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Formulation, Development and Evaluation of Novel Dosage Form Containing Silk Fibroin for Elderly Patients

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ABSTRACT

Dysphagia (difficulty in swallowing) is common among all age groups, especially in elderly and pediatrics. Dysphagia can result in compromising the nutritional status, complicating the administration of solid medications and undermining quality of life. Persons suffering from dysphagia may get choked even when they consume liquid formulation, thus to alleviate such problems silk fibroin gel formulation was prepared. Formulation of oral gel was carried out using silk fibroin as a gelling agent, pharmaceutical gelatin as a stiffening agent and DrotaverineHCl as a model drug. A different concentration of silk fibroin ranging from 2-7% w/v was used to prepare the gel. The prepared formulations were evaluated for appearance, texture, pH, syneresis, drug content, viscosity, and *in vitro* release. Sucralose was used as a sweetening agent, Sodium benzoate as a preservative, tartrazine as a coloring agent and pineapple flavor. Formulation F2 with 2% silk fibroin concentration not only showed more than 90% drug release in 75 minutes but also the desired organoleptic properties. The optimized formulation F2 showed substantial stability when subjected to short term stability study ($5 \pm 3^{\circ}\text{C}$). Problem of dose measurement was outweighed as oral gel to be taken using teaspoon. Dose of the drug is adjusted in such a way that one teaspoonful of gel contains one dose of the drug.

Key words: silk fibroin, drotaverineHCl, oral gel, elderly patients, dysphagia

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INTRODUCTION

Dysphagia (difficulty in swallowing) is relatively common in the general population, but the prevalence increases with age and poses particular problems in the elderly patients, potentially compromising nutritional status, complicating the administration of solid medications [1]. Elderly people are particularly prone to develop dysphagia for several reasons. Its prevalence can be seen in several diseases like Advanced Alzheimer's disease, acute stroke, head and neck cancer and in myasthenia gravis [2-4]. Patients with dysphagia find it difficult to swallow the tablets and hard gelatin capsules thus do not comply with the prescription, which results in high incidence of non-compliance and ineffective therapy [5]. Convenience of administration and patient compliance are gaining significant importance in design of dosage forms. Changing the formulation may help to overcome the problems of administering medicines to such patients like prescribing liquid formulations and oval tablets [1]. Persons suffering from dysphagia may get choked when they consume liquid formulations. Taking into consideration of the disadvantages of solid and liquid dosage forms, this research work was planned with the objective to formulate, develop and evaluate an oral gel of silk fibroin for elderly patients. This gel formulation will ease the swallowing and helps in administration of drugs.

Silk cocoons are made up of two proteins sericin and fibroin. Sericin constitutes 25-30% of silk proteins, remaining is constituted by fibroin [6]. Fibroin has been explored as a biomaterial for various applications like controlled release tablets, gels and microspheres [7,8]. Silk fibroin is a natural polymer and is used in the present study as gelling agent.

DrotaverineHCl was used as a model drug in the study. It is an antispasmodic and used in the treatment of smooth muscle spasms of gastrointestinal origin. It was proved effective in the treatment of acute colicky pain caused by renal and ureteric stones and no serious side effects were reported. Dose of the DrotaverineHCl in adults ranges from 120 to 240 mg per day in 2-3 divided doses. Oral bioavailability of DrotaverineHCl is highly variable and it ranges from 25% to 91%. DrotaverineHCl and its metabolites are 80-95% protein bound and has volume of distribution of 193 to 195 litres. Drotaverine undergoes extensive first-pass metabolism. It is readily metabolized in the liver by O-deethylation to mono- and di-phenolic compounds and their corresponding glucuronic acid derivatives and is excreted in the urine and faeces. The half-life of the DrotaverineHCl ranges from 7 to 12 hours [9].

MATERIALS AND METHODS

DrotaverineHCl provided as a gift sample by Sanofi-Aventis, Gujarat. Silk fibroin extracted in house, Sucralose Sodium Benzoate and Tartrazine was purchased from Ranbaxy SAS Mumbai. All other reagents used were of analytical grade.

Extraction of silk fibroin

Silk fibroin was extracted by Ajisawa's method. Cocoon shells were degummed by boiling them in 0.05% sodium carbonate solution for 60 minutes, washed 4 times with warm water and air-dried. This degummed silk was added to Ajisawa's reagent in the ratio of 1:15 and stirred at temperature of 75 °C for 3 hours. Fibroin solution was dialyzed against running water until dialyzate tested negative for chloride ion using silver nitrate and lyophilized to obtain fibroin powder.

