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FORMULATION DEVLOPMENT AND EVALUATION OF MUCOADHESIVE MICROSPHERE OF LOSARTAN POTASSIUM BY USING NATURAL POLYMER

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Keywords:

Losartan potassium, Katira Gum, Babul Gum, *in vitro* dissolution

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ABSTRACT: The present work is regarding formulation, development and evaluation of losartan potassium microspheres using natural polymer. The mucoadhesive microspheres of losartan potassium were successfully developed by W/O emulsion solvent evaporation technique using two different natural polymers katira gum and babul gum. Total 12 batches were formulated. Six formulations were prepared by using each natural polymer i.e. LKM1 to LKM6 using katira gum (KG) and LBM1 to LBM6 using babul Gum (BG). All the formulations were evaluated for micromeritic properties, physical evaluation, which includes particle size analysis, percentage yield, drug content, drug entrapment efficacy, percent moisture loss and swelling index, *in vitro* dissolution studies, *in vitro* mucoadhesion, scanning electron microscopy, *in vitro* mucoadhesion and drug polymer interaction studies. The Optimized batch LKM5 was found to release the drug for 12 h (99.78%) and follows Higuchi Matrix model in dissolution studies, indicating the matrix-forming potential of natural polymer and diffusion controlled release mechanism.

INTRODUCTION: A drug delivery system is defined as a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy and safety by controlling the rate, time, and place of release of drugs in the body. The efficiency of any drug therapy can be described by providing a therapeutic amount of drug to the proper site of action to achieve the desired concentration of the drug in blood or tissues, for the desired therapeutic response which is therapeutically effective and non-toxic for a prolonged period of time.

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Recently the novel dosage forms which can control the release rate and target the active drug molecule to a particular site have attained a great formulation interest. Microspheres are one of the novel drug delivery system which possess several applications and are made up of assorted polymers [2].

Microspheres can be defined as solid, approximately spherical particles ranging in size from 1 to 1000 µm range in diameter having a core of drug and entirely outer layers of polymers as coating material. They are made up of polymeric, waxy or other protective materials i.e. biodegradable synthetic polymer and modified natural products such as starches, gums, proteins, fats and waxes.

However, the success of these microspheres is limited due to their short residence time at site of absorption.

It would, therefore be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membrane. This can be achieved by coupling bioadhesion characteristics to Microspheres [3]. Microspheres constitute an important part of such particulate drug delivery systems by virtue of their small size and efficient carrier capacity [4]. They have varied applications and are prepared by using various polymers. Mucoadhesive drug delivery system are delivery system which utilizes the property of bioadhesion of certain polymers which become adhesive on hydration and can be used for targeting a drug to a particular region of the body for extended periods of time.

Losartan Potassium is an effective antihypertensive drug but it undergoes hepatic first pass metabolism. Its half-life ($t_{1/2}$) is 1.5-2 hrs and it should be administered 3-4 times to maintain plasma drug concentration [5, 6]. So, it requires control release of a drug. Hence an alternative drug delivery system is needed for increasing therapeutic efficacy, reducing the dosing frequency of drug and improving its half-life and bioavailability [7].

Therefore, it is necessary to develop a newer formulation which releases the drug in a sustained release manner. Thus losartan potassium would become promising candidate for mucoadhesive microspheres in the management of hypertension.

Thus, the present study reports a novel attempt to prepare microspheres of antihypertensive drug Losartan potassium by using natural polymers Katira gum (*Cocholospermum religiosum)* and Babul gum (*Acacia niloctica*) as carriers by using W/O emulsion solvent evaporation Technique.

MATERIALS AND METHODS: Losartan Potassium was obtained as a gift sample from Vasudha Pharma and Chem Limited, Hyderabad, AP. Katira Gum and Babul Gum was purchase from local market, Nagpur. Liquid paraffin, Tween 80 was purchased from Loba chemicals, Mumbai and iso propyl alcohol was purchased from SD Fine Chemicals Ltd., Mumbai. All other reagents used were of analytical grade.

Preparation of Losartan potassium Microspheres: Mucoadhesive microspheres containing Losartan potassium were prepared by the water-in-oil (W/O) emulsification solvent evaporation technique. The drug was dissolved in each polymeric aqueous solution. The solution was poured in 200ml liquid paraffin contains 0.5 % tween 80 as an emulsifying agent. The aqueous phase was emulsified into oily phase by stirring the system in a 1000 ml beaker (**table 1**).

Constant stirring at 1000 rpm was carried out using mechanical stirrer and its content was heated by a heating mantle at 20°C. Stirring and heating were maintained for 2 hours until the aqueous phase was completely removed by evaporation. The light oil was decanted and collected microspheres were washed three times with 100 ml aliquots of isopropyl alcohol, filtered through Whatman filter paper, dried in oven at 50°C for 2 hours and stored in desiccators at room temperature [8] (**fig. 1**).

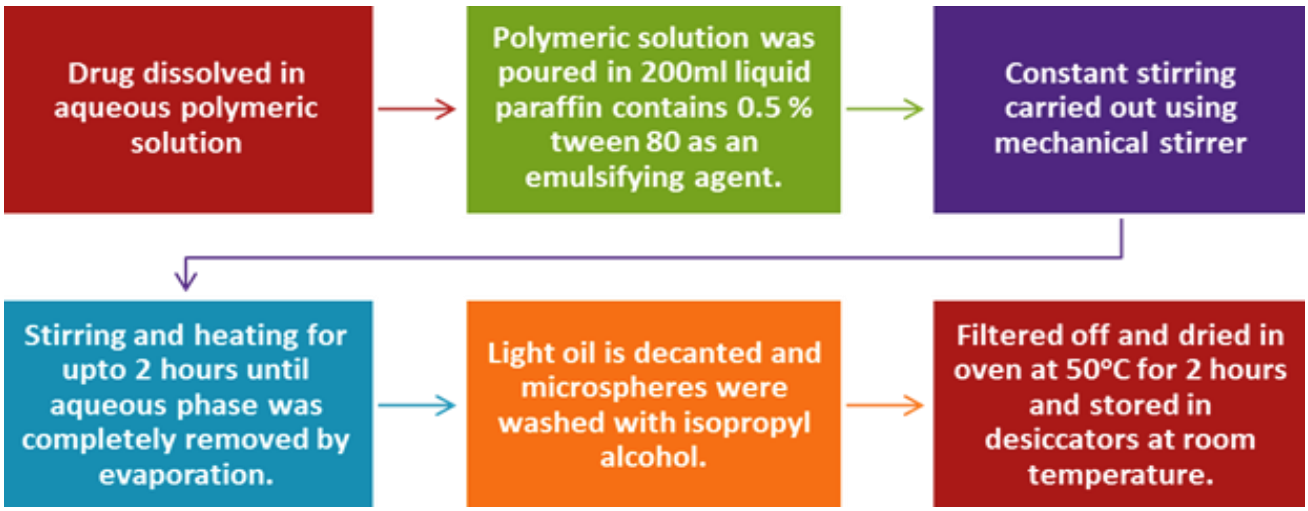

FIG. 1: SCHEMATIC REPRESENTATION OF PREPARATION OF MUCOADHESIVE MICROSPHERES OF LOSARTAN POTASSIUM BY W/O EMULSION SOLVENT EVAPORATION TECHNIQUE

TABLE 1: FORMULATION DESIGN OF MUCOADHESIVE MICROSPHERES OF LOSARTAN POTASSIUM USING DIFFERENT NATURAL POLYMERS.

