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DESIGN AND DEVELOPMENT OF HYDROXYZINE HYDROCHLORIDE CONTROLLED RELEASE TABLETS BASED ON MICROSPONGE TECNOLOGY

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ABSTRACT

The purpose of the present study aims to design novel drug delivery system containing hydroxyzine hydrochloride microsponges and to prepare controlled release microsponge tablets. Hydroxyzine hydrochloride is an anti-histaminic drug used in the treatment of urticaria and pruritus. It has a half-life of about 3-4hrs. The Microsponge Delivery System is a unique technology for the controlled release of active agents, and it consists of porous polymeric microspheres, typically 10–50 µm in diameter. Microsponges of the drug were prepared by using polymer Methocel 10000cps and in combination with Eudragit –S 100, Eudragit-L 100, Eudragit-RL 100 and Eudragit-RS 100. These are prepared by oil in oil emulsion solvent diffusion method using acetone as dispersing solvent and liquid paraffin as the continuous medium. Magnesium stearate was added to the dispersed phase to prevent flocculation of polymeric microsponges. Compatibility of the drug with adjuncts was studied by FT-IR. Production yield, loading efficiency, particle size analysis, surface morphology and invitro release studies were carried out. The microsponge formulation (F8) was found to be stable at 40^{O}C and 75% relative humidity with respect to particle size, loading efficiency and invitro drug release. The optimized microsponge formulation F8 was compressed into tablets by using different diluents (Microcrystalline cellulose, directly compressible lactose and directly compressible dicalcium phosphate dihydrate). Mechanically strong tablets were obtained owing to plastic deformation of sponge like structure of microsponges. Thickness, hardness, friability, % drug content and invitro release studies were done on microsponge tablets. The results were kinetically evaluated and the release rate of hydroxyzine hydrochloride was found to be zero order. The microsponge tablet formulation, F11 showed controlled release of hydroxyzine hydrochloride for 12hrs.

INTRODUCTION

Oral administration remains the most popular route for drug delivery and tablets are the preferred dosage form. Tablets offer safe and convenient method of active pharmaceutical ingredients (API) administration with excellent physicochemical stability and accurate dosing. They are mass produced with robust quality control and provide different branding possibilities through pigmented film coating, shapes, sizes, printing and logos .The dissolution rate of drug from its dosage form is considered as an important parameter in the bioavailability. The rate determining step in the absorption of orally administered hydrophilic drugs is the rate of drug permeation through the biomembranes. [1, 2] Pharmaceutical products designed for oral delivery are mainly conventional drug delivery systems, which are designed for immediate release of drug for rapid absorption. These have limitations such as atypical peak- trough plasma concentration-time profile, drugs with short half life require frequent administration, unavoidable fluctuations in the drug concentration. In order to overcome the drawbacks of conventional drug delivery systems, several technical advancements have led to the development of controlled drug delivery systems. A microsponge delivery system (MDS) is highly crosslinked, patented, porous, polymeric microspheres that acquire the flexibility to entrap a wide variety of active ingredients such as emollients, fragrances, sunscreens, essential oils, anti-infective, anti-fungal and anti-inflammatory agents etc that are mostly used for prolonged topical administration and recently for oral administration [3]. It is a unique technology for the controlled release which consists of microporous beads normally 10-50 microns in diameter, loaded with active ingredients that subsequently release them over a time in a controlled manner. The objective of the present study was to design and optimize the microsponges of hydroxyzine hydrochloride by oil in oil emulsion solvent diffusion method using methocel 10000cps and in combination with eudragit-S100, eudragit-L100, eudragit-RL100, eudragit-RS100, acetone as dispersing solvent and liquid paraffin as the continuous medium. Magnesium stearate was added to the dispersed phase to prevent flocculation of polymeric microsponges. The optimized microsponge formulation was compressed into tablets by using different diluents (Microcrytalline cellulose, directly compressible lactose and directly compressible dicalcium phosphate dihydrate). Hydroxyzine hydrochloride is a piperazine derivative and has been shown to have potent anxiolytic, sedative and antihistaminic activities. Hydroxyzine hydrochloride is used for the treatment of urticaria and pruritus. Urticaria is caused by the release of histamine and other mediators of inflammation (cytokines) from cells in the skin [4].

The skin lesions of urticarial disease are caused by an inflammatory reaction in the skin, causing leakage of capillaries in the dermis, and resulting in an edema which persists until the interstitial fluid is absorbed into the surrounding cells. This process can be the result of an allergic or nonallergic reaction, differing in the eliciting mechanism of histamine release [5]. Hydroxyzine hydrochloride is rapidly absorbed through the gastrointestinal tract. The daily dose of hydroxyzine hydrochloride was taken 3-4 times in divided doses for every 6hrs. Hence, to maintain the plasma drug concentration and to reduce the frequency of drug administration hydroxyzine hydrochloride microsponge tablets were prepared.