Method of preparation of silk fibroin gel

Required quantity of silk fibroin (1 to 7%), drug and all other ingredients were taken separately in distilled water and mixed them with constant stirring. pH of the mixture was adjusted to 5 using citric acid to get the gel. The obtained silk fibroin gel was stored between 2-8 °C to prevent putrefaction of the protein. The quantity of ingredients taken in the formulation is given in Table. 1. Dose of the drug was adjusted in such a way that each teaspoonful (5ml) of silk fibroin gel contains one dose of drug (40mg), i.e. 1 dose=5ml of gel.

Table 1: Formulation chart for 100ml oral gel

Formulation code	Ingredients						Distilled Water (Upto 100ml)
	DrotaverineHCl (mg)	Silk Fibroin (%)	Gelatin (%w/v)	Sucralose (%w/v)	Sodium Benzoate (%w/v)	Tartrazine (%w/v)	
F1	800	1	0.5	0.2	0.5	0.001	q.s
F2	800	2	0.5	0.2	0.5	0.001	q.s
F3	800	3	0.5	0.2	0.5	0.001	q.s
F4	800	4	0.5	0.2	0.5	0.001	q.s
F5	800	5	0.5	0.2	0.5	0.001	q.s
F6	800	6	0.5	0.2	0.5	0.001	q.s
F7	800	7	0.5	0.2	0.5	0.001	q.s

In this formulation, silk fibroin was used as a gelling agent. Gelatin was used as stiffening agent and to minimize the syneresis. Sucralose and sodium benzoate were used as sweetening agent and preservative respectively. Tartrazine color and pineapple flavor was used.

Evaluation of The Silk Fibroin Gel

The formulated silk fibroin gel was subjected to evaluation for the following parameters.

Texture Evaluation

Stickiness and grittiness represents the texture of the gel. The formulation was evaluated for the same by mildly rubbing between two fingers.



pH Of The Oral Gel

Silk fibroin produces gel in acidic pH. The pH of the formulation was adjusted to 5.0 using citric acid. pH of the formulation has influence on stability as well as on the taste of the formulation. The pH of the oral gel was measured using Digital pH meter at room temperature.

Syneresis

The contraction of gel on storage and separation of water from it is known as syneresis. Syneresis is observed more often in the gels where low concentration of the gelling agent is used. All the formulations were kept under observation for signs of syneresis for 90 days during storage at $5 \pm 3^{\circ}\text{C}$. The formulations free from signs of syneresis were considered for further studies.

Drug Content

The prepared formulations were analyzed for drug content by taking 5 ml of gel in 100ml volumetric flask and the volume was adjusted with pH 1.2 HCl buffer. Solution was kept for 1 hour after vigorous shaking and filtered to remove the gel material. 4 ml above solution was transferred into 10 ml volumetric flask and volume was adjusted with pH 1.2 HCl buffer. Absorbance was measured at 241 nm by UV spectrophotometer. The formulations comply with the test if not more than one of values thus obtained is outside the limits of 85-115% of the average value and none is outside the limits 75-125%.

Drug - Excipient Compatibility Studies

DrotaverineHCl and the silk fibroin were subjected for FTIR spectroscopic analysis, to ascertain possible interaction between them.

Determination of Viscosity

The viscosity of all the formulations was measured using Brookfield DV II+ viscometer using spindle no: 94 at 50 rpm at room temperature.

In Vitro Release Study

In vitro dissolution studies were carried out using dissolution apparatus (USP XXII, apparatus type I) with 900 ml of pH 1.2 HCl buffer as dissolution medium maintained at $37 \pm 0.5^{\circ}\text{C}$. Gel equivalent to 40 mg of DrotaverineHCl (one dose) was placed in basket and stirred at 100 rpm. Aliquots were withdrawn at regular intervals (5 min) of time and replacing the same volume with pH 1.2 HCl buffer. The absorbance of the sample solution was measured at 241 nm after suitable dilutions using UV spectrophotometer.

Stability Studies

Stability is defined as the ability of particular drug or dosage form in a specific container to remain within its physical, chemical, therapeutic and toxicological specification. Drug decomposition or degradation occurs during stability, because of chemical alteration of the active ingredients or due to product instability, leading to lower concentration of the drug in the dosage form. The stability of pharmaceutical preparation should be evaluated by stability studies.

Formulation F2 was stored in refrigerator between the temperature ranges of $5^0 \pm 3^0$ C for 90 days. Oral gel was observed for pH, viscosity, appearance and drug content at an interval of 15 days.