Formulation code	Drug concentration (%w/w)	Katira Gum (%w/w)	Babul Gum (%w/w)
LKM1	1	1	-
LKM2	1	2	-
LKM3	2	1	-
LKM4	2	2	-
LKM5	3	1	-
LKM6	3	2	-
LBM1	1	-	6
LBM2	1	-	7
LBM3	2	-	6
LBM4	2	-	7
LBM5	3	-	6
LBM6	3	-	7

Evaluation of Drug loaded Mucoadhesive Microspheres:

1. **Micromeritic Properties:** The flow properties of drug loaded microspheres were studied by determining various parameters like the Bulk density, Tapped density, Angle of repose, Carr's consolidation Index, Hausner's Ratio [9, 10, 11].

2. **Physical characterization of prepared Microspheres:**

   a. **Particle size analysis [12]:**

   - All the microspheres were evaluated with respect to their size and shape using MOTIC PLUS – 2.0 (ml) instruments. The average sizes of the microspheres were calculated.

   - **Scanning electron microscopy (SEM):** The shape and surface morphology of the microspheres was studies by using scanning electron microscopy.

   b. **Percentage yield [12]:** The percentage yield of each batch was calculated on weight basis with respect to the weight of starting material. All experiments were carried out in triplicate. The percent yield of prepared microsphere was calculated by following formula;

Percentage Yield=

$$\frac{\text{Weight of Microspheres}}{\text{Weight of Drug + Weight of Polymer}} \times 100$$

   c. **Drug content estimation [12]:** The drug content of the prepared microspheres was determined by the method of extraction of drug present in microspheres. Drug loaded microspheres (100mg) were powdered and extracted in 100 ml Phosphate buffer 6.8 P^H for 24 hrs. Then the resultant dispersion of microspheres was sonicated for 30 minutes for complete mixing and filtered through a Whatman filter paper. The concentration of drug present in filtrate was determined spectrophotometrically at 206.3 nm using 6.8 P^H phosphate buffer as blank. Each determination was made in triplicate. The drug content of prepared microsphere was calculated by following formula;

Drug Content=

$$\frac{\text{Calculated Drug Content}}{\text{Total amount of Microspheres}} \times 100$$

   d. **Drug Entrapment efficiency [12]:** The drug entrapment efficacy of the prepared microspheres was determined by the method of extraction of drug present in microspheres. Drug loaded microspheres (100mg) were powdered and extracted in 100 ml Phosphate buffer 6.8 P^H for 24 hrs.

   Then the resultant dispersion of microspheres was sonicated for 30 minutes for complete mixing and filtered through a Whatman filter paper. The concentration of drug present in filtrate determined spectrophotometrically at 206.3 nm using 6.8 pH phosphate buffer as blank. Each determination was made in triplicate.

The drug entrapment efficiency (DEE) was calculated by the following formula;

Drug Entrapment Efficiency=

$$\frac{\text{Calculated Drug Content}}{\text{Theoretical Drug Content}} \times 100$$

e. **Theoretical Drug Content** was determined by calculation assuming that the entire LP present in polymer solution used get entrapped in LP microspheres, and no loss occurs at any stage of preparation of LP microspheres.

f. **Practical drug Content** was analyzed by using following procedure;

Weighed amount of LP microspheres equivalent to 100 mg microspheres was dissolved in 100 ml of phosphate buffer P^H 6.8. This solution was kept for 2 hours for the complete dissolution of the LP in Phosphate buffer pH 6.8. This solution was filtered and further diluted to make the concentration of 10ug/ml solution. The absorbance of the solution was measured at 206.3 nm using double beam UV -Visible Spectrophotometer against Phosphate buffer pH 6.8 as a blank and calculated for the percentage of drug present in sample.

g. **Swelling Index [13]:** Swelling index illustrate the ability of the mucoadhesive microspheres swell at the absorbing surface by absorbing fluids available at the site of absorption, which is a primary requirement for initiation of mucoadhesion.

A weighed amount of microspheres (100mg) was placed in 100 ml of 6.8 P^H phosphate buffer and swelling was allowed at 37^{o}C and change in weight variation between initial weight of microspheres and weight due to swelling is measured by taking weight periodically and socking with filter paper. The percent swelling value can be determined using following equation.

Swelling Index=

$$\frac{\text{Mass of Swollen Microspheres} - \text{Mass of Dry Microspheres}}{\text{Mass of Dried Microspheres}} \times 100$$

h. **Percent of Moisture loss [14]:** The LP loaded microspheres of different polymers were evaluated for percentage moisture loss which gives idea about its hydrophilic nature. The microspheres were weighed initially and kept in desiccators containing calcium chloride at 37^{o}c for 24 hrs. The final weight was noted when there was no further change in the weight of sample.

$$\% \text{ Moisture Loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

3. ***In vitro* drug release studies:** Prepared Microspheres equivalent to 100 mg were accurately weighed and filled into tight in the Muslin cloth. In vitro drug release study was carried out in USP paddle type dissolution test apparatus using phosphate buffer pH 6.8 as dissolution medium, Volume of dissolution medium was 900 ml and bath temperature was maintained at 37±1°C throughout study. Paddle speed was adjusted to 50 rpm. An interval of 1 hour, five ml of sample was withdrawn with replacement of five ml fresh medium and analyzed for losartan potassium content by UV-Visible spectrophotometer at 206.3 nm.

4. ***In vitro* drug release kinetics:** Analysis of drug release from microspheres was performed with a flexible model that can identify the contribution to overall kinetics, mechanism of drug release and the dissolution data obtained for optimized formulation was treated with the different release kinetic equations [14]. In order to study the exact mechanism of the drug release from microspheres, drug release data was analyzed according to Zero Order, First Order, Higuchi square root, Hixon Crowell, Koresmeyer model. The criterion for selecting the most appropriate model was chosen on the basis of goodness to fit test [15, 16].

