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A STUDY ON FORMULATION AND EVALUATION OF MICROSPHERES OF BENAZEPRIL ANTI HYPERTENSIVE DRUG

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ABSTRACT

Benazepril HCl is an ACE inhibitor which is used in treatment of high blood pressure. Microspheres of Benazepril HCl were prepared using Chitosan and Xanthan gum by solvent evaporation technique. Compatibility study was carried out by using FTIR and results showed that there was no significant interaction between drug and excipients. Microspheres thus obtained were found to be pale yellow in color and showed free flowing character. Micromeritic studies showed that prepared microspores showed excellent to good flow properties. The Scanning electron microscopy results indicate that microspheres are of smooth and spherical in shape. Particle size was performed by using optical microscope and particle sizes are found in the range 152.8±8.35to 276.2±5.47µm and particle size depends up on the concentration of Xanthan gum i.e. formulation containing higher concentration of Xanthan are larger size when compared to Chitosan containing batches. Entrapment efficiency results showed that formulation F10 containing 1:9 ratio of Chitosan: Xanthan gum showed highest percentage of entrapment (95.90%) and showed 59.74% of drug loading. *In-vitro* drug release showed decreases as concentration of Xanthan gum increases the dissolution profile of Benazepril HCl fromprepared formulation. Formulation F2 containing 9:1 ratio of Chitosan: Xanthan gum showed94.86% of drug release at the end of 12 hours. Short term stability study showed that prepared Benazepril microsphere formulation was physicochemically stable throughout stability period. The study has been conclude that microspheres offer a practical and suitable approach to prepare controlled release dosage form of Benazepril HCl with natural occurring gums like Chitosan and Xanthan gum.

KEYWORDS: Benazepril HCl; Chitosan; Xanthan gum; microspheres; Solvent evaporation.

INTRODUCTION

Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000µm.They are made of polymeric, waxy or other protective materials that are biodegradable synthetic polymers and modified natural products such as starches, gums, proteins, fats and waxes. The solvents used to dissolve the polymeric materials are chosen according to the polymer and drug solubility, process safety and economic considerations. Microspheres are small and have large surface-to-volume ratio. At the lower end of their size they have colloidal properties. The interfacial properties of microspheres are extremely important, often including their activity.[1-3]

- The potential use of microspheres in pharmaceutical industry
- The conversion of oils and other liquids to solids for ease of handling
- Taste and odour masking
- To delay the volatilization
- Separation of incompatible materials.
- Improvement of flow properties of powders
- Safe handling of toxic substances
- Improve the solubility of water insoluble substances by adding in dispersion of such materialin aqueous media
- Production of sustained, controlled release and targeted medications.
- Reduce the dose dumping potential compared to large implantable devices.

Advantages

- Microspheres provide constant and prolonged therapeutic effect.
- Reduces the dosing frequency and thereby improve the patient compliance.

- They could be injected into the body due to the spherical shape and smaller size.
- Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effects.
- Microsphere morphology allows a controllable variability in degradation and drug release.

Disadvantages

- Dosage forms of this kind should not be crushed or chewed.
- Criteria for the Preparation of microspheres
- Preparation of microspheres should satisfy certain criteria:
- The ability to incorporate reasonably high concentrations of the drug.
- Stability of the preparation after synthesis with a clinically acceptable shelf life.
- Controlled particle size and dispersability in aqueous vehicles for injection.
- Release of active reagent with a good control over a wide time scale.
- Biocompatibility with a controllable biodegradability and
- Susceptibility to chemical modification.

Hypertension

Hypertension is the state of increase in blood pressure than normal tension of 120/80 mm Hg. WHO-ICH guidelines (2003) have defined it to be 140 mm Hg systolic and 90 mm Hg diastolic pressure. Epidemiological studies have confirmed that higher the pressure greater is the risk of cardio vascular disease. Hypertension is usually asymptomatic until complication develops in target organs. Dizziness, flushed face, headache, fatigue, epistaxis, and nervousness are not caused by uncomplicated hypertension. Severe hypertension can cause severe cardiovascular,neurologic, renal and retinal symptoms (e.g. symptomatic coronary atherosclerosis, HF, hypertensive encephalopathy, renal failure).

Primary hypertension: Hemodynamic and physiologic components (eg. plasma volume, activity of the renin angiotensin system) vary indicating that primary hypertension is unlikely to have a single cause.[4-5]

Secondary hypertension: Secondary hypertension Causes include renal parenchymal disease (eg, chronic glomerulonephritis or pyelonephritis, polycystic renal disease, connective tissue disorders, obstructive uropathy), renovascular disease, pheochromocytoma, Cushing's syndrome, primary aldosteronism, congenital adrenal hyperplasia, hyperthyroidism, myxedema and coarctation of the aorta. Excessive alcohol intake and use of oral contraceptives are common causes of curable hypertension.

Anti-hypertensive drugs

Depending upon their mechanism of action they are broadly classified as

Angiotensin converting enzyme inhibitors

- Benazepril
- Enalapril
- Captopril
- Ramipril

Angiotensin-II receptor type 1 (AT1) Antagonists

- Losartan
- Candesartan
- Irbesartan

Calcium channel blockers (CCB)

- Verapamil
- Diltiazem
- Amlodipine
- Nifedipine

Diuretics

- Thiazide:- Hydrochlorothaizides, chlorthaizides
- High ceiling:- Furosemide
- K + Sparing:- Spironolactone, amioloride

ß- adrenergic blockers

- Propranolol
- Atenolol
- Metoprolol

ß +α- adrenergic blockers

- Labetolol
- Carvedilol

α -adrenergic blockers

- Prazosin
- Terazosin
- Phenoxybenzamine

Central sympatholytics

- Methyldopa
- Clonidine

Vasodilators

- Arteriolar: Minoxidil, hydralazine
- Arteriolar + venous: Sodium nitroprusside

MATERIALS AND METHODS

Table 2: Chemicals.

Sl. No.	Chemical name	Source
1.	Benazepril HCl	Yarrow chem products, Mumbai
2.	Xanthan gum	Yarrow chem products, Mumbai
3.	Chitosan	S D Fine Chem Limited, Mumbai
4.	Sodium lauryl sulfate	S D Fine Chem Limited, Mumbai
5.	Methanol	S D Fine Chem Limited, Mumbai
6.	Dichloromethane	S D Fine Chem Limited, Mumbai
7.	Sodium hydroxide	S D Fine Chem Limited, Mumbai
8.	Dihydrogen ortho phosphate	S D Fine Chem Limited, Mumbai

*Distilled water was used throughout the study

Table 3: List of Instruments Used with Manufacturer.

Sl. No	Instruments	Manufacturer
1	Electronic analytical balance	ACCULAB, EUROPE, Germany
2	UV-Visible spectrometer	Shimadzu UV-1800, Japan
3	FTIR	THERMO NICOLET
4	Dissolution apparatus	Lab India, Mumbai, India
6	Magnetic stirrer	Almicro, Bangalore
7	pH meter	Techno scientific products, Bangalore
8	Centrifuge	Remi Elektrotechnik Limited, Thane, India.
9	Hot air oven	Kadavil electro mechanical industries, Kerala
10	Microscope	Pilot products, Bombay
11	Scanning electron microscopy	HITACHI, Japan

Pre-Formulation Studies

Pre-formulation testing is the first step in the rational development of dosage forms of a drug.It can be defined as an investigation of Physical and chemical properties of drug substance, alone and when combined with excipients. The goals of the program therefore are:

- To establish the necessary physico-chemical characteristics of a new drug substance.
- To determine its kinetic release rate profile.[6]
- To establish its compatibility with different excipients.
- Hence, pre-formulation studies on the obtained sample of drug include physical tests and compatibility studies.