RESULTS AND DISCUSSION

Table 2: Evaluations of the oral gel formulations

Test parameters	Formulations					
	F2	F3	F4	F5	F6	F7
Texture	NS and NG	NS and NG	NS and NG	NS and SG	NS and SG	NS and SG
pH	5.02	5.04	5.01	5.06	5.02	5.05
Syneresis	No	No	No	No	No	No
Drug content (%)	99.52	99.54	99.42	99.14	99.22	98.77
Viscosity (cps)	8234	9545	11565	14940	17679	20646

NS: non sticky, NG: non gritty, SG: slightly gritty, cps: centipoises

The following results (Table.2) were obtained after the evaluation of the oral gel. The formulation containing 1% silk fibroin (F1) did not form complete gel of required consistency and shown signs of syneresis. It was rejected and not considered for further studies.

Appearance

Tartrazine was used to match the yellow color of the DrotaverineHCl in the formulation. All the formulations were light yellow colored and translucent in appearance.

Texture evaluation

Formulation F2, F3 and F4 were Non-sticky and non gritty. Formulation F5, F6 and F7 were non sticky but slightly gritty. The non gritty nature of F2, F3, and F4 may be due to suitable concentration of silk fibroin and slightly gritty nature of the F5, F6 and F7 may be due to higher concentration of silk fibroin.

pH

pH of the formulation has influence on stability as well as on the taste of the formulation. Silk fibroin produces gel in acidic pH and it was adjusted using citric acid. All the formulations produced gel at pH 5.0.

Syneresis

There was no sign of syneresis in any of the formulations during the storage period of 90 days. This might be due to right concentration of silk fibroin and gelatin. Formulation (F1) containing low concentration of silk fibroin showed the syneresis and had no required consistency. It was rejected and not considered for further studies.

Drug Content

Drug content of all the formulations were around 99%, which is well within the acceptable limits.

Drug - Excipient Compatibility Studies

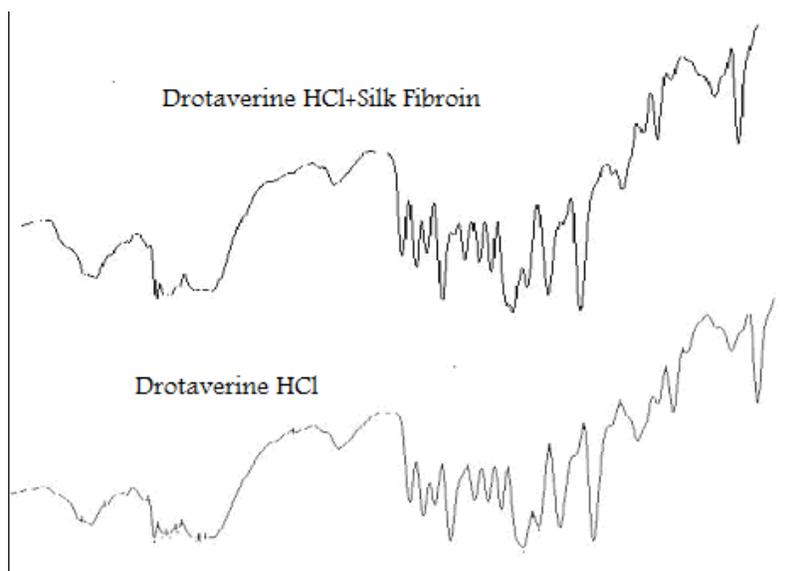


Fig.1: Compilation of FTIR spectra of DrotaverineHCl and DrotaverineHCl + Silk fibroin

From the FTIR data (Fig.1), it was observed that similar characteristic peaks with minor differences were present indicating that there was no chemical interaction between the drug and polymer. Hence it can be concluded that the drug has no interaction with silk fibroin.

Viscosity

Viscosity is the one important parameter which provides vital information during the optimization of the silk fibroin gel. There was increase in viscosity with the increase in concentration of silk fibroin in the formulation (Fig.2). The increased concentration caused more inter protein hydrogen bonding and formation of thick three dimensional structure of the silk fibroin gel. This proved the excellent gelling ability of the silk fibroin at pH 5. Viscosity of the formulation F2, F3 and F4 were near to the in house specification. This may be due to the right concentration of silk fibroin in the formulation. Formulation F5, F6 and F7 were thick and slightly gritty. This may be due to higher concentration of silk fibroin in the formulation. The effect of gelatin and other ingredients on the viscosity was same in all the formulations because their concentration in all the formulations was same.