5. ***In-vitro* mucoadhesion study** [17, 18]: A piece of intestinal mucosa (2x2 cm) was mounted on to glass slide of (3x1 inch) using elastic bands. Weighed microspheres were spread onto wet rinsed tissue specimen and immediately thereafter the slide was hung onto the arm of a USP tablet disintegrating test apparatus. The disintegration machine containing tissue

specimen was adjusted for up and down movement in 6.8 pH Phosphate buffer at 37°C in a beaker. Numbers of microspheres still adhering on to the tissue were weighed at hourly intervals upto 8-10 hrs. The weight of microspheres leached out at different intervals is measured. The % mucoadhesion is calculated by the following equation

$$\% \text{ Mucoadhesion} = \frac{Wa - W1}{Wa} \times 100$$

Where, Wa is the weight of microspheres applied W1 is the weight of microspheres leached out

6. **Compatibility Studies [19]:**

a. **FTIR Studies:** In the preparation of microspheres, the drug and polymer may interact as they are in close contact with each other, which could lead to the instability of preformulation studies. FTIR spectra of pure drug (LP), pure polymers and formulations (Drug loaded microspheres) containing both drug and polymers were performed to study the drug polymer interaction. FTIR study was performed by using Fourier transformed infrared spectrophotometer (Bruker, Vertex 70)

7. **Accelerated stability studies for the optimised formulation [20, 21]:** The purpose of stability testing is to provide evidence on how the quality of drug substance or drug product varies with time under the influence of a variety of environmental factor such as temperature, humidity and light and enables recommended storage condition. Accelerated stability studies of the optimized drug loaded mucoadhesive microspheres were carried out as per ICH guidelines, at 40 ± 2°C /75 ± 5% RH or 25°C ± 2°C /60 % RH ±5 for 60 days. The samples were investigated for particle size analysis, particle size, drug content, drug release, and appearance at regular intervals (0 days, 15 days, 30 days, 45 days, and 60 days).

RESULT AND DISSCUSSION:

Evaluation of Mucoadhesive Microspheres:

1. Micromeritic Properties of Prepared Mucoadhesive Microspheres

TABLE 2: MICROMERITIC PROPERTIES OF KATIRA GUM LOADED MICROSPHERES (MEAN ± S.D., N=3)

Batch No	Bulk density (g/ml)	Tapped density (g/ ml)	Angle of repose (θ)	Carr's Index	Hausner's Ratio
LKM1	0.19±0.001	0.21±1.33	23.56±0.18	10.19±0.04	1.09±0.02
LKM2	0.10±0.001	0.12±0.002	20.32±0.1	12.08±0.02	1.12±0.01
LKM3	0.08±0.004	0.10±0.001	21.00±0.02	14.51±0.04	1.16±0.01
LKM4	0.19±0.007	0.23±0.004	22.53±0.11	17.26±0.1	1.2±0.01
LKM5	0.25±0.002	0.29±0.02	22.33±0.1	12.03±0.2	1.1±0.03
LKM6	0.24±0.002	0.28±0.003	21.62±0.1	13.58±0.16	1.15±0.01

TABLE 3: MICROMERITIC PROPERTIES OF BABUL GUM LOADED MICROSPHERES (MEAN ± S.D., N=3)

Batch No	Bulk density (g/ml)	Tapped density (g/ ml)	Angle of repose (θ)	Carr's Index	Hausner's Ratio
LBM1	1.29±0.004	1.50±0.004	25.89±0.3	14.12±0.19	1.14±0.02
LBM2	0.56±0.004	0.65±0.004	20.25±0.1	14.96±0.02	1.14±0.02
LBM3	1.26±0.002	1.45±0.04	24.3±0.1	13.49±0.2	1.09±0.01
LBM4	1.164±0.01	1.41±0.02	25.41±0.03	19.16±0.04	1.21±0.01
LBM5	0.62±0.001	10.71±0.001	21.04±0.04	12.4±0.14	1.12±0.01
LBM6	0.64±0.002	1.04±0.02	23.21±0.1	18.5±0.14	1.21±0.02

2. Physical Characterization of Prepared Microspheres

Particle Size and Shape:

