RESEARCH ARTICLE

Fabrication and evaluation of oral tablets using natural mucoadhesive agent from seeds of *Caesalpinia pulcherrima* (L.) SW

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Oral mucoadhesive sustained drug delivery systems of salbutamol sulfate were formulated using an isolated natural agent from the seeds of *Caesalpinia pulcherrima*. The isolated material was evaluated for various parameters, such as, melting point, viscosity, pH, elemental analysis, swelling index, phytochemical constituents, and solubility studies. The mucoadhesive characters of the isolated substance were identified by a comparative study with hydroxyl propyl cellulose and sodium alginate, by various *in vitro* methods, such as, Shear stress measurement, Wilhelmy's method, Falling sphere method, and Detachment force measurement. Formulation and evaluation of mucoadhesive oral tablets of salbutamol sulfate (100 mg), using isolated natural materials in different proportions, and *in vitro* release studies, were carried out for three different formulations according to the U.S.P apparatus two (paddle method). Each 100 mg tablet was taken in 900 ml of acid buffer 1.2 and maintained at 37°C. After two hours the filtrate was collected and replaced in buffer 7.4. *In vitro* releases of three different formulations for nine hours were studied, which showed the sustained action of drug release with increasing the concentration of the isolated natural mucoadhesive agent in the formulations.

Key words: *Caesalpinia pulcherrima, salbutamol sulfate, natural mucoadhesive agent*

INTRODUCTION

Mucoadhesive controlled drug delivery systems are very beneficial, as they provide a controlled drug release over time and localize the drug to a specific site in the body. The prolonged residence time of the drug in the body is believed to prolong the duration of action. Mucoadhesive drug delivery devices can be applied to any mucous tissue in the body including the gastrointestinal, ocular, respiratory, buccal, nasal, rectal, urethral, and vaginal pathways. Oral administration of drugs is the preferred administration route for this article. As the GI tract is covered by a mucus layer; localization of a mucoadhesive drug delivery system to a specific site is very beneficial.

To overcome the limitations of first generation 'off-the-shelf' mucoadhesive materials, new types of materials have been investigated that allow specificity, or prolong and strengthen the mucoadhesion process. Sometimes, the existing mucoadhesive polymers have been modified, while in others, new materials are developed. One approach to produce improved mucoadhesives has been to modify the existing materials. For example, the thiol groups (by coupling cysteine, thioglycolic acid, cysteamine) have been placed into a range of mucoadhesive polymers such as the carbomers, chitosans, and alginates.[1,2] The concept is that *in-situ* they will form disulfide links not only among the polymers themselves, thus inhibiting overhydration and formation of the slippery mucilage, but also with the mucin layer / mucosa itself, thus strengthening the adhesive joint and leading to an improved adhesive performance. This interesting approach appears to be meeting with some success.

Natural pharmaceutical excipients[3]

Today we have several pharmaceutical excipients of plant origin, like starch, agar, alginates, carrageenan, guar gum, xanthan gum, gelatin, pectin, acacia, tragacanth, and cellulose. These natural excipients find applications in the pharmaceutical industry as binding agents, disintegrates, sustaining agents,

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protectives, colloids, thickening agents, gelling agents, bases in suppositories, stabilizers, and coating materials. The advantages of natural plant-based excipients include that they are of low cost, natural origin, fairly free from side affects, biocompatible, and bioacceptable, with a renewable source, environmental friendly processing, local availability, better patient tolerance, as well as public acceptance. They improve the national economy by providing inexpensive formulations to people, by using locally available material.

Pharmaceutical applications of plant agents

The plant agents may have a linear or branched structure. They may have acidic, basic or neutral characteristics. Among them the hydrocolloids, with basic characteristics, have limited commercial importance, whereas, acidic and neutral polymers have wide pharmaceutical applications. They are ingredients in dental and other adhesives and are used as bulk laxatives. These hydrophilic polymers are useful as tablet binders, disintegration mediums, emulsifiers, suspending agents, gelling agents, stabilizing agents, thickening agents, and sustaining agents.

Hence, in the present study, a natural mucoadhesive agent from the seeds of *Caesalpinia pulcherrima* has been used as a release modifying agent, for the development of the oral drug delivery of salbutamol sulfate, in the form of oral mucoadhesive tablets.

