Physicochemical characterization and evaluation of suspending properties of arabinoxylan from Ispaghula *(Plantago ovata)* husk

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Abstract: The purpose of this study is to evaluate the use of arabinoxylan as potential suspending agent, an effective alternative to commercially used excipients for the preparation of pharmaceutical suspensions. Alkali extraction was done to separate arabinoxylan from ispaghula (*Plantago ovata*) seed husk by alkali extraction its physicochemical characterization was done and the suspending properties of arabinoxylan isolated were evaluated comparatively with those of bentonite at different concentration ranges of 0.125,0.25,0.5 and 1% in Zinc oxide suspension. The parameters employed for evaluation were sedimentation volume, degree of flocculation, flow rate, density, pH, redispersibility, microbiological evaluation and particle size analysis. Physicochemical characterization of arabinoxylan indicates its suitability as excipient as it has fair flow properties, low moisture content and almost neutral pH. Arabinoxylan at low conc. 0.125% showed sedimentation volume comparable to commercially used suspending agents such as bentonite 1% while suspensions containing higher concentrations such as 0.25% (sedimentation volume 92%), 0.5% (sedimentation volume 94%) and 1% conc. (sedimentation volume 98%) of arabinoxylan remained almost completely suspended during study period of 7 days. Formulations containing 0.125% and 0.25% arabinoxylan as suspending agents are easily redispersible as compared to bentonite containing formulation while formulation containing 0.5% arabinoxylan are moderately redispersible while formulation containing 1% suspending agent gel upon storage and was not redispersible. Furthermore arabinoxylan produces stable, highly flocculated suspension, which fulfilled microbiological, and particle size specifications, however the formulations containing higher arabinoxylan 1% concentration gel upon storage. So it is concluded that arabinoxylan could be used as effective suspending agent at low concentrations in Zinc oxide suspension.

Keywords: Arabinoxylan, *Plantago ovata,* seed husk, suspending agent, zinc oxide.

INTRODUCTION

Pharmaceutical dosage forms comprise of pharmacologically active compounds and certain inactive compounds called excipients. Excipients not only facilitate the manufacturing of the dosage form but also affect its certain properties like bioavailability and stability. Excipients are derived from three basic sources, natural, semi-synthetic and synthetic (Ryan *et al*., 2005).

Some of the naturally occurring swellable materials are *Plantago ovata*, *Mimosa pudica, Ocimum basilicum, Salvia aegyptiaca* and *Astragalus gummifer.* The major constituent of these biomaterials is polysaccharide. These naturally occurring biomaterials are being used as folk medicines from centuries back. These naturally occurring swellable materials in aqueous media could be useful as drug carrier due to their biocompatibility, bio-degradability (Saima *et al*., 2009).

Members of the plant genus *Plantago* are known by the common name Psyllium (Singh, 2007). The ispaghula husk is derived from the dried ripe seeds of *Plantago ovata* Forsk. From the past Ispaghula seed husk is used as dietary supplement to treat large bowel disorders. In some recent studies it has been found as blood cholesterol lowering agent (Moreyra *et al*., 2005). It has been used in folk medicines as demulcent and emollient (Ramkumar and Rao, 2005).

Arabinoxylans are linked covalently or non covalently to each other and other structural components in the cell wall matrix, and are insoluble in aqueous environment. Different methods have been reported for arabinoxylans isolation from various plant tissues. Aqueous and alkali extractions are the two most commonly used methods for arabinoxylan extraction from different plants (Fincher and Stone, 1974; Izydorczyk *et al.,* 1991; Faurot *et al,* 1995). The ability of natural polysaccharide to increase solution viscosity accounts for their use as suspending agents in oral pharmaceutical (Rao *et al.,* 2011). Arabinoxylans form highly viscous solutions and present work is to evaluate the arabinoxylan as potential suspending agent in Zinc oxide formulation.

