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Investigation of *Corchorus olitorius* mucilage as a potential mucoadhesive agent in developing *in situ* mucoadhesive nasal gel

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ABSTRACT

The present study aimed to formulate a mucoadhesive *in situ* gel with mucilage extracted from *Corchorus olitorius* for the intranasal route. Propranolol HCl used as a model drug. For the comparative study, the mucoadhesive *in situ* gels were prepared using two different concentration 0.4% and 0.5% of *C. olitorius* mucilage, Carbopol P-934 and hydroxypropyl methylcellulose. The prepared *in situ* gel was subjected to characterization test, such as pH, appearance, gelation temperature, spreadability, viscosity, mucoadhesive strength, and *in vitro* study. From the comparative study, 0.5% mucilage containing *in situ* gel formulation can be considered as optimum formulation.

INTRODUCTION

An intranasal delivery route is a non-invasive approach that could provide adequate systemic delivery of therapeutic compounds. From the pharmacokinetic point, the intranasal route helps to avoid the first-pass metabolism, which leads to improving bioavailability (Dhuria *et al*., 2010). Nevertheless, mucociliary clearance becomes a significant disadvantage (Arora *et al*., 2002). To address this limitation, different dosage form designs have been introduced, such as the use of bioadhesive polymer, adding absorption enhancers, or the use of sophisticated delivery strategy, which may include like nasal gel and powder forms. Ordinarily nasal application of conventional gels or powders are difficult to administer and can be irritative to the mucosal tissue (Upadhyay *et al*., 2011). To achieve better residence time, a mucoadhesive *in situ* gelling system appears to be a desirable approach because of its ability to rapid phase transition at the administration site (Singh *et al*., 2013). These systems are reported to increase the retention time of the specific dosage form within the nasal cavity (Singh *et al*., 2013). This expedites the drug absorption from this route that leads to improved bioavailability.

The *in situ* gelling system is a liquid formulation which changes its physicochemical state, liquid/sol to gel under certain physiological conditions. This polymeric system depends upon different stimuli which may include pH, modulated temperature, exchange of solvent, and ionic change (Kaur *et al*., 2016). In majority, two methods are considered for the development of *in situ* gel, viz., cold method and hot method. Temperature-sensitive or thermoresponsive *in situ* gel has been extensively explored compared to other approaches because it has a low adverse effect on local tissues (Wang *et al*., 2013). The phase transition from a solution to a gel form generally found to be occurred above the lower critical solution temperature, for particular delivery systems, which may be found between 25°C and 37°C.

Numerous methods have been suggested to explain the mucoadhesion mechanism. Mucoadhesion is generally indicated by the contact period (connection duration) between the mucoadhesive polymer and the mucous membrane. Depending on the moisture present in the mucosal layer, the mucoadhesive polymer may tend to attach with the help of its chemical bonds and induce hydrogen and van der Waals bonds with the mucous layer. For better adhesion, mucoadhesive molecules should be capable

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of favouring both mechanical and chemical interaction (Carvalho *et al*., 2010, Rahamatullah *et al*., 2011)

This study aims to obtain a mucoadhesive material from a natural source and exploring it in the development of an *in situ* nasal gel. Mucilage isolated from *Corchorus olitorius* (jute plant) was explored as a mucoadhesive agent which was compared with two synthetic mucoadhesive polymers—Carbopol and hydroxypropyl methylcellulose (HPMC). Pluronic F-127 was used in this study, to act as a base of the thermosensitive polymeric gel due to its properties as a thermoresponsive gelling agent with no-toxicity, non-irritating, and biocompatibility (Majithiya *et al*., 2006). To ascertain the quality of the developed formulation of *in situ* nasal gel, the formulated gels were evaluated for their pH, rheological characteristics, sol–gel transition temperature, adhesion strength, drug content, *in vitro* release profiles along with the release kinetics.

Propranolol HCl, a synthetic beta-adrenergic receptor blocker, was used as a model drug in this study. This drug is widely recommended in the treatment of angina pectoris, myocardial infarction, cardiac arrhythmia, and hypertension. It has a biological half-life of about 5–6 hours and is subjected to a comprehensive hepatic first-pass effect. The oral bioavailability of propranolol HCl is reported to be around 26%–30% (Gaikwad *et al*., 2017). The low bioavailability issue can be addressed by delivering this drug through the intranasal route.