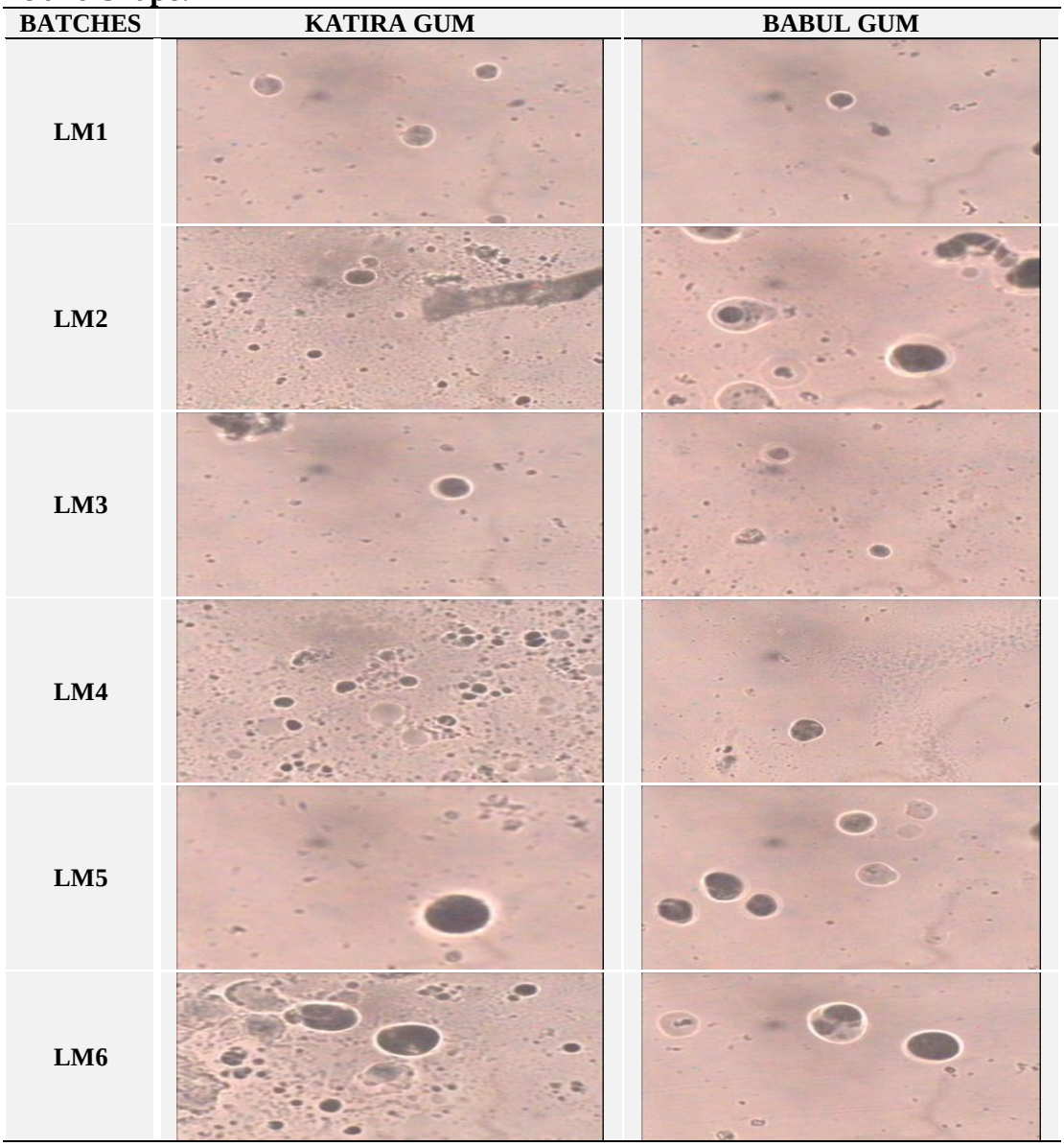

FIGURE 2: PHOTOGRAPHS SHOWING MICROSPHERES PREPARED FROM KATIRA GUM AND BABUL GUM

TABLE 4: PARTICLE SIZE ANALYSIS OF KATIRA GUM LOADED MICROSPHERES (MEAN ± S.D., N=3)

Sr. No.	Batch No	Particle Size in radius	Shape
1.	LKM1	17.07 ± 0.04	Spherical
2.	LKM2	23.45 ± 0.57	Spherical
3.	LKM3	20.31 ± 0.14	Spherical
4.	LKM4	20.30 ± 0.25	Spherical
5.	LKM5	32.33 ± 1.41	Spherical
6.	LKM6	37.21 ± 0.07	Spherical

TABLE 5: PARTICLE SIZE ANALYSIS OF BABUL GUM LOADED MICROSPHERES (MEAN ± S.D., N=3)

Sr. No.	Batch No	Particle Size in radius	Shape
1.	LBM1	19.48 ± 0.45	Spherical
2.	LBM2	13.45 ± 0.29	Spherical
3.	LBM3	16.09 ± 0.11	Irregular
4.	LBM4	15.22 ± 0.24	Spherical
5.	LBM5	20.80 ± 0.14	Oval
6.	LBM6	17.96 ± 0.21	Spherical

Percentage yield, Drug Content and Percent Drug Entrapment:

TABLE 6: PERCENTAGE YIELD, DRUG CONTENT AND PERCENT DRUG ENTRAPMENT OF KATIRA GUM LOADED MICROSPHERES (MEAN ± S.D., N=3)

Sr. No.	Formulation Code	Percent Yield (%)	Drug Content (%)	Entrapment Efficiency (%)
1	LKM1	53.71±0.17	49.74±0.24	80.73±0.12
2	LKM2	42.06±0.091	33.03±0.077	76.01±0.25
3	LKM3	28.28±0.49	66.24±0.021	85.04±0.16
4	LKM4	45.48±0.070	50.22±0.24	63.90±0.10
5	LKM5	42.04±0.58	74.97±0.04	96.26±0.29
6	LKM6	27.83±0.049	60.15±0.07	94.71±0.24

TABLE 7: PERCENTAGE YIELD, DRUG CONTENT AND PERCENT DRUG ENTRAPMENT OF BABUL GUM LOADED MICROSPHERES (MEAN ± S.D., N=3)

Sr. No	Formulation Code	Percent Yield (%)	Drug Content (%)	Entrapment Efficiency (%)
1	LBM1	51.89±0.04	13.98±0.11	40.48±0.11
2	LBM2	70.67±0.70	12.63±0.28	53.08±0.05
3	LBM3	69.27±0.035	25.20±0.014	62.19±0.13
4	LBM4	67.61±0.19	22.24±0.021	68.53±0.24
5	LBM5	45.40±0.007	33.24±0.028	78.37±0.28
6	LBM6	54.4±0.15	30.003±0.084	80.40±0.14

Percent Moisture Loss and Swelling Index:

TABLE 8: PERCENT MOISTURE LOSS AND SWELLING INDEX OF KATIRA GUM LOADED MICROSPHERES (MEAN ± S.D., N=3)

Sr. No.	Formulation Code	% Moisture Loss	Swelling Index (%)
1	LKM1	2.53±0.03	1.35±0.2
2	LKM2	3.00±0.14	1.72±0.24
3	LKM3	2.72±0.02	1.80±0.02
4	LKM4	3.66±0.03	1.71±0.04
5	LKM5	2.15±0.14	1.94±0.02
6	LKM6	2.43±0.02	1.88±0.02

TABLE 9: PERCENT MOISTURE LOSS AND SWELLING INDEX OF BABUL GUM LOADED MUCOADHESIVE MICROSPHERES (MEAN ± S.D., N=3)

Sr. No.	Formulation Code	% Moisture Loss	Swelling Index (%)
1	LBM1	2.96±0.01	0.79±0.02
2	LBM2	3.97±0.02	0.86±0.12
3	LBM3	2.30±0.01	1.01±0.007
4	LBM4	2.81±0.01	0.81±0.03
5	LBM5	2.15±0.02	1.3±0.04
6	LBM6	3.19±0.02	1.02±0.10

In vitro drug release studies:

TABLE 10: *IN VITRO* DRUG RELEASE DATA OF KATIRA GUM LOADED MUCOADHESIVE MICROSPHERES (MEAN ± S.D., N=3)

Time (Hrs)	LKM1 (%)	LKM2 (%)	LKM3 (%)	LKM4 (%)	LKM5 (%)	LKM6 (%)
1	22.79±0.02	23.67±0.04	27.18±0.02	28.35±0.03	27.77±0.04	21.62±0.01
2	24.70±0.01	25.87±0.01	31.40±0.04	28.49±0.02	32.27±0.01	26.45±0.07
3	26.88±0.02	32.09±0.04	34.99±0.02	28.91±0.03	40.49±0.01	32.96±0.02
4	29.03±0.01	32.20±0.02	41.13±0.01	34.22±0.01	45.74±0.03	37.10±0.01
5	30.87±0.04	44.05±0.04	47.49±0.07	36.68±0.2	49.49±0.02	49.49±0.03
6	33.83±0.07	46.08±0.07	50.92±0.04	36.89±0.02	59.18±0.2	59.18±0.02
7	38.17±0.03	50.91±0.02	57.42±0.03	39.87±0.07	67.34±0.01	68.18±0.07
8	39.92±0.02	58.22±0.01	63.29±0.01	45.83±0.04	76.24±0.07	76.24±0.04
9	43.05±0.01	58.44±0.03	68.80±0.1	50.05±0.03	83.08±0.04	81.40±0.01
10	45.86±0.1	63.11±0.02	72.57±0.02	62.00±0.01	94.84±0.03	87.88±0.03
11	46.14±0.07	71.04±0.04	77.68±0.03	66.62±0.02	98.16±0.01	95.39±0.02
12	47.25±0.03	72.00±0.01	81.08±0.04	70.35±0.02	99.78±0.02	97.03±0.01

