Original article

The pharmaceutical applications of a biopolymer isolated from *Trigonella foenum-graecum* seeds: Focus on the freeze-dried matrix forming capacity

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A R T I C L E   I N F O

A B S T R A C T

The aim of the present study was to evaluate the funtion of fenugreek seed mucilage (FSM) as potential matrix forming agent for orodispersible pharmaceutical lyophilisates. The FSM was isolated and characterized. FSM colloidal dispersions were prepared and the rheological evaluation was performed. Oral lyophilisates (OLs) with different FSM concentrations, containing meloxicam as model drug were prepared by freeze drying method. The OLs were characterized and compared to gelatin containing tablets, prepared under the same conditions.

The FSM dispersions revealed shear thinning flow type. Based on colloidal dispersions' rheological properties, five FSM concentrations were taken forward to the lyophilization step. Completely dry and elegant tablets were obtained. Texture analysis indicated highly porous structures, confirmed by SEM analysis, which explain the fast disintegration properties. All the prepared tablets disintegrated in less than 47 s. The disintegration process was prolonged by the increase in FSM content, due to the high viscosity the polymer creates in aqueous media. FSM tablets presented longer disintegration times, as compared to gelatin tablets, but also higher crushing strength. Considering the fast disintegration and the high crushing strength, FSM is a good candidate as matrix forming agent for fast disintegrating dosage forms or other freeze-dried preparations.

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1. Introduction

Orally dispersible tablets are solid dosage forms that quickly disintegrate in the oral cavity, without the need of water for swallowing. Several techniques were used to obtain ODTs, having as common objective the production of porous or low strength matrices, which would easily disintegrate in aqueous media. Among these, lyophilization delivers high porosity matrices, called oral lyophilisates (OL), which disintegrate in the mouth in a few seconds (AlHusban et al., 2011, Lai et al. 2011, 2014). In recent years, many of the freeze-drying studies have focused on finding and testing excipients that could provide convenient mechanical strength, fast drying rates, long stability in time and fast dissolution of the active principle (Kasper et al., 2013). Actually, the greatest challenge concerning oral lyophilisates formulation involves finding proper types and ratios of excipients that could achieve simultaneous fast disintegration and high mechanical strength. The matrix forming agents are excipients that determine both the disintegration capacity and the mechanical strength. Several polysaccharides like xanthan gum, dextran and alginic acid salts

and protein derivatives like gelatin were studied for this purpose (Prajapati et al., 2013).

On the other hand, lately, many novel natural polymers had drawn the attention of the scientific community for the diverse panel of pharmaceutical applications such as binders, disintegrants, thickeners, gelling agents, being used for the development of sustained release dosage forms, mucoadhesive dosage forms, suspensions, emulsions and gels (Prajapati et al., 2013). The fenugreek (*Trigonella foenum graecum*) seed mucilages and gums consist of polysaccharides - galactomannans, namely linear chains of $(1 \rightarrow 4)$ linked β-D-mannose, with single units of α-D $(1 \rightarrow 6)$ D-galactose attached as side chains (Chang et al., 2011, Mishra et al., 2006, Youssef et al., 2009). The galactomannans differ by the mannose:galactose (M/G) ratio, from 1.54 for guar gum, to 3.75 for locust bean gum. Apparently, the one extracted from fenugreek presents the highest percentage of galactose, with a M/G ratio close to 1, which determines its high water solubility (Doyle et al., 2009, Kamble et al., 2013). The polysaccharides isolated from fenugreek seeds have been studied for their disintegrant properties in ODTs (Kumar et al., 2009), mucoadhesive properties (Datta and Bandyopadhyay, 2005, Nayak et al., 2013, Nayak and Pal, 2014) and suspending capacity (Nayak et al., 2012). They have also been mentioned in several studies for their hypoglycemic effect (Kamble et al., 2009, Kumar et al., 2005) as well as for antioxidant and anti-inflammatory actions (Sindhu et al., 2012).

Up to date, there has been no report in the literature on the development of a freeze dried dosage form, using the fenugreek seed mucilage (FSM) as matrix former agent. Therefore, the present study aimed to prove the utility of FSM as matrix forming agent at the manufacture of OLs, having meloxicam as model drug and mannitol as cryoprotectant.

Meloxicam is an oxicam derivative, part of the non-steroidal anti-inflammatory drug (NSAID) group, used for the treatment of rheumatoid arthritis, osteoarthritis and postoperative pain. Considering the conditions for which is prescribed, the fast onset of effect could be helpful. In addition to that, the target population is often consisting of elderly, who may also suffer of disphagia; both hypothesis justify the formulation of OLs containing meloxicam, which could provide fast disintegration and increase patient compliance (Loza, 2008, Ochi et al., 2014).

The first stage of the study concerned the isolation and characterization of the mucilage obtained from indigenous cultures of fenugreek. Secondly, colloidal dispersions with various concentration of FSM were prepared and their rheological behavior was tested. Considering the results, the experiment went further to the optimization of the freeze drying process and then to the preparation of tablets. The variable factor in the tablet formulation was the FSM concentration. In order to be able to state objectively the FSM's properties as matrix forming agent, the tablets containing FSM were compared to tablets containing gelatin – the standard polymer used as matrix forming agent in OLs - prepared under the same conditions.

2. Material and methods

2.1. Materials

Meloxicam (Uquifa SA, Barcelona, Spain) and mannitol (Pearlitol 200M, Merck, Germany) were used. Fenugreek seed mucilage was isolated from fenugreek seeds that were purchased from a local plant material producer. All other chemical reagents used were analytical grade. Xylose, Arabinose, Fructose, Glucose, D-Galactose (Gal) and D-Mannose (Man) were purchased from Fluka and trifluoroacetic acid from Merck, Germany.

2.2. Isolation and characterization of FSM

2.2.1. Isolation of FSM

FSM was isolated according to a literature reported method (Nayak et al., 2013). Briefly, 100 g of fenugreek seeds were soaked in distilled water (0.75 l) for 12 h at room temperature and then boiled until the formation of slurry. The slurry was kept in the refrigerator for another 12 h, the upper clear solution was decanted and concentrated on water bath to 1/3 of its original volume. The solution was cooled at room temperature and then it was poured into acetone, under continuous stirring. The precipitate was washed several times with acetone, then dried at room temperature and kept over sillica gel, in desiccators until further use.