MATERIALS AND METHODS

The materials used include zinc oxide (Merck), glycerol (Riedel-deHaen), benzoic acid (Merck), bentonite (Riedel-deHaen). *Plantago ovata* (ispaghula) seed husk was purchased from local market of Sargodha, Pakistan.

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Isolation of arabinoxylan

Arabinoxylan was isolated from the husk by method of (Saghir *et al.,* 2008). 100g of Ispaghula seed husk was soaked in 5 liters 0f distilled water over night. (2.5%) of aqueous NaOH solution was added to the mixture to adjust the pH at 12 and after stirring of two to three minutes the husk was separated from the gel by vacuum filtration. Sample was coagulated with concentrated acetic acid at pH 3. The gel obtained was washed several times with distilled water over a period of 2-3 days until the pH remained constant and freeze dried for 1 week. The yield of gel was about 45%.

Physicochemical characterization of arabinoxylan

Solubility test

Isolated arabinoxylan was tested for solubility in water, methanol, ethanol, chloroform, acetone, dimethyl-sulfoxide and sodium hydroxide (British Pharmacopeia, 2004).

Swelling Index

Swelling behavior of arabinoxylan was studied. It was allowed to swell for 24 h in distilled water for this, 1g of arabinoxylan was added in graduated cylinder then volume occupied by the arabinoxylan was measured, volume was made 100 with distilled water It was left overnight for swelling and swelling index calculated from the initial and final volume of arabinoxylan.

Loss on drying

The method used was a modification of that specified in the (British Pharmacopeia, 1994) for acacia. 1.0 g of the arabinoxylan was transferred into a petri dish and then dried at 105°C in an oven until a constant weight was obtained. Loss on drying is the difference of the weights of arabinoxylan before and after drying.

Total ash

Total ash was determined by the method of (United state Pharmacopeia, 2006). 2g of the arabinoxylan sample was transferred to a tarred crucible, and incinerated, gently at first, and the transferred to a graphite furnace and temperature was gradually increased to 675±25, ash was cooled in a desiccator and weighed. Then percentage of ash was determined as:

$$\frac{\text{Weight of Ash}}{\text{Weight of original sample}} \times 100$$

PH determination

pHof 1% w/v aqueous dispersion of arabinoxylan was determined using a pH meter (Jenway 3510) (Emeje *et al*., 2004).

Bulk density, Tapped density

Bulk density was measured by method I of (United State Pharmacopeia, 2006) according to this method 100g of arabinoxylan test sample passed through a sieve no 18 and then added in a dry 250-mL cylinder without compacting. Powder was leveled without compacting and apparent volume, Vo, to the nearest graduated unit was noted. Bulk density was then calculated in g per ml, by the formula:

(M) / (Vo)

Tapped density was measured according to method I of (United State Pharmacopeia, 2006) Arabinoxylan powder was passed into a dry 250-mL glass graduated cylinder (readable to 2mL) without compacting, apparent volume Vo was noted.

Cylinder was tapped initially 500 times by raising the cylinder and allowing it to drop under its own weight and tapped volume Va was noted. The tapping was repeated additional 750 times and noted the tapped volume, Vb. Vb is considered the final tapped volume Vf if the difference between two volumes is less than 2%. The tapped density was calculated in g per mL, by the formula:

(M) / (Vf)

Measures of arabinoxylan compressibility

The Compressibility Index and Hausner Ratio are the simple, fast and most commonly used methods for powder flow determination.

