International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-3, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Design, Development and *In Vitro* Evaluation of Controlled Release Microspheres of Losartan Potassium

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Manuscript No: IJPRS/V3/I3/00349, Received On: 23/07/2014, Accepted On: 31/07/2014

ABSTRACT

Microspheres are well accepted technique to control the drug release from the dosage form to improve bioavailability, reduce absorption difference in patients, reduce the dosing frequency and adverse effects during prolong treatment. The main objective of the present study is to prepare and evaluate Losartan potassium microspheres by Emulsification internal gelation method, with water soluble polymers such as Sodium alginate, Guar gum, Xanthan gum and $Baco_3$ as crosslinking agent, using as carrier for oral administration in view to achieve oral controlled release of the drug. Losartan potassium is a Angiotensin II receptor blocker selectively and specifically antagonize the action of angiotensin II, a potent vasoconstrictor impacting BP regulation. Angiotensin II receptor blocker are becoming increasingly popular for the treatment of hypertension because they are effective and well tolerated. It has short biological half-life of 1.5-2 hrs. This necessitates multiple daily dosing for maintenance of its plasma concentration within the therapeutic index, hence there is impetus for developing controlled release dosage form that maintains therapeutic plasma drug concentration for long period. Compatibility studies revealed there was no interaction between the drug and polymers. The formulations were evaluated for particle size distribution analysis, flow properties like Angle of repose, bulk density, tapped density, Hausner's Ratio, Carr's index, microencapsulation efficiency, Scanning electron microscopy and *in-vitro* release studies. The optimized formulation showed good *in-vitro* controlled release activity of the drug Losartan potassium.

KEYWORDS

Controlled Release Microspheres, Sodium Alginate, $Baco_3$, Cross Linking Agent, Emulsification Internal Gelation Method

INTRODUCTION

Controlled drug delivery systems designed to deliver drug at predetermined rates for predefined periods of time and have been used to overcome the shortcoming of conventional drug formulations.

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The term microspheres describes a monolithic spherical structure with the drug or therapeutic agent distributed throughout the matrix either as a molecular dispersion or as a dispersion of particles (in the 1 - 1000µm size ranges) for use as carries of drugs and other therapeutic agents. Microspheres are well accepted technique to control the drug release from the dosage form to improve bioavailability, reduce absorption difference in patients, reduce the dosing frequency and adverse effects during prolong

treatment[1-2]. Losartan potassium is a Angiotensin II receptor blocker selectively and specifically antagonize the action of angiotensin II (AT_1 receptor) in vascular smooth muscle and the adrenal gland, producing decreased BP. Angiotensin II receptor blocker are becoming increasingly popular for the treatment of hypertension because they are effective and well tolerated. Losartan potassium is the first orally active angiotensin II receptor antagonist, losartan is extensively metabolized in liver. It is widely prescribed in the treatment of hypertension. It undergoes extensive biotransformation and has an elimination half-life of 1.5 – 2 hr[3]. The rational of this study is to design and evaluate an oral site-specific, controlled drug delivery system of Losartan potassium microspheres for treatment of hypertension.

MATERIALS AND METHOD

Materials

Losartan potassium was obtained as a gift sample from Aurobindo pharma Ltd (Hyderabad). $Baco_3$, Sodium alginate, guar gum and Xanthan gum was gifted by Himedia Lab's Pvt. Ltd.

Preparation of Losartan Potassium Microspheres by Emulsification Internal Gelation Method

Microspheres containing Losartan potassium were prepared employing sodium alginate alone and in combination with xanthan gum and guar gum, $Baco_3$ as crosslinking agent. The homogeneous polymer(s) solution was prepared in distilled water stirred magnetically with gentle heat.

The drug and cross-linking agent were added to the polymer solution and mixed thoroughly by stirring magnetically to form a viscous dispersion which was then extruded through a syringe with a needle of size no. 23 into light liquid paraffin containing 1.5% Tween 80w/v and 0.2%v/v glacial acetic acid being kept under magnetic stirring at 100 rpm. The microspheres were retained in the light liquid paraffin for 30 min to produce rigid discrete particles.

They were collected by decantation and the product thus separated was washed with chloroform to remove the traces of paraffin oil[3]. The microspheres were dried at 40^OC under vacuum for 12h. The compositions of the microspheres formulations are listed in Table 1.

