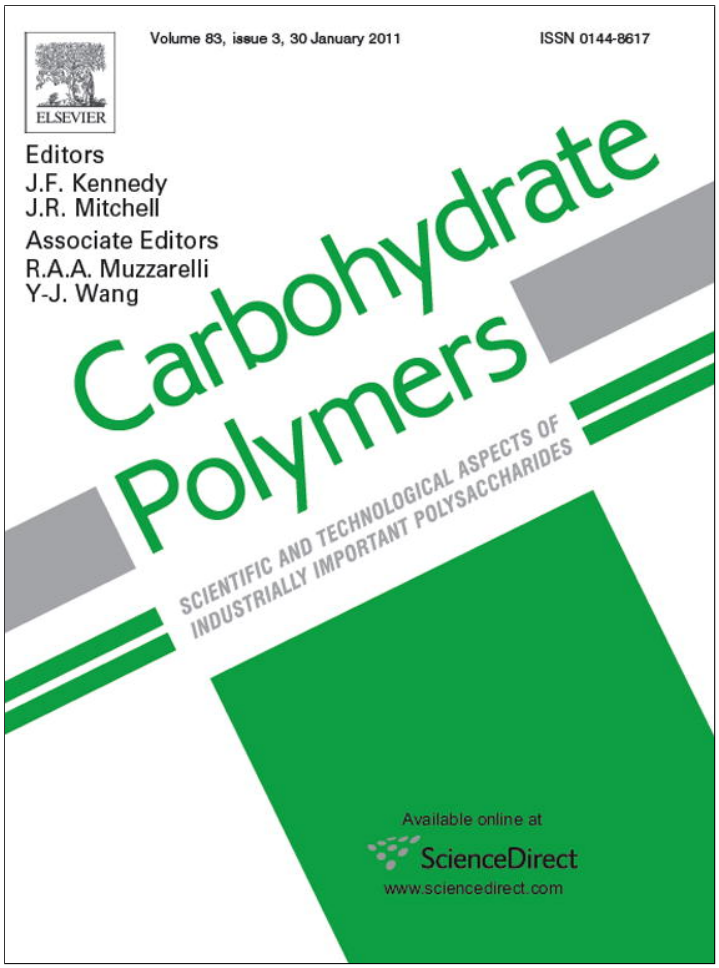

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Evaluation of hot-water extracted arabinoxylans from ispaghula seeds as drug carriers

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ABSTRACT

This work evaluates the potential of a hot water extracted arabinoxylan from ispaghula (*Plantago ovata*) seeds as a safe, effective and economical drug carrier. In thermal analysis integral procedural decomposition temperature and comprehensive index of thermal stability were found to be 308 °C and 0.47 respectively. Gel permeation chromatography showed the existence of two components in the fraction. Atomic force microscopy indicated a surface having nanostructure. The tablets incorporating caffeine and diclofenac sodium formulated by the use of arabinoxylan exhibited sustained release profiles in various media. A pH-independent release pattern was observed for caffeine, while for diclofenac sodium the profile was found to be pH-dependent. The arabinoxylan provided a better sustained release as compared with that of Voltral®, a standard diclofenac sodium preparation. The release data suggest Power Law an appropriate model for this system.

1. Introduction

Solvent-swellable materials have gained much interest for their use as controlled drug delivery materials. The controlled release devices range from simple oral, buccal and bioadhesive systems to advanced stimuli-sensitive and biomimetic hydrogel systems (Lin & Metters, 2006; Peppas, Bures, Leobandung, & Ichikawa, 2000). These materials absorb water on contact with biological fluid, swell, and release the incorporated drug by complex phenomena involving diffusion, dissolution and erosion, etc. The water-swellable materials are polymeric hydrogels. The hydrogels used as drug carriers may be natural or synthetic polymers. Natural materials are preferable over synthetics due to their greater biocompatibility and lower toxicity.