Corchorus olitorius, an annual crop, is an erect, herbaceous plant of Malvaceae family, reaching a height of 2.4 m. It is a significant source of natural fibres, mainly in Asian and Latin American countries and as an essential green leafy vegetable in many areas. The cultivation of *C. olitorius* is done in many parts of the world, such as Syria, Lebanon, Palestine, and Egypt, as a potherb and its culinary use go back at least as far as the Ancient Egyptians. It is also cultivated and eaten in the Caribbean and Brazil, in the Middle East and India, Bangladesh, Japan, and China (Loumerem *et al*., 2016). Different parts of these plants are used as folk medicines. Certain phenolic compounds isolated from *C. olitorius* jute showed great antioxidation, inflammatory inhibition, and cytotoxic activities (Hassan *et al*., 2019).

Moreover, new flavonol glycosides, isolated from the leaves of *C. olitorius*; corchorusides A and B exhibited α-glucosidase inhibitor activity. *Corchorus olitorius* exhibited diverse biological activities, including antioxidant, antitumor, hypoglycemia, antimicrobial, anti-inflammatory, analgesic, antiobesity, gastroprotective, and wound healing effects (Hassan *et al*., 2019).

MATERIALS

Propranolol HCl was obtained from Yarrow Chem, Mumbai. Pluronic F-127 was purchased from Sigma-Aldrich, India. Carbopol P-934, HPMC were purchased from SDFCL, Mumbai. All the other chemicals and ingredients were used in the study were of analytical grade.

METHODS

Extraction of the mucoadhesive agent from plant source

Corchorus olitorius leaves were collected from the local vendor and washed thoroughly. Then, the leaves were cut into small pieces and dried under shaded conditions. Then, three times the volume of distilled water was added to the leaves and soaked for 6 hours and then boiled for 1 hour and left undisturbed for an hour. The liquid extract was separated from the marc by passing through a multi-layer muslin cloth without squeezing. The filtrate was kept undisturbed for some time and concentrated by heating on a water bath at 60°C. After cooling, the extract was added to three times its volume of acetone and stirred. Separated masses were collected and dried at 60°C in a hot air oven. Dried masses were powdered and stored in desiccators (Assi *et al*., 2017; Azubuike *et al*., 2017).

Phytochemical characterization of mucilage

The extracted mass was treated with ruthenium red solution to determine the presence of mucilage. The mucilage was treated with α-naphthol and concentrated sulphuric acid for carbohydrates. Fehling's solution A and B was used for detecting the presence of reducing sugar in extracted mucilage. The mucilage was again treated with ferric chloride and lead acetate solution for the detection of tannins. The extract was also treated with 2% copper sulphate solution and ethanol, along with potassium hydroxide pellets for the detection of proteins (Raaman, 2006).

Determination of pH

pH of 1% w/v aqueous solutions of the mucilage was measured by digital pH meter (Multipara, MP-5).

Study of swollen volume

Aliquot of 300 mg of the mucilage powder was put in 6 ml of water in a measuring cylinder for 24 hours. Swollen volume was recorded and determined with the following equation:

$$\text{Swollen Volume} = V_2 - V_1$$

where, V_1 is the initial volume and V_2 volume is the final volume.

Loss on drying

The powdered mucilage sample was taken in a petri dish and dried in a hot air oven at 110°C until a constant weight was obtained.

$$\text{Loss on drying (\%)} = (W_1 - W_2)/W_1 * 100$$

where, W_1 = Original weight of sample (mg); W_2 = Constant weight of the sample attained (mg).

Drug-polymer interaction study

Fourier-transform infrared spectroscopy (FT-IR) study was done to identify any possible drug-polymer interaction. IR spectrum of pure of Propranolol HCl and extracted mucilage were analyzed in Attenuated total reflectance (ATR) -FT-IR Spectrophotometer (Agilent Technology, CDR-630). Also, a physical mixture of the drug:mucilage (1:1) and drug:polymer (1:1) was made by the trituration process, in a mortar and pestle, also analyzed. The mixtures of drug and polymer samples were kept for 15 days before taking the IR spectrum. The samples were scanned from 500 to 4,000 cm^{-1} wave number.