MATERIALS AND METHODS

Materials

Hydroxyzine hydrochloride was obtained as a gift sample from Symed laboratories. Methocel 10000cps, eudragit-S100, eudragit-L100, eudragit-RL100, eudragit-RS100, acetone, magnesium stearate and talc were procured from Research lab fine chem. Industries, Mumbai. Liquid paraffin was procured from Jiangsu Huaxi International Trade Co.Ltd, China. Microcrystalline cellulose, directly compressible lactose and directly compressible dicalcium phosphate dihydrate were procured from S.D.Fine Chemicals, Mumbai. All the reagent and materials were of analytical or pharmacopoeia grade.

Drug polymer compatibility studies

Compatibility of drug with excipients was determined by carrying out IR studies. Infrared spectrum of Hydroxyzine hydrochloride and physical mixture of drug and polymer was determined on Fourier Transform Infrared spectrophotometer (8400 S Shimadzu) using KBr pellet method. The results were shown in figure I&II.

Procedure for the preparation of hydroxyzine hydrochloride microsponges by oil in oil emulsion solvent diffusion method

A specific weight of polymer was dissolved in acetone. Once a clear solution was obtained, 0.5 g of the drug was added in addition to magnesium stearate (3% *w/v* of solvent), and the whole mixture was kept in the ultrasonic bath of 70-kHz frequency for 2 min where homogenous dispersion was obtained [6,7,8].

The mixture was then poured into 300 ml of liquid paraffin previously cooled to 10 ± 0.5°C while it was stirred by a mechanical stirrer for 45 min. The oil in oil emulsion prepared was gradually heated to 35 ± 2°C and was stirred at this temperature for

another 30 min. During this time, the acetone was completely removed by diffusion into liquid paraffin and evaporation through the air/liquid interface.

The solidified microsponges were filtered, washed six times with 50 ml of n-hexane, air-dried at room temperature for 12 h, and stored in a desiccator for further investigations [9]. The same procure was followed for all the preparations and the composition of various formulae was represented in table I.

Table I. Composition of different formulation blends of microsponges

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Internal phase									
Hydroxyzine.Hcl	500	500	500	500	500	500	500	500	500
Methocel 10000Cps	60	120	180	180	180	180	180	180	180
Eudragit-S 100	-	-	-	40	-	-	-	-	-
Eudragit-L 100	-	-	-	-	40	-	-	-	-
Eudragit RL-100	-	-	-		-	40	-	-	-
Eudragit RS-100	-	-	-	-	-	-	40	60	80
Polymer: Acetone(w/v)	1:5	1:5	1:5	1:5	1:5	1:5	1:5	1:5	1:5
% w/v of Mg.stearate	3	3	3	3	3	3	3	3	3
External phase									
Liquid Paraffin	300	300	300	300	300	300	300	300	300

CHARACTERIZATION OF PREPARED MICROSPONGES

Fourier transform infrared analysis

Infrared spectroscopy was conducted using FT-IR spectrophotometer and the spectrum was recorded in the wavelength region of 4000 to 400 cm^{-1}. The procedure consisted of dispersing the sample (drug alone, mixture of drug and excipients and the optimized formulation) in KBr and compressed into discs by applying a pressure of 5 tons for 5 minutes in a hydraulic press. The pellet was placed in the light path and the spectrum was recorded.

Photomicroscopic Analysis

A sample of microsponge powder was examined for morphological characteristics under a binocular image analyzer microscope (Leica DMLB, Berlin, Germany) and photographed (figure III) at a magnification of ×50.

Scanning Electron Microscopy

The detailed surface topography of the selected microsponges was observed using a scanning electron microscope (Scimadzu Corporation, Japan). The microsponge sample (figure IV) was attached to the specimen holder using a double-coated adhesive tape and was gold-coated (~20-nm thickness) under vacuum using a sputter coater for 5–10 min at 40 mA and then investigated at 30 kV [10].

Particle Size Analysis

Particle size and size distribution of microsponge particles was determined using optical microscope. The values were given for the formulations in the form of mean particle size.

Determination of percentage yield

The production yield of the microsponges was determined by calculating accurately the initial weight of the raw materials and the weight of the microsponge obtained,

Production yield= $(W_{Pr} / W_{Th}) \times 100$

W_{Pr} = Practical mass of microsponges

W_{Th} = Theoretical mass (polymer + drug)

Determination of Encapsulation Efficiency

An accurately weighed amount of loaded microsponges (20 mg) was dissolved in 10 ml of methylene chloride, 30 ml of distilled water was added, and the mixture was stirred for 24 h till complete solvent evaporation. The solution was filtered in a volumetric flask; the volume was completed with water [11, 12]. The concentration of hydroxyzine HCl in water was determined spectrophotometrically (Schimadzu 1800, JAPAN) at λ_{max} = 233 nm after appropriate dilution. Unloaded microsponges produced no significant absorbance values at the same wavelength. Each tested formulation was analyzed in triplicate. The encapsulation efficiency was calculated through the following relationship:

Encapsulation Efficiency

$$(\text{Practical drug loading}/\text{Theoretical drug loading}) \times 100$$

The results were represented in the table II

Flowability Testing

The flowability of the prepared powder microsponges was tested by measuring their angle of repose and the calculation of compressibility index and Hausner's ratio. The results of characterized microsponges were represented in the table III

***In Vitro* Release Studies**

The microsponges, equivalent to a 50-mg drug were subjected to invitro release studies. The *in vitro* release of hydroxyzine HCl from the microsponges was studied using USP basket apparatus with a stirring rate of 50 rpm. The temperature was maintained at 37 ± 0.5°C. Drug release was carried out in 900ml of phosphate buffer (pH 7.4).

An aliquot of 5-ml sample was collected over a period of 12 h and was assayed spectrophotometrically for the drug at λ_{max} = 233 nm. Each time sample was withdrawn, a fresh buffer was added to keep the dissolution medium volume constant. A kinetic treatment of the data was performed to determine the mechanism of the release of the drug. The cumulative release profiles of the formulations were represented in figureV.

Stability Studies

Stability study has been defined as the ability of a particular formulation, in a specific container, to remain within its physical, chemical, therapeutic and toxicological specifications.

Stability testing of pharmaceutical products is done for the following purposes

1. To ensure the efficacy, safety and quality of active drug substance and dosage forms.

2. To establish shelf-life or expiration period and to support tablet claims.

Haynes divided the countries of world into 4 zones namely

Zone = I (Moderate)

Zone = II (Mediterranean)

Zone = III (Hot, Dry)

Zone = IV (Very hot, moist)

India falls under Zone-III & IV

As per ICH guidelines following storage conditions are specified for solid oral dosage form to conduct stability study under accelerated condition for zone III and IV countries.

Accelerated study: 40^{O}C/ 75%RH sampling were done after 3 months.

Procedure: In the present study, stability studies were carried out at 40^{O}C/ 75%RH

Formulation was analyzed for following parameters

- Particle size analysis
- Drug entrapment efficiency
- Invitro dissolution study of hydroxyzine hydrochloride microsponge formulation

The results were shown in the table IV

FORMULATION DEVELOPMENT AND EVALUATION OF HYDROXYZINE HYDROCHLORIDE MICROSPONGE TABLETS

Preparation of hydroxyzine hydrochloride microsponge tablets

Tablets containing 55mg of hydroxyzine hydrochloride microsponges were prepared by direct compression technique. Diluents, binder, talc and magnesium stearate were sieved through Sieve no #80.The tablets were prepared by direct compression technique. Lubricant was added during blending. During blending total mass was taken in a laboratory designed blender and mixed for 30 minutes. Attention was given to ensure thorough mixing and phase homogenization [13, 14]. The appropriate amounts of the mixtures were accurately weighed in an electronic balance for the preparation of tablets and finally compressed using 16 station rotary tablet punching machine. The compositions of formulations were shown in (Table V).

Table V. Composition of different tablet formulations of Hydroxyzine hydrochloride microsponges

INGREDIENTS (mg)	**F10**	**F11**	**F12**
Microsponge powder	55	55	55
Starch	40	40	40
Microcrystalline cellulose	101	-	-
Directly compressible lactose	-	101	-
Directly compressible Dicalcium phosphate	-	-	101
Mg.Stearate	2	2	2
Talc	2	2	2
Total weight	200	200	200

Micromeritic properties of microsponge formulation powder blends [15]

Determination of bulk density and tapped density:

About 5 gms of formulation powder blend was weighed and transferred to a 25 ml measuring cylinder. The bulk volume was noted. The bulk density was calculated by the formula:

Bulk density = $\frac{WEIGHT\ OF\ POWDER}{BULK\ VOLUME\ OF\ POWDER}$

Tapped density = $\frac{WEIGHT\ OF\ POWDER}{TAPPED\ VOLUME\ OF\ POWDER}$

Angle of Repose:

Angle of repose was measured by using open cylinder method. Two side open cylinder was taken and cleaned thoroughly, accurately weighed 5 gms of formulation powder blend was transferred to the open cylinder. Then the cylinder was lifted up. Height of pile and radius of the base of the pile was measured with ruler. Then the angle of repose was measured with the following formula.

The angle of repose (θ) was calculated by using the formula,

$$\theta = \text{Tan}^{-1}\frac{h}{r}$$

Where h= height of pile

r= radius of pile

θ = angle of repose

Compressibility index:

Accurately weighed, 5 gms of formulation powder blend was transferred to a 25 ml measuring cylinder and bulk volume was measured. Then it was subjected to 100 tapings. The tapped volume was measured. Carr's index was calculated by the following formula:

Compressibility index (%) = $\frac{TAPPED\ DENSITY - BULK\ DENSITY}{TAPPED\ DENSITY} \times 100$

Hausner ratio

Accurately weighed formulation powder blend was transferred to a 25ml measuring cylinder. The bulk volume was measured and then subjected to 100 tapings. The tapped volume was measured. Hausner ratio was calculated by the following formula:

$$\text{Hausner ratio} = \frac{TAPPED\ DENSITY}{BULK\ DENSITY}$$

The results of micromeritic properties of microsponge formulation powder blends were represented in table VI.

