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Original Article

Isolation and Functioning Investigation of *Salvia hispanica* Seed Mucilage as a Potential Sustained Release Carrier for Water Soluble Drug

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ABSTRACT

Background: Natural biopolymers or hydrogels that are biodegradable and safe from biological point of view along with their capability to retain active principle within their network thus prolonging the release. The present work was undertaken to explore the potential of mucilage from mature seeds of Chia (*Salvia hispanica*) to sustain the release of water-soluble drugs. Losartan potassium was used as a model to prepare the gelispheres. **Materials and Methods:** The mucilage was isolated and their physicochemical properties like viscosity, phytoconstituent, FTIR, hydration index were studied. Gelispheres loaded with active drug Losartan Potassium (LP) were prepared using sodium alginate, pectin and CSM in various ratios by polyelectrolyte complexation (Ionic Gelation) method. Micrometric properties, surface contour analysis, *ex vivo* mucoadhesion study, *in vitro* drug release study, compatibility, XRD, stability study, statistical analysis was carried out on the prepared gelispheres. **Results:** The isolated mucilage of mature Chia (*Salvia hispanica*) seeds possesses immense properties like viscosity modification capacity, consistency, excellent hydration and mucoadhesive nature that can be explored for developing active principal delivery devices. **Conclusion:** The developed drug loaded gelispheres exhibited good mucoadhesion which enabled sustaining the release of drug for prolonged duration advocating the use of mucilage to design a twice daily losartan potassium sustain release system for management of hypertension.

Keywords: Chia seed mucilage, Gelisphere, Mucoadhesion, Losartan potassium, Ionic gelation.

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INTRODUCTION

Polymers derived from the botanical source have diverse application right from food, cosmetics to pharmaceutical industries.[1,2] Natural biopolymers or hydrogels that are biodegradable and safe from biological point of view along with their capability to retain active principle within their network thus prolonging the release of active constituent, imbibing huge amount of biological fluids or mimicking bio tissues are gaining attention due to wide biomedical applications in recent years.[3] Research involving identification, isolation and utilization of potential carriers either natural or chemically modified polysaccharides for developing controlled, sustained, delayed or other modified drug delivery systems has been accelerated since past few years.[4,5] This acceleration for natural polymeric carriers may be attributed to its sustainability, low cost, abundant availability equated to the harmful effects of chemically developed polymers mostly attributable to residual organic solvents retained in its structure post manufacturing. Among diverse polysaccharides from plant origin the broad-spectrum application of mucilage-based polymers find various applications as pharmaceutical carriers. The physico-chemical and three-dimensional network of these hydrogels can be regulated for designing a modified drug release system. Few problems associated with single unit dosage form like dose dumping, frequency of administration can be overcome by multi-particulate sustain release systems.[6] The multi-particulate carriers or gelispheres can also deliver bioactive molecule for prolonged duration in the entire gastrointestinal region with additional property of mucoadhesion when developed with mucilagenous polymer of natural origin along with enhanced bio availability. Sometimes, hydrophillic swellable polymers of biological origin alone are not capable to sustain the drug release, consequently it becomes necessary to club polymers to achieve desired characteristics of bio-molecule release from the dosage forms. Hence, in order to design a twice daily prolong delivery dosage form of losartan potassium, an amalgamation of polymers is required.

In management of hypertension angiotensin II (type-I) receptor antagonist play a predominate role. Losartan potassium

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belonging to imidazole derivative among various active molecules of this category is employed widely for the above-mentioned purpose.[7] But, this active molecule demonstrates a low bioavailable level (33%) from gastrointestinal tract with 1.5 to 2.5 hr of plasma elimination.[8] Both nature of Losartan potassium i.e., low bioavailability and short biological half-life makes it perfect candidate for formulating in to Sustained Release (SR) formulation. So, the present experimental work focuses to design and develop multi-particulate gelisphere SR formulation of Losartan potassium that can substantiate drug release for an extended duration.

Various methods have been probed for developing the gelisphere sustain release systems to deliver the active principle.[9] Most preferred technique to formulate these particulates system loaded with active ingredient is Ionic gelation technique basically due to its low cost of developing the final product and non-utility of organic solvents as found in other techniques of encapsulation. Therefore, it can be aptly said that mucoadhesive gelispheres dosage form is a novel approach for drug delivery that can deliver active bio-molecule to target area in an optimal amount for the required period of time to provide maximum therapeutic efficacy. The core venture of this research is to design and develop oral sustained release mucoadhesive gelisphere dosage form of losartan potassium by ionic gelation technique using different polymeric blends to evaluate the effect of polymeric system and optimize a suitable polymeric combination so as to obtain a prolong discharge profile for the drug.

MATERIALS AND METHODS

Materials

Mature seeds of Chia (*Salvia hispanica*) were purchased from daily mart of Berhampur, Odisha, India. Isolated CSM (Chia Seed Mucilage) from the seeds were stored in air tight container. Losartan Potassium (LP) was procured from Cipla Limited., Goa, India. While sodium salt form of alginic acid (SA; 3500cps ≈ viscosity) was received as a generous sample and Pectin (high methoxylated, of molecular weight 1.758 x 10^6 in terms of Dalton) was received from CP Kelco, Denmark. Rest all chemicals and solvents used for the work were highly purified grades of analytical reagent.