Identification of Pure Drug

The selected pure drug Benazepril was subjected for investigation of physical characterization parameters such as:

IR Spectroscopy

Compatibility Study using FT-IR: To establish drug-excipients compatibility, binary powder mixtures were prepared in 1:1 ratios with excipients. The binary mixtures were ground in a mortar, screened and the mixtures were filled individually in amber colored vials and sealed. After specific time period the mixture were subjected to assay and all binary mixtures showed desired drug concentration ranges. FT-IR scanning was also done to establish drug- excipients- interactions.

Solubility Analysis

Pre-formulation solubility analysis was done to select a suitable solvent system to dissolve the drug as well as various excipients used for formulation of microspheres based formulation.

Melting Point Determination

Melting point determination of the obtained drug sample was done as it is a first indication of purity of the sample. The presence of relatively small amount of impurity can be detected by a lowering as well as widening in the melting point range. The melting point of Benazepril was measured by Thiele‟s tube apparatus.

Analytical Method used in the determination of Benazepril HCl

The UV spectrophotometric method was developed for the analysis of the drug using Shimadzu 1800 spectrophotometer**.**

Determination of λ_{max}

Accurately weighed 10 mg of drug was dissolved in 100 ml of 0.1 N HCl in 100 ml of volumetric flask and prepare suitable dilution to make it to a concentration of 100µg/ml make adequate of sample with concentration range of 5-25µg/ml. The spectrum of this solution was run in 200-400 nm range in U.V spectrophotometer.

Standard Curve for Benazepril HCl

100 mg of Benazepril HCl was accurately weighed and dissolved 50 ml of 0.1 N HCl. The solution was sonicated for 5 min and final volume was adjusted to 100

ml to give stock solution-I (1000 µg/ml concentration). 10 ml of stock solution-I was placed in 100 ml volumetric flask and volume was adjusted with methanol to give stock solution-II of 100µg/ml concentration. Stock solution-II was further diluted with methanol to get working standard solution of 5, 10, 15, 20 and 25 µg/ml of 0.1 N HCl to construct Beer‟s law plot for the pure drug. The absorbance of the solutions was measured at 254 nm using UV-visible spectrophotometer. A graph of concentration VS absorbance was plotted.

Preparation of Benazepril HCl microspheres by solvent evaporation technique Formulation of Benazepril HCl Microspheres

Benazepril HCl microspheres were prepared using Chitosan, Xanthan gum and distilled wateras continuous phase by solvent evaporation technique. Initially dichloromethane (DCM) and methanol was mixed uniformly at room temperature, then HPMC and Xanthan gum in various proportions was dissolved in the above solution. To this mixture, a drug solution corresponding to 40 mg was added and mixed thoroughly and injected drop wise in to the continuous phase consisting of 100mL of 0.2% (w/v) SLS (sodium lauryl sulphate) at 1500 rpm for 210 min using a stirrer and heated by a hot plate at 50^{o}C. The microspheres obtained was washed for 2-3 times with distilled water and dried at room temperature.[7-9]

Table 4: Formulation of Benazepril HCl Microspheres.

Formulation Code	Chitosan (Mg)	Xanthangum (mg)	BenazeprilHCl (mg)	Dichloromethane (ml)	Methanol (ml)	Sodium lauryl sulphate (mg)
F1	1000	--	40	10	10	200
F2	900	100	40	10	10	200
F3	800	200	40	10	10	200
F4	700	300	40	10	10	200
F5	600	400	40	10	10	200
F6	500	500	40	10	10	200
F7	400	600	40	10	10	200
F8	300	700	40	10	10	200
F9	200	800	40	10	10	200
F10	100	900	40	10	10	200
F11	--	1000	40	10	10	200

Compression evaluations

- Micromeritic Studies
- Determination of angle of repose
- Determination of Bulk Density and Tapped Density
- Hausner's Ratio:
- Compressibility index (Carr's Index):
- Particle Size Determination
- Morphological Study using SEM
- Percentage Yield
- Drug Loading and Drug Entrapment
- Release kinetic studies
- Zero Order Kinetics
- First Order Kinetic
- Stability Studies
- Importance of stability studies
- Purpose of stability studies

Aim and Objectives

Aim

The study aimed to formulation and evaluation of microspheres ofan anti hypertensive drug.

The objectives include

- Preparation of standard calibration curve for Benazepril HCl.
- To perform drug and polymers compatibility studies using FTIR.
- To prepare mucoadhesive microspheres of Benazepril HCl using, Xanthan gum, EudragitRS-100 and polycarbophil in combinations by emulsion cross-linking technique using glutaraldehyde as cross-linking agent.
- Evaluation of the formulation consists of
- Physical characterization of microspheres which includes
- Particle size analysis
- Determination of particle shape and surface morphology
- Percentage yield
- Drug loading
- Drug entrapment efficiency
- *In-vitro* drug release studies
- *In-vitro* drug release kinetics
- To carry out the accelerated stability studies on selected formulations.

RESULTS AND DISCUSSION

Melting point

Melting point of Benazepril HCl was determined by capillary method and result was found to be 220-221^{o}C.

Hence complies with USP standards (221°C), thus indicating the purity of the drug sample.

Determination of λ_{max} of Benazepril HCl

The λ_{max} of the Benazepril HCl was found to be 254 nm in 0.1N HCl.

Calibration curve of Benazepril HCl

The absorbance of Benazepril HCl was measured in a UV spectrophotometer at 254 nm against 0.1N HCl. The absorbance so obtained was tabulated and graph was obtained by plotting absorbance VS concentration.

Table 1: Spectrophotometric data of Benazepril HCl in 0.1N HCl.

SL.NO.	Concentration (µg/ml)	Absorbance			Average	Standard Deviation
		Trial 1	Trial 2	Trial 3		
1	0	0	0	0	0	0
2	5	0.102	0.108	0.109	0.103	0.0040
3	10	0.182	0.185	0.183	0.184	0.0015
4	15	0.280	0.279	0.281	0.281	0.0032
5	20	0.355	0.349	0.357	0.355	0.0027
6	25	0.459	0.448	0.448	0.448	0.0021

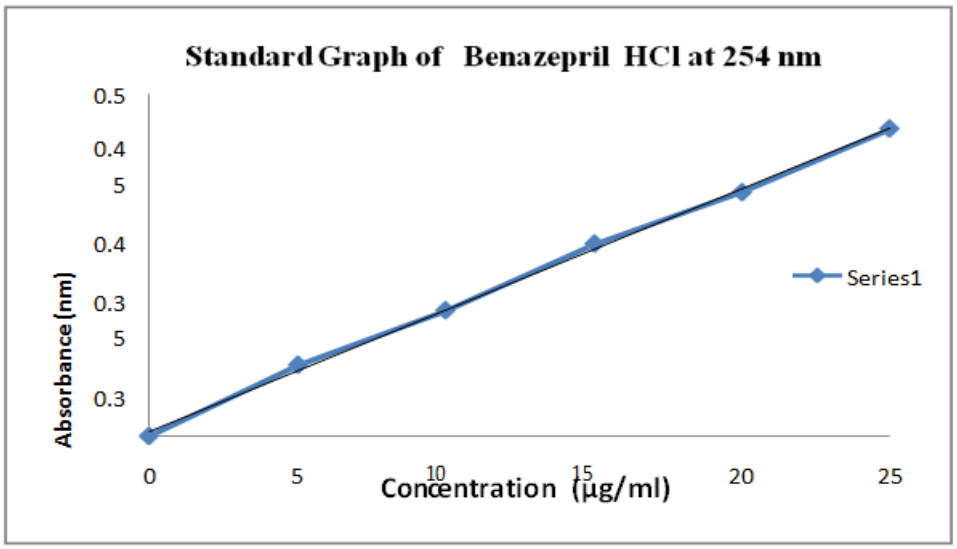

Figure 1: Calibration Curve of Benazepril HCl at 254 nm.