EVALUATION OF PREPARED MATRIX TABLETS

The following evaluation tests were conducted for the prepared matrix tablets

Physical characteristics evaluation of prepared matrix tablets

Thickness:

The thickness of the tablets was determined using a vernier calliper. Five tablets from each formulation were used, and average values were calculated.

Tablet thickness=V.S.R+ (M.S.RXL.C)

V.S.R= vernier scale reading

M.S.R= main scale reading

L.C= least count

Weight variation test:

Twenty tablets were collected and were weighed collectively and individually. From the collective weight, average weight was calculated. The percent weight variation was calculated using the formula

% weight variation = $\frac{Average\ weight - individual\ weight}{Average\ weight}$ X 100

Hardness test:

Hardness of the tablet was determined using the Monsanto hardness tester. The lower plunger was placed in contact with the tablet and a zero reading was taken. The lower plunger was then forced against a spring by tuning threaded bolts until the tablet fractured.

Then the final reading was recorded. The hardness was calculated by deducting the initial pressure from the final pressure. The results were expressed as an average of 5 readings in terms of $kg/cm^{2.}$

Friability:

This test was performed to know the effect of friction and shock on tablets. Twelve tablets from each formulation were collected and accurately weighed. Then the tablets were collected and placed in Roche friabilator and operated for 100rpm (25 rpm speed).Tablets were dusted and reweighed. The test complies if tablets not lose more than 1% of their weight. Friability Percentages of the tablets were calculated using the formula

%Friability= $\frac{w_1 - w_2}{w_1}$

W1=initial weight of tablets

W2=weight of tablets after friabilation

Drug content determination of prepared microsponge tablets

Twenty tablets from each formulation were selected for the estimation of drug content. The tablet was weighed,triturated and transferred the powder to a 100 ml flask containing 50 ml of pH 7.4 phosphate buffer . The content of the flask was filtered through a filter, kept in a 100 ml volumetric flask. The residue was washed with another 40 ml of pH 7.4 phosphate buffer and the volume was made up to the mark . The sample was suitably diluted and analysed spectrophotometrically against blank (pH 7.4 phosphate buffer) at 233 nm using double beam uv visible spectro photometer. The results of evaluation studies were represented in table VII.

***In vitro* drug release studies [16, 17]**

The in vitro dissolution studies were carried out using USP dissolution apparatus-II which was set at 50 rpm. Dissolution test was carried out in 900 ml of pH 7.4 phosphate buffer at 37±0.5^{o}c. Sampling was done for every one hour. Then at each interval of time, 5 ml of samples were collected and replaced with the same amount of dissolution medium. The samples withdrawn were analyzed spectro photo metrically at 233 nm using *UV* visible double beam spectro photometer. Five tablets from each formulation were used for the *in-vitro* dissolution study. The drug release profile was fitted into several mathematical models to get an insight of the release mechanism of the drug from the dosage form. The zero order and peppas plots of optimized formulations were represented in figures VI & VII. Model fitting release profile of optimized formulations were shown in the table VIII.