Pharmacokinetic Profile:

TABLE 11: PHARMACOKINETIC PROFILE OF KATIRA GUM LOADED MICROSPHERES

Batch No.	r^2					n	Best Fit Model
	Zero Order Equation	First Order Equation	Higuchi's Matrix Equation	Korsemeyer Peppas Equation	Hixson-Crowell's Equation		
LKM1	0.6941	0.8481	0.9734	0.9632	0.8058	0.3289	Matrix
LKM2	0.9123	0.9831	0.9874	0.9696	0.9724	0.4953	1st order
LKM3	0.9031	0.9876	0.9918	0.9775	0.9796	0.4760	Matrix
LKM4	0.8680	0.9376	0.9485	0.8817	0.9294	0.3842	Matrix
LKM5	0.9567	0.8303	0.9759	0.9752	0.9487	0.5676	Matrix
LKM6	0.9783	0.9233	0.9662	0.9808	0.9774	0.6768	Peppas

In vitro Mucoadhesion test:

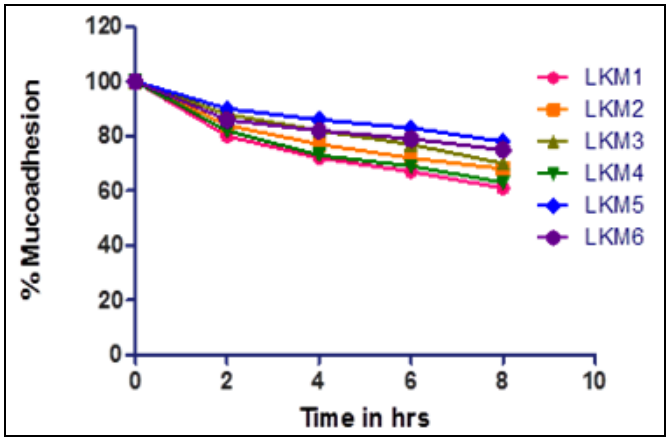

FIG. 4: MUCOADHESION BEHAVIOR OF KATIRA GUM LOADED MUCOADHESIVE MICROSPHERES FORMULATIONS IN pH 6.8

Drug-polymer interaction studies:

FTIR Studies: The FTIR spectrum of Losartan Potassium and Katira Gum alone and the optimized batches of Katira Gum loaded Microspheres were presented in figures:

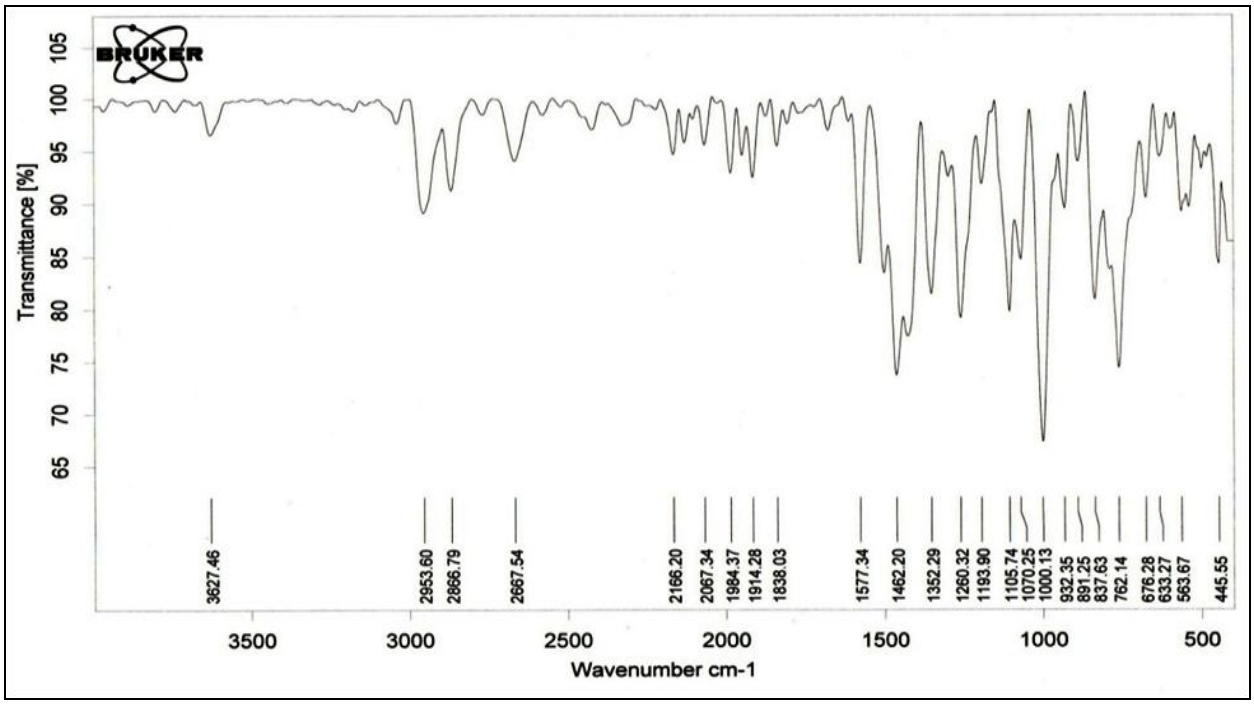

FIGURE 5: INFRARED SPECTRUM OF LOSARTAN POTASSIUM

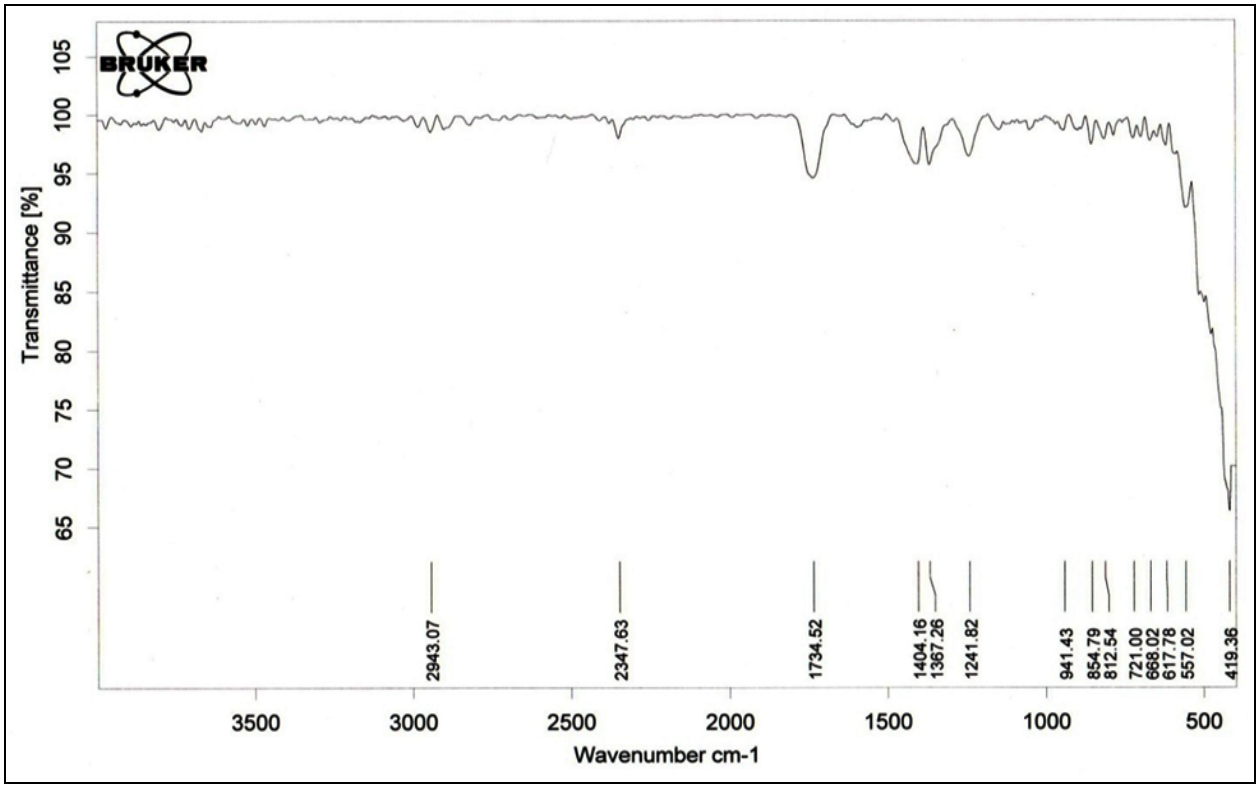

FIGURE 6: INFRARED SPECTRUM OF *COCHOLOSPERMUM RELIGIOSUM* (KATIRA GUM)