Preparation of *in situ* gels

In situ gels were produced based on the formula in Table 1, using the cold method. (Basu and Bandyopadhyay, 2010;

Table 1. Formulation codes.

Formulation code	F0	F1	F2	F3	F4	F5	F6
Propranolol HCL	-	4.8 mg/ml	4.8 mg/ml	4.8 mg/ml	4.8 mg/ml	4.8 mg/ml	4.8 mg/ml
Pluronic F127 (%w/v)	18%	18%	18%	18%	18%	18%	18%
Carbopol 934P (% w/v)	-	0.5%	0.4%	-	-	-	-
COM (% w/v)	-	-	-	0.5%	0.4%	-	-
HPMC (% w/v)	-	-	-	-	-	0.4%	0.5%
Propylene Glycol (% w/v)	1%	1%	1%	1%	1%	1%	1%
Benzalkonium Chloride (% w/v)	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%	0.01%

Schmolka, 1972). Briefly, an 18% w/v Pluronic F-127 was weighed and dissolved in a solution of propylene glycol:water and then the mucoadhesive agent (synthetic or natural) was added to it with thorough mixing using stirrer at 200 rpm Propranolol HCl was added to the homogenous solution with continuous stirring and was kept at 4°C overnight. Formulation codes are depicted in Table 1.

Appearance

The appearance of the formulations was determined by visual inspection.

pH of formulation

The pH of all formulations was measured using a digital pH meter, and the pH meter was calibrated with buffers of pH 4 and pH 7 before the measurements (Jagdale *et al*., 2016).

Measurement of gelation temperature and time

The sol–gel transition temperatures of the formulations were determined using the technique described by Altuntaş and Yener (2017). Briefly, a pre-weighted amount of formulation in solution form was transferred into a glass beaker containing a magnetic bar that was allowed to rotate within, with the help of a magnetic stirrer (Tarsons Spinot Digital, MC 02). The beaker was heated at an increasing rate of 1°C per minute, with constant stirring at 80 rpm. The temperature at which the magnetic bar stopped its rotation was considered as the gelation temperature and time taken for converting the solution into gel was recorded.

Spreadability test

To determine the spreadability of *in situ* gel, approximately 0.5 g of the gel was placed at the centre of the glass plate (15 cm × 15 cm). One glass plate was covered with another glass plate. Next, the weight of 300 g was applied on the upper side of the plate; as a result, the gel was spread out in between the two plates. After 5 minutes, the weight was removed, and the diameter of the spread area (cm) was measured (Chaudhary and Verma, 2014). The study was done in three replicates.

Viscosity studies

The viscosity was measured at 37°C using Brookfield viscometer using spindle number 64 at 100 rpm.

Drug content estimation

One milliliter formulation was taken in a 10 ml volumetric flask and diluted with distilled water. The diluted solution was filtered, and 1 ml of filtered solution was again diluted to 10 ml with distilled water. Drug content was estimated by measuring the absorbance of the above solution at 289 nm using a UV visible spectrophotometer (Shimadzu 1800) (Jagdale *et al*., 2016). The % drug content was calculated using the following equation

$$X = Y - C/M$$

where X is conc. in µg/ml, Y is the absorbance of the solution, C is the intercept of the standard curve, and M is the slope of the standard curve, which was plotted between the absorbance of known concentration of drug dissolved distilled water. The absorbance was measured at 289 nm using a UV visible spectrophotometer (Shimadzu,1800).

$$\% \text{ Drug Content} = \frac{\textit{Concentration of drug in sample solution}}{\textit{equivalent concentration of drug taken}} * 100$$

In vitro drug release study

Franz diffusion cells were used for the study with a dialysis membrane. Dialysis membrane (molecular weight 12,000–14,000 Dalton.) was placed in between the donor and the receptor compartment. Aliquots of 1 ml of the formulation containing drug equivalent to 4.8 mg were applied on the surface of the membrane. It was in contact with the receptor compartment containing 100 ml of phosphate buffer pH 6.4. The cell was agitated by a magnetic stirrer at 60 rpm and maintained at 37°C. Aliquots of 1 ml of diffusional media were withdrawn at specific intervals and were replaced with an equal volume of fresh phosphate buffer pH 6.4. The absorbance of the withdrawn media was measured at 289 nm.