Among the several natural hydrogels, polysaccharide-based materials have been the focus of research for a long period of time. Some of these are: xanthan gum (Talukdar & Plaizier-Vercammen, 1993), karya gum (Sujja-areevath, Munday, Cox, & Khan, 1996), guar gum (Khullar, Khar, & Agarwal, 1998), tamarind seed polysaccharides (Sumathi & Ray, 2002), gum cordia (Mukherjee, Dinda, & Barik, 2008) and ispaghula husk (Bharadia & Gohel, 2006). The hydrogels from ispaghula (*Plantago ovata*) seeds or husk, isolated by various methods, are well characterized arabinoxylans (Fischer et al., 2004; Laidlaw & Purcival, 1949, 1950; Saghir, Iqbal, Hussain, Koschella, & Heinze, 2008). They have been reported to stabilize foams (Izydorczyk, Biliaderis, & Bushuk, 1991) and emulsions (Carvajal-Millán et al., 2007; Yadav, Johnston, Hotchkiss, & Hicksa, 2007). They also possess very good film-forming properties with films having comparable strength with those of hydroxypropyl methylcellulose, a widely used coating material in pharmaceutical tablets (Chanliaud, 1995). Arabinoxylan gels have potential applications for colon-specific protein delivery of other drugs (Niño-Medina et al., 2010).

Ispaghula seeds and husk are abundantly available throughout the world. Arabinoxylans can be isolated as fibrous hydrophilic materials at a very low cost. The husk is widely used for treatment of constipation (Bouchoucha, Faye, Savarieau, & Arsac, 2004; Ramkumar & Rao, 2005), diarrhea (Washington, Harris, Mussellwhite, & Spiller, 1998), ulcerative colitis (Fernandez-Banares et al., 1999) and hypercholesterolemia (Moreyra, Wilson, & Koraym, 2005; Rodriguez-Moran, Guerrero-Romero, & Lazcano-Burciaga, 1998). Isolated arabinoxylans have also shown their potential in reduction of serum cholesterol and improving adsorption of calcium and magnesium (Hopkins et al., 2003; Lopez et al., 1999). The husk itself has been used in formulation of several hydrogels. Physically modified (Gohel & Patel, 1997), succinic acid treated (Gohel, Amin, Chhabaria, Panchal, & Lalwani, 2000), tartaric acid treated (Gohel, Patel, & Amin, 2003) and polyacrylamide mod-

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ified husks (Singh, Chauhan, Sharma, & Chauhan, 2007a, 2007b; Singh et al., 2006; Singh, Chauhan, Kumara, & Bala, 2008) have been evaluated as controlled drug delivery agents. The seeds are in use in Unani medicine for various abdominal disorders. These are very cheap materials, for example, the seeds cost about US$ 0.5/kg in Pakistan. Although the use of the husk has been studied as formulation aid for sustained release preparations but no attempt has been made to evaluate the isolated arabinoxylan, the really useful material for the purpose. The aim of present study was to develop an economical process for extraction of arabinoxylan from ispaghula seeds, study its properties and evaluate its use in controlled drug delivery.

2. Materials and methods

2.1. Materials

The materials used in this study were: ispaghula seeds (local market); diclofenac sodium (a gift from Standpharm Pakistan (Pvt) Ltd., Lahore, Pakistan); caffeine (Serva Feinbiochemica GmBH, Germany); sodium acetate (E. Merck, Germany); hydrochloric acid (Riedel-de Haën, Germany); sodium hydroxide (Riedel-de Haën, Germany); potassium chloride (Fluka, USA); potassium dihydrogen phosphate (Fluka, USA); acetic acid (E. Merck, Germany). All the chemicals were used without further purification. Double-distilled water was used throughout this study.

2.2. Extraction of arabinoxylan from ispaghula seeds

Seeds (15 g) were washed with distilled water and transferred to a 2-L beaker containing distilled water (500 cm^3) and heated gently to boiling and kept at that state for 5 min. The solution became thick due to separation of a gel-like material from seeds. The mixture was allowed to stand till seeds settled down. The arabinoxylan was separated, air-dried (loss on drying 8–10%) for a week and powdered. Yield: 30% of the dry seed weight.

