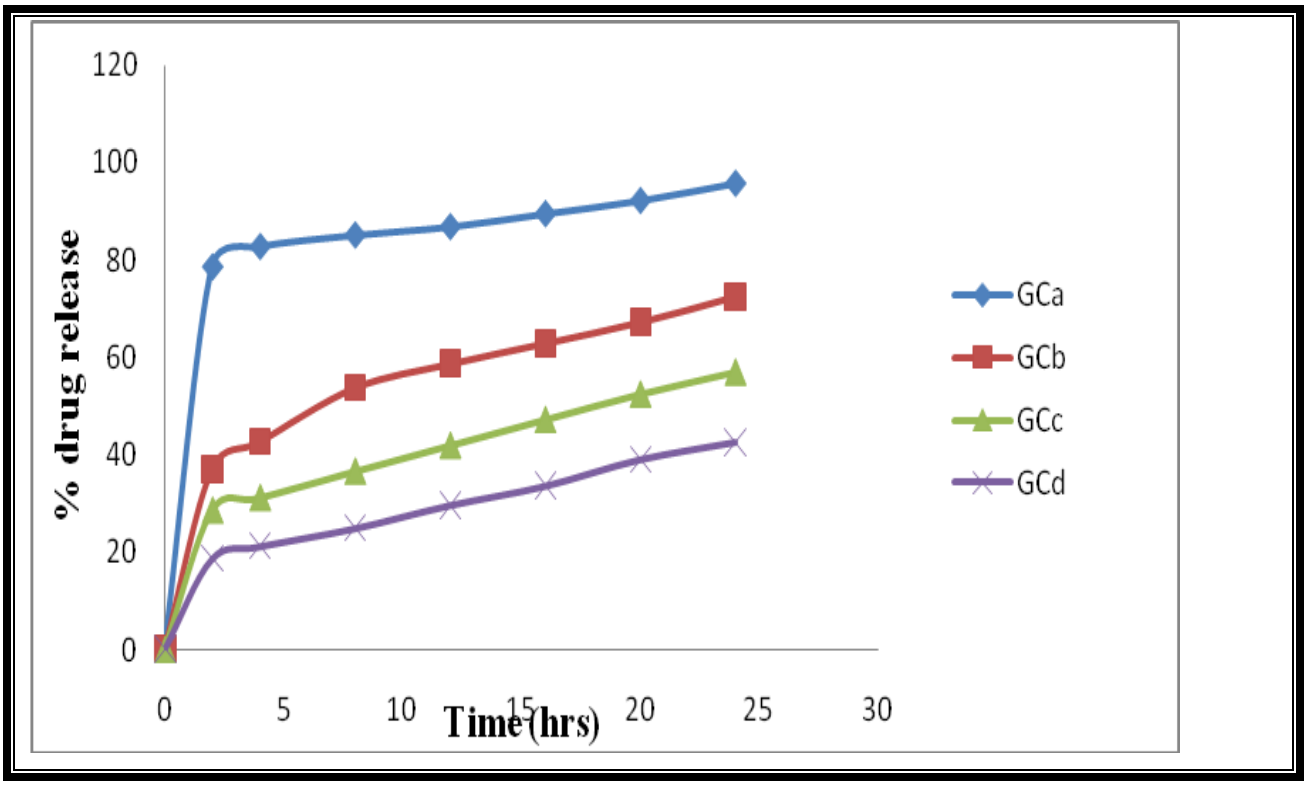
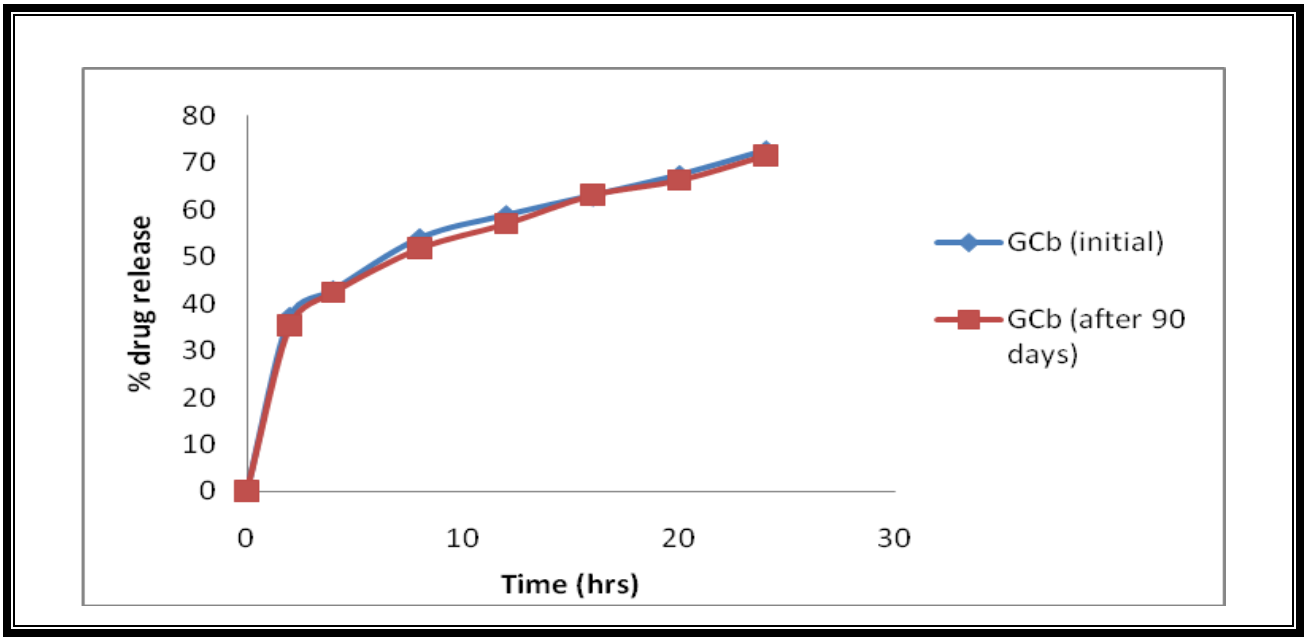