Table 1: Composition of different microspheres formulations of Losartan potassium

Formula	Drug + Sodium alginate			Drug + Sodium alginate + Guar gum			Drug + Sodium alginate + Xanthan gum		
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
	1:4	1:6	1:8	1:4	1:6	1:8	1:4	1:6	1:8
Losartan potassium (mg)	500	500	500	500	500	500	500	500	500
Sodium alginate (mg)	2000	3000	4000	1000	1500	2000	1000	1500	2000
$Baco_3$ (mg)	500	500	500	500	500	500	500	500	500
Guar gum (mg)	-	-	-	1000	1500	2000	-	-	-
Xanthan gum (mg)	-	-	-	-	-	-	1000	1500	2000

Evaluation of Microspheres[4-6]

Flow Properties of Microspheres

The prepared microspheres were evaluated for Angle of repose, Bulk density, Tapped Density, Carr's Index, Hausner's Ratio.

Size Distribution and Particle Size Analysis

The particle size of microspheres was determined by using optical microscopy method in which 100 particles were measured using light microscope.

Estimation of Losartan Potassium Content (Drug Content)

The drug content in each formulation was determined by triturating 100mg microspheres and powder equivalent to average weight was added in 100ml of 6.8 pH phosphate buffer, followed by stirring. The solution was filtered, diluted suitably and the absorbance of resultant solution was measured spectrophotometrically at 234 nm using 6.8 pH phosphate buffers as blank.

Entrapment Efficiency

Entrapment efficiency was calculated using the formula.

$$\text{Entrapment efficiency} = \frac{\text{Amount of drug entrapped in microspheres}}{\text{Total amount of drug}} \times 100$$

SEM Analysis

The samples for the SEM analysis were prepared by sprinkling the microspheres on one side of the double adhesive stub. The stub was then coated with fine gold dust. The microspheres were then observed with the scanning electron microscope.

%Yield of Microspheres

Microspheres recovered at the end of preparation were weighed and the yield was calculated as a percentage of the total amounts of polymer and drug added during the preparation of microspheres.

$$\%\text{Yield} = \frac{\text{Practical yield of microspheres}}{\text{Theoretical yield of microspheres}} \times 100$$

In-Vitro Release Profile Losartan Potassium Microspheres[7-8]

In-vitro release profile of Losartan potassium microspheres studies were performed, using USP Type II dissolution test apparatus. Microspheres weighted equivalent to 100mg of Losartan potassium wrapped in parchment paper and placed in the dissolution vessel containing 900mL of dissolution medium. The medium was maintained 37 ±0.5 0C and stirred at 100 rpm. The in-vitro dissolution studies were performed at pH 6.8 phosphate buffer (artificial small intestinal fluid). The sample (5 mL) was withdrawn at each hour interval, withdrawn solution filtered through a 0.45µm membrane filter and replaced with the same volume of test medium and withdrawn sample were diluted if required and then estimated for Losartan potassium at 234 nm spectrophotometrically using UV- Visible spectrophotometer. Corresponding concentrations in sample were calculated from standard plot and calculated cumulative percentage of drug release from each formulations.

In Vitro Drug Release Kinetics[9]

For understanding the mechanism of drug release rate kinetics of the drug from dosage forms, the data obtained during *in-vitro* dissolution studies were subjected to kinetic treatment to obtain the order of release and release mechanism.

Drug Excipient Compatibility Studies[10]

Fourier Transform Infrared (FTIR) Spectroscopy studies were used for the evaluation of physicochemical compatibility and interactions, which helps in the prediction of interaction of the drug with Xanthan gum, Guar gum and $Baco_3$ used in microspheres formulations. In the present study 1:1 ratio was used for preparation of physical mixtures and analyzed for compatibility studies.

Stability Studies of Losartan Potassium Microspheres[11]

The optimized Losartan potassium microspheres were separated in to two groups. Each group of formulations were placed separately in stability

chamber which is maintained at 25±5ºC/60% RH and 40±5ºC/75% RH respectively for three months and every month the formulations from each group were subjected to dissolution studies and % drug release was calculated.

RESULTS AND DISCUSSION

In the present study, it was aimed to develop Losartan potassium microspheres using water soluble polymers as a carrier for oral administration to extend the period of the dosage form.

This process produced uniform microspheres. These microspheres were characterized for size analysis, flow properties, % Drug Content, % Entrapment efficiency. All the formulations offered good flow property. The technique also showed good entrapment efficiency. The micrometric parameters like angle of repose, bulk density and tapped density of all microspheres confirms better flow and packaging properties. All the formulations showed good flow ability represents in terms of angle of repose, Carr's index, and Hausner's ratio.