Methods

Isolation of Chia Seed Mucilage (CSM)

For isolating the mucilage form Chia seeds, the seed sample were first hydrated in distilled water in a ratio of 1:20 with temperature set at 25°C. The contents were agitated for 2 hr on a magnetic stirrer in order to completely hydrate the seeds. On complete swelling of the seeds small aliquots of the sample were centrifuged at 6000 rpm for 1 hr as described by Linda *et al.*,[10] for expressing out the gel like contents. Post centrifugation it was observed that distinct strata existed in the test tube with the upper layer comprising of excess water. This was followed by a thick viscous slurry zone of mucilage that was removed very carefully without disturbing the layer of seeds below. At the extreme bottom of tube exhausted seed were present that was discarded off upon completely separation of the mucilagenous layer. The isolated mucilage was oven dried at 40°C on a glass petri dish for 6 hr. After drying the off-white product obtained was removed from the dish, pulverized, shifted and stored in air tight container.

Determination of properties of Isolated Polymer

Yield Value of the mucilage

The amount of mucilage extracted was calculated based on the formula described below and detail of the same is represented in Table 1a.

$$\text{Yield (in terms of percentage)} = [\text{Mass of final product}/\text{Mass of raw materials taken at first}] \times 100 \quad (1)$$

Characterization based on chemical composition

Determination of presence of various phytoconstituents in CSM by chemical tests

Isolated mucilage of mature Chia (*Salvia hispanica*) seeds was subjected to various test to identify the phytoconstituents present in it as per the standard process described by Gokhale *et al.*,[11] The details of the study is depicted in (Table 1a).

FTIR of isolated Polymer

The IR spectral data of CSM was obtained using infrared spectrophotometer instrument of model Prestige-21 and make Shimadzu FT-IR from Japan the spectral data of which is represented in Table 1b. The pulverized, shifted, dry powder sample of the extracted mucilage was mixed with KBr of IR grade and pellets of same was produced using pellet press designed by Kimaya Engineers of India and examined over the range of 4000-200 cm^{-1}.

Table 1a: Results of tests carried out on isolated CSM for determination of various phytoconstituents.

Phytoconstituent	Test methods	Chia Seed Mucilage (CSM)	CSM Yield %
Carbohydrates	Molish's Test	P	72.85%
Polysaccharides	Iodine Test	P	
Proteins	Millon's test	P	
Mucilage	Ruthenium Red	P	
Tannins	Detection by using Acetic acid	A	
Alkaloids	Mayer's test	A	
Phenolic compounds	Ferric chloride test	P	

'P' represents Presence of the ingrdient, 'A' stands for absence of the ingredient in CSM: Chia seed mucilage isolated.

Table 1b: Infrared Spectral Peak Data.

Spectral Peak value	Functional Group
3422.54	Presence of hydroxyl bond
2928.64	Presence of C-H bond symmetric
2859.77	Presence of C-H bond asymmetric
1656.62	Presence of C=O
1545.00	Presence of C=O, C=C
1153.29	Presence of C-O-C Stretching Vibration

Table 2: Functional attributes of isolated CSM.

Viscosity		Hydration Index
Concentration of CSM (% w/v)	Viscosity in cP	
1%	7.98 ± 1.02	6.34 ± 0.78
2%	17.78 ± 1.06	
3%	21.78 ± 1.32	
4%	26.43 ± 2.04	
5%	30.87 ± 2.18	

Preparation of Gelispheres

Determination of functional attributes of CSM

Viscosity

Various concentrations (1-5%) of CSM gel was prepared and the viscosity was determined at 25°C temperature employing viscometer of Brookfield model designed by Bruker from Berlin, Germany (Brookfield DV-1 Prime). The results are given in Table 2.

Hydration index

For the estimation of hydration index weighed quantity of CSM sample was added to 100 mL of double distilled water and kept undisturbed for 24 hr. Swelled material was cautiously isolated from the water phase and surplus of media removed. The hydration index value of the mucilage was estimated using the mathematical equation as noted below and data is represented in Table 2.

$$\text{Hydration index} = (\text{Mass of the hydrated sample} - \text{Initial mass of dry sample})/\text{Initial mass of dry sample} \quad (2)$$

Gelispheres loaded with active drug Losartan Potassium (LP) were prepared using sodium alginate, pectin and CSM in various ratios by polyelectrolyte complexation (Ionic Gelation) method (Table 3). All three polymeric agents were individually mixed with distilled water and then algino-pectinate slurry was prepared by homogenizing both sodium alginate and pectin system at 3000 rpm for 30 min. Then to the above combination of system CSM solution was added and mixed well for another 30 min at same speed of rotation, care was taken to see that no air was entrapped in the polymeric base solution. To this carrier polymeric blend LP was added and amalgamated for 5 min at speed of 5000 rpm. The active molecule loaded matrix forming solution was extruded out drop by drop using hypodermic needle of 22 G to a 5% solution of barium chloride with uninterrupted stirring for half-an hour for absolute curing of formed gelisphere. Following this the gelispheres were collected and dried over night at room temperature. Prepared gelispheres were stored in air tight glass vessel for succeeding usage.

Table 3: Composition of LP loaded CSM-Algino-Pectinate gelispheres.

Batch code	Polymeric base (% w/w)	Drug (g)
F1	Chia Seed Mucilage (CSM) 1% + Sodium Alginate 0.5% + Pectin 0.5%	1
F2	Chia Seed Mucilage (CSM) 2% + Sodium Alginate 0.5% + Pectin 0.5%	
F3	Chia Seed Mucilage (CSM) 3% + Sodium Alginate 0.5% + Pectin 0.5%	
F4	Chia Seed Mucilage (CSM) 4% + Sodium Alginate 0.5% + Pectin 0.5%	
F5	Chia Seed Mucilage (CSM) 5% + Sodium Alginate 0.5% + Pectin 0.5%	
F6	Chia Seed Mucilage (CSM) 6% + Sodium Alginate 0.5% + Pectin 0.5%	

Table 4: Physico-chemical Characterizations of Losartan Potassium loaded CSM-Algino- Pectinate Gelispheres.