Drug release kinetic study

The drug release mechanism from the formulation, drug release data obtained from *in vitro* drug release studies for the formulations were analyzed by fitting the data into different kinetic models (Altuntaş and Yener, 2017).

Texture profile analysis

Texture profile analysis of all formulations was evaluated by TA-XT2 texture analyser (Stable Microsystems) equipped with a 5 kg load cell. Formulations were initially transferred into a jacketed glass vial (20 ml) at 37°C. An analytical probe was inserted into each formulation to a defined depth of 15 mm and at a defined rate of 2 mm/seconds, allowing a period of the 30 seconds between the end of the first and the beginning of the second compression. The probe holding the mucosa was lowered onto the surface of the gel with a constant speed of 0.5 mm/seconds until in

contact with the gel surface, and a contact force of 4.0 g was then applied for 2 minutes. From the time curve, the mucoadhesive property of the gel was determined (Baloglu *et al*., 2011).

RESULTS AND DISCUSSION

Phytochemical characterization of mucilage

Chemical identification of extracted mass isolated from *C. olitorius* (jute plant) leaves was performed, as shown in Table 2. The treatment of the extracted mass with ruthenium red solution confirmed its mucilaginous property. The mucilage was treated with α-naphthol and concentrated sulphuric acid, which gives a reddish-pink colour ring, which revealed that it consists of carbohydrates. The mucilage, when treated with ferric chloride solution for detection of tannins, showed dark green colour indicating the presence of tannin. A white precipitate was observed when the mucilage was treated with lead acetate solution, which also confirms the presence of tannins. By treating the mucilage with Fehling's solution A and B, the mucilage solution gave a brick-red precipitate after heating, which indicates the presence of reducing sugars. Furthermore, the mucilage when tested with the Biuret test, the result indicates the absence of proteins (Raaman, 2006).

Determination of $_{p}$H

pH of *C. olitorius* (jute plant) mucilage was found to be 6.105 ± 0.369 (n = 3), which is within the pH range of nasal route, that is 5.5–7.5.

Study of swollen volume

Swellability data is presented in Table 3. *Corchorus olitorius* mucilage swellability was found to be higher than formulation containing HPMC but lower than the formulation containing Carbopol as a mucoadhesive material. For optimum adhesion, swellability is a valuable property.

Loss on drying

A 19.5% loss in weight was recorded on drying.

Drug-polymer interaction study

Drug-polymer interaction was determined by comparing the IR spectra of the pure drug along with the drug-polymer mixture. As shown in Figure 1A, Propranolol HCl gives the peaks in the IR spectrum at 3,271.07 cm^{-1} due to the hydroxyl group (O-H), at 2,802.637 cm^{-1} due to the presence of an amine group (N-H), at 1,265.158 cm^{-1} due to aryl alkyl ether group (R-O-R′). At 795.795 cm^{-1} due to a-substituted naphthalene group. Figure 1B shows the IR Spectrum of *C. olitorius* (jute plant) mucilage. Figure 1C–E revealed the presence of peaks at 3,271.07, 1,265.158, 2,802.637, and 795.795 cm^{-1} that indicated that the frequencies of functional groups of pure drugs did not change in the mixture containing different polymers.This result shows that there was no primary interaction between the drug and the polymer.

Table 2. Chemical identification.

Name	Presence
Carbohydrates	++
Tannins	++
Proteins	--
Reducing sugars	++
Mucilage	++

Table 3. Result of swollen volume.

Mucoadhesive agent	Swollen volume(cm/mg)
Corchorus (jute plant) mucilage	1.8 ± 0.16
HPMC	1.3 ± 0.34
Carbopol 934	2.9 ± 0.11

All values were expressed as the mean ± SD, n = 3.