2.2.2. HPLC analysis of monosaccharides

1.0348 g of FSM was hydrolyzed with 20 ml trifluoroacetic acid (TFA) 6M, for 6 h in a sealed glass tube. After evaporation for complete removal of TFA, the hydrolysate was dissolved in distilled water then assessed for monosaccharide composition using a Shimadzu Prominence system (Kyoto, Japan) equipped with a refractive index detector (model RID 10A). Monosaccharides were separated isocratically at 80 °C, using a Shodex SP-0810 column $(80 \times 300$ mm) and water (Millipore, Bedford, MA, USA) as eluent. The injection volume was 20 μl. A monosaccharide standard mixture consisting of xylose, arabinose, mannose, fructose, glucose and galactose was used at a concentration between 2 and 10 mg/mL in order to get the equation of calibration curve. Monosaccharides were quantified from peak area measurements using response factors obtained with standard monosaccharides.

2.2.3. Preparation of colloidal dispersions

Colloidal dispersions, with concentrations of 0.01%, 0.02%, 0.05%, 0.1%, 0.2%, 0.25%, 0.5%, 0.75%, 1%, 1.25%, 1.5% and 2% (w/w) were prepared. FSM was hydrated with distilled water for 30 min, at room temperature, maintained in water bath at 50 °C, for 2 h and kept under stirring at 1000 rpm, for 30 min, until complete homogenization.

2.2.3.1. Determination of the polymer size, Zeta potential and conductivity of the FSM colloidal dispersions. The average size measurement of polymers in dilute FSM dispersions was performed by dinamic light scattering, at a backscattering angle of 90°, using a Malvern Zetasizer Nano ZS. The size measurements were performed on dilute FSM dispersions, with concentrations ranging from 0.01% to 0.2% (w/v). At least 3 measurements, at 25 °C, were done for each dispersion and the average size was calculated.

2.2.3.2. Determination of the rheological behavior of FSM colloidal dispersions. The rheological behavior was evaluated using the Brookfield DV III Ultra viscosimeter on the concentrated dispersions, with FSM content starting from 0.25% to 2% (w/w). The viscosity was measured at increasing rotation speeds, from 0.3 rpm to 100 rpm, then decreasing to 0.3 rpm again. All measurements were done in triplicate, at room temperature and the plots for dynamic viscosity vs. shear stress were recorded. For a better characterization of the rheological behavior, Power-law model was used, according to Eq. (1):

$$\tau = K\gamma^n \tag{1}$$

where τ is the shear stress (mPa), γ is the shear rate (s^{-1}), K is the consistency index (mPasn) and n is the flow behavior index (dimensionless). In order to calculate K and n values, log τ vs. log γ were plotted and K was determined as the resulting straight line's intercept, while n was the slope of the resulting line.

2.3. Suspension preparation

Mannitol 5% (w/v) was dissolved in each of the 5 chosen dispersions: 0.5%, 0.75%, 1%, 1.25% and 1.5% (w/w). 0.45 g of meloxicam was suspended in the obtained mixtures, yielding a content of 7.5 mg meloxicam in 0.5 ml suspension (Table 1).

2.3.1. Primary drying temperature optimization

Differential scanning calorimetry (DSC 822e system, Mettler Toledo) was used to determine the glass transition temperatures (Tg') and the crystallization event of the formulations in its frozen state (before freeze drying). From each liquid formulation (Table1), 15–25 mg was loaded into aluminium pan with pierced lid, cooled from 25 °C to −55 °C, at a rate of 10 °C/min and then heat back to 25 °C at 20 °C/min. This cycle was repeated three times. The glass transition temperature was evaluated using STARe software (v.12.0).

2.3.2. Freeze-drying process

0.5 ml of each homogenous suspension were transferred in 50 blister sockets and lyophilized using Virtis Advantage Plus (SP Scientific, Gardiner, USA) freeze dryer, by an automatic cycle (Table 2). An annealing step was applied before the drying step.

2.3.3. Pharmaceutical characterization of tablets

2.3.3.1. Texture analysis.
The mechanical properties of the orodispersible tablets were determined by texture analysis, using a Brookfield TexturePro CT V1.5 texture analyzer (Brookfield Engineering, USA). The freeze-dried tablets were removed from the blister sockets, and compressed with an acrylic probe (TA10), to a constant deformation of 80%, at a rate of 0.05 mm/s and a trigger load of 10 g. The results were presented as load (N) vs. distance (mm) curves, as average values for three replicate determinations.

2.3.3.2. In vitro disintegration time.
The in vitro disintegration time was determined according to the European Pharmacopoeia 8.0 (Eur.Ph.8.0) method, for six tablets. The tablets were placed one at a time, in Erlenmeyer containing 200 ml distilled water kept at 20 ± 0.5 °C. The time necessary for the complete disintegration,

until no solid residue was seen, was recorded, using a digital stopwatch. The average disintegration time and standard deviation were calculated.

2.3.3.3. Wetting time and water absorption ratio.
In order to measure the wetting time, two pieces of tissue paper were placed in a Petri dish containing 4.5 ml distilled water. The tablet was placed on top of the tissue paper and the time until the dissolution media reached the upper surface of the tablet was recorded. The tablets used in the wetting time test were weight before (W1) and after the test (W2) and the water absorption ratio was calculated according to the following equation: $r = 100*(W2-W1)/W1$.

2.3.3.4. In vitro dissolution studies.
In vitro dissolution studies were performed according to the Eur.Ph.8.0 using the paddle method. The dissolution media was 900 ml phosphate buffer pH 7.4, kept at 37 °C and a rotation speed of 50 rpm. At predetermined time intervals of 5 10, 15, 20 and 30 min, 5 ml samples were withdrawn and replaced with the same volume of fresh media. The samples were filtered and the amount of dissolved drug was determined spectrophotometrically, at 360 nm using a UV/VIS spectrophotometer Specord Plus (Anallytik Jena, Germany).