RESULTS

Figure I: IR spectra of pure hydroxyzine hydrochloride

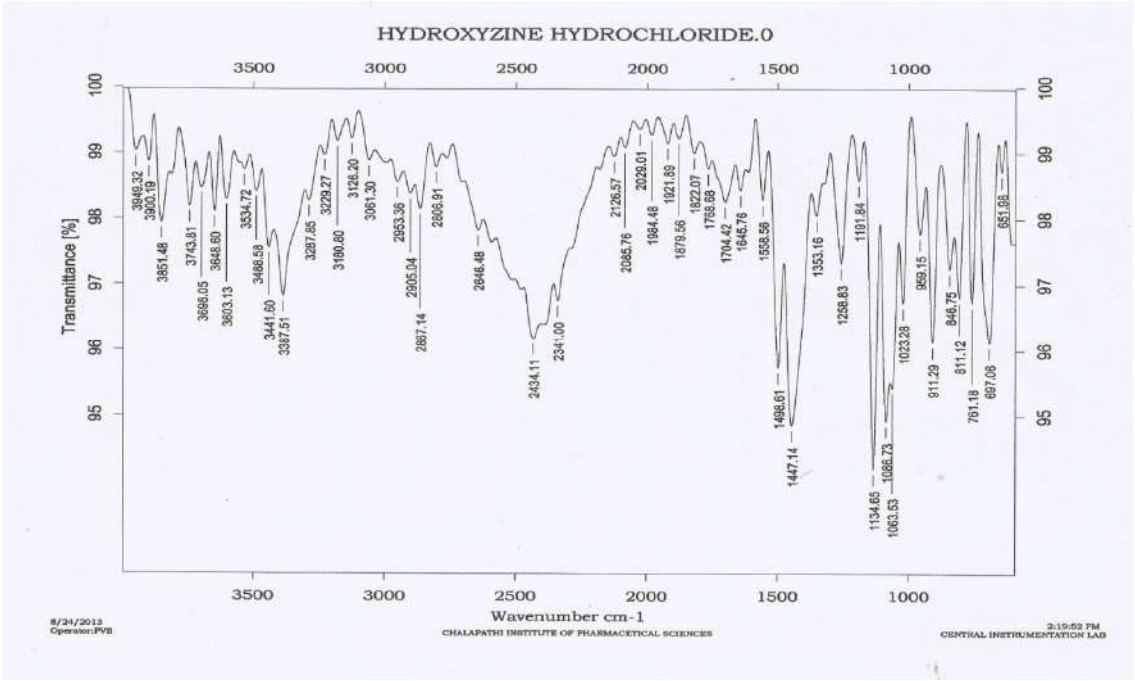

Figure II: IR spectra of Hydroxyzine hydrochloride, Methocel 10000Cps and Eudragit RS100 (optimized formulation F8)

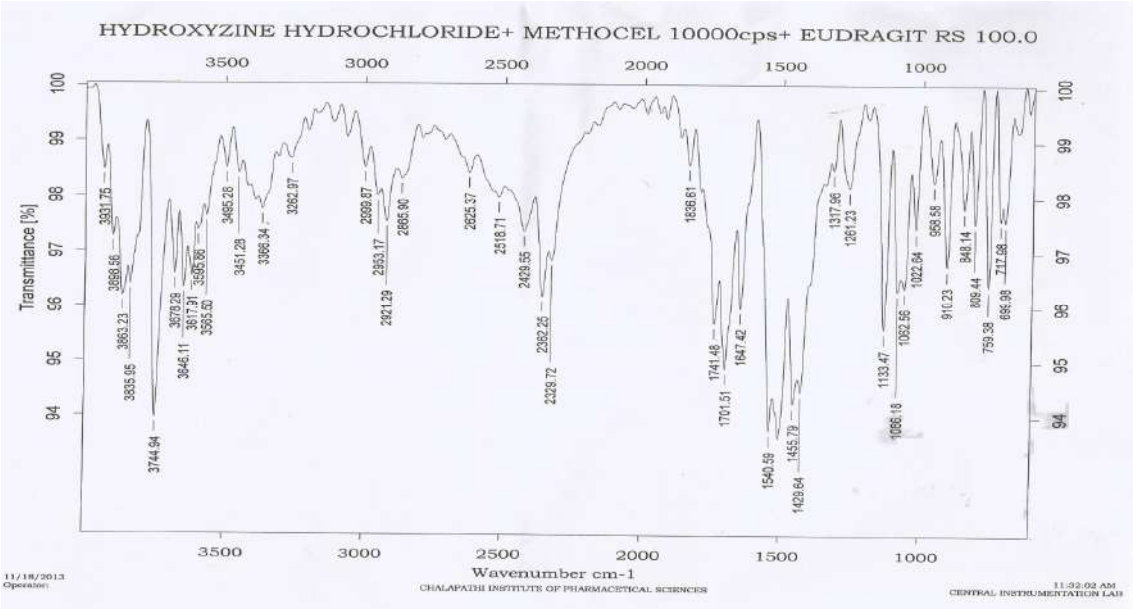

Figure III. Photomicrograph of the Hydroxyzine hydrochloride microsponges of formulation F8

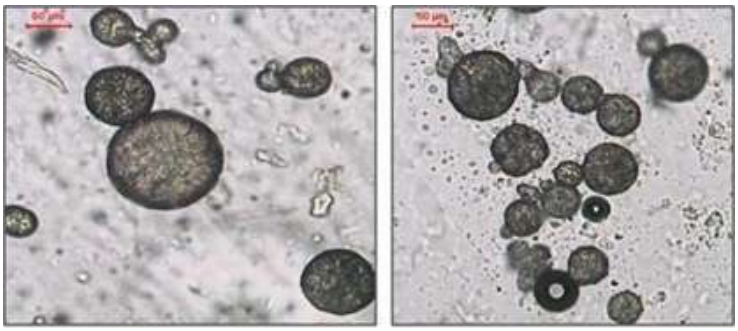

Figure IV. SEM photograph of Hydroxyzine hydrochloride microsponges formulation F8