Compatibility studies using FT-IR

Infra-red spectrum of drug, polymers and mixture of both were determined by KBr disks method. All the characteristic peaks of Benazepril HCl were present in the spectrum of drug and polymer mixture, indicating compatibility between drug and polymer. The spectrum confirmed that there is no significant change in the chemical integrity of the drug. There is no change in functional group peaks [C=O(s), C=C (s), C-H (b), C-N(s), C-O (s), =C-H (b)] of Benazepril HCl in all the IR-spectra.[10]

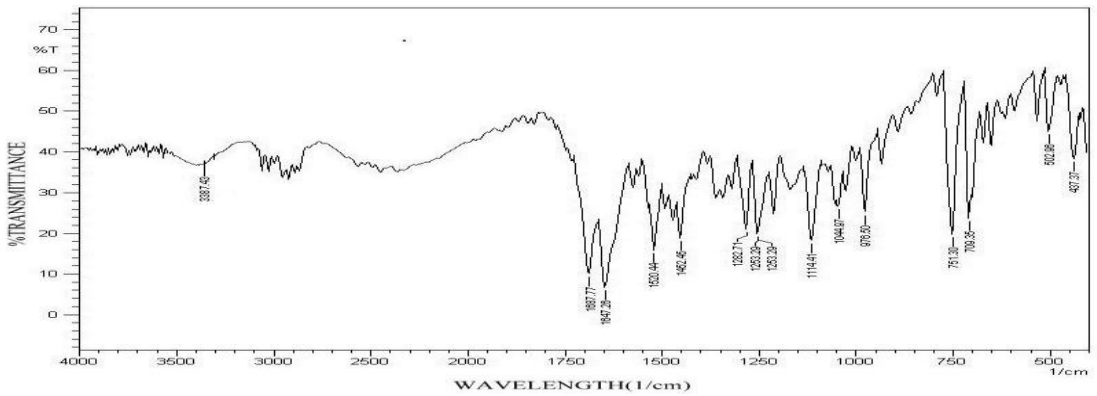

Figure 2: FT-IR spectrum of pure drug (Benazepril HCl).

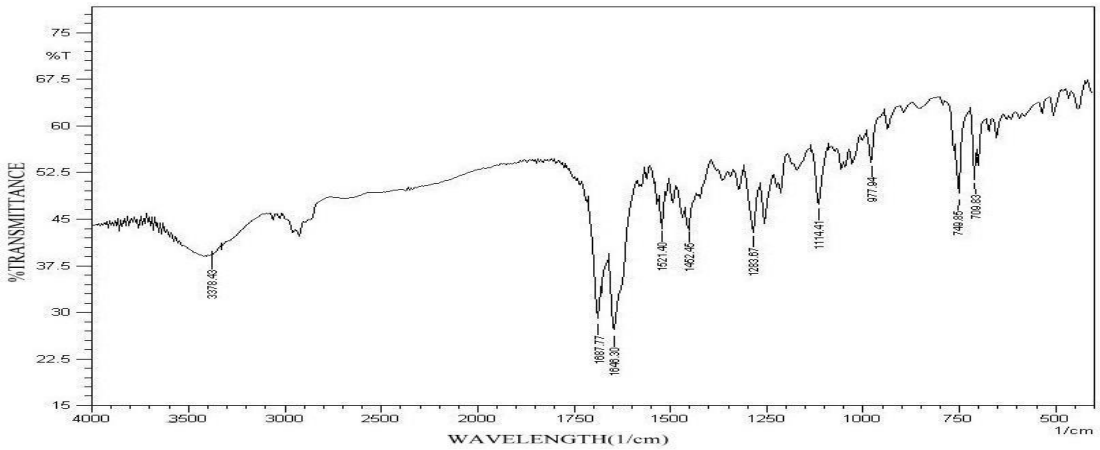

Figure 3: FT-IR spectrum of drug+ Chitosan.

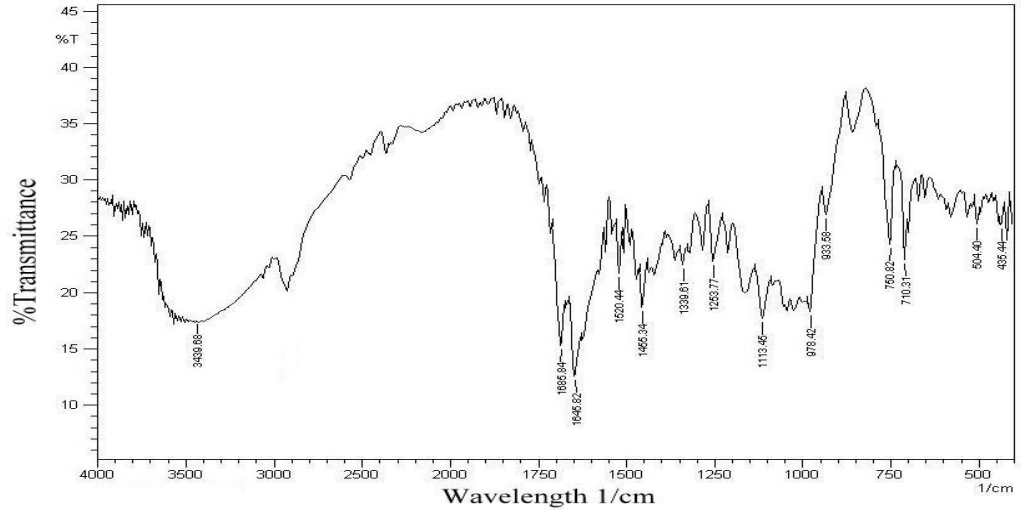

Figure 4: FT-IR spectrum of drug + Xanthan gum.

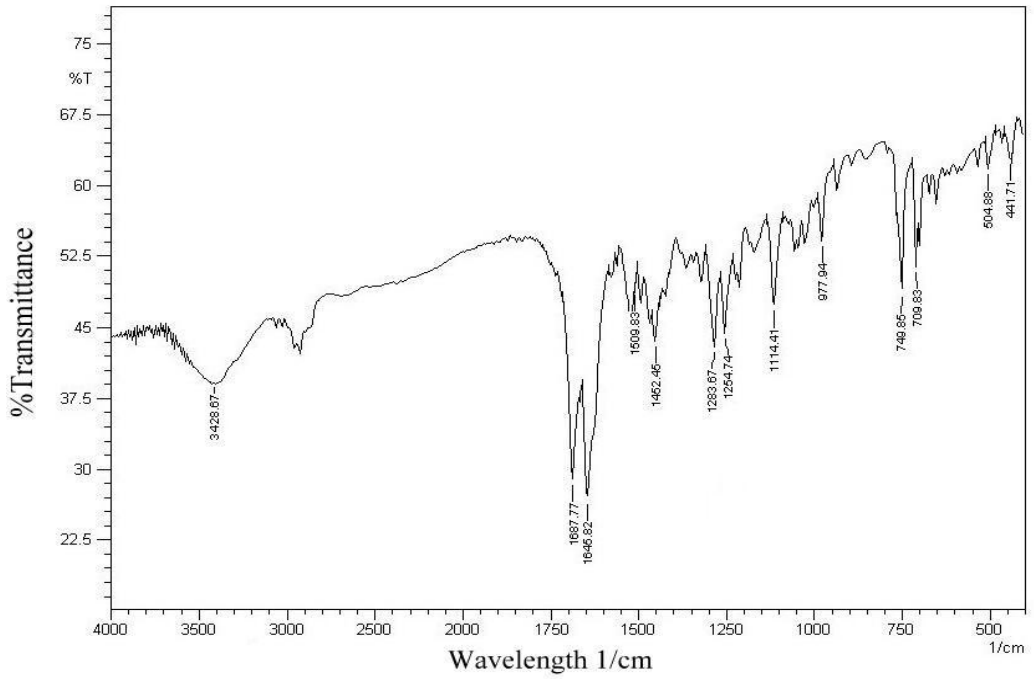

Figure 5: FT-IR spectrum of Benazepril HCl microsphere formulation.