Formulation	Yield (%)	Enmeshment efficiency (%)	Grain size (µm)	Flow Rate (gms/second)
Pure Drug	-	-	-	No flow
F1	72.19 ± 2.12	57.93 ± 2.01	589 ± 10.02	4.56 ± 0.19
F2	79.93 ± 3.76	61.52 ± 1.77	672 ± 13.36	4.78 ± 0.24
F3	83.36 ± 1.80	64.90 ± 2.73	698 ± 12.66	5.68 ± 0.14
F4	87.74 ± 3.61	68.38 ± 3.22	758 ± 14.03	5.87 ± 0.34
F5	91.02 ± 2.61	71.92 ± 1.76	782 ± 9.07	6.98 ± 0.38
F6	88.79 ± 1.87	76.10 ± 2.70	812 ± 7.69	No flow

Mean ± SD, $n = 3$.

Yield of gelispheres

The % of final amount of gelispheres formulated in each lot was estimated based on weight of final product versus weight of raw materials used initially to develop the formulations. The following mathematical equation was used to calculate the % yield and data of same is depicted in Table 4.

$$\%\ \text{yield} = [\text{Wt. of final product}/\text{Wt. of raw materials}] \times 100 \quad (3)$$

Grain size estimation (μm)

The formulated gelisphere was analyzed for the size of grain by sieving method.[12] Each batch of the formulations were segregated in to various size fractions based on weight by shifting it using specified sieves of minimum mesh apertures (sieve no. 12, 14, 16, 18 and 22). Specified sieves were stacked in such a pattern that grittiest particle is present on top mesh and finest at the bottom of setting, post 5 min mechanical shaking, each fraction of the gelispheres held back on each sieve were weighed. The study was carried out in triplicate and mean diameter of the grain was calculated with the help of mathematical equation written below and data of the above study is represented in Table 4.

$$\text{Mean Grain Size} = \frac{\Sigma\,(\text{Mean grain size of the fraction} \times \text{Weight fraction})}{\Sigma\,(\text{Weight Fraction})} \quad (4)$$

Enmeshment Efficiency (%)

Approximately weighed quantity of gelisphere (100 mg) was added to 100mL of pH 6.8 buffer media and agitated for a duration of 4 hr with ultra sonicator. After sonicating the sample, the resultant fluid was passed through Whatman filter paper (0.45 μm). The resultant left over debris was washed for two times using fresh aliquotes of buffer, so that any clinging drug can be infused out. The drug content of the resulting strained out liquid was subjected to spectrophotometric determination at 252 nm (UV-2450, Shimadzu, Japan).[13] The procedure was carried out consecutively for three times and enmeshment efficiency was calculated as per the formula,

$$\text{Enmeshment efficiency} = \frac{\text{Quantity of drug in gelisphere}}{\text{Quantity of drug added initially}} \times 100 \quad (5)$$

The data of the study is represented in Table 4.

Flow rate

The flow rate of the prepared gelispheres was determined by Hall flow principle were around 50g of the formulated particles were allowed to pass through a funnel with geometric dimension of 60° cone angle and 2.5 mm of outlet aperture.[14] The triplicate study data is depicted in Table 4.

$$\text{Flow rate} = \frac{\text{Weight of sample (g)}}{\text{Time in seconds}} \quad (6)$$

Derived property characterization

The derived properties of gelispheres were determined based on various parameters the details of which is depicted in Table 5.[15] The determination of aerodynamic behaviour of the gelisphere was based on determination of angle of repose study done by fixed funnel method. The fluff and consolidated densities were estimated using digital bulk density instrument (Electrolab, India).[16] The data is depicted in Table 5.

Table 5: Micromeritic Study of Losartan Potassium loaded CSM-Algino-Pectinate Gelispheres.

Formulation	Fluffdensity (g/mL)	Consolidated density (g/mL)	Angle of Repose (°)	Carr's index (%)	Hausner's ratio
F1	0.62 ± 0.029	0.77 ± 0.06	21.91 ± 3.38	19.23 ± 1.90	1.24 ± 0.07
F2	0.60 ± 0.091	0.69 ± 0.07	19.03 ± 9.09	18.06 ± 2.57	1.22 ± 0.15
F3	0.55 ± 0.087	0.64 ± 0.09	17.69 ± 1.61	16.92 ± 2.55	1.20 ± 0.17
F4	0.67 ± 0.049	0.78 ± 0.04	15.06 ± 2.36	13.92 ± 2.49	1.16 ± 0.06
F5	0.57 ± 0.029	0.66 ± 0.07	14.22 ± 1.77	13.95 ± 2.90	1.16 ± 0.09
F6	0.55 ± 0.091	0.63 ± 0.17	12.98 ± 2.94	12.91 ± 2.16	1.15 ± 0.10
PD	0.53 ± 0.029	0.96 ± 0.06	43.98 ± 3.21	43.16 ± 2.13	1.76 ± 0.31

Mean ± SD, n = 3, PD: Pure Drug.