2.3.4. Solid state analysis

2.3.4.1. Differential scanning calorimetry.
Differential scanning calorimetry (DSC) was used to evaluate the thermal behavior of the samples (meloxicam, FSM, mannitol and 1% FSM OLs – formulation C). 4–6 mg of the individual samples and their binary mixtures, as well as from the OLs were sealed in aluminium pans and heated in the differential scanning calorimeter (Mettler-Toledo, GmbH, Switzerland) under a dynamic nitrogen flow from 20 to 400 °C, at a rate of 10 °C/min, under dynamic nitrogen atmosphere at a flow of 80 mL/minute, using an empty aluminium pan as a reference. Temperature was calibrated using indium (melting point of 156.6 °C) as standard. The thermal events were evaluated using STARe software (v.12.0).

2.3.4.2. FT-IR analysis.
FT-IR spectra of the samples (meloxicam, FSM, mannitol and 1% FSM OLs – formulation C) were obtained

Table 1
The composition and Tg' of the suspensions prepared for freeze drying.

Components	Formulations						
	A	B	C	D	E	F	G
Meloxicam (g)	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Mannitol (g)	1.50	1.50	1.50	1.50	1.50	1.50	1.50
0.50% FSM dispersion	q.s.	–	–	–	–		
0.75% FSM dispersion	–	q.s.	–	–	–		
1% FSM dispersion	–	–	q.s	–	–		
1.25% FSM dispersion	–	–	–	q.s.	–		
1.50% FSM dispersion	–	–	–	–	q.s.		
1% Gelatin dispersion	–	–	–	–	–	q.s.	–
5% Gelatin dispersion	–	–	–	–	–	–	q.s.
Final volume (ml)	30	30	30	30	30	30	30
Tg' (°C)	−25.66	−26.62	−29.29	−29.90	−29.55	−20.77	−20.25

q.s. = quantum satis – completed as necessary until the final volume.

Table 2
Freeze drying and annealing processes parameters.

Step	Thermal treatment			Primary drying		
	Temperature (°C)	Time (min)	Ramp/Hold (R/H)	Temperature (°C)	Time (min)	Vacuum (mTorr)
1	−55	120	H	−20	1200	300
2	−12	180	H	Secondary drying		
3	−55	120	H	+5	600	50

on a Jasco FT-IR 4100 using the attenuated total reflection technique, on a ZnSe ATR crystal. The spectra were recorded between 4000 and 600 cm^{-1} at an optical resolution of 4 cm^{-1} and interpreted using the Jasco Manager 2 software.

2.3.4.3. Scanning electron microscopy (SEM) analysis. Fresh fracture surfaces of samples were sputter-coated with 7 nm Pt/Pd in an Agar Automatic Sputter Coater (Agar Scientific, UK) and images were recorded at 30 kV in a scanning electron microscope Quanta 3D FEG (FEI, USA).

2.4. Statistical analysis

Descriptive statistics was applied for all measured parameters, for all formulations. ANOVA test was performed, with a level of significance p = 0.05. A p-value below 0.05 was considered statistically significant. The sample size was n = 3 for all determinations, except for the disintegration time (n = 6). The results were presented as average value and standard deviation of all determinations.

3. Results and discussions

3.1. Isolation of the mucilage and HPLC analysis of Monosaccharides

The isolation yield of mucilage from fenugreek (*Trigonella foenum graecum*) seeds was 15.04% w/w. Previous studies reported slightly higher isolation efficiencies, which can be due to the variability between plants cultivated in different geographical areas (Kumar et al., 2009, Nayak et al., 2013).

From the total amount of analyzed FSM via HPLC (1.0348 g), 76.36% was hydrolysable, containing 41.66% galactose (Gal) and 34.69% mannose (Man), while 23.64% was non-hydrolysable material. The percentage of Gal in the hydrolyzate was 54.56%, while Man was 45.44%, meaning 1.2:1 ratio. Youssef et al. (2009) studied the composition of purified fenugreek gum, reporting a higher percentage of Man (41.57%) and less Gal (33.91%), a ratio of 1:1.23, from a total percentage of identified sugar of 75.48%; the disagreement of results could be due to structural differences between the linkage sequence of some of the monosaccharide residues, which will be the object of further analytical studies (Youssef et al., 2009).

3.2. Evaluation of the colloidal dispersions

Zeta potential, conductivity and Z-average diameter values are listed in Table 3, for each colloidal dispersion. The measurements were performed on dilute solutions, with concentrations ranging from 0.01 to 0.2% (w/v) FSM. Zeta potential is a measure of the stability of disperse systems. High absolute values (both positive and negative) indicate strong repulsion between macromolecules, which leads to good stability. On the contrary, low absolute values, usually lower than 30, show weak repellent forces and high aggregation tendency, thus the formation of unstable colloidal dispersions (Kaewmanee et al., 2014, Wang et al., 2010). All the 5 dispersions were negatively charged, with values between

−20.4 mV and −14.8 mV, exceeded by those reported by Haddarah et al. (2014), from −12.95 mV to −2.67 mV for the locust bean galactomannan. The results indicated that the mucilage dispersions were all unstable and there was no significant difference in Zeta potential values among each other (p > 0.05). However, FSM content increase determined a slight decrease of stability and a growing capacity to form macromolecular aggregates. This hypothesis was confirmed by the polymer sizes, that increased significantly (p < 0.05) from 761.13 nm in 0.01% (w/v) FSM solution, to 2801.33 nm in 0.2% (w/v) FSM solution. These results are in agreement with those obtained by Carneiro-da-Cunha et al. (2011), which showed increasing Z-average values for galactomannan polymers from different plant sources, from 2070 nm, in 0.2% (w/v) polymer dispersion, to 7467 nm, in 0.6% (w/v) polymer dispersion. This behavior can be explained by the neutral structure of galactomannans and the high number of available hydroxyl groups in its structure that determine polymer bonding (24). The conductivity was significantly influenced by the mucilage concentration increase (p < 0.05), ranging from 0.0248 mS/cm to 0.1703 mS/cm. This raise could be determined by the presence of electrolytes in the solution that can produce important changes in zeta potential values, with variations in the dispersion's stability, therefore their presence in pharmaceutical preparations should be rigorously controlled (Kaewmanee et al., 2014).