The microspheres were found to be discrete, spherical and free flowing. The % yield was found to be in the range of 88%-93%. The mean particle size of the various formulations was found to be in the range of 625.34-718.68µm.The results are given in Table 2 and figure 1.

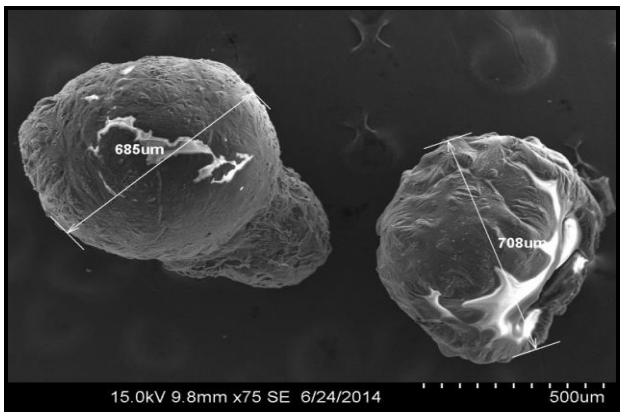

Figure 1: SEM Photograph showing particle size of microspheres

Microspheres prepared with Losartan potassium and Sodium alginate in 1:4, 1:6 and 1:8 ratios shown controlled drug release for a period of 7 hours, 8 hours and 10.5 hours respectively.

Table 2: Evaluation Tests of Losartan potassium microspheres Formulated with Sodium alginate alone and as combinations with Guar gum and xanthan gum in Different Ratios

Formu-lation	Angle of repose (θ)	Carr's Index (%)	Hausner's Ratio	Average Particle Size(µ)	% yield	%Encapsulation Efficiency	% Drug content
F_1	33.42	14.43	1.12	632.34	90	98	19.6
F_2	32.53	12.67	1.14	702.32	92	97.05	13.86
F_3	34.78	13.81	1.18	662.53	89	97.03	10.79
F_4	31.7	11.45	1.14	654.19	93	98.5	19.7
F_5	32.53	13.52	1.17	692.27	91	95.09	13.58
F_6	33.69	14.76	1.15	705.47	88	94.33	10.49
F_7	31.54	11.65	1.17	635.26	91	98.40	19.68
F_8	32.67	13.49	1.15	718.68	89	96.21	13.74
F_9	34.54	15.79	1.16	698.52	93	98.65	10.97

Microspheres prepared with Losartan potassium and Sodium alginate + Guar gum in 1:4, 1:6 and 1:8 ratios shown controlled drug release for a period of 7.5 hours, 8.5 hours and 11 hours respectively.

Microspheres prepared with Losartan potassium and Sodium alginate + Xanthan gum in 1:4, 1:6 and 1:8 ratios shown controlled drug release for a period of 8.5 hours, 10.5 hours and 12 hours respectively.

Table 3: Pharmacokinetic release of Losartan potassium microspheres with Sodium alginate, Sodium alginate + Guar gum and Sodium alginate + Xanthan gum

Formul--ation	RELEASE MODEL								
	Zero order		First order		Higuchi Matrix		Koresmeyer-Peppas		
	K_0	R_0	K_1	R_1	K_H	R_H	n	K_K	R_K
F1	15.8899	0.9843	-0.4237	0.9257	34.6694	0.9520	0.8515	20.2264	0.9904
F2	14.0913	0.9792	-0.3761	0.9319	32.7951	11.394	0.9061	16.7701	0.9893
F3	9.1120	0.9949	-0.2196	0.7773	23.9437	0.9202	0.7948	13.2071	0.9877
F4	13.0543	0.9958	-0.3487	0.7502	29.1133	0.9224	0.8205	17.0273	0.9879
F5	12.4032	0.9960	-0.3262	0.8478	29.5647	0.9416	0.8561	15.9069	0.9944
F6	9.7992	0.9943	-0.2738	0.8429	26.5186	0.9432	0.8414	13.4192	0.9919
F7	12.1905	0.9908	-0.3261	0.7739	29.1950	0.9532	0.7679	18.2429	0.9900
F8	10.0233	0.9973	-0.2673	0.8310	26.4426	0.9907	0.8478	13.3381	0.9907
F9	8.1693	0.9981	-0.1973	0.7690	22.8997	0.9216	0.8505	10.8800	0.9909