Solubility analysis

The Benazepril HCl was freely soluble in water; and also soluble in methanol and ethanol; practically insoluble in acetone. It was soluble in 0.1N HCL (pH 1.2) and phosphate buffer (pH 7.4). Solubility analysis is important because the drug has to dissolve in the solvents and also in the dissolution medium used.

Micromeritic Studies

Various micromeritic properties were evaluated for various parameters such as bulk density, tapped density, % Compressibility index, Hausner's ratio and angle of repose. The % Compressibility index was in the range of 10.16-17.94 for all the formulations F1 to F11 indicating good flow property. The values of angle of repose for all microsphere formulations was found to be in the range of 19.88-27.94 which indicated the excellent to good flow properties of prepared microspheres.[11-14]

Table 2: Micromeritic properties of captopril mucoadhesive microspheres.

Formulation Code	Bulk Density ($g/cm^{3)}$	Tapped Density(g/cm^3)	Compressibility Index (%)	Hausner'sRatio	Angle of Repose (θ)
F1	0.5426±0.015	0.6126±0.009	12.65±1.21	1.158±0.023	21.93±0.23
F2	0.4986±0.028	0.6814±0.004	14.24±1.32	1.166±0.051	24.74±0.24
F3	0.5234±0.015	0.7243±0.008	10.16±1.27	1.193±0.011	27.94±0.17
F4	0.4813±0.009	0.6446±0.005	11.94±1.34	1.131±0.019	23.81±0.14
F5	0.5418±0.013	0.6183±0.001	12.36±1.04	1.141±0.020	24.67±0.36
F6	0.5168±0.011	0.7136±0.012	13.56±1.02	1.156±0.08	27.08±0.16
F7	0.4576±0.014	0.7228±0.008	12.47±1.21	1.142±0.031	23.61±0.64
F8	0.5754±0.013	0.6845±0.011	15.24±1.03	1.229±0.013	24.54±1.07
F9	0.5438±0.016	0.6432±0.014	15.45±0.84	1.183±0.026	25.12±1.51
F10	0.558±0.0130	0.6743±0.018	17.94±1.34	1.122±0.03	19.88±0.14
F11	0.5418±0.013	0.6643±0.075	15.94±1.34	1.129±0.02	22.±430.16

Particle Size Analysis

Particle size of Benazepril microspheres was determined by optical microscopy by using stage micrometer and ocular micrometer. The mean particle size for the microsphere formulations was found to be in range of 152±4.35µm to 276±5.47µm. Formulation F1 containing Chitosan alone showed 152±4.37 µm size. Formulation containing Chitosan: Xanthan gum in 1:9 ratio showed highest particle size (256±8.44 µm). It was noticed that the particle size of the microspheres increased with increased concentration of Xanthan gum and this may be due to high viscosity of Xanthan gum which increases the droplet size and results in increase in particle size. Formulation containing mixture of Chitosan and Xanthan gum in 9:1 ratio showed lower particle size (F2; 168.77±6.11) which might be low viscosity of Chitosan compared to Xanthan gum.[15-16]

Table 3: Average Particle Size of Benazepril Microspheres.

Formulation code	Average particle size (µm)±SD
F1	152.8±8.35
F2	168.3±6.11
F3	176.6±9.42
F4	198.8±7.14
F5	210.9±10.73
F6	223.6±12.24
F7	240.2±8.69
F8	247.3±11.46
F9	253.9±12.51
F10	256.6±8.44
F11	276.2±5.47

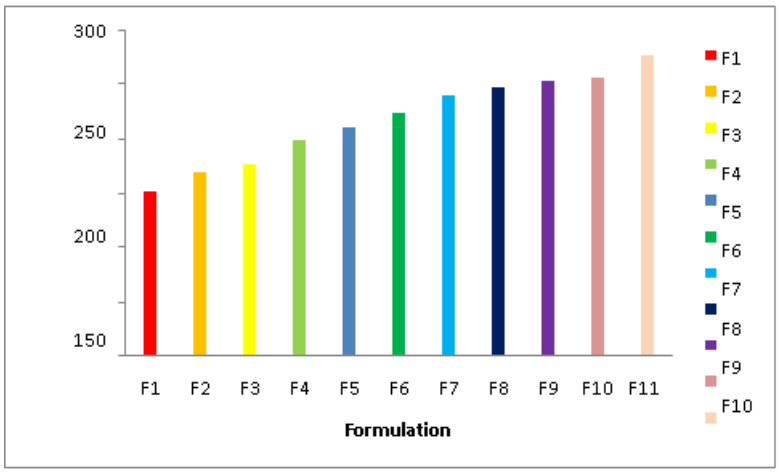

Figure 6: Comparison of average particle size of the prepared microspheres.

Scanning Electron Microscopy

The shape and surface morphology of prepared Benazepril HCl microsphere was done by scanning electron microscope. Image obtained from SEM analysis of microsphere samples revealed that all microspheres prepared were smooth, almost spherical in shape and non porous. The microspheres containing higher concentration of Xanthan gum were smooth, spherical and slightly aggregated particles when compared with the microspheres of Benazepril with higher concentration of Chitosan which were porous, rough, grossly, discrete spherical.