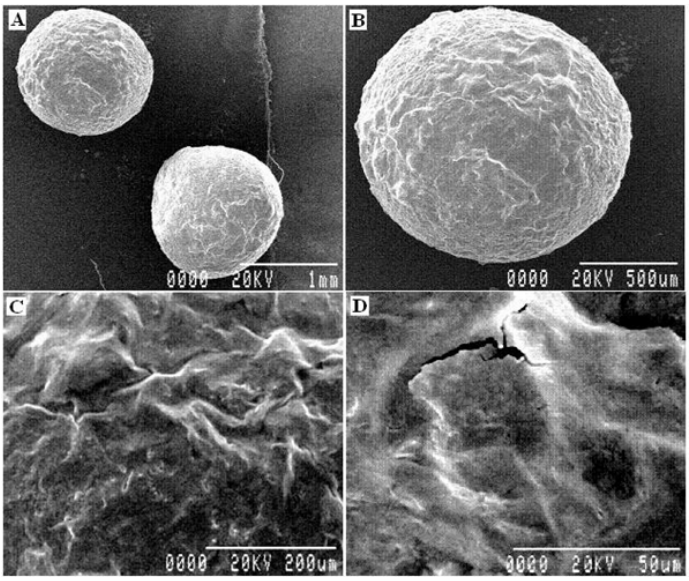

Figure 1: Scanning Electron Micrographs of Optimized CSM-Algino-Pectinate Gelisphere Formulation (F5) (A=30X;B=60X;C=200X;D=1000X).

Surface Contour Analysis

The surface contour of the completely dried gelisphere was determined by scanning electron microscope bearing model number S-530, HITACHI, Japan. The photomicrographs are depicted in (Figure 1).

Performance characterization of the gelispheres *Ex ivo* mucoadhesion study

Ex vivo mucosal adhesion of the fabricated gelispheres was carried out in simulated gastric media as well as simulated enteric media by *in vitro* wash off test[17] using excised fresh intenstinal mucosa collected from local mart slaughter house Figures 2 and 3. The freshly cut goat intestine mucosal tissues section of dimension (2 × 3 cm) was precisely sliced, washed with ringer solution and then fixed on glass slide with help of acrylate glue. On the affixed mucus membrane, 100 nos. of gelisphere from

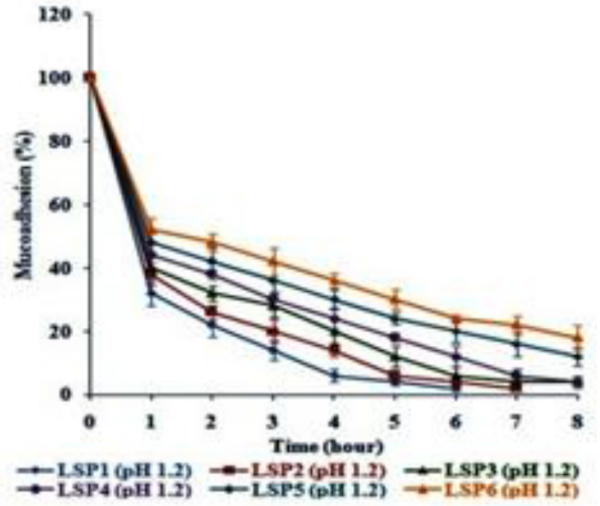

Figure 2: Muadhesion of gelisphere in 0.01 N HCl.

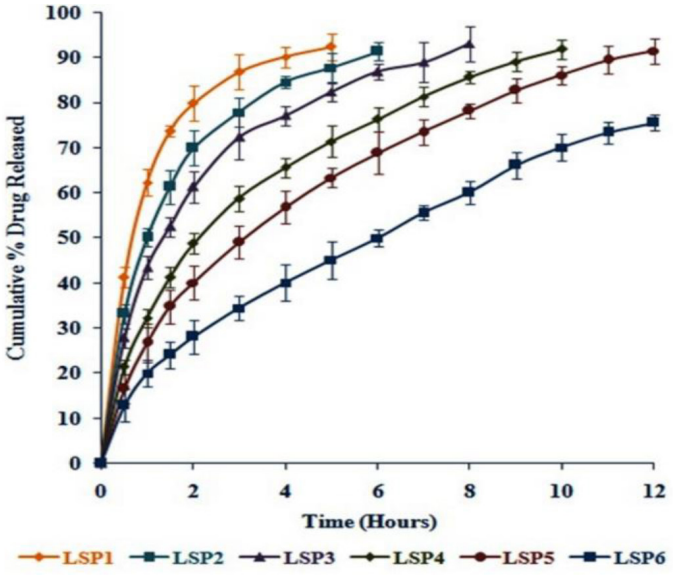

Figure 3: Mucoadhesion of gelisphere in phosphate buffer pH 6.8.

each batch was spread in such a pattern that the edge of the membrane was avoided. Then the slides set up was hanged to the lever of tablet disintegrating test apparatus and dipped in 1L of media successively at 37 ±0.5°C and moved in to and fro direction vertically. At pre-set time gaps, the count of gelisphere still clinging on surface of tissue were determine by counting. Each of the finding was determined in triplicate and % mucoadhesion was computed using the mathematical equation as follows.

$$\% \text{ Mucodhesion} = \frac{\text{No. of gelisphere adhering at time } (tf)}{\text{No. of gelisphere spreaded at time } (to)} \times 100 \quad (7)$$

In vitro drug release study and comparison analysis of various release profiles

The expulsion out of active principle (Losartan Potassium - LP) from the formulated gelispheres was determined at 50 rpm with help of 6 stage dissolution apparatus type II model number DISSO 2000, make LABINDIA, India (IP., 2007) (Figure 4).[18] A quantified amount of gelisphere (equated to 50 mg of pure LP) was exposed in 900 mL media of pH 6.8 simulated enteric media (phosphate buffer)[19] with stirring speed of (50 rpm) and temperature 37 ± 0.5°C. At periodic gaps of time, 5 mL of sample was drawn out, dribbled through Whatman filter paper and examined for drug content using UV-2450 Shimadzu, Japan at 252 nm. Throughout the experiment sink condition was maintained by replacing equivalent number of fresh media and the release studies of LP were recorded in triplicate.