Compressibility Index is calculated by the formula:

$$\frac{100(v_o - v_f)}{v_o}$$

Hausner Ratio is calculated by the formula:

$$\frac{V_o}{V_f}$$

Angle of repose

The angle of repose, of arabinoxylan was measured according to the fixed funnel and cone method (Ohwoavworhua and Adelakun, 2005). According to this method graph paper placed on a flat horizontal surface and a funnel was clamped with its tip 2 cm above paper. The powder was carefully poured through the funnel until the cone thus formed just reached the tip of the funnel. The height (h), of the powder cone and the mean diameter (D), of the base of the powder cone was determined and the angle of repose calculated using the equation:

Tan □= 2h/D

Preparation of zinc oxide suspension

Bentonite powder 1gm and 20gm of zinc oxide were triturated together with 20ml of glycerin to form a smooth paste. Benzoic acid (0.1gm) was added. 50ml of chloroform water was added gradually. The mixture was transferred into 100ml volumetric flask; volume was made with distilled water and then shaken vigorously for 2 minutes. The procedure was repeated using different concentration of arabinoxylan 0.125g, 0.25g, 0.5g and 1g.

Evaluation of suspensions

The suspensions were evaluated for sedimentation volume, pH, density, particle size, flow rate, degree of flocculation, microbial load and redispersibility (Elijah *et al*., 2011; Ravi *et al* 2009).

Sedimentation volume

Sedimentation volume was measured by carefully introducing the suspension in a measuring cylinder of 100ml and observing rate of sedimentation after every 1 hour for seven hours and then after 24 hours for seven days.

Density and flow rate measurement

Density was measured using specific gravity bottle. The time required to flow through a 10ml pipette for each suspension sample was determined and the flow rate was calculated using the equation

$$\text{Flow Rate} = \frac{\text{Volume of pipette ml}}{\text{Flow time seconds}}$$

Determination of the pH and Redispersibility of the suspensions

At weekly intervals the pH of each of the prepared suspensions was measured using pH meter for four weeks. 10 ml of each suspension was poured into four calibrated tubes, and were stored at room temperature. At the end of storage period, each tube was inverted at ninety degree and no of inversions to redisperse the sedimented suspension was noted.

Particle size analysis

150 ml of distilled water was added into 200ml cylinder then 10 ml of each suspension was separately transferred into cylinder and mixed by shaking then 10 ml aliquot was removed at a distance of 10 cm below the surface of the mixture at fixed intervals of 1, 5, 10, 15, 20, 25 and 30 min. The aliquots were added to evaporating dish and evaporated to dryness in an oven at 105^{o} C and the residue so obtained was weighed. The particle diameter (d in cm) was then calculated using the Stoke's equation

$$d = \frac{18\text{nh}}{(ps - po)gt}$$

h is the distance of fall of the particle (cm)
t is the time (s)
ρ0is the viscosity of the dispersion medium (poise)
ρs – ρ0 is the density gradient between the dispersed particles and the liquid (gcm-3)
g is the gravitational constant (cms-2)

Degree of flocculation (β) Determination

The degree of flocculation was determined by forming flocculated suspensions with potassium dihydrogen-phosphate (0.004 mol) (Emit *et al*., 2010).

The degree of flocculation (β) was determined from the following equation:

β = (Vu) floc / (Vu) defloc

(Vu) floc sedimentation volume in the flocculated suspension
(Vu) defloc is the sedimentation volume in the deflocculated suspension.

Microbial load determination

The microbial load and/or growth in suspension formulations (containing 1.0% w/v of suspending agent) was evaluated on day 0 and on day 21 of storage by colony count method on nutrient agar media.

RESULT

The percentage yield of arabinoxylan isolated from *Plantago ovata husk* was found to be 45%. The arabinoxylan obtained was subjected to physicochemical characterization as shown in (table 1). Arabinoxylan was soluble in DMSO at 80°C and in aqueous NaOH (2.5%) it was insoluble in ethanol, methanol chloroform and acetone. The swelling characteristic of arabinoxylan was studied. The results show that it had higher swelling index (88%) of its original volume suggesting that it may perform well as matrixing agent, binder and disintegrant.) pH of 1% solution was 6.76, it had low moisture content with loss on drying value 0.1± 0.01% and flow characteristic designated the powder passable.