The flowing curves of the studied aqueous dispersions of FSM with concentrations ranging from 0.25% to 2% were plotted and shown in Fig. 1, representing the viscosity versus shear stress (up and down curves). The results showed that the viscosity decreased with the increase of the shear rate, without thixotropy.

The Power-law model coefficients and the viscosities recorded at 10 rpm are presented in Table 4. The viscosity values increased with the FSM content increase, from 32 mPa s for 0.25% FSM dispersion, to 12200 mPa s for 2% FSM dispersion. The parameters listed in Table 4 indicated that all the dispersions exhibited non-Newtonian, shear-thinning behavior, as evidenced by n < 1. The flow behavior index decreased with the FSM content increase, while the consistency index (K) increased, which indicates a maximum thickening effect at a FSM content of 2% (Wang et al., 2012). In order to use the FSM colloidal dispersions at the preparation and lyophilization of meloxicam suspensions, they had to be consistent enough to prevent meloxicam settlement and fluid enough to be easily and accurately poured into blister sockets, therefore, 0.5%, 0.75%, 1%, 1.25% and 1.5% FSM were chosen to continue the study with the freeze-drying step.

3.3. Primary drying temperature optimization

Before the solid dosage form preparation, the compatibility between meloxicam and the new excipient, FSM was tested and no incompatibility issues were revealed (results not shown).

Determination of glass transition temperature or eutectic temperature for crystalline components is important to establish the freeze drying temperature, in order to obtain tablets with a stable morphological structure and avoid collapse. Collapse phenomena were reported when drying at temperatures above the Tg', when the mobility in the frozen solutions increases with the breakage of the superior dried structure (AlHusban et al., 2010). Therefore, we measured Tg' for five suspensions with FSM and two with gelatin (Table 1). The FSM concentration increase determined a 4 °C Tg' drop. The lower the freeze-drying temperature is, the longer it takes to complete freeze-drying. The annealing process was shown to have an important effect on increasing the drying rate (Abdelwahed et al., 2006, Shi et al., 2013). It produces a system relaxation into a lower free energy configuration, this way avoiding the formation of metastable glass, which could affect the matrix stability by crystallization. In addition to that, it was reported that

Table 3
Polymer size, polydispersion index and Zeta potential.

FSM content (%)	Zeta Potential[*] (mV)	Conductivity[*] (mS/cm)	z-Average diameter[*] (nm)
0.01	−20.4 ± 3.20	0.0248 ± 0.0006	761.13 ± 64.89
0.02	−20.26 ± 0.97	0.0262 ± 0.0013	860.33 ± 59.25
0.05	−16.1 ± 0.62	0.0481 ± 0.0013	1139.33 ± 64.60
0.1	−14.8 ± 1.74	0.0922 ± 0.0001	1767.66 ± 36.14
0.2	−16.93 ± 0.58	0.1703 ± 0.0005	2801.33 ± 96.47

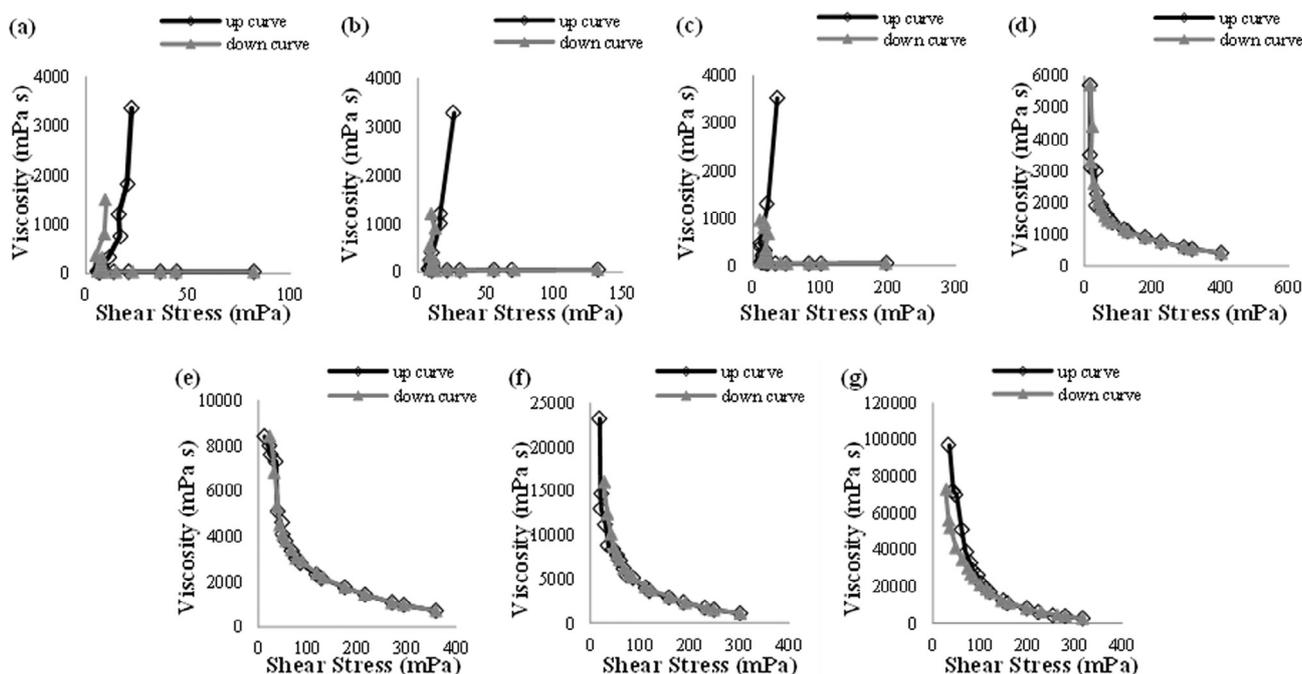Values represent means ± standard deviation from triplicate.

Fig. 1. The flowing curves of fenugreek seed mucilage (FSM) colloidal dispersions containing increasing FSM concentrations, as it follows: (a) 0.25% FSM, (b) 0.5% FSM, (c) 0.75% FSM, (d) 1% FSM, (e) 1.25% FSM, (f) 1.5% FSM, (g) 2% FSM. Values represent means of three determinations; for clarity, the error bars were not shown.