Fig.2: Viscosity of the formulation

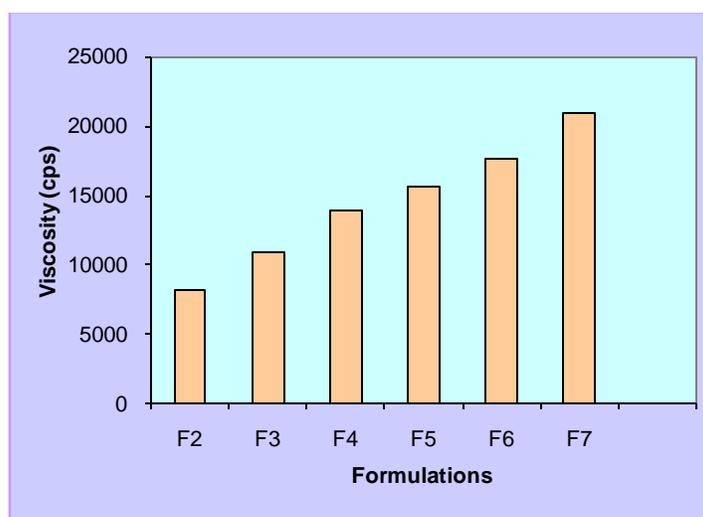
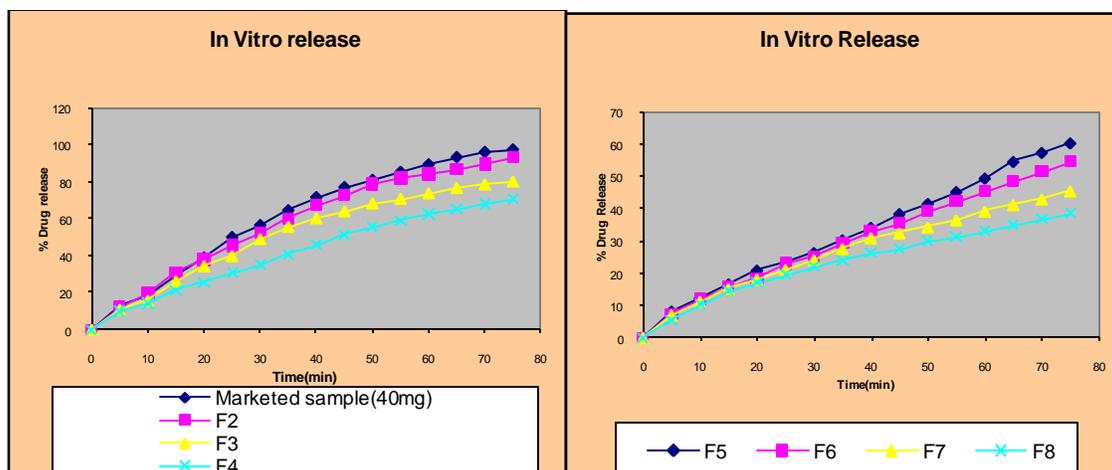


Fig.3: In vitro release profile of Marketed sample and formulations F2-F8



In vitro drug release

The releases of drug from formulations F2 to F7 were compared with the marketed tablet of DrotaverineHCl 40mg. It was found that release from formulation coded F2 was comparable with the marketed tablet. It was also observed that with the increase in concentration of silk fibroin in the formulation, decreased the drug release (Fig.3). Order of drug release from the formulations was F2>F3>F4>F5>F6>F7

This was due to increased concentration of silk fibroin caused more inter protein hydrogen bonding and formation of thick three dimensional structure of the gel. This could possibly prevent the drug to come out easily from the gel.

Stability Studies

Table.3: Stability studies of the oral gel

Sampling Interval	% Drug content	pH	Viscosity
	5 ⁰ C±3 ⁰ C	5 ⁰ C±3 ⁰ C	5 ⁰ C±3 ⁰ C
15 Days	98.902 ± 0.21	5.02	8284
45 Days	98.354± 0.24	5.04	8288
60 Days	97.802 ± 0.36	5.03	8290
75 Days	97.754 ± 0.42	5.05	8294
90 Days	97.710 ± 0.89	5.04	8295

*Standard deviation n=3

Results of short-term stability studies (Table.3) of the optimized formulation F2, indicated insignificant changes in pH, viscosity and drug content. Precipitation and syneresis were not observed in any of the samples during the study. There was no change in appearance of the gel.

If formulation is stored at room temperature, putrefaction may result. Therefore it is recommended that oral gel should be stored in refrigerator at the temperature range of 2-8⁰C.

CONCLUSION

From the above results it was found that the formulation F2 showed acceptable texture and viscosity. The percentage drug release was above 90% in 75 minutes. The formulation was stable when it is stored between the temperature ranges of 2-8⁰C. Consistency of the gel was sufficient enough to take it in the teaspoon. It can be concluded that novel dosage form can be formulated using silk fibroin gel, which can ease the administration of medicaments in dysphagic patients.



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