Preparation of *in situ* gels

Prepared *in situ* gels formulations are coded as provided in Table 1. Pluronic F-127 was used as a base thermosensitive polymeric gel. The concentration for the Pluronic F-127 was kept constant at 18% w/v as this concentration showed reversal thermal gelation, transforming from a liquid into a semisolid gel in 1–2 minutes at the characteristic sol–gel transition temperatures (Nie *et al*., 2011). *Corchorus olitorius* mucilage was compared to Carbopol P-934 and HPMC as a mucoadhesive agent. Considering the different concentrations of the mucoadhesive polymers, six formulations were made. Compared to Carbopol P-934 and HPMC based *in situ* gel, the *C. olitorius* mucilage-based formulation F3 (Fig. 2) had a gelation temperature of 28°C, which was acceptable for nasal application as it was nearer to the nasal physiological temperature range 29°C–34°C with least gelation time (Wang *et al*., 2017)

Appearance

The mucilage based formulations were coloured turbid solutions (Table 4).

pH of formulation

The mean pH values of all the formulations (Table 5) from F1 to F6 were recorded in the range of 5.2 to 6.23, which is nearer to average baseline human nasal pH which was reported to be around 6.3 (Washington *et al*., 2000).

Measurement of gelation temperature and time

Gelation temperature and time range were shown in Table 6. Gelation temperature for all the formulations was found in the range of 26°C–32°C and time range 58 seconds to 2 minites. It was observed that depending upon the concentration of mucoadhesive polymer material, an alteration in the gelation temperature occurs in the formulations.

Spreadability test

The spreadability test result was documented in Table 7. The mean spreadability for all the formulations from F1 to F6 was recorded between the range of 8.3–9.9 cm. From the study, it was noticed that spreadability was dependent upon the viscosity range for all the formulations.

Viscosity studies

Viscosity of all the formulations from F1 to F6 is between the range of 4,010 and 5,000 cps shown in Table 8. The

