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EXPLOITING NATURAL POLYMER REINFORCED MICROSPHERES AND INVESTIGATING ITS CHARACTERISTICS FOR SUSTAINED RELEASE

Ujjwala Y. Kandekar

Department of Pharmaceutics, PES Modern College of Pharmacy (For Ladies), Moshi, Pune - 412105, Maharashtra, India.

Keywords:

Mucilage, *Colocacia esculenta*, Dexibuprofen, Sodium alginate

Correspondence to Author:
Ujjwala Yadav Kandekar

Assistant Professor,
Department of Pharmaceutics,
PES Modern College of Pharmacy (For Ladies), Moshi, Pune - 412105, Maharashtra, India.

E-mail: ujja2303@gmail.com

ABSTRACT: The objective of current research work is exploring the use of natural mucilage for sustained release characteristics for the formulation of microspheres. Orifice ionic gelation method was used to formulate the microspheres by using complex-forming polymer sodium alginate in combination with isolated mucilage. Mucilage from *Colocacia esculenta* corms were isolated and combined with sodium alginate to fabricate in the form of microspheres. Resultant batches were characterized by differential scanning calorimetry, Fourier transforms infrared spectroscopy, scanning electron microscopy, X-ray diffractometry, swelling capacity, flow properties, particle size, and *in-vitro* dissolution behavior. Isolated mucilage was found to be swellable in water and amorphous in nature. FTIR and DSC study indicates compatibility between drug and selected polymer. All the formulations exhibit better flow properties. Particle size was found in the range of 780-880 micron. The optimized formulation is releasing the drug for the period of 12 h. *Colocacia esculenta* corms mucilage along with sodium alginate can be efficiently utilized to retard the drug release and minimize the side effects of the drug, so as to get maximum utilization of the desired dose.

INTRODUCTION: Recent inclination in pharmaceutical research is to design and develop new formulations, thereby enhancing the therapeutic efficacy of existing drugs. Sustained release technology has rapidly emerged over the past three decades as a new interdisciplinary science that offers novel approaches for the delivery of the drug into systemic circulation at a predetermined rate [1].

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Recently, carrier technology offers a sharp approach for drug delivery by entrapping the drug into a carrier such as microspheres, nanocarriers, liposomes which are responsible to change the release and absorption characteristics of the drug.

Out of above-mentioned examples, microspheres are considered as reliable means to deliver the drug at the desired site along with specificity and are able to maintain the expected therapeutic level of the drug without untoward side effects if modified as per the need [2-5]. These offer effective protection of encapsulated agent against degradation, the possibility of controlled and local delivery of the drug over periods and ease of administration [6-7]. Variety of natural and synthetic polymers are used for the preparation of microspheres [8-12].

Several pharmaceutical excipients from natural resources have been proved to be efficient as thickening agents, disintegrants, gelling agents, binding agents, drug release retardants, *etc*. They exhibit precise advantages such as low cost, natural origin, and improved compatibility, environmental friendly, minimum side effects, greater local availability, *etc*. [13] Current research work is dealing with the formulation of microspheres by isolating mucilage from *Colocacia esculenta* corms and coupling it with sodium alginate.

Microspheres were prepared by orifice ionic gelation technique by using calcium chloride to form the complex of calcium alginate; this complex provides rigidity to the formulation. *Colocacia esculenta* mucilage is a water-swellable heterogeneous polysaccharide containing mannose, arabinose and galactose. It also contains amino acid such as aspartic acid and glutamic acid [14]. Sodium alginate is sodium salt of alginic acid that is a mixture of polyuronic acid mainly consisting of D-mannuronic acid and L- glucoronic acid residues.

Both of these polymers are biodegradable in nature, swell in the water to get viscous colloidal solution with increasing concentration. Physicochemical properties of *Colocacia esculenta* mucilage were studied prior to incorporate it into microspheres.

Dexibuprofen belonging to the category of NSAIDs was chosen as a model drug. It is s- ibuprofen, propionic acid derivative. It acts on (COX-1) and (COX-2) which are responsible to inhibit the prostaglandin synthesis. It is having a half-life of 1.8 to 2 h. This necessitates formulating it into sustained release dosage form for a reduction in dosing frequency and prolonging the drug release. It is having the drawback of gastric irritation when given in conventional dosing units, this problem is minimized by converting into microspheres [15-17].

MATERIALS AND METHODS:

Materials: Dexibuprofen was obtained as a gift sample from Emcure pharmaceuticals. *Colocacia esculenta* corms were purchased from the local market in Pune and authenticated from Agharkar Research Institute Pune. Sodium alginate and calcium chloride were procured from Lobachem Pvt. Ltd. All the other reagents and chemicals used were of analytical grade.

Methods:

Isolation of Mucilage: *Colocacia esculenta* corms were collected from the local market and washed thoroughly with distilled water to remove the contaminant. Firstly 500 grams of corms were soaked in 1000 ml distilled water overnight. The thick solution was separated and boiled for 30 min. The mass was filtered through a muslin cloth.

The retained residues were boiled at 60 ± 1 °C with 500 ml distilled water for 15 min and the combined liquid was passed through eight folds of muslin cloth. The polymer was precipitated from the filtrate by adding acetone. The precipitated mucilage was dried in an oven at 45 °C for 3 h, checked for flow properties and stored in the desiccator for further use [18].

Characterization of Mucilage

Organoleptic Properties of Mucilage: The mucilage was observed visually using watch glass for its texture, appearance, color, and odor.

Determination of Percentage Yield: It was calculated from the ratio between the initial weight and the final weight of the extracted mucilage.

Solubility: Solubility of the mucilage was checked in different solvents such as water, hot water, ethanol, methanol, acetone, chloroform, ether, and DMSO.

Preliminary Phytochemical Screening: Tests for different phytochemical constituents such as carbohydrates, reducing and non-reducing sugars, starch, proteins, tannins, alkaloids, glycosides, steroids, and phenol were carried out.

Physicochemical Analysis:

Determination of pH: The pH was measured by using digital pH meter at room temperature.