MATERIALS AND METHODS

Materials

Salbutamol sulfate and Hydroxyl propyl cellulose, were obtained as gift samples from Fourrts India Laboratories Pvt. Ltd., Chennai, India, Sodium alginate was purchased from the Indian Research Products, Chennai, India. Seeds of *Caesalpinia pulcherrima* were collected from the surrounding areas of Erode, Tamilnadu, India. Magnesium stearate, Dicalcium phosphate, Talc, Acetone, and all other chemicals were of Analytical Grade.

Methods

Isolation of the natural mucoadhesive agent[4,5]

After cleaning the seeds of *caesalpinia pulcherrima* (500 mg), they were soaked separately in distilled water (2500 ml) for 24 hours and then boiled for an hour with stirring, using a mechanical stirrer, at 2000 rpm, and kept aside for the release of the natural agent into the water. The soaked seeds were taken and squeezed using several folds of muslin cloth to separate the marc from the filtrate. Next, an equal quantity of acetone was added to the filtrate, to precipitate the natural mucoadhesive agent, which was then separated by filtration. The marc was not discarded, but was used for multiple extractions, with decreasing quantity of extracting solvent (water) for each successive cycle of extraction (that is 2000, 1500, and 1000 ml of water for the second, third, and fourth extractions, respectively). The second, third, and fourth extracts were pooled and concentrated under vacuum at 40˚C to half the volume, and then the agent was precipitated using acetone. The separated mucoadhesive agent was dried at 40˚C in a hot air oven. The dried mucoadhesive agent was powered, passed through sieve No. 100 and stored in airtight containers at room temperature till further use.

Elemental analysis

Carbon (C), Hydrogen (H), Nitrogen (N), Sulfur (S) and Oxygen (O) present in the isolated natural mucoadhesive agent was reported from the C and M laboratory, Central Electrochemical Research Institute, Karaikudi, India.

The elements such as Nitrogen (N), Carbon (C), Sulfur (S), and Hydrogen content percentage was analyzed and is shown in the Table 1.

Comparative mucoadhesive characterization of natural agent with existing polymers, shear stress measurement[6]

Different concentrations of the mucoadhesive agent solution, such as, 1, 2, and 3%w/v, using hydroxyl propyl cellulose (HPC), Sodium alginate, and a natural isolated mucoadhesive agent from *Caesalpinia Pulcherrima* were prepared. A specified amount of prepared solution was spread on three glass plates. Another clean slide was placed over the first plate and made to spread the polymer solution uniformly in between the two glass plates, by placing 100 g weight on the glass plates.

Wilhelmy's method[9]

The glass plates were coated by dipping them into a 1%w/v solution of test materials and dried at 60˚C for three hours. One end of a glass rod was tied to a pan in which a beaker was placed. The polymer-coated glass plate was suspended from one end of the rod and made to penetrate the mucus, which was kept in a 50 ml vial. Next this assembly was kept undisturbed. After keeping it for a time interval of 5, 10, 15, and 30 minutes, the water required to pull out the glass plate from the mucus represented the force required to break the mucus-polymer contact against adhesion.

Falling sphere method[7,8]

A clean burette was taken and filled with 10% mucus solution and fixed in a stainless steel stand. A polymer-coated mustard grain was taken and slowly placed at the top of the mucus layer. The time taken by the grain to cover 50 divisions in the burette was noted, and the values were tabulated.

Table 1: Elemental analysis result

	Weight (mg)	C/N ratio	Content (%)	
Natural Mucoadhesive Agent (C.P)	4.6810	34.86	N	1.127
			C	39.28
			S	0.550
			H	6.468

*C.P - *caesalpinia pulcherrima*

Detachment force measurement[6]

This is the method used to measure the *in vitro* mucoadhesive capacity for different polymers. It is a modified method developed by Martti Marvola,[6] to assess the tendency of mucoadhesive materials to adhere to the esophagus. The assembly of this apparatus consists of two glass slides, one modified physical balance, weights, thread, goat intestine, tyrode solution, distilled water, and a beaker to hold the water.