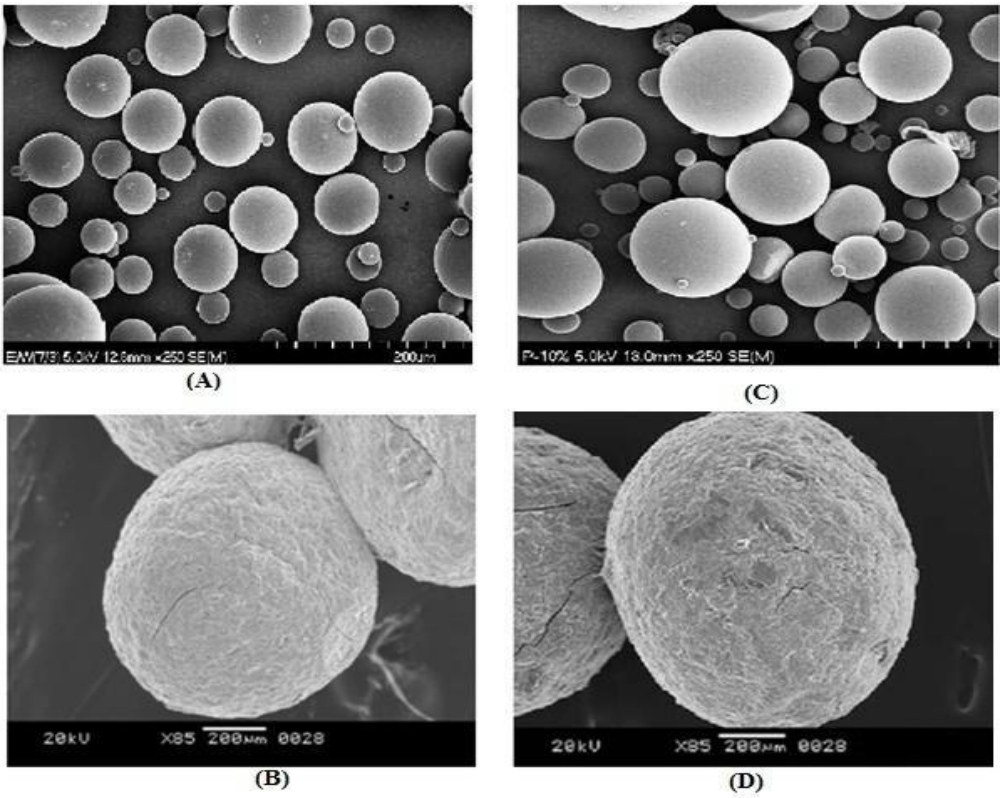

Figure 7: SEM images of F2 and F10 formulation.
(A) and (B) are images of F2 formulation, (C) and (D) are images of F10 formulation

Drug Loading, Drug Entrapment and Percentage yield

The results of drug loading capacity and entrapment efficiency are shown in **Table 12** Formulation F1 and F11 containing Chitosan and Xanthan gum alone respectively showed less entrapment capacity. Formulation F10 containing 9:1 ratio of Xanthan gum: Chitosan showed highest entrapment. As the Xanthan gum concentration was increased, the percentage drug loading decreased and percentage entrapment efficiency was increased due to increase viscosity of the Xanthan gum. The entrapment efficiency was increased with higher Xanthan gum concentration and this may be due to the diffusion of drug into the aqueous phase because of decrease in interfacial tension by Xanthan gum between drug and aqueous phase. The percentage drug loading of all formulation was ranged between 48.54±0.13 and 97.78±0.01% and the entrapment efficiency is between 68.3±0.11 and 95.9±0.22%. Percentage yield of different formulation F1 to F11 were calculated and the yield was foundin the range of 89.21%-52.68%. The percentage yield was higher for formulation F1 which contains Chitosan alone. Percentage loading was decreased gradually when the concentration of Xanthan gum was increased.[17]

Table 4: Drug Loading and Drug Entrapment of Microspheres.

Formulation Code	Percentage Drug Loading(%)	Percentage DrugEntrapment (%)	Percentage yield (%)
F1	48.54	68.30	89.21
F2	97.78	82.48	86.38
F3	68.81	90.40	79.53
F4	71.73	78.12	75.88
F5	77.88	83.62	71.20
F6	82.86	91.44	69.98
F7	85.44	78.88	65.14
F8	76.32	84.94	63.56
F9	63.12	92.48	58.91
F10	59.74	95.90	54.44
F11	52.30	70.71	52.68

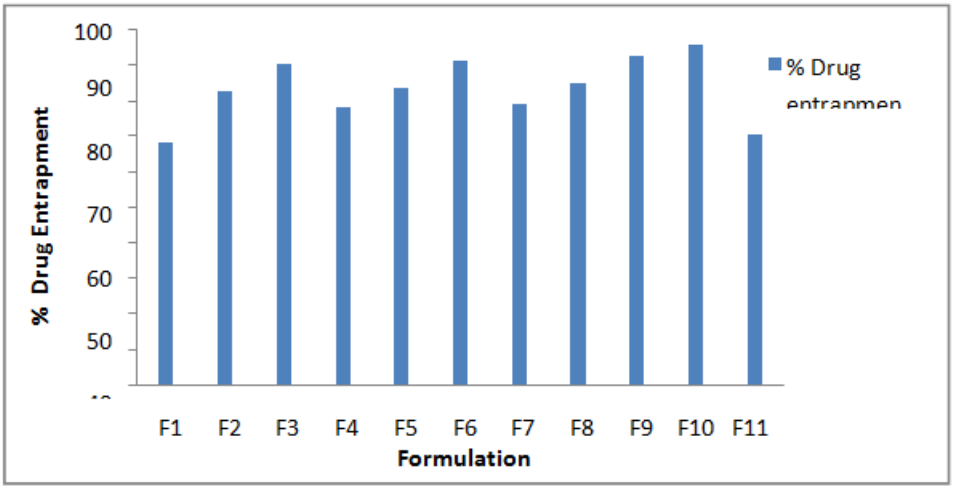

Figure 8: Comparison of % drug entrapment of the prepared microspheres.

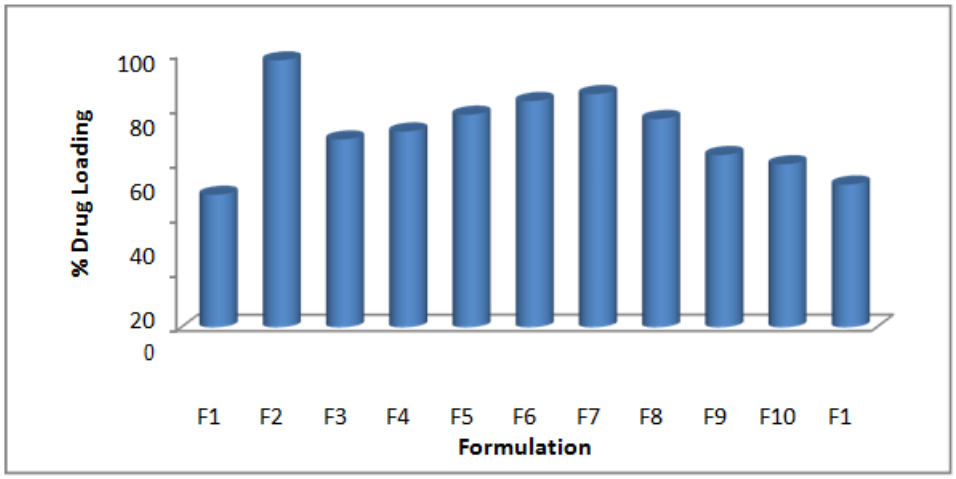

Figure 9: Comparison of percentage drug loading of the prepared microspheres.

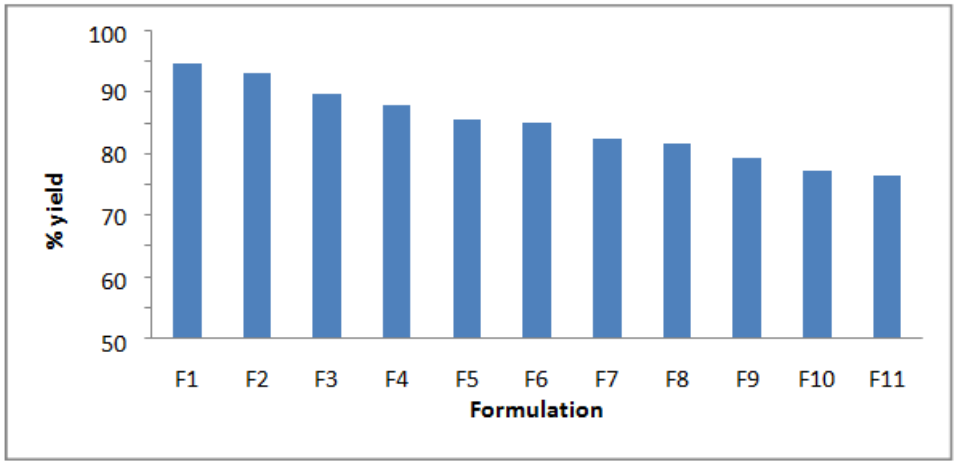

Figure 10: Comparison of percentage yield of the prepared microspheres.