Table: 4 Stability studies of best formulation according to ICH guidelines

S.NO	Time (hrs.)	% Drug release (mg)						
		Initial	25±5°C/60% RH			40±5°C/75% RH		
			1st month	2nd month	3rd month	1st month	2nd month	3rd month
1	1	09.25	09.19	08.45	08.45	09.12	08.07	08.99
2	2	16.91	18.53	17.50	18.46	17.48	18.42	18.38
3	3	25.02	26.90	27.86	28.81	26.84	25.78	22.73
4	4	32.04	33.96	35.91	33.87	35.88	34.85	36.81
5	5	43.94	45.91	46.87	44.84	44.86	45.82	43.77
6	6	49.76	49.67	48.63	49.60	49.61	48.57	49.53
7	7	57.51	59.50	57.45	58.41	56.42	58.39	58.36
8	8	64.82	68.77	64.72	67.69	65.71	66.68	66.63
9	9	73.17	76.12	73.10	76.07	73.09	74.05	73.99
10	10	83.90	83.79	84.75	85.70	84.73	82.69	82.65

The correlation coefficient values (Table 3) clearly indicate that dissolution profiles followed Zero order kinetics and mechanism of drug release was governed by Koresmeyer-Peppas model. The diffusion exponent values (n) were found to be in the range of 0.7679-0.9061 and it followed the non-fickian transport diffusion mechanism.

Comparative *In-vitro* Zero order and Koresmeyer-Peppas drug release profiles plot of Losartan potassium microspheres prepared with Sodium alginate, Sodium alginate + Guar gum, Sodium alginate + Xanthan gum in different ratios by Emulsification internal gelation method were shown in the figure 2-7.

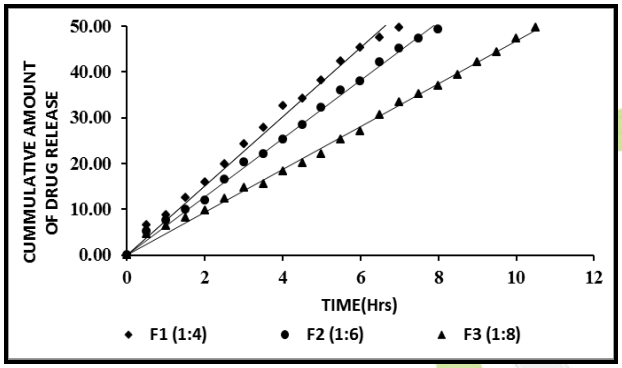

Figure 2: In-vitro Zero order release profile plot of Losartan potassium microspheres prepared with Sodium alginate in different ratios

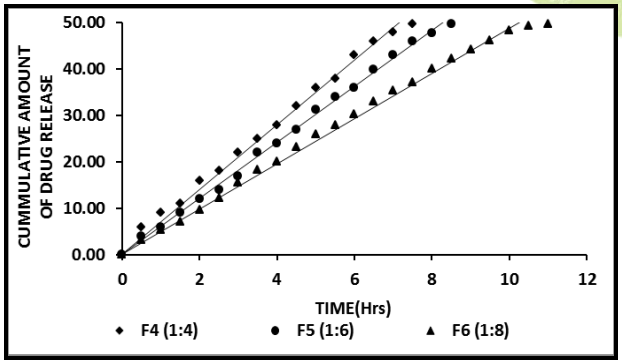

Figure 3: In-vitro Zero order release profile plot of Losartan potassium microspheres prepared with Sodium alginate and Guar gum in different ratios

The results indicated that the drug release from the microspheres was not changed significantly when stored at varying conditions and the release data was given in table 4, Thus the drug release from microspheres was found be quite stable.

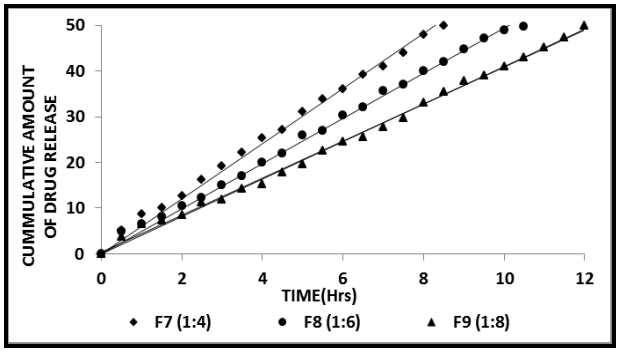

Figure 4: In-vitro Zero order release profile plot of Losartan potassium microspheres prepared with Sodium alginate and Xanthan gum in different ratios