Table 4
Power-law model parameters and viscosity values for studied FSM colloidal dispersions.

FSM colloidal dispersion concentration (%)	Rheological parameters			
	K^* (mPa s^n)	n^* (dimensionless)	r^2	Viscosity at 10 rpm * (mPa s)
0.25	0.4181 ± 0.0155	0.9487 ± 0.0066	0.9761	32 ± 1
0.5	0.4212 ± 0.0442	0.9967 ± 0.0225	0.9631	42 ± 2.6457
0.75	1.1826 ± 0.1239	0.8337 ± 0.0181	0.9773	55 ± 1.7320
1	28.1190 ± 0.0656	0.5947 ± 0.0013	0.9942	1153.33 ± 5.7735
1.25	44.8339 ± 0.3133	0.5646 ± 0.0012	0.9936	2320 ± 0.00
1.5	62.1794 ± 0.5970	0.5137 ± 0.0042	0.9937	4096.66 ± 55.0757
2	134.039 ± 0.1083	0.3845 ± 0.0036	0.996	12200 ± 200

* Values represent means ± standard deviation from triplicate.

thermal treatment increases the pore size in the freeze-dried matrix, which is important in this particular case for both the improvement in the drying rate and fast disintegration. It involves holding the samples at a constant temperature above the measured *Tg'*and below the ice melting temperature (Abdelwahed et al., 2006, Ayensu et al., 2012). The annealing step during DSC testing consisted in cooling the sample to −55 °C, then heating back to −12 °C and maintaining constant temperature for 10 min, cooling again to −55 °C and finally warming to 25 °C. Thermal treatment had as result the disappearance of glass transition in the heating phase (Fig. 2), therefore it was included into the freeze-drying cycle, as shown in Table 2, and the primary drying was performed at −20 °C, instead of −30 °C, which should have been the maximum temperature of the product allowed the primary drying without an annealing step. The chosen freeze-drying cycle led to dry cakes, with no signs of shrinkage or collapse phenomena.

3.4. Characterization of OLs

The preparation of OLs requires the use of a matrix forming agent, that gives shape and hardness to the tablets. This study aimed to demonstrate that FSM in concentrations ranging between 0.5 and 1.5% has a matrix forming capacity. For this purpose, we compared the 5 OL formulations prepared with FSM with tablets containing the standard matrix former – gelatin, in concentrations of 1% and 5%. 5% gelatin is the usual content in ODTs and the 1% gelatin formulation (in the same range FSM) was considered, for a better comparison (Chandrasekhar et al., 2009). As cryoprotectant, mannitol 5% was used in all formulations. Mannitol content was chosen to be half of its solubility limit in water and it can be improved in further studies, in order to increase meloxicam solubility or mechanical strength. For low water solubility drugs, as meloxicam, a suspending agent is necessary, to provide high viscosity and maintain the stability of the suspension until the freezing step. In this particular study, the addition of a suspending agent was considered inappropriate, since the FSM colloidal dispersions present high viscosities, described in the previous chapter. So, FSM was expected to play both roles: matrix forming agent and suspending agent.

3.4.1. Physical characterization and mechanical strength

All formulations resulted in completely dried and elegant tablets, with light, porous aspect and pale yellow colored, due to meloxicam. They were all strong enough to handle, except for F tablets, which had to be manipulated with care, in order not to damage their structure. The weight and size are presented in Table 5. The settlement of meloxicam prior to complete freezing
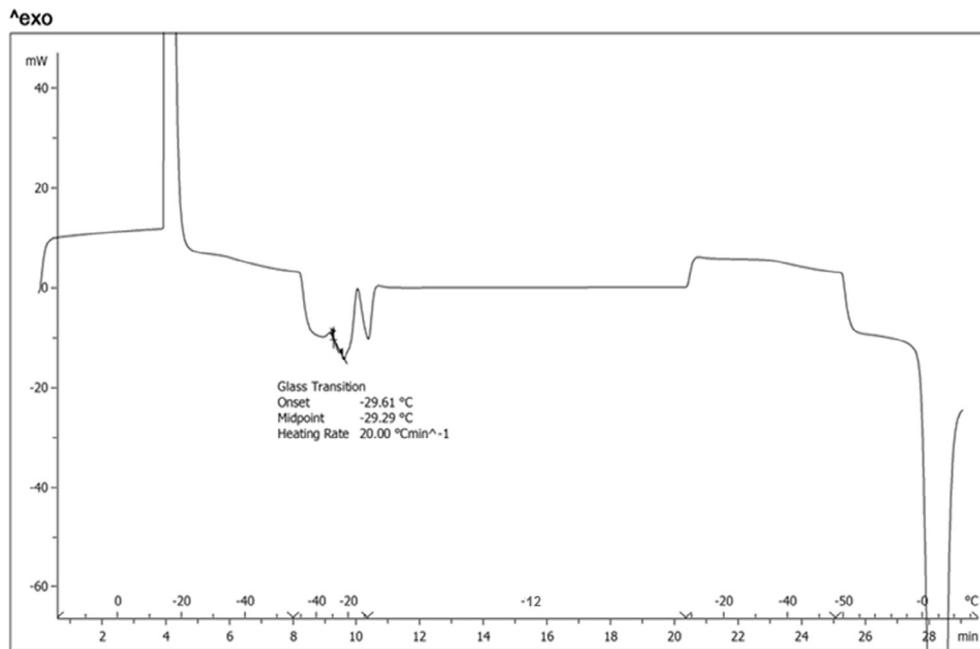
Fig. 2. DSC thermogram of meloxicam suspension containing FSM 1%, showing the effect of the annealing step.

Table 5
Physical characterization of oral lyophilisates (homogeneity, weight, diameter and thickness) and disintegration properties (disintegration time, wetting time, water absorption ratio).