Swelling Index: 1 gm mucilage powder was taken in a 25 ml measuring cylinder. 25 ml of distilled water was added and a measuring cylinder was shaken thoroughly every 10 min for 1 h and allowed to stand for 24 h. The volume filled by the mucilage powder was noted. The swelling index (SI) was calculated using the formula given below.

Swelling Index = Final volume – Initial volume / Initial volume

Loss on Drying (LOD): Accurately weighed 1 gm sample was heated at 105 °C to get a constant weight in a hot air oven and percent loss of moisture on drying was calculated using the formula given below:

LOD (%) = Initial volume – Final volume × 100 / Final volume

Ash Value:

Determination of Total Ash Value: Silica crucible weighed and ignited. About 2 gm. of powdered polymer weighed into a crucible. The dish supported on a pipe clay triangle placed on a ring of retort stand. Heated with a burner, till vapors almost cease to be evolved; carbon was burnt off. Cooled, the ash weighed and the percentage of total ash calculated with reference to the air-dried sample of the crude polymer.

Total ash value of the sample = 100 (a-b) × 100 / c

Where,
b = Wt. of empty crucible
c = Wt. of Polymer taken
a = Wt. of Crucible + Ash

Determination of Acid-Soluble Ash Value: Proceed as per the steps mentioned in the procedure for the determination of total ash value. Further-using 25 ml of dil. HCl the ash from the dish used for total ash washed into 100 ml beaker. Wire gauze placed over a bunsen burner and boiled for 5 min. filtered through an ashless filter paper and the residue washed twice with hot water. A crucible ignited in the flame, cooled and weighed. The filter paper and residue placed together into the crucible; heated gently until the vapors cease to be evolved and then more strongly until all carbon has been removed. Cooled, the residue weighed and acid-insoluble ash of polymer calculated with reference to the air-dried sample of a crude polymer.

Acid insoluble ash value of the sample = 100 X x ×100 / y

Where,
x = wt. of the residue;
y = wt. of polymer taken

Determination of Water-Soluble Ash: This was determined in a similar way to acid insoluble ash, using 25 ml of water, instead of dilute hydrochloric acid [19-20].

Micromeritic Properties:

Angle of Repose: A funnel with the end of the stem cut perpendicular to its axis of symmetry was fixed at a height near about 2.5 cm above the graph paper placed on a flat horizontal surface. The powder was cautiously filtered through the funnel till the peak of the pointed heap just touched the tip of the funnel. The radius (r) of the base of the pile and height (h) of the pile was determined. The angle of repose (θ) was calculated using the formula given below:

$$\theta = \tan^{-1} (h/r)$$

Bulk Density: Microspheres were accurately weighed. Weighed quantity of microspheres was poured into a graduated measuring cylinder via funnel and volume occupied by microspheres without tapping was recorded. Bulk density was calculated by using the formula:

Bulk Density (Db) = Mass of Microspheres (M) / Volume of Microspheres (Vb)

Tapped Density: The weighed quantity of microspheres was poured in a graduated measuring cylinder. The measuring cylinder was placed on mechanical tapper to give 100 taps. The volume occupied by microspheres after tapping was recorded. Tapped density was calculated by the formula:

Tapped density (Dt) = Mass of the Microspheres (M) / Tapped volume of Microspheres (Vt)

Carr's Index: Compressibility index or Carr's index value computed according to the following equation:

Carr's index = Tapped density – Bulk Density × 100 / Tapped Density

Hausner's ratio: Hausner's ratio was calculated using the formula given below [21].

Hausner's ratio = Tapped density / Bulk density

Determination of Viscosity: Viscosity of aqueous solution was measured by Brookfield's Viscometer (Model-LV DV II + Pro) using spindle number 64 at 37 ± 1 °C at different speeds of at 2, 4, 10, 20, 30, 50, 60 and 100 rpm. Stability of paste viscosity at 20 rpm was observed after 1, 2, 3, 4, 5, 10, 15, 20 and 30 min [22].

X-ray Diffraction Studies: The physical state of drug, the polymer was assessed by performing XRD studies. Powder X-ray diffraction (PXRD) patterns were traced by using the X-ray diffractometer D_8 advanced model of BrukerAxs Company fitted with a copper target, a voltage of 40 kV, and a current of 30 mA. The scanning rate was 1^0/min over a 2θ range of 5°-50° [23].

FT-IR Spectroscopy: FT-IR spectroscopy was carried out to check the compatibility between the drug and the polymer. A physical mixture of drug and polymers was prepared and mixed with anhydrous potassium bromide. This mixture was scanned over a wavenumber region from 4000 to 400 cm^{-1} in an FTIR spectrophotometer (Shimadzu, Japan) [24].

Differential Scanning Calorimetry: Differential scanning calorimeter equipped with a computer analyzer (JAPE DSC, Perkin Elmer, USA) was used for scanning. Each sample weighing 1 mg was placed into standard aluminum pans and sealed, the bare aluminum pan was used as a reference, Scan was carried out at a heating rate of 10 °C/min under a nitrogen atmosphere over a temperature range of 30 °C-300 °C [25].

Formulation of Microspheres: Sodium Alginate solutions were prepared in various concentrations with the addition of distilled water and soak for 30 min. Various concentrations of isolated mucilage were soaked in distilled water overnight and triturated to get smooth slurry, this mass is added in the sodium alginate solution. The drug is added in this solution and triturated thoroughly. Initial screening was done to formulate the batches in different ratios of Sodium alginate and isolated mucilage. Different batches were prepared as given in **Table 1**. The central composite design was used to formulate microspheres by varying the concentration of sodium alginate and mucilage.

TABLE 1: FORMULATION OF VARIOUS BATCHES

Formulations	Drug (gm.)	Sodium Alginate (%)	Mucilage (%)
F1	1	4	1.5
F2	1	4	2.21
F3	1	3	1
F4	1	5.41	1.5
F5	1	5	2
F6	1	5	1
F7	1	2.59	1.50
F8	1	4	0.79
F9	1	3	2

Evaluation of Microspheres:

Surface Morphology: The morphology details of microspheres were analyzed by using Scanning electron microscopy (SEM). The microspheres were secure on support with carbon-glue and coated with gold using the gold sputter module in the high vacuum evaporator. Samples were then seen with the Scanning electron microscope (JEOL JSM- 6360A scanning microscope, Tokyo, Japan) at 10 kV.