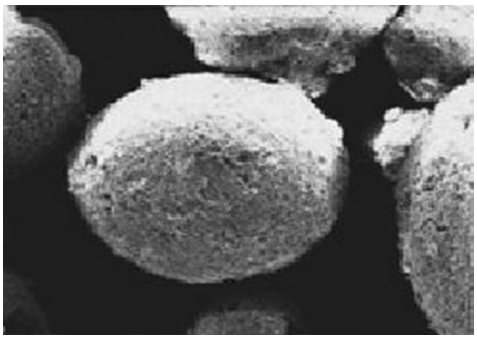

Table II. Evaluation of Hydroxyzine hydrochloride microsponges

Parameter evaluated	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Production yield(%)	62.38	65.41	67.42	71.85	74.36	72.94	78.94	82.46	81.59
Mean particle size(µm)±S.D	71.40±1.6	69.30±2.4	64.70±1.7	63.50±1.9	61.30±3.8	59.40±7.4	55.80±6.2	49.60±3.4	47.40±2.5
Loading efficiency (%)	82.31±0.3	85.46±0.5	90.57±1.2	94.32±0.08	93.67±0.6	95.81±0.9	96.44±1.4	98.29±0.4	98.43±0.09

Table III. Micromeritic properties of microsponge formulation

Property evaluated	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
Bulk density g/cm^3	0.54	0.54	0.53	0.53	0.53	0.53	0.56	0.53	0.53
Tapped density g/cm^3	0.68	0.65	0.62	0.61	0.60	0.62	0.65	0.59	0.60
CI (%)	19.5	17.5	15.3	13.1	11.8	15.3	13.2	10.8	11.9
Hausner's ratio	1.25	1.21	1.17	1.15	1.13	1.17	1.16	1.11	1.13
Angle of repose(^{O})	25	26	23	24	21	19	16	15	16.3

Figure V. Invitro drug release profiles of prepared microsponges (F1 to F9)

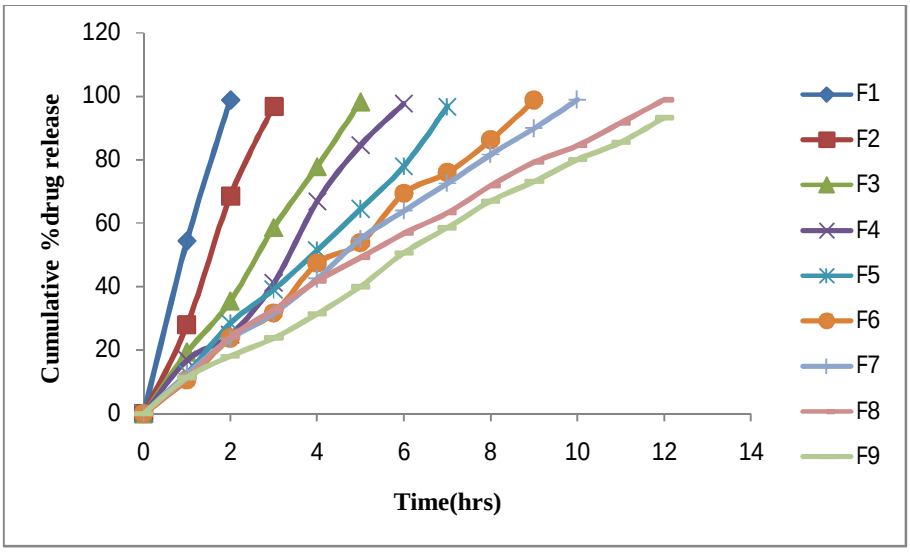

Table IV. Stability study data of optimized formulation of microsponges (F8)

S.No	Parameter	Optimized formulation F8	Stability study data of F8 40^{o}C and 75% relative humidity(after 3 months)
1	Particle size	49.60±3.4	48.99±3.4
2	Loading efficiency	98.29±0.4	97.98±0.4
3	Invitro release study: cumulative %drug release at the end of 12th hr	98.81%	98.02%

Table VI. Micromeritic properties of powder blends containing hydroxyzine hydrochloride microsponges

Formulation code	Bulk density g/cm^3	Tapped density g/cm^3	Hausner ratio	Compressibility Index (%)	Angle of repose (^{0})
F10	0.56	0.65	1.16	13.84	27.89
F11	0.66	0.75	1.16	13.84	25.68
F12	0.64	0.73	1.16	14.28	24.32

Table VII. Physical characteristics of hydroxyzine hydrochloride microsponge tablets prepared by employing direct compression techniques

Formulation code	(Mean ± S.D)				
	Thickness (mm)	Average weight(mg)	Hardness (kg/cm^2)	%Friability	%Drug content
F10	6.01±0.01	200±0.02	5±0.00	0.08±0.001	99.89±0.02
F11	6.07±0.01	200±0.01	5±0.02	0.06±0.003	99.56±0.04
F12	6.05±0.02	200±0.01	5±0.03	0.07±0.002	99.64±0.05

Figure VI. Zero order plots of optimized formulations F8 (microsponge) and F11 (controlled release microsponge tablet)