Table 1: Physicochemical characterization of Arabino-xylan from *Plantago ovata* husk

Parameters	Results
Solubility	Soluble in DMSO at 80°C and soluble in aqueous NaOH (2.5%) Insoluble in water, ethanol, methanol, chloroform
Swelling index in distilled water	88± 1%
Loss on drying	0.1± 0.01%
Bulk density (g/cc)	0.1 30± 0.003
Tapped density (g/cc)	0.174 ±0.013
Compressibility index	25
Hausners quotient	1.33±0.12
pH (1% Soln)	6.76 ± 0.03
Total ash	3.05 ± 0.03
Angle of repose	40.4 ± 1.17º

The sedimentation volumes measured for the Zinc oxide formulations at the different concentrations of suspending agents are shown in (fig. 1). Arabinoxylan at low conc. 0.125% showed sedimentation volume comparable to commercially used suspending agents such as bentonite 1% while suspensions containing higher concentrations such as 0.25% (sedimentation volume 92%), 0.5% (sedimentation volume 94%) and 1% conc. sedimentation volume 98%) of arabinoxylan remained almost completely suspended during study period of 7 days.

Effect of type and concentration of suspending agents on the flow rate and density of Zinc oxide suspensions is shown in (table 2). Density of Zinc oxide formulation was directly proportional to concentration of arabinoxylan while it was inverse in case of flow rate.

Table 2: Effect of type and concentration of suspending agents on the flow rate and Density of Zinc oxide suspensions

Suspending agents	Concentration (%w/v)	Flow rate (ml s-1)	Density
Bentonite	1	Too viscous to determine	1.27
Arabinoxylan	0.125	1.16	1.08
	0.25	1.06	1.16
	0.5	0.65	1.24
	1	Too viscous to determine	1.28

The pH of the Zinc oxide suspension formulations is shown in (table 3). The result showed that all the suspension formulations containing arabinoxylan had slightly acidic pH, which was stable over one month of observation, while bentonite containing formulations had slightly basic pH. Zinc oxide formulations containing 0.125% and 0.25% arabinoxylan were easily redispersible as shown in table 2, while formulation containing 0.5% arabinoxylan was moderately redispersible while formulation containing 1% arabinoxylan gel upon storage The microbial load and/or growth in suspension formulations containing 1.0 % w/v of suspending agent was evaluated on day 0 and on day 21 of storage and the results are recorded as the no of colony forming unit/ ml of formulation as shown in (table 5) all Zinc oxide formulations on day 21 have growth within the specification i.e.> 10^3 [USP, 1999]. Particle size analysis was done on day 30 of storage. All formulations had particle size > 5µm shown in (table 4) so these are free from grittiness.

DISCUSSION

Search for natural materials such as gums and mucilages as excepients is major research era now days. Physiochemical characterization of arabinoxylan was done and physiochemical properties of arabinoxylan justify its use as suspending agent in paracetamol suspension.

The presence of moisture in the material is undesirable, as it will lead to activation of enzymes and proliferation of microorganisms. The moisture content of arabinoxylan was low as calculated from its loss on drying value (table 1), suggesting its suitability as excipient in formulations containing moisture sensitive drugs. The knowledge of compressibility and flow indices is important for justifying the use of this material as an excipient in a pharmaceutical formulation. According to USP its flow is fair aid not needed (USP, 2006).