***In-vitro* drug release studies**

Dissolution studies on all the eleven formulations of Benazepril microspheres were carried out using a USP dissolution apparatus. Dissolution test was carried out first 2 hours with 0.1N HCl (pH 1.2) and after 2 hours experiment was conducted by using pH 7.4 phosphate buffer as the dissolution medium. The cumulative percent drug release was found to be in the range of 64.56% to 94.86% at the end of 12 hours. First 4 hours the cumulative % of drug release from prepared microsphere was higher than drug release was slower. This might be the fact that gradually microspheres start swelling which in turns controls the drug release from the microsphere formulations. The cumulative drug release depends upon the combination of polymer used. Formulation F1 and F11 showed less than 65% of drug released at the end 12 hours, whereas formulation containing 9:1 ratio of Chitosan and Xanthan gum showed 94.86% drug release at the end of 12 hours. The increased density of the polymer matrix at higher concentrations Chitosan results in an increased diffusional path length. This may decrease the overall drug release from the polymer matrix. Furthermore, smaller microspheres are formed at this ratio and have a larger surface area exposed to dissolution medium, giving rise to faster drug release.[18]

Table 5: *In-vitro* drug release for Benazepril Microspheres formulation.

Time(hrs)	Cumulative % drug release of formulations										
	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11
1	16.47	14.38	13.46	14.98	14.10	13.18	13.76	13.11	12.59	11.04	10.50
2	30.18	28.25	24.70	27.55	24.83	24.15	22.94	20.56	18.37	19.37	20.37
3	42.46	40.38	38.74	40.62	38.35	36.87	36.29	31.90	27.50	27.50	28.50
4	58.84	55.46	53.12	51.82	50.65	48.18	47.74	46.58	45.34	42.34	35.87
5	64.30	59.43	57.58	58.01	56.21	54.06	53.49	51.41	46.98	47.85	41.05
6	71.29	66.85	64.34	65.64	62.73	61.74	57.50	55.28	54.47	50.47	48.76
7	75.94	71.81	68.53	71.26	68.47	65.93	62.78	60.49	60.86	56.52	52.54
8	79.84	76.52	72.25	73.72	71.58	68.71	70.18	68.52	57.41	60.41	55.41
9	83.23	80.14	74.31	77.16	74.69	71.18	75.26	72.76	62.50	62.50	57.50
10	85.01	81.89	75.53	80.59	77.93	73.68	77.39	74.84	65.57	65.57	59.57
11	86.89	83.11	77.04	82.43	79.85	74.81	80.61	77.60	68.83	69.83	61.83
12	88.38	94.86	78.29	84.30	81.12	75.90	82.32	79.72	74.69	72.69	64.56

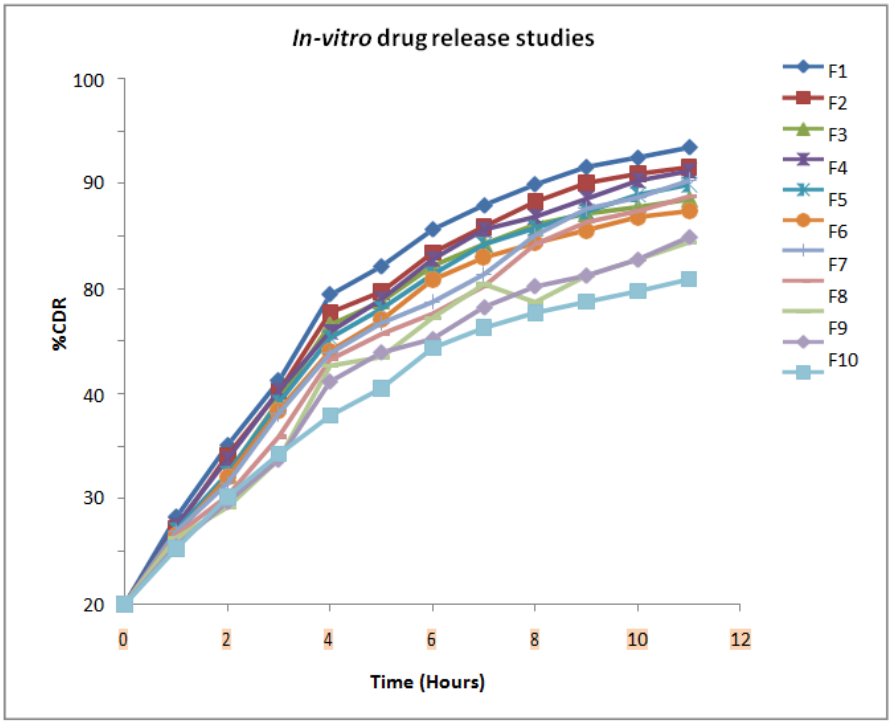

Figure 11: Comparative cumulative percentage drug Profile of Benazepril HClMicrospheres.

Drug Release Kinetics

More often, kinetics of drug release studies allows the measurement of some important physical parameters, such as the drug diffusion coefficient and resorting to model fitting on experimental release data. Thus, mathematical modelling has very important value in the process optimization of all the phenomena affecting drug release kinetics from the formulation. The pattern of the drug release from the Benazepril HCl microspheres was investigation by different kinetic equations (Zero order, First order, and Higuchi's equation). The release mechanism was understood by fitting the obtained data to Korsmeyer-Peppas model. The *in-vitro* release profile of drug from all microsphere formulations could be expressed by Higuchi model, as the plots shows high linearity **(R^2 = 0.992-0.999)** in comparison to zero order **(R^2 = 0.882-0.991)** and Higuchi's **equation (R^2 = 0.982-0.997)**. So, it was understood that Higuchi release kinetic release pattern was followed by all formulations. According to this model, the drug releases from these batches may be controlled by diffusion through the microspores. To confirm diffusion mechanism the data were fitted into Korsmeyer- Peppas model. All microsphere formulations F1 to F11 showed high linearity **(R^2 = 0.976-0.996)** with slope (n) ranging from **0.632 to 0.847** indicating all formulations followed **non-Fickian** release mechanism as their **'n'** values are lies between **0.45-0.89**.

Table 6: Mathematical modelling and drug release kinetics of formulation F0 to F9.

Formulationcode	KINETIC MODELS				
	Zero order	First order	Higuchi	Korsmeyer-pappas	
	R^2	R^2	R^2	n	R^2
F1	0.939	0.879	0.991	0.754	0.996
F2	0.916	0.866	0.982	0.847	0.989
F3	0.882	0.908	0.995	0.632	0.993
F4	0.883	0.811	0.997	0.820	0.990
F5	0.926	0.903	0.986	0.694	0.989
F6	0.922	0.896	0.994	0.810	0.991
F7	0.991	0.905	0.997	0.793	0.994
F8	0.889	0.912	0.985	0.802	0.987
F9	0.887	0.906	0.997	0.768	0.976
F10	0.983	0.869	0.994	0.798	0.992
F11	0.932	0.885	0.992	0.762	0.987