Percentage Yield: Dried Microspheres were accurately weighed. The percentage yield was calculated as per the formula:

Percentage yield = (Practical yield / Theoretical yield) × 100

Where,
Practical yield – the weight of dried microspheres
Theoretical yield – the weight of drug + weight of the polymer

Particle Size: Particle size examination of drug-containing microspheres was analyzed by optical microscopy using a compound microscope. 10 ml of distilled water was taken and a small quantity of microspheres was added into it. Form this suspension few drops were added on a clean glass slide. The slide was mounted on the stage of the microscope and the diameter of at least 100 particles was measured using a calibrated ocular micrometer.

Percent Entrapment Efficiency: 10 mg of microspheres were exactly weighed, crushed by using mortar and pestle, and then moved to a conical flask containing 100 ml of phosphate buffer (pH 6.8). The flask was placed on a rotary shaker at 100 rpm for 24 h. After 24 h, a 1 ml sample was withdrawn and volume was made up to 10 ml by phosphate buffer (pH 6.8). Absorbance was taken by using UV spectrophotometer at 221 nm [26]. Then percent entrapment efficiency was calculated by the formula:

% Entrapment efficiency = (Practical content / Theoretical content) × 100

Swelling Study: 500 mg of dried microspheres were accurately weighed and placed in a petri plate containing 5ml of phosphate buffer (pH 6.8). The swelling was allowed to occur at room temperature for 8 h. After 8 h, microspheres were removed from

medium and blotted with filter paper to remove adsorbed solvent on the surface and weighed immediately [27].

Percent degree of swelling was calculated by using the formula:

% Degree of swelling = (Weight of swollen Microspheres - Initial weight / Initial weight) × 100

Flow Properties: Flow properties like Bulk density, Tapped density, Angle of Repose, Carr's index, Hausner's ratio were determined as explained in the previous section.

***In-vitro* Drug Release Studies:** *In-vitro* drug release studies were carried out for all formulations by using USP type II dissolution test apparatus (paddle apparatus) occupied with 900 ml of 0.1 N hydrochloric acid followed by phosphate buffer pH 6.8 and weighed quantity of microspheres were filled into capsules and coated with eudragit L100 and added to dissolution medium. The study was continued for 12 h. At the interval of each hour, 5 ml of dissolution media was withdrawn and an equal volume of dissolution medium was replaced to maintain sink condition and solution was analyzed for the drug content spectrophotometrically at 221 nm against a blank. All the determinations were made in triplicate [28].

Stability Studies: According to ICH guidelines, an accelerated stability study has to be carried out at 40 ± 2 °C / 75 ± 5% RH for 6 months. The formulation testing was carried out at an interval of 0, 3 and 6 months. Percent entrapment efficiency, Particle size, and *in-vitro* release studies were carried out as per ICH guidelines.

Tested parameters were compared with formulations before subjecting to stability study [29].

RESULTS AND DISCUSSION: Mucilage is isolated from *Colocacia esculenta* corms, percentage yield was found 9.5% w/w. Obtained mucilage is off white in color with an irregular shape, free from odor and sticky in taste, rough and hard in nature. The solubility of the mucilage was checked in different solvents, it indicates that isolated mucilage is swellable in hot water and forms viscous colloidal solution quickly as compared to cold water, where it takes overnight to form viscous colloidal solution.

Preliminary Phytochemical Screening of Isolated Mucilage: For the determination of chemical constituents, different phytochemical tests were carried out, which indicated the presence of carbohydrates, proteins, and mucilage. Starch, alkaloids, glycosides, steroids, tannins and phenolic compounds were found to be absent. This data indicates that isolated mucilage is free from other phytochemicals which may interfere with the formulation. It can be also concluded that obtained mass is lacking any pharmacologically active component, which will be responsible for any pharmacological effect.

Physicochemical Analysis: Physicochemical properties like pH, swelling index, LOD, total ash value, acid insoluble ash, and water-soluble ash and micrometric properties are shown in **Table 2**.

TABLE 2: PHYSICOCHEMICAL ANALYSIS OF ISOLATED MUCILAGE

S. no.	Test	Result
1	pH	6.7 ± 0.01
2	Swelling index (ml)	10.2 ± 1.27
3	Loss on drying (%)	5 ± 1.76
4	Total ash value (%)	6.6 ± 1.56
	Acid insoluble ash (%)	0.06 ± 2.87
	Water soluble ash (%)	0.08 ± 2.54
5	Bulk density (gm./ml)	0.65 ± 0.37
	Tapped density (gm./ml)	0.9 ± 0.22
	Angle of repose (degree)	28.36 ± 0.26
	Carr's index	11.71 ± 0.34
	Hausner's ratio	1.38 ± 0.47

*All the reading were taken in triplicate (n=3)

a pH of mucilage was found to be neutral. It indicates noteworthy flow properties which may be necessary for large scale processing. These could be further improved by adding glidant. The ash values are at a low level; suggest that mucilage was free from contamination during processing and handling [30].

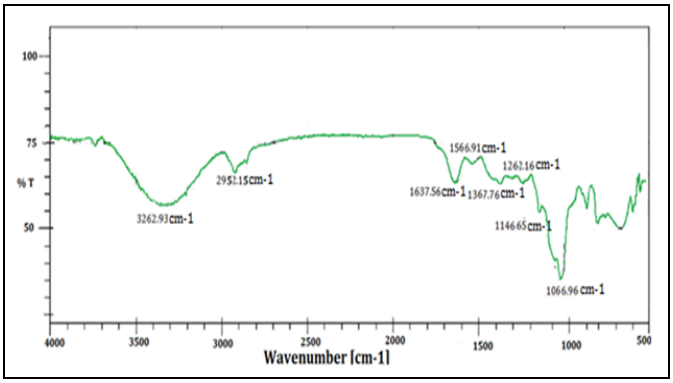

FIG. 1: VARIOUS PEAKS OBSERVED WERE SHOWN IN ABOVE SPECTRA

Infrared Spectra of Isolated Mucilage: FTIR spectra were carried out to find out characteristic functional groups present in the mucilage. Isolated polymers were scanned in the range of 4000-400 cm^{-1} **Fig. 1** shows the infrared spectra.