Immediately after slaughter, the intestines were removed from the goat and transported to the laboratory in tyrode solution (g/litre); sodium chloride 8; potassium chloride 0.2; calcium chloride $2H_2O$ 0.134; sodium bicarbonate 1.0; sodium dihydrogen phosphate 0.05, and glucose H_2O 1. During this experiment, the intestine was taken from a specified area, placed on one glass slide, and tied. The glass slide with the intestine was affixed on one side of the floor, below the modified physical balance. The already prepared 200 mg plain polymer tablet was pasted on another glass slide and it was balanced on the assembled physical balance, with a beaker on the other side, which was used to hold the water. Now the balance was calibrated.

Recording of adherence

The plain polymer tablet in the slide was left on the intestine segment slide, lightly pressing the intestine segment with a forceps. The assembly had to be kept undisturbed for a fixed time interval, 5, 10, 15, and 30 minutes. Then water was added slowly, dropwise, to the beaker side. The amount of water required to pull out the tablet from the intestinal segment represents the force required to pull the tablet against the adhesion. The procedure similar to the above-mentioned procedure is repeated for the comparative study among HPC, Sodium alginate, and an isolated natural mucoadhesive agent.

The force in Newton's is calculated by the equation, $F = 0.00981\ W/2$

Preparation of granules for oral tablets using isolated natural agent[10,11]

For the preparation of granules, the wet granulation method was used. Accurately weighed quantities of the ingredients were mixed in a glass mortar and the required quantity of warm water was added to the powder mass and mixed thoroughly. The granules were prepared by passing the wet mass through British standard sieve (BSS) No. 16. The wet granules were dried in a hot air oven for 30 minute at 60°C and then passed through BSS No. 22. Finally, the granules were collected to do the evaluation. Each tablet weight was calculated and finally the required quantity of ingredients was used to prepare the mass, for the granules to formulate sufficient tablets.

With reference to Table 6 the formula for three different formulations by mentioning the proportions of active and inactive Pharma ingredients, which are used for the formulated tablets.

Evaluation of blend characteristics of salbutamol sulfate

Characteristics such as Flow property, bulk density, tapped density, Compressibility index, and Hauser's ratio were analyzed for the prepared blend.

Formulation and evaluation of oral tablets of salbutamol sulfate

1. Average thickness[12]
2. Hardness test[12]
3. Friability test[12]
4. Weight variation test[13]
5. Drug content uniformity[13]
6. Determination of surface pH[14,15]
7. Water absorption studies[11,16]
8. Mucoadhesive strength measurement[6]

***In vitro* release studies[10,17,18]**

The *in vitro* release studies of salbutamol sulfate oral mucoadhesive tablets were carried out according to the U.S.P apparatus two (paddle method). Tablets of 100 mg were taken in 900 ml of acid buffer 1.2 maintained at 37°C. After two hours the filtrate was collected and replaced in buffer 7.4. The dissolution medium was rotated at 50 rpm. A sample of 5 ml was withdrawn from the dissolution medium at specific time intervals and an equal volume of medium was replaced immediately. The withdrawn samples were then filtered and suitably diluted. The absorbance of the filtrate was determined at a wavelength of 276 nm against a buffer of pH 7.4 as the blank. The amount of drug present in the filtrate was then determined from the calibration curve and the cumulative percent of drug release was calculated. The same procedure was repeated for three formulations and a comparative study of the three formulations' drug release profiles was shown in the results and the comparative *in vitro* release of all the three formulations are shown graphically in Figure 1.

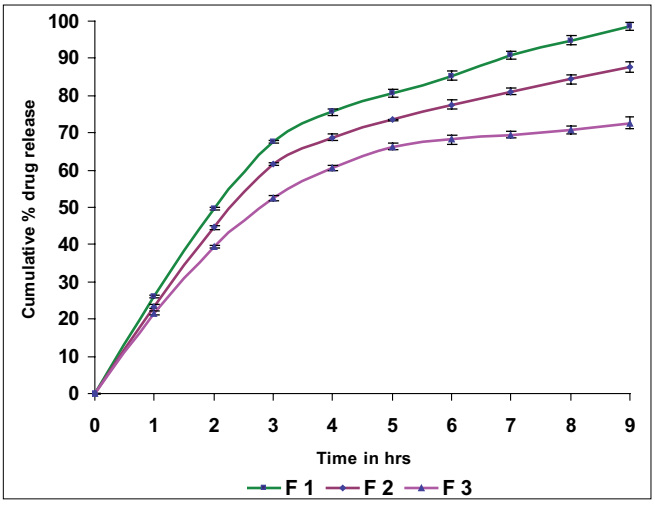

Figure 1: *Formulation-1 (F1), Formulation-2 (F2), Formulation-3 (F3). *In vitro* release comparative study for three different formulation of Salbutamol sulphate tablet using isolated natural adhesive material