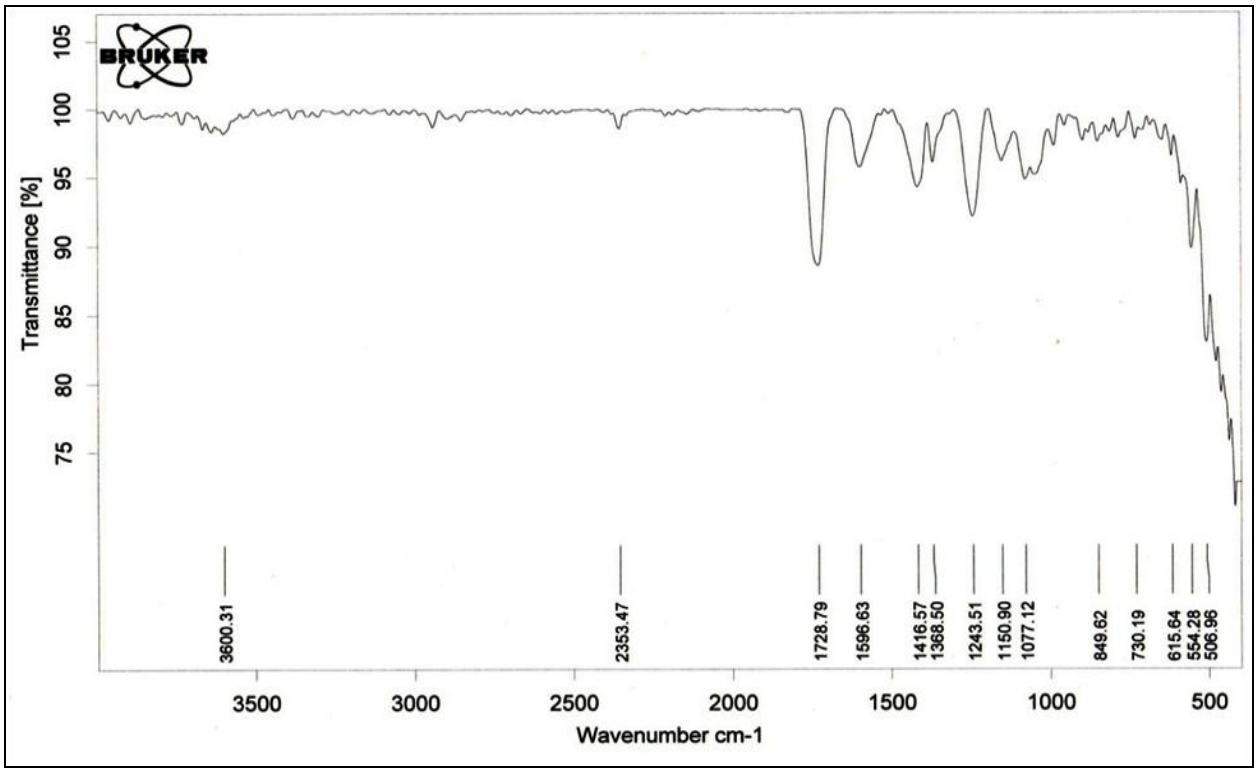

FIGURE 7: INFRARED SPECTRUM OF LKM5 BATCH

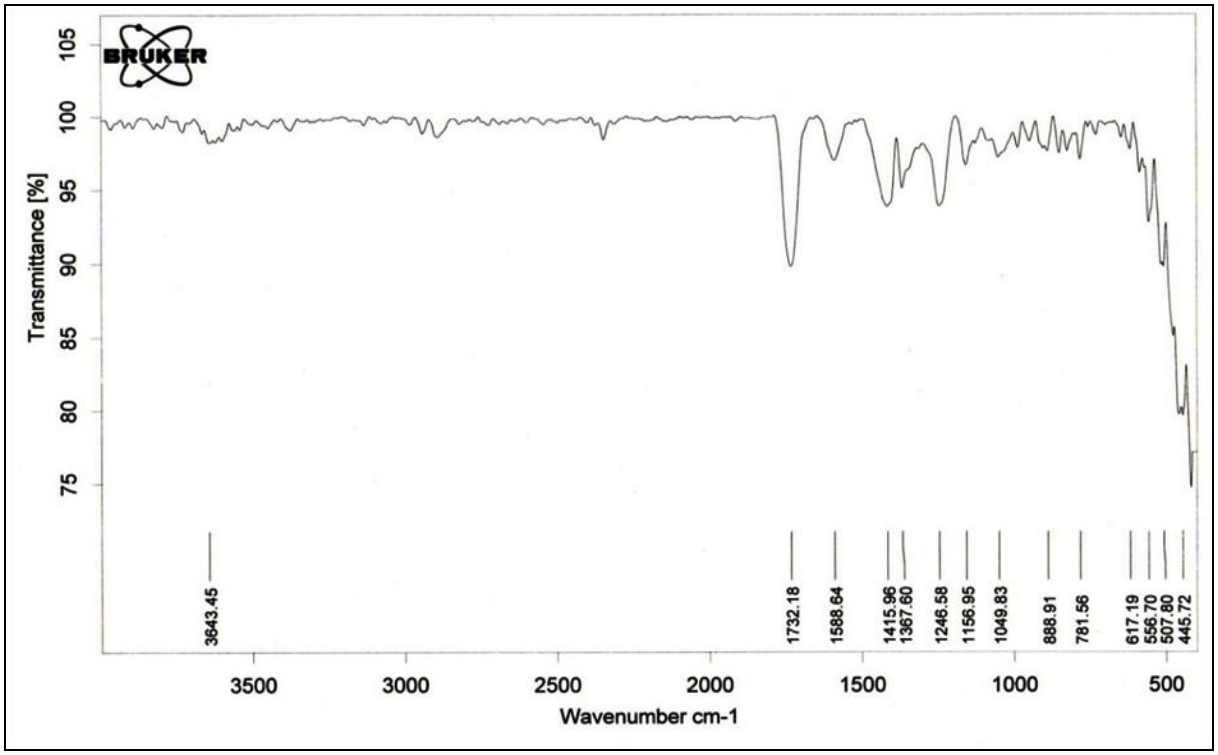

FIGURE 8: INFRARED SPECTRUM OF LKM6 BATCH

Scanning Electron Microscopy:

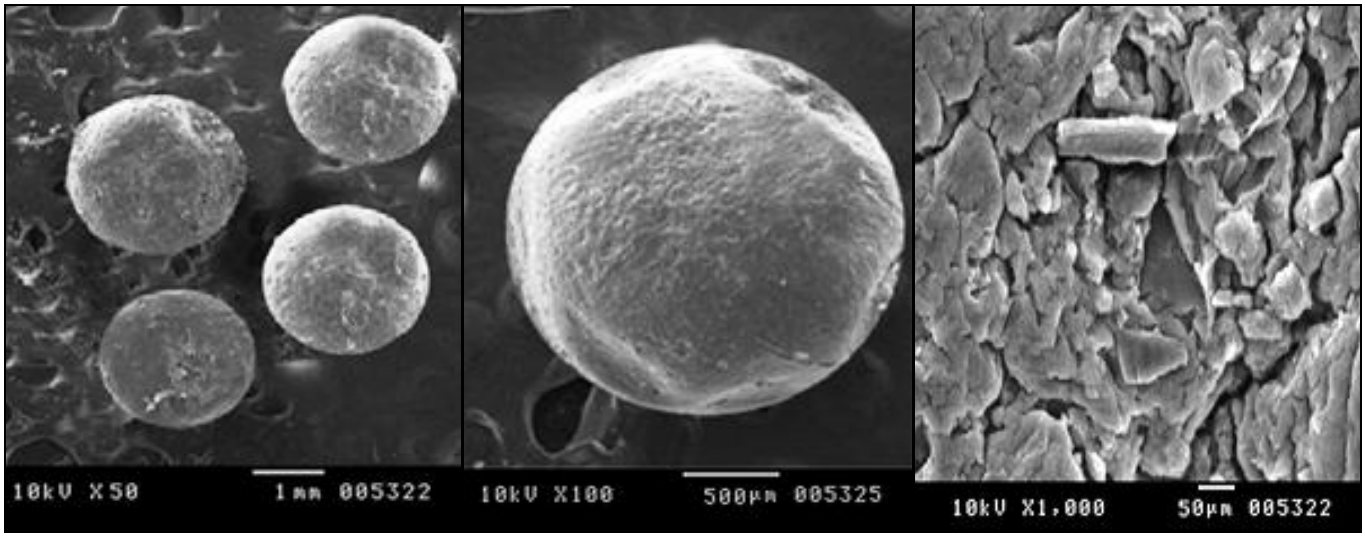

FIGURE 9: SEM PHOTOMICROGRAPH OF LKM5 BATCH

Accelerated Stability Studies:

TABLE 12: PHYSICAL STABILITY CHARACTERISTICS OF OPTIMIZED KATIRA GUM LOADED MUCOADHESIVE MICROSPHERES: (Mean ± S.D; n = 3) Condition: room temp: 40°C, Relative Humidity: 75%

Evaluation parameter	Formulation code	Sampling Intervals in Days				
		0	15	30	45	60
Particle size (µm)	LKM5	20.80 ± 0.1	20.72 ± 0.02	20.56 ±1.2	20.48 ± 0.07	20.42 ± 0.1
	LKM6	17.96 ±0.07	17.76 ± 0.04	17.56 ± 0.02	17.32 ± 0.02	17.27 ± 0.02
Drug content (mg)	LKM5	74.97 ± 0.03	74.76 ± 0.1	74.58 ± 0.1	74.44 ± 0.02	74.39 ± 0.07
	LKM6	60.15 ± 0.2	60.01 ± 0.03	59.83 ± 0.07	59.76 ± 0.07	59.71 ± 0.2
Drug release (%)	LKM5	99.78 ± 0.1	99.64 ± 0.07	99.55 ± 0.2	99.39 ± 0.01	98.26 ± 0.04
	LKM6	97.03 ± 0.05	96.88 ± 0.1	96.76 ± 0.05	96.68 ± 0.2	96.55 ± 0.03

DISCUSSION: The Losartan Potassium (LP) microspheres were prepared by W/O Emulsion solvent evaporation technique by using two different natural polymers i.e. katira gum (KG) and babul gum (BG). Total 12 formulations were prepared. Six formulations were prepared by using each natural polymer i.e. LKM1 to LKM6 using katira gum and LBM1 to LBM6 using babul gum. These formulations were prepared by using common oil phase i.e. Liquid paraffin.

Micromeritic properties: The flow properties of microspheres were evaluated by measuring the bulk density, tapped density, angle of repose, Carr's index and Hausner's ratio.

- Katira Gum loaded Microspheres show excellent flow characteristics with the angle of repose $<25^0$. The Carr's index of the maximum batches was in the range of 10.19±0.04% to 17.26±0.1, which indicate excellent packability of microspheres, whereas the Hausner's ratio of the maximum batches was less than 1.16, which indicate good flow (**Table 2**).

- Babul Gum loaded Microspheres show excellent flow characteristics with the angle of repose $<25^0$ (LBM2, LBM3, LBM5, LBM6) $>25^0$ (LBM1, LBM4). The Carr's index of the maximum batches was in the range of 12.4±0.14 to 19.16±0.04, which indicate good packability of microspheres, whereas the Hausner's ratio of the maximum batches was less than 1.21 indicate good flow (**Table 3**).