The release mechanism of LP the formulated micro matrix system of gel spheres was evaluated from the cumulative % drug release versus time curves. Mathematical equations as mentioned below were used and the results are given in Table 6.

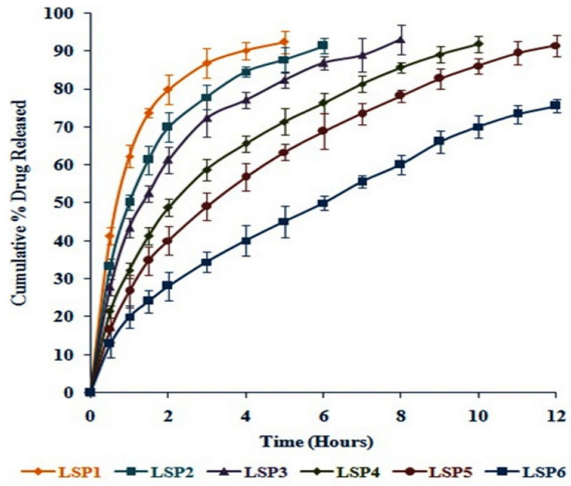

Figure 4: *In vitro* Cumulative % of drug release profile of CSM-Algino-Pectinate Gelispheres (Mean ± SD, *n* = 3).

Table 6: Analysis of Kinetics of drug release (*in vitro*) from Losartan Potassium loaded CSM-Algino-Pectinate Gelispheres*

Model		F1	F2	F3	F4	F5	F6
Zero-order r^2		0.678	0.765	0.80	0.876	0.911	0.959
First-order r^2		0.962	0.971	0.983	0.993	0.994	0.993
Higuchi r^2		0.912	0.953	0.963	0.989	0.992	0.990
Korsmeyer-Peppas	r2	0.915	0.957	0.966	0.987	0.991	0.992
	n	0.34	0.39	0.41	0.47	0.48	0.47
$t_{50\%}$ (hr)		0.72	0.97	1.68	2.26	3.11	6.07

*Analyzed by regression coefficient method, r^2 = Coefficient of determination.

The mathematical equation for Zero-order reaction,

$$Q = k_0 t \quad (8)$$

where Q represents quantity of active principle released at a specific time t and is the constant term for release rate.

The mathematical equation for First-order reaction,

$$\ln (100\ q) = \ln 100 - k_1 t \quad (9)$$

where, k_1 depicts the release rate constant for a first-order reaction.

The mathematical equation for Higuchi's square root model.[20]

$$Q = k_H t^{1/2} \quad (10)$$

where Q represents fraction (%) of active ingredient released in a specific time t, is the constant for release rate in Higuchi's square root equation.

The Korsmeyer-Peppas analysis and its mathematical equation used for determination is[21,22]

$$\frac{M_t}{M_\infty} = kt^n \quad (11)$$

The indication of various quantities in the equation are,

n is defined as release exponent that indicates of the mechanism of active principle's release from the dosage form

$\frac{M_t}{M_\infty}$ = ratio of the incremental release of the drug in time t and ∞

t = time of release of drug

Therefore, $\frac{M_t}{M_\infty}$ is the increment of drug evacuated in time t, major process of drug release while n symbolizes drug release process from the formulations during *in vitro* analysis.

Statistical Analysis

In vitro drug release data was subjected to one-way analysis of variance (one-way ANOVA) followed by Holm-Sidak multiple comparison analysis in order to identify if any substantial difference exists among the formulations. The software used for this study and measure the difference between formulations at confidence level of $p<0.05$ was PRISM software (GraphPad, San Diego, CA).

Stability Performance Characterization of the optimized formulation

Thermal Property Studies

Compatibility of LP with the excipients used for developing the gelispheres was determined using Differential Scanning Calorimetric analysis (DSC) instrument (Diamond DSC 2000, Perkin Elmer, USA). DSC reports for LP, sodium alginate, pectin and CSM mixture and the optimized gelisphere has been represented in Figure 5.

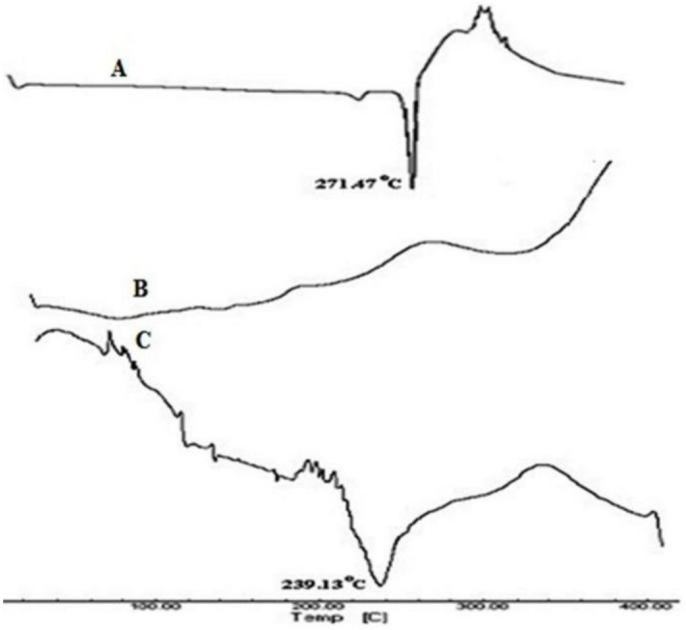

Figure 5: DSC Thermogram of Losartan Potassium (A), Polymeric Mixture (B), Optimised Gelisphere Formulation (C).