RESULTS AND DISCUSSIONS

Caesalpinia pulcherrima seeds yielded 12%w/v of natural mucoadhesive agent. The isolated mucoadhesive agent was tested for identification of hydrocolloid, and a positive result was obtained. The purity of the selected mucoadhesive agent was determined by carrying out tests for the presence of different phytoconstituents and the result showed the presence of only carbohydrates. The pH was found to be 6.5, showing that the selected natural mucoadhesive agent might not irritate the epithelium and mucus membrane of the oral route, and was found to be suitable for oral dosage forms. The high water absorption capacity showed that the polymer could form a good bond with oral mucosa in a short duration; if pH was increased the water absorption was reduced. The viscosity studies on 1%w/v solution of isolated natural mucoadhesive agent showed a decrease in viscosity with increase in temperature and the melting point range was also reported. The isolated natural mucoadhesive agent was soluble only in the hot water. The elemental analysis study showed that the isolated natural mucoadhesive agent consists of a certain percentage of carbon, nitrogen, sulfur, and hydrogen.

The observation reveals that the natural and synthetic substances subjected for physical evaluation possess adhesive characteristics. Among these the natural mucoadhesive agent from the seeds of *Caesalpinia pulcherrima* possess considerable adhesiveness in the demonstration by *Shear Stress Measurement*. The natural agent from the seeds of *Caesalpinia pulcherrima* is found to contain more adhesiveness than HPC, but less than sodium alginate, from the comparability of adhesiveness, within 60 minutes [Table 2]. In the *Wilhelmy's Method,* it has been found that 1.5 g of weight was required to detach a 1%w/v coated plate from the mucus gel. Among the synthetic polymers HPC is poor in adhesive character and needs 1.3 to detach the plate, but sodium alginate was found to have a good adhesive character, with 1.6 g, to detach the plate. While we compare these three for the mucoadhesive character the isolated mucoadhesive agent shows a medium adhesiveness toward the mucus gel [Table 3]. In the *Falling Sphere Method*, the natural mucoadhesive agent from the seeds of *Caesalpinia pulcherrima* was found to contain more considerable adhesiveness than the HPC. Between the synthetic polymers, sodium alginate was found to possess good adhesiveness. The adhesiveness increases with the increase in time and concentration [Table 4]. In case of the *Detachment Force Measurement* the plain tablet of the natural mucoadhesive agent from the seeds of *Caesalpinia pulcherrima* showed a mutual adhesive force toward the intestine in a comparative study, with the synthetic polymers (Sodium alginate and HPC), [Table 5].

The angles of repose of all formulation blends, F_1 to F_3, were in the range of 32°49'±0.480 to 35°56'±0.560. The bulk density, tapped density, compressibility index, and Hauser ratio were found in the range of 0.3906 – 0.4032 g/cc, 0.4464 – 0.4629g/cc, 12.00 – 12.90, and 1.1360 – 1.1490, respectively. It reveals that all the formulation blends were having well flow characteristics and flow rates. The thickness of all formulations F_1 – F_3 was in the range of 2.10±0.128 to 2.40±0.203 mm. The hardness of all formulations F_1 – F_3 was in the range of 4.8±0.4 to 5.4±0.5 kg/sq.cm. The percentage friability of all formulations F_1 to F_3 was in the range of 2.23 to 4.10%. The percentage weight variation for all formulations F_1 to F_3 was in the range of 2.28 to 3.67%. The percentage of drug content for all formulations F_1 to F_3 was in the range of 97.1±1.46 to 99.3±1.50 %. Surface pH of all the formulations was in the range of 7.2±0.20 to 7.8±0.40. Swelling index was high 14.5106±0.19 for the F_3 formulation after four hours and low, 2.6081±0.15, for the F_1 formulation. On different time intervals and based on the quantity of the natural mucoadhesive agent in the formulation, the swelling index might increase, to control the drug release from the dosage form. The mucoadhesive strength of all the formulations F_1 to F_3 was found to be in the range of 20.4±0.21 g to 31.3±0.28 g. Due to a higher concentration of the isolated natural mucoadhesive agent in F_3, it showed more mucoadhesive strength than F_1 and F_2.