R^2=Regression coefficient, **n=** Exponential value

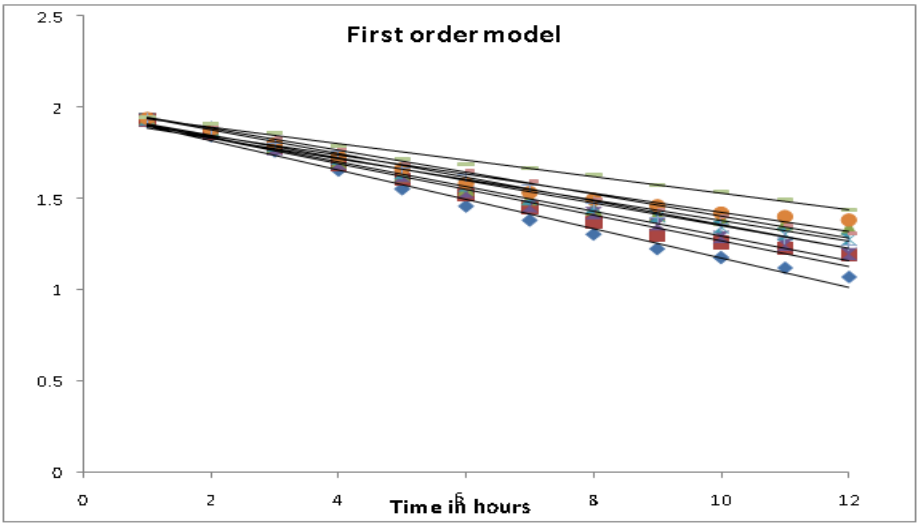

Figure 12: First order release kinetics for prepared Microspheres

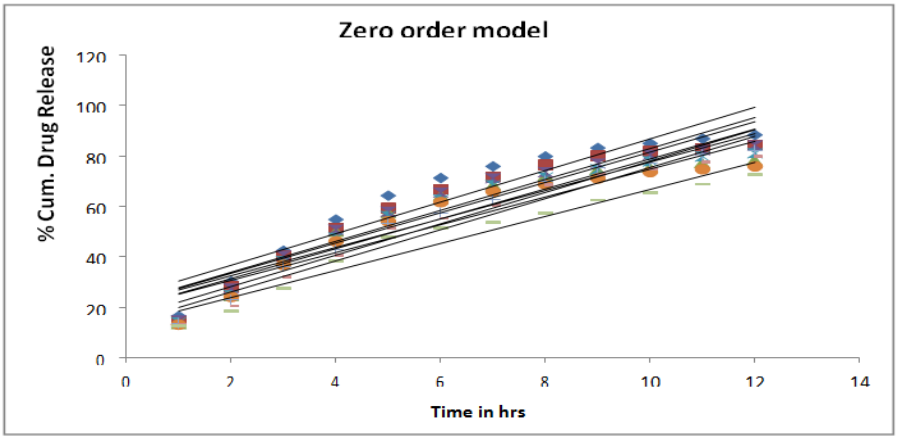

Figure 13: Zero order release kinetics for prepared Microspheres.

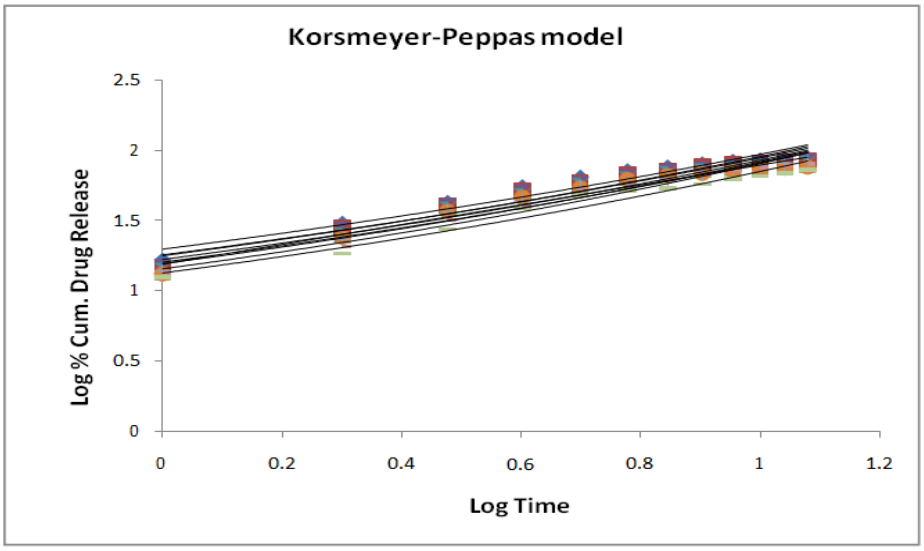

Figure 14: Korsmeyer-Peppas release kinetics for prepared Microspheres.

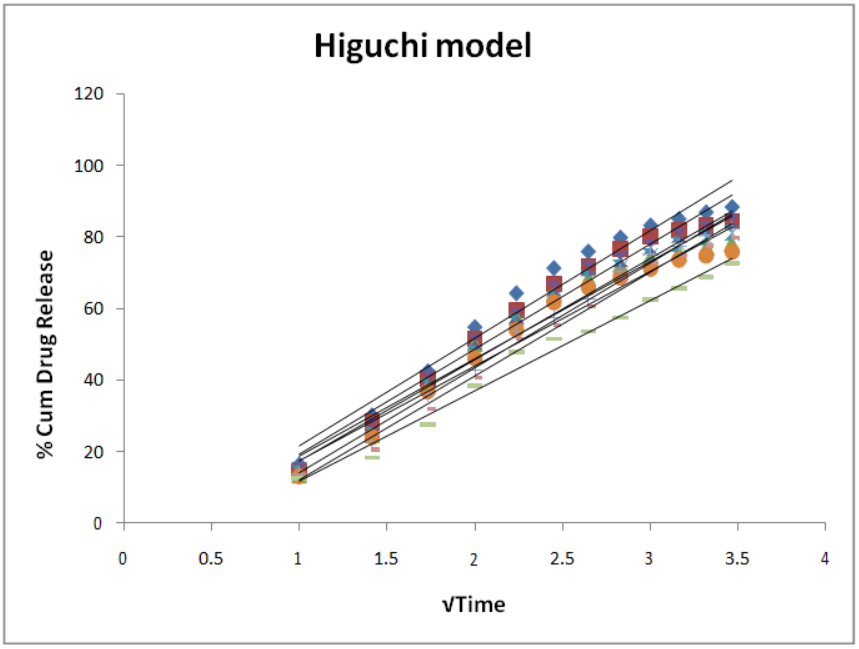

Figure 15: Higuchi release kinetics for prepared Microspheres.

Stability study: Stability study was conducted for the prepared Benazepril HCl microspheres formulation F. Formulation F2 was selected for short term stability studies based up on the in- vitro dissolution studies results. Selected formulation was stored at 40°C/75% RH respectively for a period of 90 days. Then, the sample was analyzed for physical appearance, entrapment efficiency and *in-vitro* drug release studies of the microsphere at the end of 15, 30, 45, 60 and 90 days. Results from the stability studies showed that there was no significant change in the physical appearance, drug entrapment and *in-vitro* release behaviours of the microsphere formulations. Hence prepared formulation was physicochemically stable throughout study period.[19]

Table 7: Stability studies for microsphere formulation (F2) stored at 40ºC/75% RH.