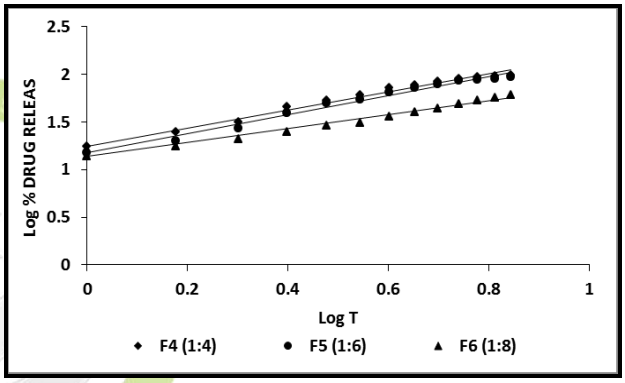

Figure 5: In-vitro Koresmeyer-Peppas release profile plot of Losartan potassium microspheres prepared with Sodium alginate in different ratios

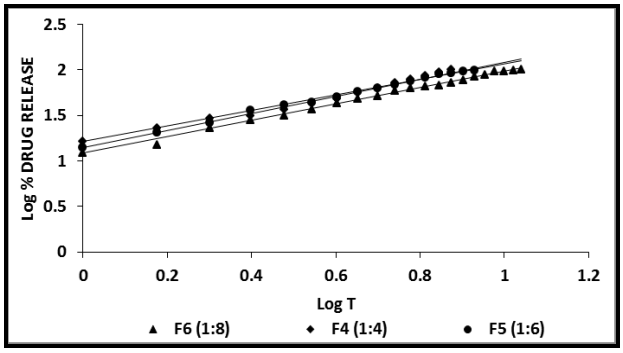

Figure 6: In-vitro Koresmeyer-Peppas release profile plot of Losartan potassium microspheres prepared with Sodium alginate and Guar gum in different ratios

Drug- excipient interactions play a vital role with respect to release of drug from the formulation amongst others.

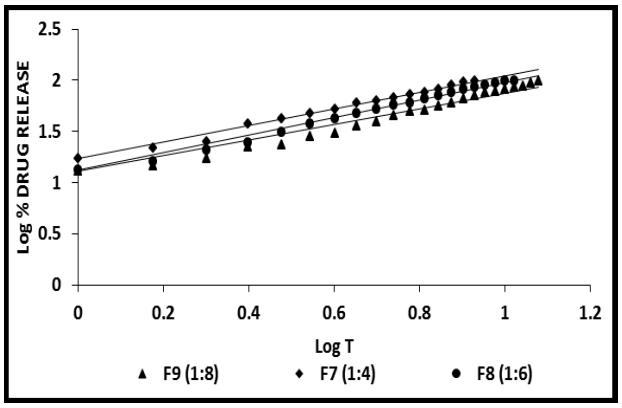

Figure 7: In-vitro Koresmeyer-Peppas release profile plot of Losartan potassium microspheres prepared with Sodium alginate and Xanthan gum in different ratios

FTIR techniques have been used here to study the physical and chemical interaction between drug and excipients used. The characteristic absorption peaks of Losartan potassium appeared at 3038.22, 3399.52, 1130.98, 1642.23, 1356.36, 1571.12 and 2926.99 denoting stretching vibration of C-H-, N-H-, O-H-, C=N, C-N, N=N and aromatic ring, respectively. Form the figures (8-11), it was observed that same peaks were also reported in all drug loaded microspheres. There was no change or shifting of characteristic peaks in drug loaded microspheres suggested that there was no significant drug polymer interaction which indicates the stable nature of the drug in all formulations.