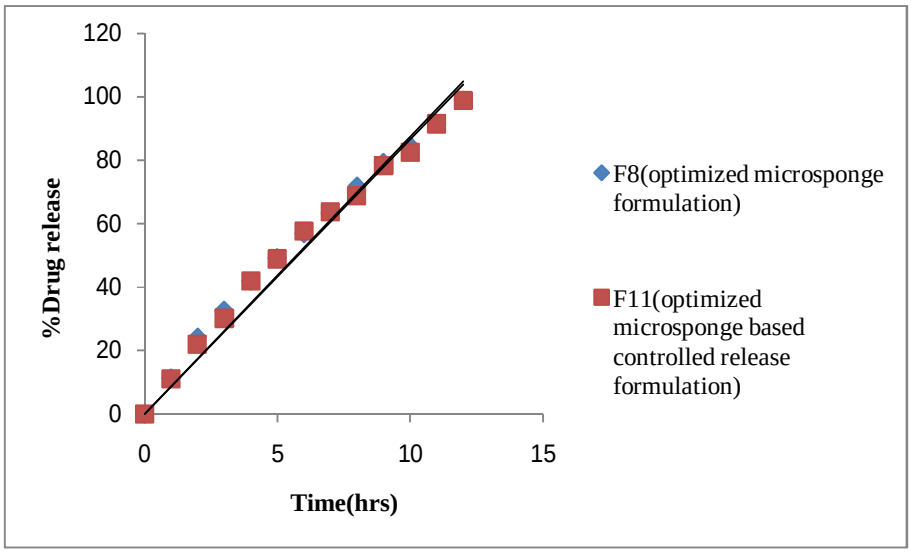

Figure VII. Peppas plots of optimized formulations F8 (microsponge) and F11 (controlled release microsponge tablet)

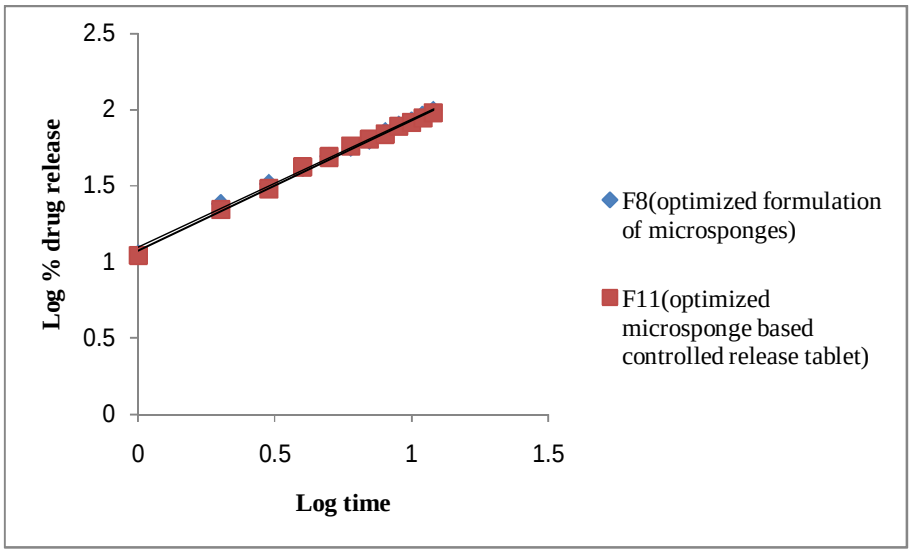

Table VIII. Model fitting release profile of optimized formulations (hydroxyzine hydrochloride microsponges (F8) and hydroxyzine hydrochloride microsponge tablets)

Formulation code	Mathematical models(kinetics)					
	Zero order R^2	First order R^2	Higuchi R^2	Korsemeyer Peppas		Best fit model
				R^2	n	
F8	0.997	0.745	0.909	0.997	0.38	Peppas
F11	0.990	0.849	0.959	0.998	0.36	Peppas

DISCUSSION

FTIR studies (figures I & II) of drug and excipients revealed that there was no interaction between the selected drug and polymers.

Photomicroscopic Analysis (figure III) revealed that the microsponges had regular, spherical shape with roughness on the surface and several pores. The microsponges of Hydroxyzine hydrochloride with Eudragit-RS100 and Methocel 10000cps were smooth, porous, glossy and discrete spherical (Scanning Electron Microscopy (figure IV)**)**.

For the prepared formulations F1 to F9 production yield was in the range of 62.38% to 82.46%., the mean particle size was in the range of 47.40±2.5 µm to 71.40±1.6 µm, Loading efficiency (table II) was in the range of 82.31±0.3 to 98.43±0.09%.