Matrix forming agent	Content (%)	Formulation	Homogeneity[1]	Weight[2] (mg)	Diameter and thickness (cm)	Disintegration time[3] (s)	Wetting time[3] (s)	Water absorption ratio[3] (%)
FSM	0.5	A	–	30.25 ± 1.11	1.17 and 4.64	4.00 ± 0.89[a]	1.00 ± 0.00[a]	158.32 ± 3.24[a]
	0.75	B	–	41.6 ± 1.63	1.17 and 4.64	5.33 ± 0.41[b]	1.00 ± 0.00[a]	249.59 ± 18.63[b]
	1	C	+	41.8 ± 1.79	1.17 and 4.66	10.67 ± 4.03[c]	89.33 ± 4.81[b]	326.46 ± 41.52[c]
	1.25	D	+	44.1 ± 1.65	1.17 and 4.66	14.67 ± 3.72[c]	151.00 ± 20.95[c]	341.47 ± 32.91 c[d]
	1.5	E	+	38.6 ± 1.84	1.17 and 4.67	46.50 ± 6.29[d]	201.67 ± 26.50[c]	369.78 ± 24.18[d]
Gelatin	1	F	–	37.9 ± 0.91	1.17 and 4.58	2.50 ± 0.24[e]	1.17 ± 0.28[d]	25.55 ± 3.02[e]
	5	G	–	54.2 ± 1.39	1.17 and 4.62	3.50 ± 0.24[f]	2.00 ± 0.00[e]	9.24 ± 1.78[f]

Values in a column followed by different letters in superscripts show significant differences within the same column (p < 0.05).
[1] + = meloxicam has not settled at the bottom of the tablet, the aspect was homogenous; - = insoluble meloxicam has settled at the bottom of the tablet, as a yellow layer.
[2] Values of the weight of tablets represent the mean ± standard deviation (n = 20).
[3] Values represent the mean ± standard deviation (n = 3).

of the suspension was observed as a yellow layer on the bottom of the tablet, at formulations A, B, F and G. On the contrary, tablets C, D and E had a homogenous aspect. The lack of homogeneity can be explained by the low viscosity of the colloidal dispersion containing FSM 0.5% and 0.75% and gelatin 1% and 5%. On the contrary, the more consistent dispersions containing 1%, 1.25% and 1.5% FSM assured the suspension stability prior to freezing and led to homogenous tablets.

Among OL's specific characteristics is low mechanical strength. Water sublimation from the freezed suspension generates a porous matrix, whose hardness depends on the pore sizes, on their walls' thickness and elasticity. The mechanical properties were evaluated by texture analysis, which is an appropriate method to asses all the changes that appear during the crushing of a porous matrix. The load – distance curves were captured for each type of tablet and are shown in Fig. 3. Compression applying on the sample determined sharp drops in load, defined as fractures and caused by the breakage of the pore walls, explaining the jagged aspect of the curves (Harnkarnsujarit et al., 2012). Tablets type A and B gave resembling curves, with small fractures, while tablets C, D and E produced numerous fractures, with high differences between before and after the load drop. On the contrary, tablets F and G
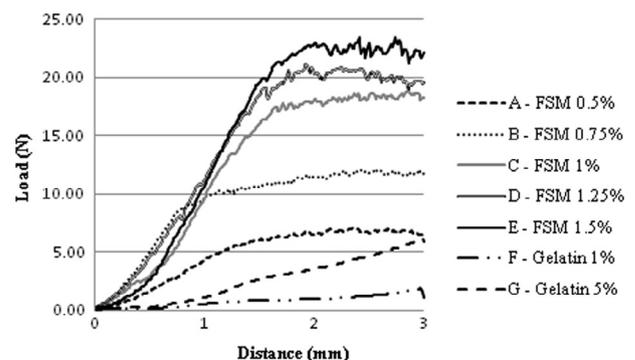

Fig. 3. Hardness profiles of the oral lyophilisates. Values represent means of three determinations; for clarity, the error bars were not shown.

gave smooth curves, with no visible fractures, indicating that wall breaking occurred at forces below the device's sensitivity. The hardness was calculated as the maximum load value of the compression cycle. For the tablets A, B, C, D and E, it increased with the FSM content, from 6.58 ± 0.6 N for tablets A, to 24.13 ± 0.7 N

for tablets E. Tablets F and G resisted to 1.8 ± 0.1 N and respectively 6.4 ± 0.2 N. Tablets F structure was so brittle, that they had to be handled with care in order to be correctly evaluated, without material loss. At the same matrix former concentration, the tablets containing 1% FSM displayed ten times bigger hardness than those containing 1% gelatin. The mechanical strength of tablets G raised at the value presented by tablets A, meaning that a content of 5% gelatin produces a matrix as resistant as 0.5% FSM.

3.4.2. Disintegration time

The main characteristic of OLs is their fast disintegration capacity in small volumes of liquid medium. Two types of determinations were performed, the disintegration test mentioned in Eur. Ph.8.0, in a large volume of water (200 ml) and the wetting time test, in a small amount of water, to better describe the tablets properties of hydration, water absorption, swelling and disintegration (Chandrasekhar et al., 2009). The disintegration and wetting properties of the seven formulations are summarized in Table 4. FSM appears to determine a very distinct disintegration profile as compared to gelatin, due to the physiochemical differences among the two. The FSM tablets (formulations A, B, C, D, E) had disintegration times ranging from 4 s to 46.5 s, that increased significantly with the raise of FSM percentage (p < 0.05). All the obtained values were below 3 min, they correspond to the Eur.Ph.8.0 demands and with one exception - formulation E, containing 1.5% FSM – they respect the FDA recommendation for a disintegration time shorter than 30 s. A sudden increase in disintegration time was observed at the tablets containing 1.5% FSM, probably due to the viscosity the mucilage creates in the liquid medium, its gelling properties and to the slow advance of the waterfront into the matrix.

On the other hand, formulations F and G presented faster disintegration, of 2.5 s for tablets containing 1% Gel and 3.5 s for those containing 5% Gel. The gelatin concentration increase led to a significant raise of disintegration time (p < 0.05), but with a much lower magnitude than FSM's concentration did. This effect was observed by other researchers as well, Shoukri et al. (2009) found that the increase in gelatin concentration determines a slower disintegration, because of the higher cohesiveness between the macromolecules and the formation of more stable gels. Formulations C and F, obtained with the same amount (1%) of the two matrix forming agents, exhibited significantly different disintegration times: 10.66 s for those containing FSM and 2.5 s for those with gelatin, due to the gelling capacity of the plant isolated polymer. The disintegration times reported in literature for gelatin OLs are slightly higher than the ones we obtained, between 5 s and 90 s, because usually it is associated with polyols, amino acids (Chandrasekhar et al., 2009, Shoukri et al., 2009) or carrageenan (AlHusban et al., 2010).