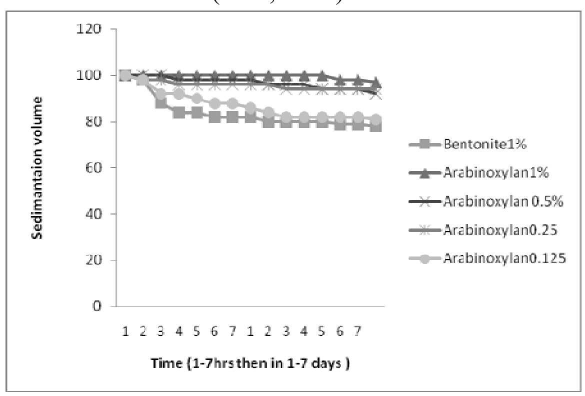

Fig. 1: Sedimentation volume of Zinc Oxide Suspension

Table 3: Determination of redispersibility and pH of Zinc oxide suspension

Suspending agent	Concentration %	Rate of redispersibility (cycles)				pH after storage			
		After 7 Days	After 14 Days	After 21 Days	After 28 Days	0th Day	7th Day	14th Day	21st Day
Bentonite	1	8	28	39	50	7.41	7.30	7.07	7.05
Arabinoxylan	0.125	6	11	19	22	6.70	6.66	6.80	6.82
	0.25	9	12	30	35	6.80	7.00	7.10	7.10
	0.5	11	50	82	147	6.71	6.82	6.96	6.96
	1	gel	Gel	gel	gel	6.84	6.88	7.00	7.03

Table 4: Particle size analysis of Zinc Oxide suspension

Formulation	Particle size in µm
Zinc Oxide suspension (1% Bentonite)	1.59 µm
Zinc Oxide suspension (1%Arabinoxylan)	1.58 µm
Zinc Oxide suspension (0.5 %arabinoxylan)	1.58 µm
Zinc Oxide suspension (0.25 %arabinoxylan)	1.58 µm
Zinc Oxide suspension (0.125 %arabinoxylan)	1.59 µm

Since the stability and physiological activity of most preparations are highly dependent on pH of an excipient so pH of arabinoxylan was determined (Luiz *et al*., 2005) pH of 1% solution is 6.76 (table 1) which is almost neutral so it may be used for formulation of both acidic and basic drugs.

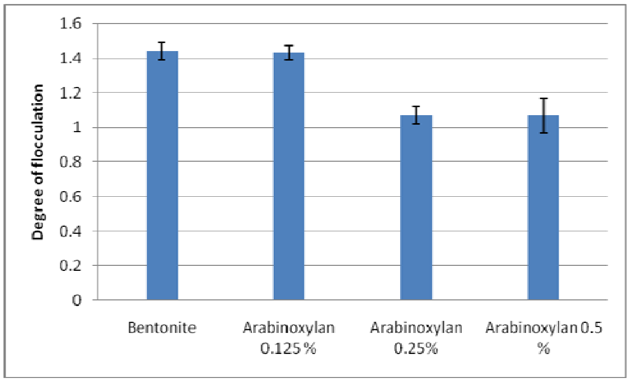

Fig. 2: Comparison of the degree of flocculation for Zinc Oxide suspension formulations containing bentonite 1% and arabinoxylan (0.5%, 0.25%, 0.125%)

In pharmaceutical suspensions, polymers play a vital role as flocculating agents with an advantage over ionic flocculating agents for their reduced sensitivity to added electrolytes; hence accommodating a wide range of excipients (Mahummad *et al*., 2010). Addition of arabinoxylan as suspending agent in Zinc Oxide formulations produce flocculated suspension so addition of flocculating agent $KMnO_4$ doesn't bring out a major change in flocculated formulations so have minimum degree of flocculation however the minimum concentration of arabinoxylan 0.125% have almost same degree of flocculation as formulation containing bentonite 1% as shown in (fig. 2).

Table 5: Microbiological evaluation of Zinc oxide suspension

Formulation	Probable number of bacteria per ml
Zinc Oxide suspension (1% bentonite)	□ 10^3
Zinc Oxide suspension (1% arabinoxylan)	□ 10^3

Arabinoxylan even at low concentration showed excellent suspending ability comparable to commercially used suspending agents as it showed low sedimentation rate, excellent redispersibility, and freedom from moisture contamination and grittiness and also maintained the stability of suspension.

CONCLUSION

From the results obtained, it is concluded that arabinoxylan from ispaghula (*Plantago ovata*) husk could be used as a suspending and flocculating agent in Zinc oxide suspension at low concentrations.

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