Characteristic functional groups were observed which represents the polysaccharide groups [31].

TABLE 3: FTIR DATA OF MUCILAGE

Frequency (cm^{-1})	Functional Group
3262.93	O-H stretch or NH stretch
2952.15	C-H stretch
1637.76, 1566.91	C-C stretch
1367.76, 1262.16	C-H bending
1146.96, 1066.96	C-O stretch, C-N stretch

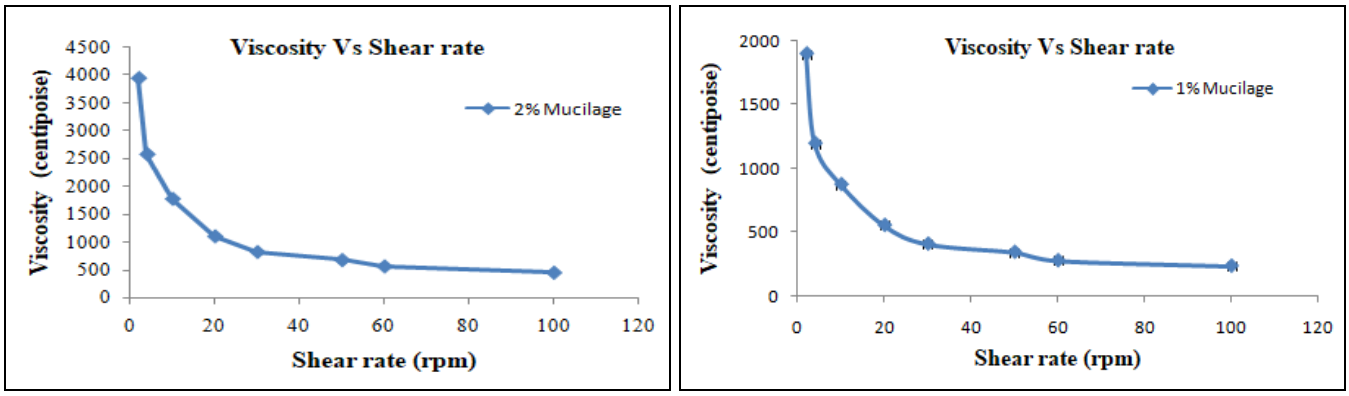

FIG. 2: VISCOSITY OF MUCILAGE AT 2% w/v AND 1% w/v

Determination of Viscosity: Viscosity measurements were necessary to study the effect of the increasing shear rate on the viscosity of the mucilage. During large scale production, this parameter plays an important role while handling the batches. Briefly, mucilage (1 and 2% w/v on dry weight basis) was cooked in a boiling bath for 15 min. and then cooled to room temperature. Viscosity was determined using spindle no. 64 at 2, 4, 10, 20, 30, 50, 60 and 100 rpm.

The above data states that there was an inverse relationship between the viscosity and shear rate. At less concentration, it follows the non-newtonian flow and as the concentration increases it shows shear thinning and followed pseudoplastic flow along with increasing the apparent viscosity [32, 33]. The reason for non-newtonian flow was the interruption in the particle aggregate mass and orientation of asymmetric particles caused by a gradual increase in the shear rate.

Powder X-Ray Diffraction Studies: P-XRD studies were carried out to determine the physical nature of the mucilage weather it was a crystalline or amorphous state that conveys the organization of the atom or molecules within the mucilage. If the atoms were structured in an orderly manner with the replication distance and rays were accustomed to similar magnitude, a precise interference pattern was produced that will give the typical geometry of the substance under observation. P-XRD pattern of the mucilage obtained as shown in the following **Fig. 3**.

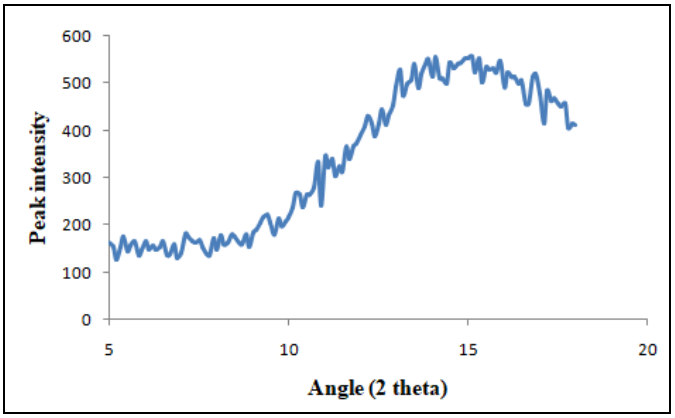

FIG. 3: X-RAY DIFFRACTION PATTERN OF MUCILAGE

Evaluation of Microspheres: Prepared formulations were evaluated for different parameters as mentioned below.

Percentage Yield: Almost all the formulation shows a comparable yield. The percentage yield is shown in **Table 4**. Various processing conditions such as transfer of mass for the trituration, filling the mass into syringe influences the yield of the product. It can be concluded that the yield of the different batches was comparable to that of pure sodium alginate.