Fig. 6: Stability study of GCb formulation initial and after 90 days at room temperature



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REFERENCES

[1] Chien YW. Novel drug delivery system, Eds. 2nd; Marcel Dekker Inc publications: New York, 1992 (50), pp: 161- 72.

[2] Khar RK, Vyas SP. Targeted and controlled drug delivery novel carrier system, Eds. 1st; CBS Publishers and Distributors: New Delhi, 2002, pp: 417- 41.

[3] www.pharmainfo.net/.../floating-microspheres-development-characterization-and-applications - United States

[4] Arora S, Ali J, Ahuja A, Khar RK, Baboota S. AAPS Pharm SciTech 2005; 6(3): 372-90.

[5] Kawashima Y, Niwa T, Takeuchi H, Hino T, Itoh Y. J Pharm Sci 1992; 81: 135–40.

[6] Kiyoyama S, Shiomori K, Kawano Y, Hatate Y. J Microencapsul 2003; 20: 497-508.

[7] Thanoo C, Sunny MC, Jayakrishnan A. J Pharm Pharmacol 1993; 45: 21-40.

[8] Banker GS, Anderson NR. The theory and practice of industrial pharmacy; Lachman, L., Lieberman, H. A., Kanig, J. L., Eds. 3rd; Varghese Pub. House: Bombay, 2003, p 297-300.

[9] Rajinikanth PS. Chem and Pharm Bulletin- Tokyo, 2009; 57(10): 1068-75.

[10] Lee JH, Park TG, Choi HK. J Microencapsul 1999; 16(6): 715-29.

[11] Luypaert J, Zhang MH, Massart DL. Analytica Chimica Acta 2003;478(2): 303–12.

[12] Mastiholimath VS. J Microencapsul 2008; 25(5): 307–14.

[13] Carstensen JT. Drug stability principles and practice. Eds. 3rd; Marcel Dekker Inc publications: New York, pp: 145-89.

[14] Hemangi S. Patil, Moreswar P.Patil, Bharat W. Tekade, Vinod M. Thakare, Vijay R. Patil. Arch Pharm Sci & Res 2009; 1(1):194-98.