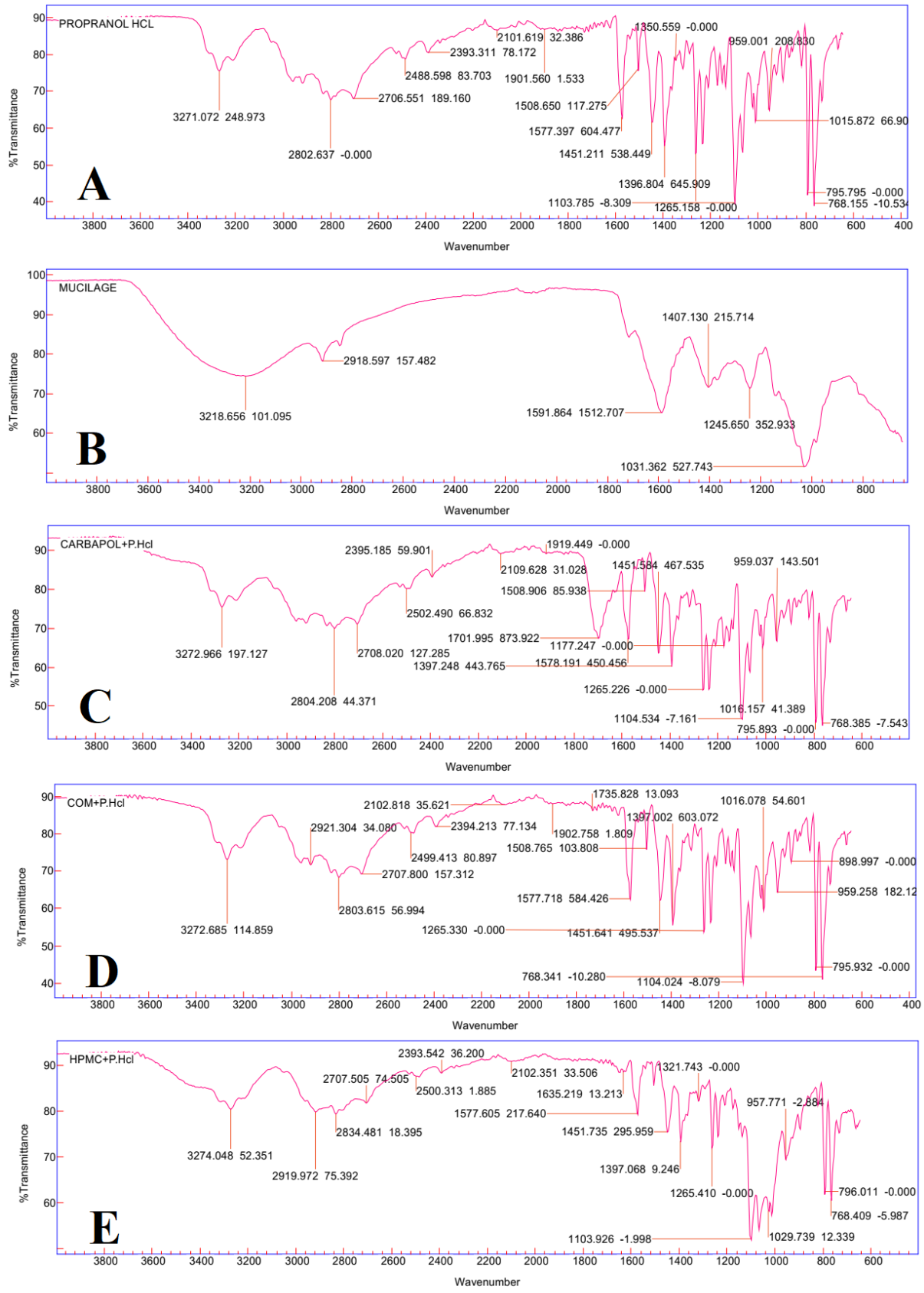

Figure 1. ATR FT-IR analysis of (A) Pure drug Propranolol HCL(P.HCL), (B) C. olitorius Mucilage (COM), (C) Carbopol +P.HCL, (D) COM+P.HCL, (E) HPMC+P.HCL.

Figure 2. Mucilage based in situ gel.

Table 4. Appearance of *in situ* gels.

Sl. No.	Formulation code	Appearance
1	F0	Transparent solution
2	F1	Turbid and viscous solution
3	F2	Turbid and viscous solution
4	F3	Coloured turbid solution
5	F4	Coloured turbid solution
6	F5	Transparent and viscous solution
7	F6	Transparent and viscous solution

Table 5. pH of *in situ* gels.

Sl. No.	Formulation code	pH
1	F1	5.279 ±0.235
2	F2	5.245± 0.536
3	F3	6.23± 0.285
4	F4	6.102 ± 0.196
5	F5	5.901± 1.023
6	F6	5.703±0.690

All values were expressed as the mean ± SD, $n = 3$.

Table 6. Gelation temperature and time.

Sl. No.	Formulation code	Gelation temp.	Time
1	F0	32°C	2 minutes 19 seconds
2	F1	30-31°C	2 minutes
3	F2	29°C	1 minute 50 seconds–2 minutes
4	F3	28°C	58 seconds–1 minute
5	F4	28°C	1 minute 6 seconds
6	F5	28°C	1 minute 10 seconds
7	F6	26°C–27°C	1 minute 11 seconds

study result showed that with an increase in the concentration of the adhesive polymer, the viscosity of the formulations also increased.

Drug content estimation

Drug content was found in the range of 93.7% to 94% (Table 9).

Table 7. Spreadability of *in situ* gel.

Formulations	Spreadability (cm)
F1	8.3 cm ± 0.20
F2	8.5 cm ± 0.23
F3	9.0 cm ± 0.50
F4	9.9 cm ± 0.50
F5	9.2 cm ± 0.25
F6	8.7 cm ± 0.25

All values were expressed as the mean ± SD, $n = 3$.

Table 8. Viscosity of *in situ* gel.

Formulation code	Viscosity (100rpm)
F1	5,000 cps
F2	4,630 cps
F3	4,801 cps
F4	4,010 cps
F5	4,500 cps
F6	4,801 cps

Table 9. Drug content of *in situ* gels.

Formulation code	Drug contain (%)
F1	94.0±0.59
F2	94.0 ±0.89
F3	94.0±0.67
F4	93.7 ± 0.91
F5	95.8 ± 0.35
F6	93.7 ± 0.46

All values were expressed as the mean ± SD, $n = 3$.