TABLE 4: PERCENT YIELD, ENTRAPMENT EFFICIENCY, BULK DENSITY, TAPPED DENSITY, CARR'S COMPRESSIBILITY INDEX AND HAUSNER'S RATIO

Sodium alginate (% w/w)	% Yield	% E. E.	B.D. (g/ml)	T. D. (g/ml)	% C.C.	% H.R.	Angle of Repose	Particle size (µ)	% Swelling
2.6	86.75	80.65	0.55	0.6	08.33	1.09	20.63	760.4	50.31
	±1.43	±2.51	±1.12	±0.15	±0.04	±0.06	±2.93	±2.32	±4.65
3	88.65	81.24	0.52	0.59	11.86	1.13	22.65	755.1	54.73
	±1.30	±3.21	±0.45	±0.07	±0.9	±0.54	±1.5	±4.58	±2.58
4	87.45	81.86	0.59	0.64	07.81	1.08	20.56	735.8	60.75
	±1.11	±2.87	±0.32	±0.04	±1.07	±0.13	±2.31	±3.47	±4.77
5	85.87	82.34	0.61	0.68	10.29	1.11	23.71	720.6	73.60
	±0.87	±3.11	±1.04	±0.78	±1.02	±0.11	±1.54	±2.76	±2.22
5.41	84.32	82.78	0.54	0.65	16.82	1.20	23.28	715.3	75.58
	±1.25	±1.86	±0.87	±0.08	±0.06	±0.03	±1.80	±3.21	±3.81

Batch no.	% Yield	% E. E.	B.D. (g/ml)	T. D. (g/ml)	% C.C.	% H.R.	Angle of Repose	Particle size (µ)	% Swelling
F1	84.78	87.88	0.50	0.63	20.63	1.16	25.1	810.5	65.32
	± 1.31	±2.34	±0.12	±0.11	±0.01	±0.03	±1.51	±5.04	±5.45
F2	84.32	86.88	0.55	0.62	11.28	1.12	26.5	845.7	70.78
	± 0.88	±3.18	±0.02	±0.23	± 0.85	±0.80	±0.13	±2.61	±2.11
F3	85.12	88.38	0.54	0.61	11.47	1.12	28.3	856.3	74.32
	± 1.23	±2.78	±0.01	±0.02	± 1.04	±0.02	±0.10	±3.06	±5.17
F4	84.76	89.47	0.54	0.64	15.62	1.18	25.7	830.8	68.02
	± 1.75	±1.87	±0.11	±0.01	± 0.84	±0.02	±0.11	±3.25	±4.18
F5	84.81	88.84	0.44	0.51	13.72	1.15	27.8	880.2	77.65
	± 1.08	±3.22	±0.04	±0.03	± 1.21	±0.02	±0.17	±0.43	±2.43
F6	85.07	81.21	0.68	0.76	10.52	1.11	26.8	880.8	78.81
	± 2.05	±2.76	±0.10	±0.01	± 0.78	±0.32	±0.07	±2.07	±2.05
F7	86.45	80.63	0.61	0.71	14.08	1.16	28.7	885.1	83.67
	± 1.88	±1.43	±0.06	±0.02	± 1.02	±0.08	±0.75	±2.83	±2.06
F8	84.23	82.07	0.48	0.58	15.51	1.18	26.3	880.4	86.43
	± 2.12	±3.51	±0.80	±0.02	± 1.02	±0.05	±0.08	±2.12	±5.38
F9	83.80	86.70	0.52	0.62	16.12	1.18	25.2	780.7	62.76
	± 1.56	±4.13	±0.07	±0.02	± 1.17	±0.01	±0.15	±3.14	±4.83

*All the reading were taken in triplicate (n=3)

***In-vitro* Drug Release Study:** Large bolus of the drug was initially released prior to attaining the stable release profile known as burst release. It was unpredictable, and the quantity of the drug release could not be efficiently accomplished. Though it was accompanied by certain advantages such as more drug release at preliminary stages helps to attain the maintenance dose early, burst release had a key advantage in the pulsatile release.

The burst release might be due to surface adsorption of the drug, porous nature of dosage forms, processing conditions, and sample geometry. During the formulation of microspheres, the burst release might be due to trapping of the drug at the surface of the polymer matrix while the formation of droplets [35].

Data obtained for the *in-vitro* study was fitted in various kinetic models such as zero order, first order, Higuchi and Korsmeyer Peppas in order to determine the kinetics of the drug release. The release rate constant (k) value and coefficient of regression (r^2), as well as the slope (n) were computed. Data indicates that drug release follows Higuchi- matrix model. n-value for sphere ranges from 0.45 to 0.85 indicated both diffusion and swelling controlled drug release (anomalous transport). Values less than 0.45 indicates fickian diffusion and values more than 0.85 indicates polymer erosion. All the formulations follow Higuchi matrix-type drug release.

Drug release from plain sodium alginate microspheres was studied; various concentrations were used to study these parameters as 5.41, 5, 4, 3 and 2.6 based on the preliminary trials. Cumulative percent drug release from plain sodium alginate batches is shown in **Fig. 4** and formulations are shown in **Fig. 5**.

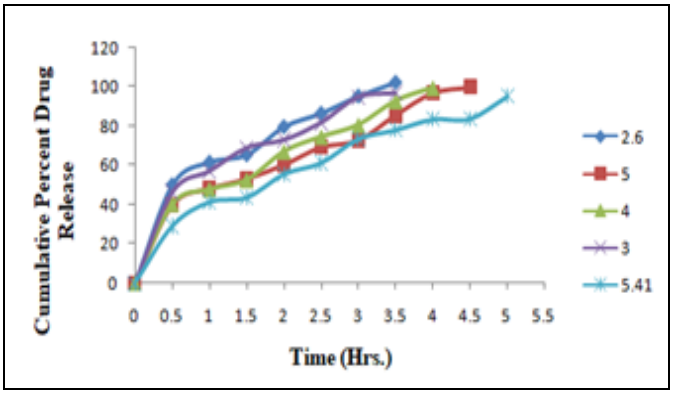

FIG. 4: CUMULATIVE PERCENT DRUG RELEASE FROM SODIUM ALGINATE MICROSPHERES

Drug release ranges from 100.21 to 95.39 percent from lowest to highest concentration for the period of 3.5 to 5 h.

Drug Release from Microspheres Containing F1-F9 Batches: Mucilage and sodium alginate forms a viscous jelly layer through which the release of the drug was delayed up to 8 h. It was observed that formulation F1 shows a 99.47% drug release. Drug release from F7 sustains for 6 h as a concentration of mucilage and sodium alginate was low when compared to other batches.

Formulation F5 shows a 92.15% drug release at the end of 8 h as it contains the highest amount of mucilage and sodium alginate, it does not release the optimum quantity of the drug at the end of 8 h.