Particle size Analysis: The particle size of katira gum loaded microspheres ranged from 17.07 ± 0.04 µm to 37.21 ± 0.07µm, and babul gum loaded microspheres ranged from 13.45 ± 0.29 µm to 20.80 ± 0.14µm. It was noticed that the microspheres prepared from BG have small particle size and they are irregular in shape as compared to KG. KG has a higher particle size and almost all the batches of katira gum loaded microspheres are spherical in shape. Babul gum loaded microspheres have moderate size range and shows spherical, oval and irregular shape of microspheres at different batches (**Table 4, 5**).

Percentage yield: The percentage yield of katira gum loaded microspheres ranged from 28.28± 0.49% to 42.04±0.58%, and babul gum loaded microspheres ranged from 45.40±0.007% to 70.67±0.70%. It was noticed that the Percentage yield of babul gum loaded microspheres has the maximum yield compared to katira gum loaded microspheres (**Table 6,7**).

Drug Content: The % drug content recovered from the katira gum loaded microspheres ranged from 33.03±0.077% to 74.97±0.04% and babul gum loaded microspheres ranged from 12.63±0.28% to 33.24±0.028%. It was noticed that the katira gum loaded microspheres shows maximum drug concentration as compared to microspheres prepared from babul gum (Table 6,7).

Drug Entrapment Efficacy: The drug entrapment efficacy of katira gum loaded microspheres ranged from 63.90±0.16% to 96.26±0.29 and babul gum loaded microspheres ranged from 40.48±0.11% to 80.40±0.14. It was noticed that the percent drug entrapment efficacy of katira gum loaded microspheres showed maximum entrapment of a drug as compared to babul gum loaded microspheres. In case of katira gum loaded microspheres, it is observed that the drug entrapment efficiency is increased as the concentration of katira gum decreased (Table 6, 7).

Percent Moisture loss: The percent moisture loss of katira gum loaded microspheres ranges from 2.15±0.03% to 3.00±0.14% and babul gum loaded microspheres ranges from 2.1±0.02% to 3.97±0.02%. It was noticed that the katira gum loaded microspheres show minimum percent moisture loss as compared to babul gum loaded microspheres. The percent moisture loss was determined for all the formulations prepared from katira gum and babul gum and tabulated in **Table 8 and 9**.

Swelling Index: The swelling index of katira gum loaded microspheres ranges from 1.35±0.2 to 1.94±0.02 and babul gum loaded microspheres ranges from 0.79±0.02 to 1.3±0.04. It was noticed that the katira gum loaded microspheres show maximum swelling index as compared to babul gum loaded microspheres.

The swelling index was determined for all the formulations prepared from katira gum and babul gum and tabulated in Table 8 and 9.

Katira gum loaded mucoadhesive microspheres formulations were optimized over babul Gum loaded microspheres, on the basis of the results obtained from the Physical evaluation of prepared mucoadhesive microspheres. For the preparation of drug loaded mucoadhesive microspheres, the concentration of gum required for micro-encapsulation is only 1-2 % in case of katira gum; whereas the concentration of gum required for microencapsulation in case of babul gum is 6-7 %.

Thus from the above parameters we optimized the Katira Gum loaded Microspheres for further evaluation. So further evaluation parameters were carried out with the Katira Gum loaded Mucoadhesive microspheres.

***In vitro* release studies:** The *in-vitro* drug release studies of katira gum loaded microspheres ranged from 47.25% to 99.78%. The maximum in *vitro* drug release was found to be 99.78% for the formulation LKM5 at 12 hrs. Formulation LKM1, LKM2, LKM3, LKM4 LKM6 showed maximum release of 47.25%, 72.00%, 81.08%, 70.35% and 97.03% respectively at 12^{th} hrs (**Table 10**).

The mechanism of Losartan Potassium release from katira gum loaded microspheres was studied by fitting the data obtained from *in vitro* release studies into zero order, first order, Higuchi's Matrix, and Korsemeyer Peppas and Hixson-Crowell's kinetic models.

The katira gum loaded microspheres formulation LKM1, LKM3, LKM4; LKM5 was good fitted to Higuchi matrix model, indicating the matrix-forming potential of natural polymer and diffusion controlled release mechanism. LKM2 was good fitted to 1^{st} order model and LKM6 was good fitted to Korsemeyer Peppas model. The value of 'n' was found to be between 0.3289 to 0.6768. For LKM5, the release exponent of 'n' was found to be 0.5676 means it follows anomalous (non-Fickian) diffusion, indicating the involvement of more than one drug release mechanism from the formulation and possibly the combination of both diffusion and erosion.

***In vitro* mucoadhesion test:** The number of microspheres adhering to the tissue was calculated after 2hr, 4hr, 6hrs and 8hr. After determination it was found that batch LKM5 showed highest percent 78% mucoadhesion than other batches (**Fig. 4**).

FTIR Studies: FTIR Spectral analysis was performed to study the drug polymer interaction. The FTIR spectra of pure drug (LP), pure polymers, *Cocholospermum religiosum* (Katira Gum) and formulations (LKM5, LKM6) containing both drug and polymers. FTIR studies show no specific interaction between drug and polymer and thus they are compatible with each other (**Fig. 5-8**).

Scanning electron microscopy: SEM is used to study the shape and surface morphology of prepared microspheres. SEM of Katira gum loaded microspheres were discrete, uniform and spherical with a smooth surface (**Fig. 9**).

Accelerated stability studies: Accelerated stability study data of the optimized batches of katira gum loaded microspheres is shown in **Table 12**. At the initial level and at the final level of the accelerated stability study, the tested microspheres showed almost similar particle size, drug content and drug release as observed at the beginning of the study. No color changes or unexpected change were observed. All the optimized batches were found stable after 2 months without much variation in particle size, drug content, drug release.

CONCLUSION:

- Katira Gum possesses all requisite qualities required for sustained drug delivery system in the form of mucoadhesive microspheres.
- Mucoadhesive microspheres of Losartan potassium were successfully formulated by w/o emulsion solvent evaporation technique using Katira gum and Babul gum.
- Katira Gum loaded Mucoadhesive micro-spheres show excellent result as compare to Babul gum loaded microspheres.
- LKM5 Batch, an optimized batch among Katira gum loaded microspheres formulation shows

excellent release rate at 99.78% at 12^{th} hours which shows its ability to sustain the drug for prolonged period of time, which leads to-

- Improve patient's compliance and convenience due to less frequent dosing of drug.
- Maximum utilization of drug enabling reduction in total amount of dose administered.
- Increased safety margin of potent drug.
- Reductions in health care cost through improve therapy, shorter treatment period and less frequency of dosing.

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