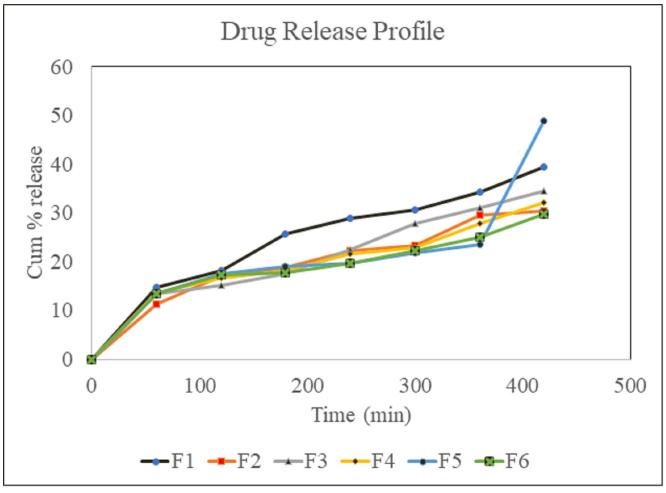

Figure 3. *In-vitro* drug release profile of different formulations.

In vitro drug release study

From the study, it was observed that polymer concentration affects the release rate (Fig. 3). The *in vitro* diffusion study shows that formulations F1, F3, and F5 had 39.55%, 35.19%,

and 48.95% release, respectively, after 7 hours. The release pattern from HPMC-based *in situ* gels showed that with the increase in the concentration of the adhesive polymer, drug release was reduced, but Carbopol and mucilage-based gels were exceptional. The *in vitro* drug release profiles of all the formulations are shown in Table 10.

Drug release kinetic study

The release kinetic profile for formulations is documented in Figure 4. On comparing the regression coefficient values, after fitting the *in vitro* release data, to different kinetic models, the R^2 value of different kinetic models was recorded in Table 11. The release data were found to be following first-order kinetics with best fitted to Korsmeyer–Peppas model. On comparing the *n* value of the Korsmeyer–Peppas model, it was found to be in between 0.47 and 0.51, indicating an anomalous or non-Fickian release of drug from the formulations.

Texture profile analysis

From the mucoadhesive test, it was found that the mucoadhesive strength increase with the increase in the concentration of the mucoadhesive agent. Compared to the Carbopol and HPMC-based gels, the mucilage incorporated gel showed the better adhesive property. Texture analysis for mucoadhesion was done and the detachment force of Carbopol, mucilage and HPMC was found to be 4.1, 5.4, and 4.8 g, respectively. The study also shows that the residence time of mucilage-based formulation F3 (Fig. 5) gives better results compared to the Carbopol and HPMC-based *in situ* gel.

Table 10. *In vitro* drug release profile.

Time (minute)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
60	14.83279396	11.23892	13.34951	13.64617	13.5383	13.34951
120	18.25782093	17.05678	15.42638	16.61597	17.47681	17.31005
180	25.76523732	18.75271	17.94203	18.28939	19.06176	17.70184
240	28.9349137	22.16188	22.95405	21.55827	19.63171	19.60808
300	30.7220658	23.3904	28.44729	22.91765	21.90802	22.28434
360	34.31675836	29.64021	31.68403	27.79824	23.46363	24.97554
420	39.5661219	30.50356	35.19913	32.10257	48.95253	29.77741