The *in vitro* release profile of all the formulations was studied for about nine hours. This study was found to have very good

Table 2: Shear stress measurement

Name of the polymer	Concentrations (%w/v)	Weight required to detach glass plate (g) At the time intervals (*n* = 6)		
		15 minutes	30 minutes	60 minutes
Hydroxy propyl cellulose	1	137.08±0.20	156.16±0.43	196.61±0.96
	2	169.64±1.67	189.89±1.50	232.13±1.49
	3	192.50±0.43	219.18±1.81	255.36±0.09
Sodium alginate	1	163.90±0.76	185.32±0.45	232.90±2.04
	2	206.12±0.83	223.26±3.72	272.92±0.04
	3	231.77±2.11	241.65±2.87	294.62±0.89
Natural mucoadhesive agent	1	141.37±1.37	163.38±1.86	203.02±2.06
	2	192.23±1.69	210.66±2.37	261.76±2.06
	3	206.92±2.86	240.00±0.58	284.39±3.35

*n – Standard deviation

Table 3: Wilhelmy's method[9]

Name of the polymer (1%w/v) solution	Weight required to detach (g) at the time intervals			
	5 min	10 min	15 min	30 min
Hydroxy propyl cellulose	0.60	0.90	1.15	1.30
Sodium alginate	0.85	1.30	1.45	1.60
Natural mucoadhesive agent	0.70	1.10	1.25	1.50

Table 4: Falling sphere method

Name of the sample	Concentration % w/v	Average time taken (seconds)
Hydroxy propyl cellulose	0.5	9.20
	1.0	9.50
	3.0	10.20
Sodium alginate	0.5	10.23
	1.0	10.42
	3.0	10.55
Natural mucoadhesive agent	0.5	9.57
	1.0	10.22
	3.0	10.50

Table 5: Detachment force measurement

Name of the polymer	Contact time	*n* = 6	
		Weight required (gram)	Standard deviation (±)
HPC	5	10.5	1.5
	10	19.5	3.5
	15	26.5	1.5
	30	29.5	1.5
Sodium alginate	5	57.7	4.0
	10	100.8	1.0
	15	113.5	2.5
	30	139.4	1.0
Natural	5	23.5	1.5
mucoadhesive agent	10	73.5	1.3
	15	96.6	3.5
	30	108.3	1.5

**n*-Standard deviation

Table 6: Formula of oral mucoadhesive tablet (each)

Ingredients	Quantity per tablet (mg)		
	F_1	F_2	F_3
Natural mucoadhesive agent	25	50	75
Dicalcium phosphate	69	44	19
Salbutamol sulphate	4	4	4
Magnesium stearate	1	1	1
Talc	1	1	1

*F-Formulation

sustaining efficacy with cumulative percent release, laying F_1 - 98.64±1.06 % (in 9 hours), F_2 - 87.72±1.42 % (in 9 hours), and F_3-72.69±1.68 % (in 9 hours). The comparative releases of all formulations showed the improvement in sustaining the property of drug release, while increasing the natural mucoadhesive agent concentration in the formulations. F_3 showed better sustaining action than F_1 and F_2, which showed the increasing concentration of natural mucoadhesive agent controls the drug release.

In vitro release comparative study for three different formulations of the Salbutamol sulfate tablet using isolated natural adhesive material

CONCLUSION

From the above-mentioned results it can be concluded that the formulation of oral tablets using salbutamol sulfate as a model drug, with an isolated natural mucoadhesive agent, from the seeds of *Caesalpinia Pulcherrima,* at different ratios, as one of the excipients, the *in vitro* release of three different formulations were studied, which showed sustained action of drug release with an increase in the concentration of the isolated natural mucoadhesive agent. At present, stability studies are going on, but this research has been conducted to utilize the mucoadhesive action of the isolated natural agent.

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