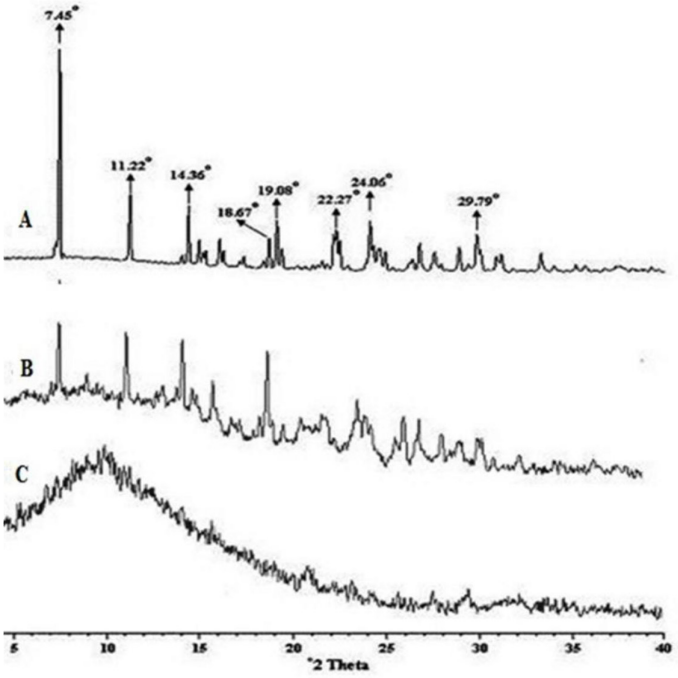

Figure 6: XRD Spectra of Losartan Potassium (A), Polymeric Mixture (B) and Optimised Gelisphere (C).

X-ray Diffraction Analysis

Physical state of Losartan Potassium, physical mixture and optimized formulation were subjected to diffraction analysis by X-ray diffractometer (X'Pert Pro, Panalytical, Netherlands) were a mono-chromatized Copper K α-1 radiation energy (of wave length equal to 1.54 Å) was used with 45 kilo voltage and

Table 7: Stability Study of best formulation of Losartan Potassium loaded CSM-Algino-Pectinate Gelisphere (F5) at Ambient Conditions.

Sampling Interval (days)	Quantity of Drug contained (%)	Physical appearance	Slope	K(days^{-1})	t90% (years)
30	99.58(1.25)	NC	-6.339 $\times10^{-5}$	1.460 $\times10^{-4}$	1.98
60	99.01(2.04)	NC			
90	98.73(1.58)	NC			
120	98.15(2.69)	NC			
150	97.89(1.97)	NC			
180	97.38(2.48)	NC			

NC = No Changes in physical appearance.

40 milli ampere of current passed in to the XRD chamber. The data was obtained in angular series from 5° to 40° (2θ) and scan acceleration of 1° per min. The XRD spectra are depicted in Figure 6.

Stability studies

Based on the ICH guideline for stability analysis of the optimized formulation of LP loaded CSM-algino pectinate gelispheres were put through 180 days of consistency examination under ambient conditions of research lab maintained at 25 ± 2°C and 60 ± 5% RH where the organoleptics features basically outer appearance and active ingredient content were screened for best formulation with 30 days interval. From the data of active principal quantity contained (%), shelf life of the best formulation was calculated the values of which are depicted in Table 7.

RESULTS AND DISCUSSION

Characterization of Isolated Polymer
Yield Value of the mucilage

The % yield of the isolated mucilage of Chia seeds was found to be 72.85%. A minor loss in yield value is observed that may be attributed to the fact that few quantities of the mucilage is lost due to sticking of it to filter paper and also might be due to handling.

Characterization based on chemical composition
Determination of presence of various phytoconstituents in CSM by chemical tests

The isolated mucilagenous substance from *Salvia hispanica* seeds on investigation for phyto constituents through various chemical tests responded positively for presence of carbohydrates, polysaccharides, proteins, mucilage and phenolic compounds whereas negative response to acetic acid and Mayer's test proved absence of tannins and alkaloids respectively (Table 1).

Fourier Transmission Infra Red Analysis of isolated polymer

The functional group determination by spectroscopic analysis using fourier transmission infrared spectrophotometer of the extracted mucilaginous polymer from Chia seeds revealed that the functional groups of O-H (stretched bond), C-H (symmetrically stretched), C-H (asymmetrically stretched), C=O (stretched bond), C=O, C=C (stretched bond) and C-O-C (Stretching vibration bonding) are present in it. Thus, indicating that the mucilage consists of polysaccharides by nature (Table 1b).

Characterization based on functional attributes of CSM
Viscosity Analysis of the segregated mucilaginous gel

The rheological evaluation of the isolated mucilage gel using Brookfield viscometer revealed that there was significant increase in viscosity values of the gel with increased concentration in a gradual pattern Table 2.

Hydration index

The Hydration index of CSM was estimated and found to be 6.34 ± 0.78 using analytical grade distill water Table 2. From the study it was observed that the mucilage had good water uptake property which may be worked upon to develop a Modified Drug Release System (MDRS) as a MDRS needs to uptake dissolution media on contact with it and release the active principal post formation of a gel layer. So, it may be concluded that by using CSM a release retardant system can be developed to sustain the active ingredients expulsion from the developed dosage form.

Characterization of Gelisphere
Physico-chemical Characterization

All the gelispheres of LP were developed using different concentration of CSM- sodium salt of alginic acid and pectin combination using polyelectrolyte complexation (Ionic Gelation) method (Table 3). The purpose behind selection of this method for present work over other methods of developing micro matrix are the simple approach to develop the formulation, easy, cost effectiveness and most importantly non utilization of organic solvents for formulating gelispheres.