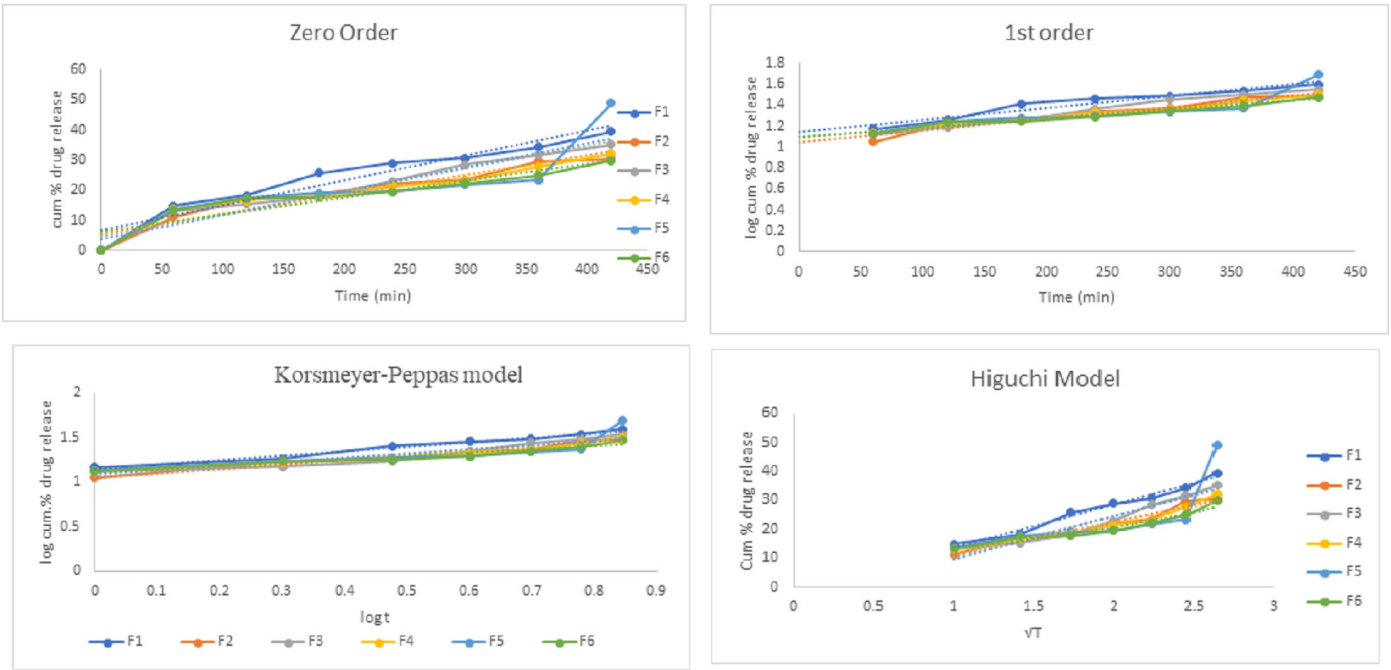

Figure 4. Release kinetic profile of different formulations.

Table 11. Release kinetic data.

Formulation	Zero order	First order	Korsmeyer–Peppas		Higuchi
	R^2	R^2	R^2	n	R^2
F1	0.9197	0.9362	0.9763	0.50	0.978
F2	0.9147	0.9278	0.9756	0.499	0.9661
F3	0.9312	0.9855	0.9462	0.51	0.9175
F4	0.9008	0.9899	0.928	0.41	0.9277
F5	0.7548	0.7784	0.6496	0.47	0.5597
F6	0.8624	0.9685	0.9159	0.36	0.9134

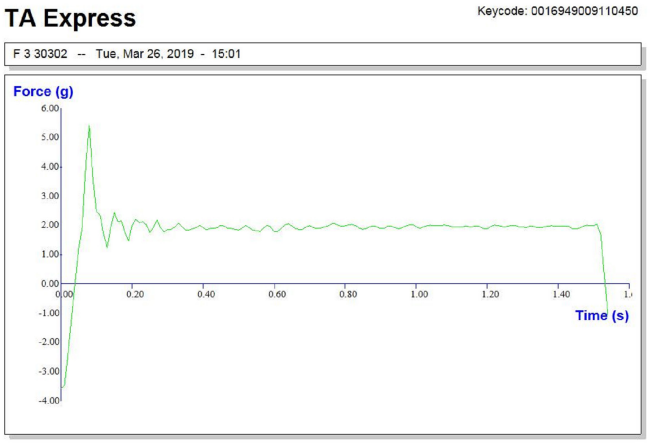

Figure 5. Texture analysis profile of formulation F3.

CONCLUSION

The objective of the proposed work was to develop an *in situ* gelling systems employing a natural mucoadhesive agent. To achieve this objective, the mucilage was extracted from the plant source, *C. olitorius* (jute plant). The mucilage extracted from *C. olitorius* showed a hopeful mucoadhesive property when compared to synthetic polymers, such as Carbopol P-934, HPMC which are commercially widely used in the preparation of nasal gels. The comparative study showed that mucilage containing gel results more or less similar to the synthetic polymer-based gels with the better mucoadhesive property. From this effect, two different formulations containing different concentration of mucilage obtained from *C. olitorius* leaves was developed and it can be concluded that among two mucilage-based formulation, F3 (0.5% w/v mucilage of *C. olitorius*) is considered as an optimum formulation. The *in situ* gel which is being prepared from natural mucilage might provide an economical dosage form for the intranasal delivery route for the upcoming future.

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AUTHOR'S CONTRIBUTIONS

All the authors contributed equally.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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