3.4.3. Wetting time and water absorption ratio

The previous results are sustained by the wetting time determinations (Table 4); for the formulations A,B,C,D and E, it increased while increasing the FSM percentage, from 1 s for 0.5% and 0.75% FSM, to 201.66 s for 1.5% FSM. Interestingly, for the formulations A and B, the wetting time is lower than the disintegration time, probably because of the cohesion forces between the hydrated macromolecules that need to be defeated for complete disintegration. On the contrary, the more concentrated formulations, C, D and E, exhibited a longer wetting time than disintegration time. That could be explained by the formation in a small volume of water of a concentrated and consistent colloidal dispersion that limits the further advance of the waterfront. The water absorption ratio was also significantly influenced by the FSM content increase (p < 0.05). It was observed that 1 % FSM tablets had high absorbing capacity, 326.46%, as compared to 1% gelatin tablets – 25.55%. The water absorption capacity of FSM in OLs was calculated at 26 g

water/1 g FSM, result supported by Chang et al. (2011) research, who found 2738 g water/100 g solid water holding capacity for fenugreek gum.

3.4.4. In vitro dissolution profile

Meloxicam release as a function of time from FSM and gelatin containing OLs is illustrated in Fig. 4. Gelatin and low concentration FSM OLs (tablets A, B and C) presented a fast disintegrating, vanishing effect from the dissolution media, while tablets D and E, due to the high viscosity created by the polymer, led to the formation of a slowly dispersing gel, with consequences on meloxicam release. After 5 min, the highest dissolved percentage was seen at tablets G, followed by tablets A and F. After 10 min, the dissolution was faster for tablets A, followed by tablets B, C and F, which exhibited percentages of dissolved drug higher than 80% after 30 min. The dissolution was favored by the presence of FSM at concentrations lower than 1%. Higher contents of FSM delivered tablets whose drug release was lower than 60% in 30 min. In the case of OLs containing FSM, the increase in polymer percentages led to prolonged dissolution, probably because of delayed disintegration and water absorption. The viscosity and stability of FSM dispersions were maintained at the dissolution test conditions (pH 7.4, 37 °C), as it is expected to happen even on a broader pH range (1–10.5), that could be partially reached in vivo (Prajapati et al., 2013). The formulations that contained low polymer percentage easily disintegrated and spread into the dissolution media, thus releasing the active ingredient, while the high FSM content tablets yielded thick gels with slow erosion and meloxicam release. As for F and G formulations, the higher gelatin percentage led to faster meloxicam dissolution.

3.5. Solid state analysis

3.5.1. DSC analysis

The thermogram of meloxicam (Fig. 5a) displayed a sharp endothermic peak at 262.89 °C (284.86 J/g), corresponding to its melting point, followed by an exothermic transformation of the substance. The DSC curve of mannitol displayed the melting endothermic event at 166.22 °C (340.70 J/g), while FSM presented dehydration phenomena between 43.12 °C and 143.66 °C (184.39 J/g). The thermogram for the OL containing 1% FSM (formulation C) showed a wide endothermic peak at 67.12 °C, corresponding to the dehydration, two sharp endothermic peaks that revealed the two mannitol polymorphs (α and β) at 143.85 °C (4.71 J/g), respectively 163.42 °C (19.92 J/g). The peak corresponding to meloxicam shifted to a lower temperature, 222.99 °C (1.73 J/g), due to the mixing of the drug with the excipients. The melting
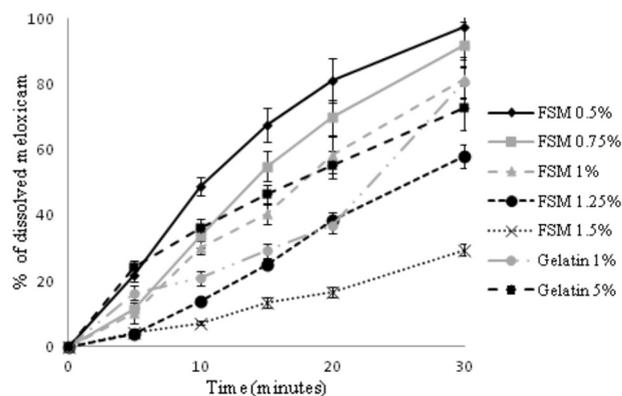

Fig. 4. Dissolution profiles of OL formulations containing different concentrations of FSM or Gelatin as matrix forming agents. Values represent means of three determinations.
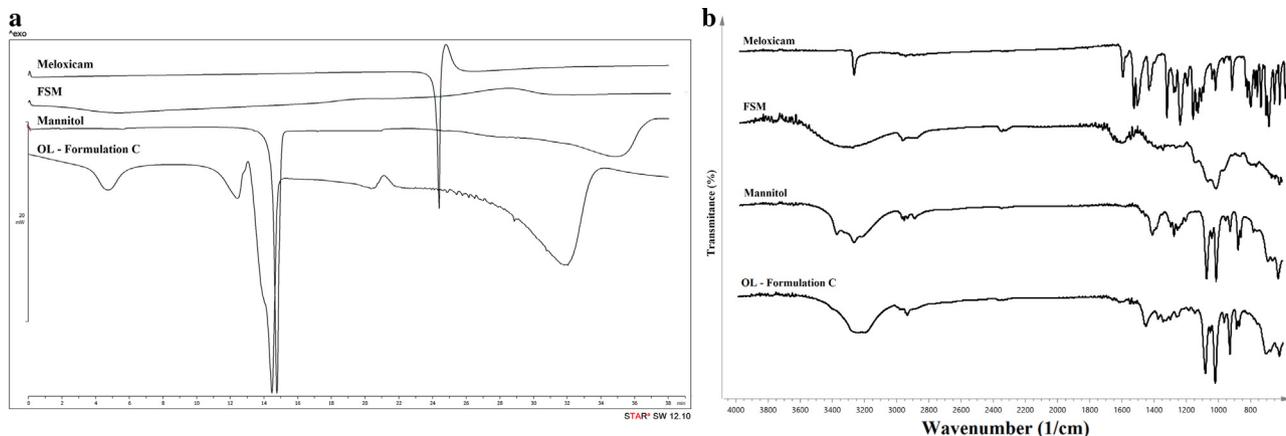
Fig. 5. (a) DSC curves showing the thermal behavior of meloxicam, FSM, mannitol and Formulation C containing 1% FSM, (b) FTIR spectra of meloxicam, FSM, mannitol and Formulation C containing 1% FSM.

enthalpy is lower than in the individual sample and the meting peak lost the sharpness showed in the individual substance scan, which can be explained by the low meloxicam percentage in the dry OL, namely 20% and by a partial loss of crystallinity during freeze-drying, that has previously been documented (Iurian et al., 2016).