Yield (%), Grain size analysis (μm) and Enmeshment efficiency (%)

The percentage yield of all the batches of formulated gelispheres were found to be in the range of 72.19 ± 2.12% to 91.02 ± 2.61% while the grain size analysis revealed that the size of the prepared gelispheres were in the range of 589 ± 10.02μm to 812 ± 7.69μm (Table 4). From the experiment on characterization of physico-chemical aspect of the prepared gelispheres it was observed that the yield value in percentage and mean size of the micro structured particles of all batches of gelispheres increased gradually. This may be ascribed to the reason that at elevated values the polymeric phase contributed enormously to the viscosity enhancement of the medium thus, increasing a greater number of calcium binding sites availability for cross linking thereby forming larger sized particles.[23] The study also revealed

that there was significant melioration in the values of enmeshment efficiency 57.93 ± 2.01% to 76.10 ± 2.70% (Table 4). This may be defined by the fact that with small incremental ascending values of polymeric quantity there is intensification the viscousness of inner phase which traps more amount of active ingredient within its large coacervate thus enhancing the enmeshment of more quantity of drugs.

Flow rate

The flow rate of gelispheres formulation when passed through a funnel revealed a significant improvement in flowability compared to pure drug. This may be described by the fact that while in virgin state the active principle might have absorbed moisture which rendered sticky property that obstructed the flow of pure drug on modifying the physical state of pure drug to a gelisphere there was a drastic change in flow rate it improved from no flow to 4.56 ± 0.19 (F1), 4.78 ± 0.24 (F2), 5.68 ± 0.14 (F3), 5.87 ± 0.34 (F4), 6.98 ± 0.38 (F5). But, in case of F6 formulation no flow was observed which can be attributed to the fact that larger sized particle or course particles block the flow through the defined orifice of the funnel. The data of the study is represented in (Table 4).

Derived property characterization

The micromeritic aspects such as aerodynamic behaviour of the formulated gelisphere in comparision to pure form of drug with respect to flow (Angle of repose), % compressibility (Carr's Index-CI) and ratio of fluff density is to consolidated density (Hausner ratio) of the prepared gelispheres are represented in Table 5. The angle of repose values and CI of pure losartan potassium was found to be 44.39 ± 3.21 degrees and 43.16 ± 2.13% respectively, defines the poor aerodynamic nature of drug whereas in case of prepared gelispheres formulations, the values were found within the order of 13.27 ± 3.25 degrees to 22.14 ± 3.48 degrees and 12.91 ± 2.16% to 19.23 ± 1.90% respectively (Table 5). The micromeritic study attributed that these developed gelispheres help to improve the flow properties and compressibility of pure losartan potassium. The betterment observed in consolidation property was further confirmed by ratio analysis of fluff density is to consolidation density. The cohesivity nature based on Hausner's calculation of the prepared formulations was found to be 1.15 ± 0.10 to 1.24 ± 0.07 whereas in case of pure drug, the value was 1.76 ± 0.31 (Table 5). So, it is confirmed that all the CSM-algino-pectin gelispheres exhibit good aerodynamic property and excellent compactibility as compared to drug in its pure state. So, the gelispheres if intended to be developed further as tablet or capsulized, may require less quantity of anti friction agents and ensure minimal production cost leading to its utilization to go ahead with mercantile production.

Surface Contour Analysis

The topography (SEM study) of the prepared CSM-algino-pectin gelisphere formulation was studied by scanning electron microscope S-530, HITACHI, Japan and the images were captured at various magnification i.e., 30X, 60X, 200X and 1000X (Figure 1). SEM analysis disclosed that the prepared gelisphere had a rigid surface texture with sphericity in appearance. At higher magnification small fissures were also observed on the surface of the dried gelisphere.

Performance characterization of the gelisphere *Ex vivo* mucoadhesion study

Mucoadhesion studies of the prepared CSM- Algino-pectinate gelispheres was done by *ex-vivo* lay-off test using simulated gastric fluid (pH 3) and simulated intestinal fluid (phosphate buffer-pH 6.8) as media. It was observed that the percentage adhesion of the gelispheres was found to be dependent on concentration gradient of the polymer. The observation from the study revealed that all the batches demonstrated better adhesivity in simulated intestinal fluid (phosphate buffer-pH 6.8) compared to gastric fluid (pH 3) indicating that mucoadhesion was pH dependent. All the formulations exhibited 2.28 ± 0.98% to 18.62 ±3.88% adherence to mucus membrane for about 8 hr in simulated gastric fluid (pH 3) (Figure 2) while in case of simulated enteric media (pH 6.8) it was 62.16 ± 4.97% to 88.82 ± 4.69% up to 8 hr (Figure 3). The results indicated that the CSM-Algino-pectinate gelispheres were having practically reduced tendency of adherence to membrane in aqueous acidic solution, whereas in enteric fluid mimicked media (phosphate buffer) its muco adherance property increased along with solubility and hydration capacity significantly this may be explained by the fact that COOH group and other active sites present in polymeric chain ionized effectively in simulated intestinal media. This reaction helps in incremental hold of fluid by the polymer and form a viscous gel structure thereby enhancing mucoadhesion. Therefore, it is suggested from the experiment that by restricting the drug release in stomach the formulations adhered to the mucosal surface of the intestine for a substantial duration of time that may be extend the drug release time before getting exhausted out.[24]