Tested days	% Drug entrapment	% CDR
	F2	F2
Initial	82.48	94.86
15	82.48	94.86
30	82.42	94.83
45	82.08	94.71
60	82.04	94.65
90	81.99	94.53

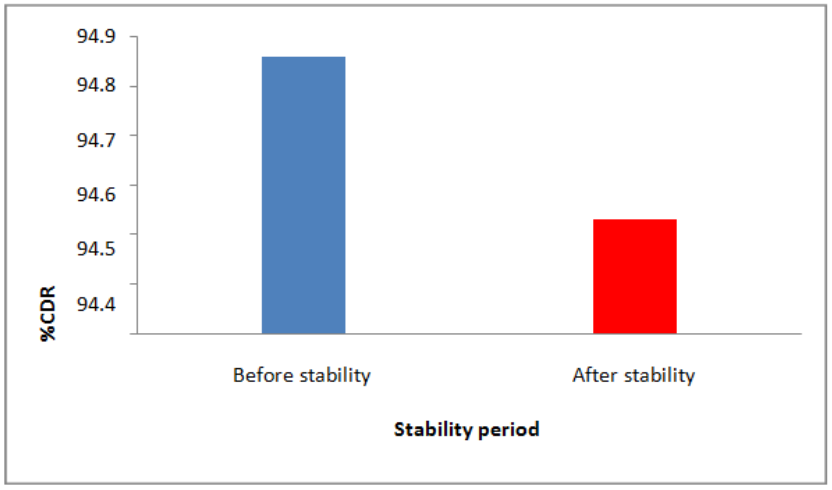

Figure 16: Comparative drug release studies before and after stability.

CONCLUSION

The precompression properties like Bulk density, tapped density; Hausner's ratio and Carr's index and angle of repose of all the formulations were found to be within the standard limits and indicate prepared microspheres showed excellent to good flow properties. Micromeritic studies revealed that the mean particle size of the prepared microspheres was within the range of 152 to 276µm. SEM analysis of the microspheres revealed that Chitosan containing microspheres were smooth, spherical and slightly aggregated particles when compared with the microspheres of Xanthan gum which were porous, rough, grossly, discrete spherical. Drug entrapment and practical yields were optimum depend upon the polymer used. As the concentration of Xanthan gum was increased the % drug entrapment was increased and loading decreased due to increase in the viscosity of the solution[20]. Cumulative percentage drug release was conducted for 12 hours and results showed formulation F2 containing 9:1 ratio of Chitosan: Xanthan gum showed highest percentage of drug release at the end of 12 hours. As the concentration of Xanthan gum was increased drug release was retarded. The formulations F1 to F11 were best fitted to Higuchi kinetic model and the drug release from the formulation was by non-Fickian diffusion mechanism. Based on the results of drug release studies, formulation F2 was selected for short term stability studies and results showed that there was no significant change in the drug entrapment, and in-vitro drug release characteristics of the microspheres. Thus, the formulated mucoadhesive microspheres seem to be a potential candidate as an oral gastro retentive controlled drug delivery system in prolonging the drug retention in stomach and increasing the bioavailability of drug[21]. FTIR results showed that polymers used were compatible with excipients used. In-vitro release studies showed that prepared microspheres has ability to release the drug for more than 24 hours in controlled manner and the mechanism of drug release was Higuchi release kinetic. Best formulation F2 was physicochemically stable throughout its stability studies.

REFERENCES

1. Patil PB, Gawali VU, Patil HN, Hardikar SR, Bhosale A V. Preparation and evaluation of mucoadhesive microspheres of atenolol and propranolol. Int J Pham Tech Res., 2009; 1(3): 639-43.
2. Shabaraya AR, Narayanacharyulu N. Design and evaluation of chitosan microspheres of metoprolol tartarate for sustained release. Indian J. Pharm. Sci., 2003; 65(3): 250-2.
3. Martinac A, Grcic JF, Voinovich D, Perissutti B, Franceschinis E. Development and bioadhesive properties of chitosan-ethylcellulose microspheres for nasal delivery. Int. J. Pharm, 2005; 291: 69-77.
4. Wang LY, Gu YH, Su ZG, Ma GH. Preparation and improvement of release behavior of chitosan microspheres containing insulin. Int. J. Pharm, 2006; 311: 187-95.
5. Palanisamy M, Khanam J, Arun Kumar N, Rani C. Chitosan microspheres encapsulated with metoprolol

succinate: Formulation and in-vitro evaluation. Res. J. Pharm. Technol, 2009; 2(2): 349-52.
6. Pavanveena C, Kavitha K, Anil Kumar SN. Formulation and evaluation of trimetazidine hydrochloride loaded chitosan microspheres. Int. J. Appl. Pharm, 2010; 2(2): 11-4.
7. Chowdary KPR, Rao YS. Mucoadhesive microcapsules of glipizide:characterization, in- vitro and in-vivo evaluation. Indian J. Pharm. Sci., 2003; 65(3): 279-84.
8. Kumar MN, Chowdary KA, Pani BK, Kumar N. Design and characterization of mucoadhesive microcapsules of metoprolol succinate. Int. J. Pharm. Pharm. Sci., 2010; 2(4): 53-7.
9. Senthil A, Hardik kumar RBT, Mehulkumar VBD, Tejas BBL, Lakshmi BB. Formulation and evaluation of mucoadhesive microspheres of venlafaxine Hcl. Int. Res. J. Pharm, 2011; 2(4): 194-9.
10. Cuna M, Alonso MJ, Torres D. Preparation and in-vivo evaluation of mucoadhesive microparticles containing amoxicillin-resin complexes for drug delivery to the gastric mucosa. Eur. J. Pharm. Biopharm, 2001; 5: 199-205.
11. Kockisch S, Rees GD, Tsibouklis J, Smart JD. Mucoadhesive, triclosan-loaded polymer microspheres for application to the oral cavity: preparation and controlled release characteristics. Eur. J. Pharm. Biopharm, 2005; 59: 207-16.
12. Yogesh ST, Vinayak SM, Shashikant CD. Use of carbomers to design mucoadhesive microspheres for an anti-H.Pylori drug, clarithromycin. Int. J. Pharm. Tech Res., 2009; 1(4): 1421-8.
13. Yadav VK, Kumar B, Prajapati SK, Shafaat K. Design and evaluation of mucoadhesive microspheres of repaglinide for oral controlled release. Int. J. Drug Delivery, 2011; 3: 357- 70.
14. Venkateswaramurthy N, Sambathkumar R, Perumal P. Controlled release mucoadhesive microspheres of clarithromycin for the treatment of Helicobacter Pylori infection. Der Pharmacia Lettre, 2012; 4(3): 993-1004.
15. Brahmaiah B, Kumar Desu P, Nama S. Formulation and evaluation of extended release mucoadhesive microspheres of Simvastatin. Int J Pharm Biomed Res, 2013; 4(1): 57-64.
16. Ershad S, Kishore VS, Sandeep M, Kartheek U, Rizwana SK, Tejaswi L. Preparation and evaluation of floating microspheres of ritonavir. Int J Res Pharm Chem. 2013; 3(4): 834- 41.
17. Godge GR. Formulation and evaluation of mouth dissolving tablet of Benazepril hydrochloride with taste masking resin. Int J Life Sci Rev., 2015; 1(7): 253-63.
18. Kumar SK. Formulation and evaluation of Benazepril hydrochloride immediate release tablets. Eur J Biomed Pharma Sci., 2015; 2(3): 452-72.
19. Barupal AK, Gupta V, Ramteke S. Preparation and characterization of ethosomes for topical delivery of aceclofenac. Indian J Pharm Sci., 2010; 72(5): 582–86.
20. Raj P, Mohammed I, Pravalika T. Controlled release Formulation and Evaluation of Idarubicin Microsphere using biodegradable hydrophilic and hydrophobic polymer mixtures. Asian J Pharma Clin Res., 2010; 3(3): 3-6.
21. Yogesh ST, Vinayak SM, Shashikant CD. Use of carbomers to design mucoadhesive microspheres for an anti-H. Pylori drug, clarithromycin. Int J Pharm Tech Res., 2009; 1(4): 1421-8.