3.5.2. FT-IR analysis

FT-IR spectra of meloxicam, FSM, mannitol and formulation C, meaning OLs containing 1% FSM are presented in Fig. 5b. The FT-IR spectrum of meloxicam exhibited distinct characteristic peaks at 3290 cm^{-1} (N—H), 1619.9 cm^{-1} (C=N), 1550.5 cm^{-1} (C—O), 1455 cm^{-1} and 1153 (S=O). The FSM spectrum showed a large band characteristic to —OH, from 3555 cm^{-1} to 3055 cm^{-1}, —CH at 2971.8 cm^{-1}, —CH stretching between 2939 cm^{-1} and 2831 cm^{-1}, ether linkage in the range 1455–1400 cm^{-1} and C—O stretching at 1016.3 cm^{-1}. Mannitol spectrum presented peaks at 1084.8 cm^{-1}, 1022.1 cm^{-1}, 932.41 cm^{-1} and 882.27 cm^{-1}. In the FT-IR spectrum of the OLs containing 1% FSM (formulation C), various characteristic peaks of the three composing substances, meloxicam, mannitol and FSM appeared without significant shifting or deviation. Therefore, the drawn conclusion is that the OLs containing FSM had significant characters of meloxicam, suggesting the absence of incompatibility between the drug and the excipients (Nayak et al., 2013, Nayak and Pal, 2014).

3.5.3. SEM analysis

Representative scanning electron micrographs of the lyophilized ODTs are shown in Fig. 6. The micrographs show the highly porous nature of the inner structure of tablets. The sublimation of the ice crystals led to the formation of heterogeneous pores, with diameters between 100 and 200 μm for formulations C and F, visibly decreasing to values below 100 μm, for formulation G, with higher amounts of polymer. Formulation F presents larger pores and thinner pore walls as compared to formulation C, which could explain the shorter disintegration and wetting obtained, but also the low mechanical strength. G tablet seemed to have a more regular structure, with small homogenous pores and thicker walls, which determined a slight increase in disintegration time and mechanical strength. Still, G tablet displays a network of elongated, cylindrical pores, a possible argument for its low mechanical strength compared to tablet C. On the other hand, the membrane's homogenous aspect, without any deposition on the fibers indicates that insoluble meloxicam was integrated completely within the polymer fiber, for FSM tablets where no sedimentation phenomena was noticed on the tablets. This might have contributed to adding extra support to the tablet structure and increasing its mechanical strength (AlHusban et al., 2010).

4. Conclusion

Fenugreek seed mucilage was isolated and tested for a novel application in the drug delivery field, as matrix forming agent at the preparation of oral lyophilisates. The FSM colloidal dispersions displayed shear-thinning behavior, with viscosities that increased with the FSM content increase. Their high consistency allowed the maintenance of suspension stability during the preparation process, with the obtention of homogenous OLs. The lyophilization
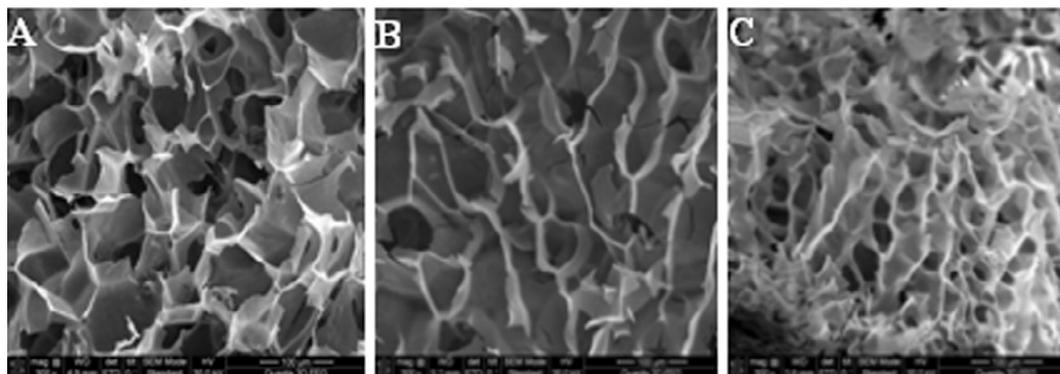

Fig. 6. SEM micrographs of oral lyophilisates: (A) tablet C – 1% FSM; (B) tablet F – 1% Gelatin; (C) tablet G – 5% Gelatin at 300x magnification.

cycle was established according to thermal analysis results and its validity was confirmed by the structure of the obtained OLs. The disintegration and water absorption properties were favored by a small FSM content, while the hardness increased at a high FSM percentage. When compared to the gelatin oral lyophilisates, the FSM tablets exhibited slower disintegration, but higher crushing strength.

A balance between fast disintegration and a mechanically strong matrix was seen with the formulation containing 1% FSM. Moreover, the polysaccharide's properties could be further improved by adding other excipients to reinforce the matrix or to hasten disintegration.

This study showed that FSM can act as a structure forming material at the preparation of lyophilized dosage forms. The results can be of high significance in the further development of freeze-dried matrices, since FSM could act like an alternative for the existing excipients.

The research could be extended from oral lyophilisates to a broader spectrum: mucoadhesive films, wound healing lyophilized products and the natural polymer could be used alone or in various combinations of matrix forming agents.

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