In vitro Drug Release studies

Release of losartan potassium from the prepared CSM-algino-pectinate gelispheres batches and effect of polymer concentration on it was investigated by *in vitro* release behaviour analysis using dissolution apparatus of type II and 900 mL of simulated enteric media (Figure 4). Formulation coded as F1, F2, F3 and F4 were able to hold up the active principle release up to 5, 6, 8 and 10 hr respectively. Formulation F1 showed 92.32 ± 3.04% drug released at 5 hr, while in case of F2 its 91.36 ± 2.07% at 6 hr, for F3 the value is 93.05 ± 3.87% active ingredient released at 8 hr and F4 measured 91.79 ± 2.04% drug release in 10 hr. Subsequently, with gradually raising the amount of polymeric system in batch F5

and F6, prolongation in drug release up to 12 hr was achieved (91.43 ± 2.87% and 75.46 ± 1.76% respectively). The $t_{50\%}$ value of all the formulations were found to be 0.72 hr (F1), 0.97 hr (F2), 1.68 hr (F3), 2.26 hr (F4), 3.11 hr (F5) and 6.07 hr (F6) (Table 6). It was found with gradual rise in polymer concentration of the prepared gelispheres there was substantial reduction in values of drug release. This may be explained by the concept that with gradient rise in the densification parameter of the polymeric network coarser particulate formation takes place which elevates the diffusion path length, that the drug molecules have to traverse. Formulations F1 to F3 exhausted out before complete hydration could take place thus, resulting in early release of active principle. Subsequently raising the concentration of CSM, sustaining effect on drug release was noticed up to 12 hr of time as the polymeric system could develop a denser envelop with divalent Ca^{2+}.[24] Thus, F5 batch being the best formulation can be used for further research studies.

Determination of release Kinetics of drug using mathematical models

Data of the active principal release from dissolution study were assessed with various mathematical models to understand the release kinetics and process of drug release from the prepared gelispheres. Based on values of regression analysis (r^2) and release exponent (n) the active principal expulsion from the gelipsheres best fitted with first order kinetics (r^2 = 0.962, 0.971, 0.983, 0.993, 0.994 and 0.993) model followed by Fickian type diffusion mechanism (n = 0.34, 0.39, 0.41, 0.47, 0.48 and 0.47) (Table 6). Hence, it is concluded that through inspissate network of polymeric phase diffusion mechanism predominately modulated the expulsion of LP from the formulated gelispheres.

Statistical Analysis

The active principles waiver from the gelispheres based on *in vitro* experimentation were subjected to one way analysis of variance accompanied by Holm-Sidak multiple comparison. The evaluation based on ANOVA intimated a substantial difference (at $p<0.05$) among all the CSM-algino-pectinate gelispheres with consideration to their $t_{50\%}$ data. Holm-Sidak multiple comparison analysis hinted that a substantial difference existed among all the formulations. Formulation F5 demonstrated the best dissolution profile where more than 90% drug was expelled out from the gelisphere in 12 hr and all batches of formulation exhibited sustaining properties based on *in vitro* active ingredient release. So, formulation F5 is selected as best formulation for further analysis.

Stability Performance Characterization of the optimized formulation
Thermal Property Studies

The effect of temperature rises and subsequent analysis using differential scanning calorimeter on optimized formulation along with pure drug and polymeric mixture revealed in the obtained thermogram (Figure 5) that LP had a strong and sharp peak at 271.47°C which tallies with the melting point value of Pure LP but, when formulated as gelispheres its value markedly diminished to 239.13°C. This indicated that in the optimized formulation probably LP was converted to amorphous state. The reflection from the study on thermo graphs indicated that the change in form of LP can be confirmed better from XRD studies.

X-ray Diffraction study

The spectra of pure losartan potassium depicted distinctive peaks at 2**θ** values of 7.45°,11.22°, 14.36°, 18.67°, 19.08°, 22.27°, 24.06°, 29.79° etc., indicating the presence of crystalline losartan potassium under XRD diffraction analysis. But in case of the best gelisphere formulation (F5), no crystalline peaks of LP were distinguishable. This result indicates that the active molecule was distributed at the molecular level thus eclipsing the crystalline features of the drug under X-ray diffraction study (Figure 6). So, it is concluded that LP exists in amorphous state in the CSM-algino-pectinate gelisphere formulation.

Stability Analysis

Losartan Potassium loaded optimized gelisphere formulation (F5) was considered for stability analysis under the ambient conditions (25°C / 60% RH) of research lab focusing few parameters like percentage of active principle contained within the gelispheres, shelf life (t_{90}) and organoleptic feature with respect to appearance of the formulation. In laboratory ambient condition, % drug content was found to be 97.38 ± 2.48% within 180 days with no prominent change in physical appearance. It was also ascertained that stable time period of the optimized formulation (F5) with respect to laboratory conditions was found to be 1.98 years (Table 7). So, it can be concluded that LP loaded within the micro matrix system of CSM-alginate-pectin polymeric system can be used commercially to deliver LP for therapeutic benefits over a prolonged duration of time.

CONCLUSION

The isolated mucilage of mature Chia (*Salvia hispanica*) seeds possesses immense properties like viscosity modification capacity, consistency, excellent hydration and mucoadhesive nature that can be explored for developing active principal delivery devices. The developed formulation of LP loaded gelispheres exhibited good adhesion property with mucus membrane which enabled sustaining the release of LP for prolonged duration. Thus, it is concluded from above experimental work that isolated mucilage from Chia seeds may be used as a promising tool to design twice daily losartan potassium sustain release system for management of hypertension.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CSM: Chia seed mucilage; **LP:** Losartan potassium; **XRD:** 'X' ray diffraction.

SUMMARY

Mucilage from mature seeds of Chia (*Salvia hispanica*) was isolated and their physic-chemical properties were studied. Gelispheres containing water soluble drug losartan potassium was prepared following ionic gelation method to sustain the release. Prepared gelispheres were studied for their micrometric properties, surface contour, *ex vivo* mucoadhesion, *in vitro* drug release, compatibility, stability. The developed drug loaded gelispheres exhibited good mucoadhesion enabling the sustained release of drug for prolonged duration.

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