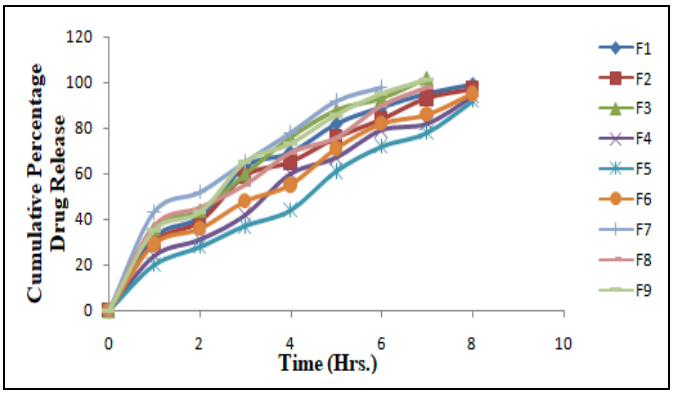

FIG. 5: CUMULATIVE PERCENT DRUG RELEASE FROM COMBINATION OF SODIUM ALGINATE AND MUCILAGE MICROSPHERES. *All the readings were taken in triplicate (n=3)

Statistical Evaluation: Statistical evaluation was carried out by design expert software. ®Results were evaluated by ANOVA test. The summary of the ANOVA is shown in **Table 5**.

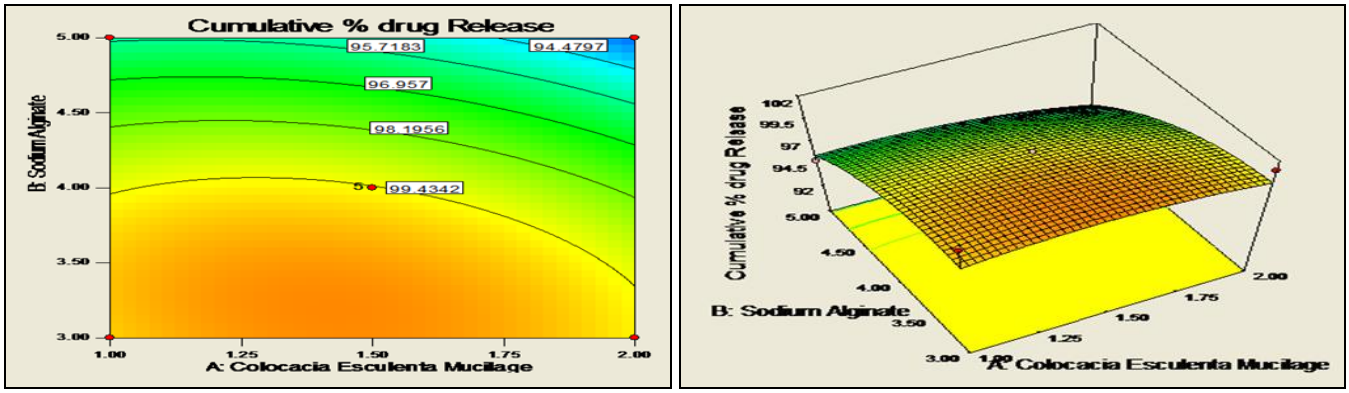

FIG. 6: CONTOUR AND 3D PLOT FOR CUMULATIVE % PERCENT RELEASE OF VARIOUS MICROSPHERES

TABLE 5: ANOVA RESULTS

Source	SS	DF	MS	F	p-value Prob > F	MS
Model	82.46	5	16.49	7.53	0.0097	Significant
A- *Colocacia esculenta* mucilage	3.66	1	3.66	1.67	0.2369	
B- sodium alginate	58.63	1	58.63	26.76	0.0013	
AB	1	1	1	0.46	0.5209	
A^2	4.42	1	4.42	2.02	0.1982	
B^2	16.66	1	16.66	7.61	0.0282	
Residual Lack of Fit	15.33	3				
Pure Error	0.000	4	0.000			
Cor Total	97.80	12				

The Model F-value of 7.53 indicates the model was noteworthy

Final Equation in Terms of Actual Factor's:

Cumulative % drug Release = +99.47-0.68 * A -2.71 * B -0.50 * A * B-0.80 * A2 -1.55 * B2

The equation obtained shows that as the amount of sodium alginate and mucilage rises there was reduction drug release. From these results, an optimized batch was finalized by design expert software by taking into account the rate of drug release. 3.70% of sodium alginate and 1% of mucilage were selected as the best concentration that will release the drug within 12 h.

Scanning Electron Microscopy: To analyze the particle morphology and surface characteristics SEM study was carried out. SEM images of sodium alginate microspheres show perfect spherical shape. As shown in **Fig. 7** and **8** surface images were taken at different resolutions, mainly 50X, 400X and 1000X. Surface morphology was studied; particles are spherical in shape and have a smooth surface.

SEM Images of Optimized Formulation: SEM of the optimized batch was carried out at 85X, 200X and 3000X, it was found that particles were near about spherical in shape and having rough surface [35].

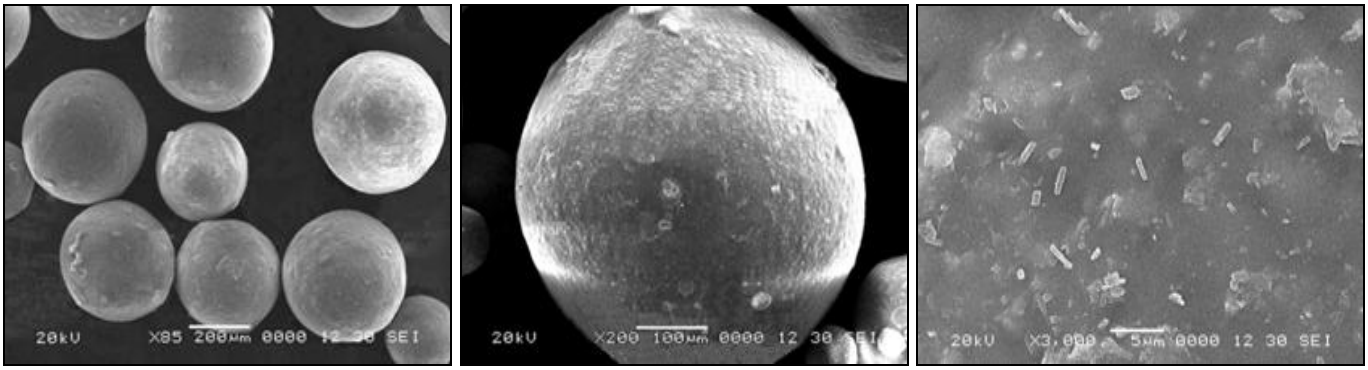

FIG. 7: SEM IMAGES OF SODIUM ALGINATE MICROSPHERES

SEM Images of Optimized Batch:

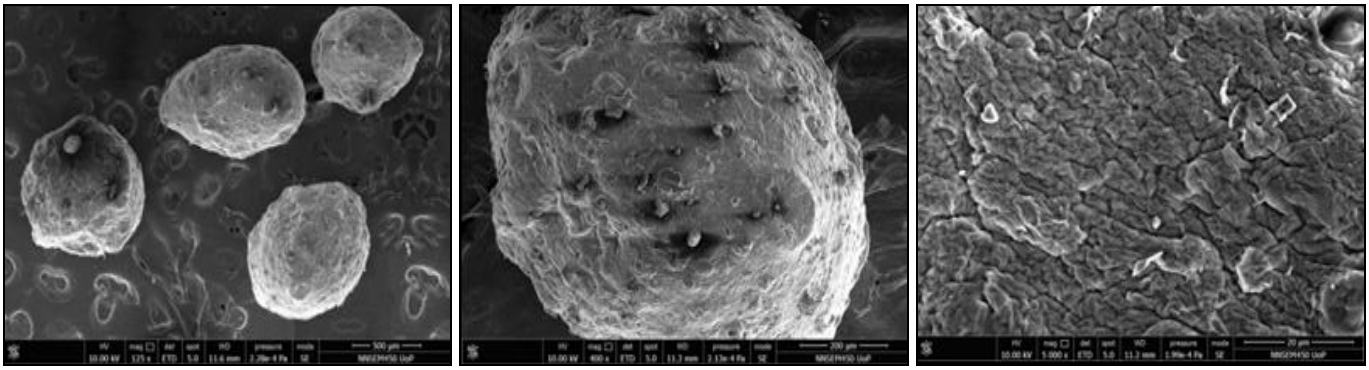

FIG. 8: SEM OF IMAGES OF OPTIMIZED BATCH

Evaluation for Percent Yield, Percent E.E., Particle Size, Percent Swelling, and Flow Properties: Optimized batch was evaluated for various parameters. The results are shown in **Table 6**. All the parameters were found satisfactory.

TABLE 6: EVALUATION OF YIELD, ENTRAPMENT EFFICIENCY, PARTICLE SIZE, SWELLING AND FLOW PROPERTIES

Evaluation parameters	Result	Evaluation parameters	Result
% Yield	84.77 ± 1.28	Bulk density (gm./ml)	0.68 ± 0.41
% E.E.	79.52 ± 2.62	Tap density (gm./ml)	0.74 ± 0.28
% Swelling	65.81 ± 2.51	Carr's compressibility index	8.10 ± 0.25
Particle Size	866.41± 3.95	Hausner's ratio	1.08 ± 0.37
Angle of repose [Degree (°)]	18.31 ± 0.51		

Drug Release Studies for Optimized Batch: 3.70% of sodium alginate and 1% of mucilage were selected as the best concentration. The obtained drug release is as shown in **Fig. 9**.

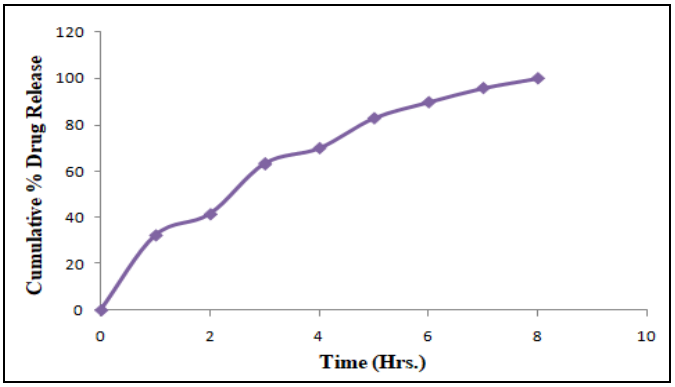

FIG. 9: CUMULATIVE % DRUG RELEASE FROM OPTIMIZED BATCH

At the end of 12 h 100% drug was released. It shows that a combination of both the polymers can retard the drug release up to 12 h.

Powder X-Ray Diffraction Study:

P-XRD Study of Pure Dexibuprofen: Pure drug shows diffraction at an angle of 7.3, 12.5, 13.3, 16.8, 18.2, 19.2, 20, 21.6, 24.8, 27.3, 30.8, 29, 32.4 and 36.7 degrees. The above spectra indicate crystalline nature of the drug [36].