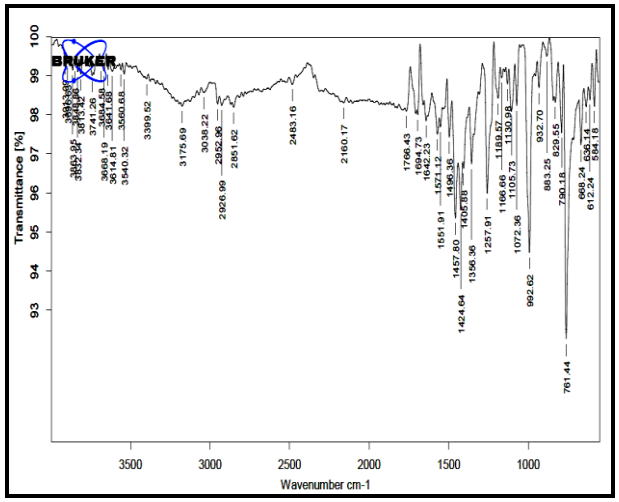

Figure 8: FTIR graph of formulation containing Losartan potassium

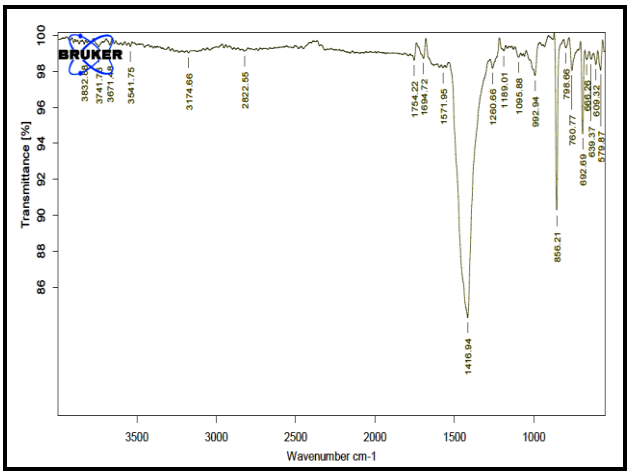

Figure 9: FTIR graph of formulation containing Losartan potassium + Sodium alginate + $Baco_3$

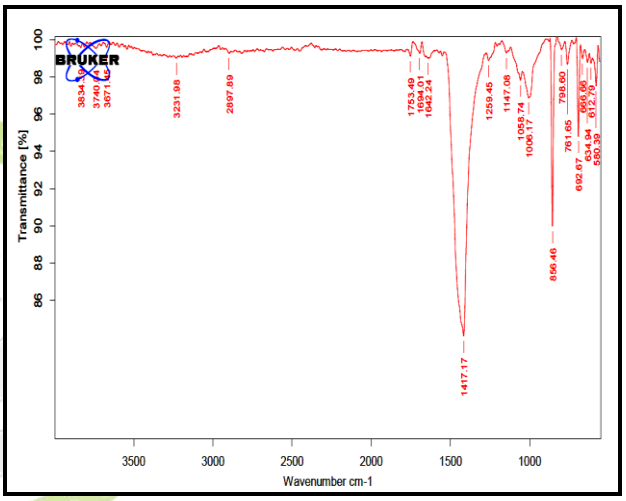

Figure 10: FTIR graph of formulation containing Losartan potassium + Sodium alginate + guar gum +$Baco_3$

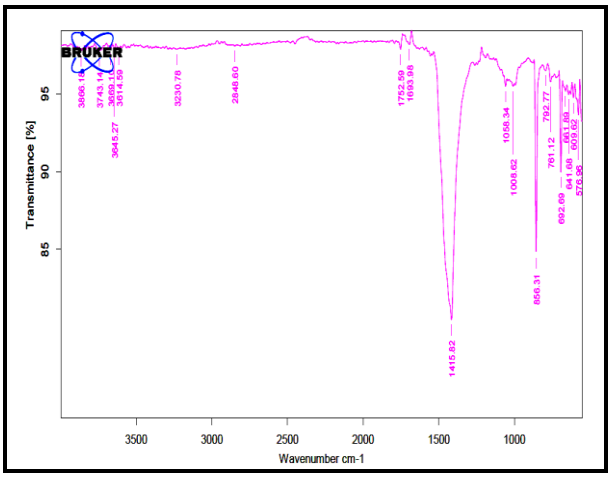

Figure 11: FTIR graph of formulation containing Losartan potassium + xanthan gum + $Baco_3$

CONCLUSION

Microspheres prepared with Losartan potassium and Sodium alginate + Xanthan gum in 1:8 ratio shown controlled drug release for a period of 12 hours. This gave a hope to the possibility of single dose treatment for patients. The formulated Losartan potassium microspheres show pharmacotechnical properties in the acceptable range. This study clearly demonstrated that one could develop a controlled dosage form of a drug having a long biological half-life as a single dose treatment and thus reduce the drug resistance in patients.

ACKNOWLEDGEMENTS

The authors are thankful to Bapatla College of Pharmacy and Dr V. Sai Kishore (guide) for his valuable support and providing facilities to carry out this research work.

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