The drug polymer ratio showed significant effect on the encapsulation efficiency of microsponges. The increased concentration of polymer showed the increased drug encapsulation efficiency. All the powder blends (table III) exhibited the desired flow properties according to pharmacopoeial requirements. It was found that after 12hrs of dissolution study the formulations F1, F2, F3, F4, F5, F6, F7, F8 and F9 (figure V) were showing 98.89%, 96.78%, 98.23%, 97.66%, 96.79%, 98.72%, 98.94%, 98.97% and 93.27% drug release respectively. The dissolution rates are inversely related to particle size as would be expected from surface area relationships. Stability studies of optimized formulation (F8) (Table IV) of prepared microsponges revealed that there was no significant change in the physical characteristics and invitro drug release profiles. Controlled release tablets were prepared

from the optimized formulation F8. The formulated tablets were subjected to various quality control tests and the results were shown in (table VI & VII). All the tablets complied with the pharmacopoeial standards. The formulation F11 showed best release retardation up to 12 hrs where as the formulations F10 and F12 showed 9hrs and 7hrs respectively. The release profiles of optimised formulations followed zero order release kinetics (VI). The data was fitted in the peppas plot (figure VII) indicating fickanian diffusion mechanism of drug release (table VIII).

CONCLUSION

Microsponge based novel drug delivery system has been developed to provide a once daily controlled release tablets for per oral delivery of hydroxyzine hydrochloride. The formulation F11 showed better release retardation of drug indicating better potential of delivery system.

REFERENCES

1. Controlled release oral products http://www.australianprescriber.com/oral extended release products/22/4/88/90. Accessed on 7-04-2011.
2. 2. R. Hendrickson (Ed.), Remington. The Science and practice of pharmacy. Vol.1, 21 st edition. Lippincott Williams &Wilkins, Hong Kong: published by Wolters Kluwer Health Pvt.Ltd; 2007.P. 917.
3. Embil K, Nacht S, The microsponge delivery system (MDS): A topical delivery system with reduced irritancy incorporating multiple triggering mechanisms for the release of actives, J Microencapsul, 13, 1996, 575-588.
4. James, William; Berger, Timothy; Elston, Dirk (2005). *Andrews' Diseases of the Skin: Clinical Dermatology* (10th ed.). Saunders. p. 150. ISBN 0-7216-2921-0.
5. [www.abouturticaria.com/types-of-urticaria-hives more information about acute urticaria: triggers, treatment options, pictures]
6. 6.Goto S, Kawata M, Nakamura M, Maekawa K, Aoyama T. Eudragit E, L and S (acrylic resins) microcapsules as pH sensitive release preparations of ketoprofen. J Microencapsulation. 1986; 3(b):305–316. doi: 10.3109/02652048609021800.
7. Kawata M, Nakamura M, Goto S, Aoyama T. Preparation and dissolution pattern of Eudragit RS microcapsules containing ketoprofen. Chem Pharm Bull. 1986; 34:2618–2623.
8. Yuksel N, Baykara T. Preparation of polymeric microspheres by the solvent evaporation method using sucrose stearate as droplet stabilizer. J. Microencapsulation. 1997; 14:725–733. doi: 10.3109/02652049709006822.
9. Bhardwaj SB, Shukla AJ, Collins CC. Effect of varying drug loading on particle size distribution and drug release kinetics of verapamil hydrochloride microspheres prepared with cellulose esters. J Microencapsulation. 1995; 12:71–81. doi: 10.3109/02652049509051128.
10. Gibaly, Abdel-Ghaffar SK. Effect of hexacosanol on the characteristics of novel sustained release allopurinol solid lipospheres (SLS): factorial design application and product evaluation. Int J Pharm.2005; 294:33–51. doi: 10.1016/j.ijpharm.2004.12.027.
11. Iannuccelli V, Sala N, Tursilli R. Influence of liposphere preparation on butyl-methoxydibenzoyl methane photostability. Eur J Pharm Biopharm. 2006; 63:140–145. doi: 10.1016/j.ejpb.2006.01.007.
12. Tursilli R, Casolari A, Iannuccelli V, Scalia S. Enhancement of melatonin photostability by encapsulation in lipospheres. J Pharm Biomed Anal. 2006;40:910–914. doi: 10.1016/j.jpba.2005.08.025.
13. Gurdeep R Chatwal, Sham K. Anand, Instrumental methods of chemical analysis. 5th edition, Mumbai: Himalaya publishing house; 2010. P. 2. 160-2.161.
14. Roger E Schirm, Modern methods of pharmaceutical analysis. 2nd edition, vol 1, CRC publishers; 2010.P. 59-61.
15. Gilberts. Banker, Christopher T. Rhodes. Modern Pharmaceutics. 3 rd edition, New York: Marcel Dekker INC; 2005. P.23-7
16. The United States Pharmacopoeia.USP27/ NF22. The Official Compendia of Standards, Asian edition. Rockville: The United State Pharmacopoeial Convention, 2004. P. 1204.
17. Indian pharmacopoeia, Monographs on dosage forms, The Indian Pharmacopoeia Commission, Ghaziabad, 2007. P. 487.