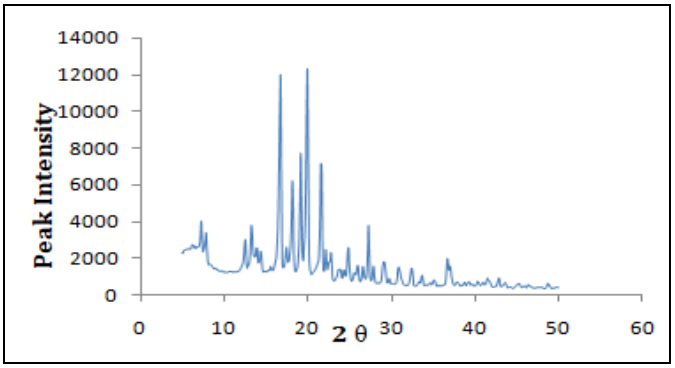

FIG. 10: PXRD OF PURE DEXIBUPROFEN

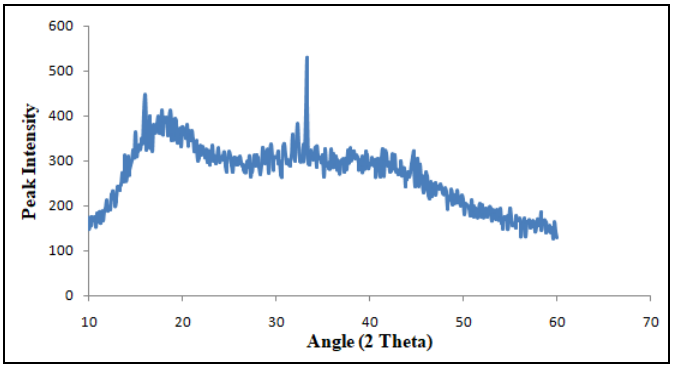

FIG. 11: P-XRD OF OPTIMIZED BATCH

P-XRD of Optimized Batch: Peaks are clumped together reveals that the drug was converted from the crystalline to amorphous structure. This might be due to the vigorous trituration of the drug along with the polymer.

FTIR Study for Optimized Batch: Characteristic peaks were observed at 2871.49, 2985.55, 1420.82, 1230.30, 1707.66, 944.94 cm^{-1} corresponding to C-H, O-H, C-C, C-O, C=O stretching and OH-bending for the pure drug. As shown in **Fig. 12** formulation containing drug shows the respective value of different groups in the range of 3031.56, 2851.56, 1685.46, 1456.96, 1285.38, 851.06 cm^{-1}. Actual peaks of the drug of corresponding functional group and the drug present in the formulation falls in the same range C=O stretch observed for the pure drug was slightly shifted to lower wavenumber this may be due to formation of ionic bind between Ca^{2+} ions and carboxyl group of sodium alginate and partial covalent bond with ether group [37].

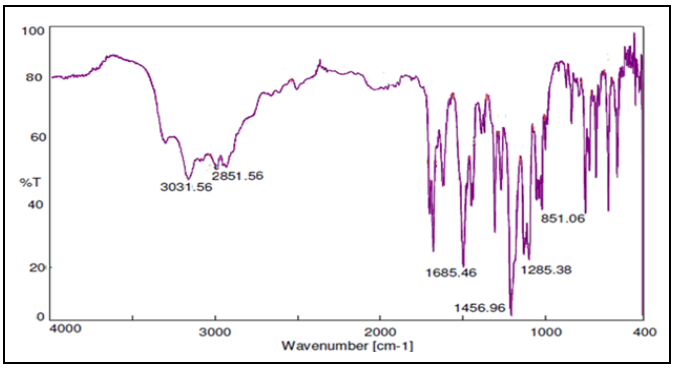

FIG. 12: FTIR SPECTRA FOR OPTIMIZED BATCH

TABLE 7: PEAKS OBTAINED FOR FTIR SPECTRA FOR OPTIMIZED BATCH

Frequency (cm^{-1})	Functional Group	Frequency (cm^{-1})	Functional Group
3031.56	O-H stretch (acidic hydroxyl)	1456.96	C=C stretch
2851.56	C-H stretch	1285.38	C-O stretch
1685.46	C=O stretch	851.06	O-H bend

DSC Study: These studies were carried out to detect the effect of experimental variables on the drug. The results of an optimized batch are shown below.

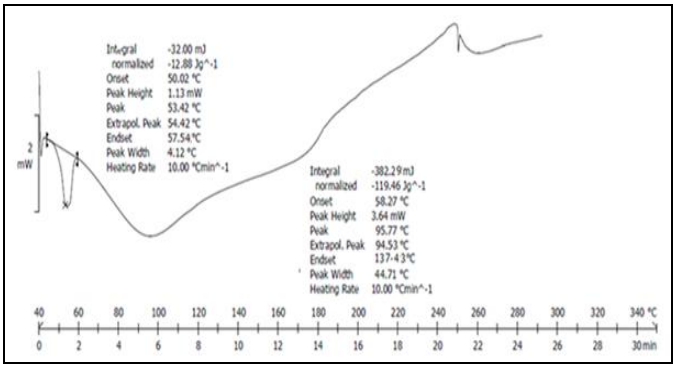

FIG. 13: DSC CURVE OF OPTIMIZED BATCH

The above curve showed a drug peak at 53.42 °C, which starts from 50.02 °C and ends at 57.54 °C, ΔH was found to be 12.88J/gm. It showed that the drug was not affected by experimental variables as a melting point of the drug was not much deviated than the standard value.

Stability Analysis: There might be a reduced risk of quality defects when stability was carried out. In accelerated stability testing, microspheres were subjected to different temperatures and humidity conditions.

For current research work, stability testing was carried out as per ICH guidelines at a temperature of 40 °C ± 2 °C and 75% RH ± 5% RH for 6 months. **Table 8** indicates the stability data for optimized formulation.

TABLE 8: STABILITY DATA

Evaluation parameters								
% CDR			% Entrapment efficiency			Particle Size		
Initial	Third month	Six month	Initial	Third month	Six month	Initial	Third month	Six month
100.95 ±3.26	100.65 ±2. 85	99.95 ±2.31	80.23 ± 2.16	80.35 ±2.41	80.15 ±4.73	867.45 ±3.65	872.48 ±3.61	872.33 ±3.61

CONCLUSION: The objective of the current research work was to explore the use of natural mucilage for sustained release characteristics. Efforts were mainly focused on the application of natural polymer to formulate a novel dosage form microspheres. Mucilage was isolated from *Colocacia Esculenta* corms and evaluated. Ionic orifice gelation method was used to prepare microspheres. According to trial results, by varying composition of mucilage and sodium alginate, various batches were prepared, subjected for evaluation and compared with plain sodium alginate.

All the formulations give better yield. Entrapment efficiency was directly proportional to the concentration of polymer. The swelling study was carried out for 8 h. The optimized formulation could retard the drug release up to 12 h. It was found that drug release was inversely proportional to polymer concentration.

From these result, it could be concluded that isolated mucilage was having the potential to retard the drug release.

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