2.3. Characterization

2.3.1. Elemental analysis

Elemental (CHNS) analysis of the air-dried arabinoxylan was performed by the use of Vario Micro CHNS Analyzer (Elementar analysensysteme, Germany).

2.3.2. FTIR spectra

FTIR spectrum of the dry arabinoxylan was recorded on IR-prestige-20 spectrophotometer (Schimadzu, Japan) in the range 4000–400 cm^{-1} using KBr disc method.

2.3.3. Loss on drying

The loss on drying was determined by drying the isolated arabinoxylan (2 g) at 105 ± 2 °C as per British Pharmacopeia 2009 method.

2.3.4. Thermal analysis

Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were carried out with a simultaneous thermal analyzer QA-600 (TA instruments, USA) in the range ambient −600 °C, using nitrogen gas at a flow rate of 100 cm^3 min^{-1} and the heating rate was 10 °C min^{-1}. The activation energies (E_a) for each major stage of decomposition were calculated by Broido method (Broido, 1969) (Eq. (1)).

$$\ln \ln \left(\frac{1}{y}\right) = -\frac{E_a}{RT} + \text{constant} \quad (1)$$

where $y = (w_t - w_\infty)/(w_0 - w_\infty)$, w_t is the weight of the sample at any time t, w_0 the initial weight and w_∞ the final weight. Thermal stability of the isolated arabinoxylan was determined by determination of its integral procedural decomposition temperature (IPDT) and comprehensive index of thermal stability (ITS) calculated by Doyle's method (Doyle, 1961). The data were analyzed by the use of Universal Analysis 2000 software, version 4.2E (TA Instruments, USA) and MS Excel® 2003.

2.3.5. Gel permeation chromatography

An aqueous solution of the isolated arabinoxylan, obtained on boiling, was subjected to gel permeation chromatography (GPC) by Agilent 1200 series (Agilent, Germany) equipped with Quat pump (G1311A) and refractive index detector (G1362A) using water as eluent (flow rate 1.0 cm^3 min^{-1} at 70 °C) and injection volume of 10 μL. The parameters calculated were molar mass averages (M_w, M_n and M_z), molar masses at peak top (M_p), volumes at peak top (V_p) and polydispersity index (PDI). The data were analyzed by the use of ChemStation GPC Data Analysis software Rev. A.02.02 (Agilent, Germany).

2.3.6. Microscopy

AFM image was taken by the use of Scanning Probe Microscope, model 9500 J3 (Shimadzu, Japan) in the contact mode at room temperature. SEM image of the gold-coated sample was taken with a JSM-6480 LV scanning electron microscope (JEOL, Japan). An accelerating voltage of 10 kV was applied.

2.3.7. Swelling index

The dry arabinoxylan (10 mg) was soaked in distilled water (100 cm^3) for 24 h. The swollen material was then removed and weighed after drying externally by the use of a blotting paper. The swelling index was calculated as

$$\text{swelling index} = \frac{w_f - w_i}{w_i}$$

where w_f is the weight of swollen material and w_i is the initial weight of the dry material.

2.4. Interaction of arabinoxylan with the immune system and its toxicity study

2.4.1. Complement fixing activity

Complement fixation test is an in vitro test for the ability of the extract to interact with the complement cascade reaction. It is based on inhibition of haemolysis of antibody sensitized sheep red blood cells by the complement from human sera as described by Michaelsen (method A) (Michaelsen, Gilje, Samuelsen, Høgåsen, & Paulsen, 2000). AX was subjected to this test using polysaccharide fraction PMII from Plantago major as a positive control (Samuelsen et al., 1996). Inhibition of haemolysis induced by the test sample was calculated by the formula: Inhibition = $(A_c - A_s/A_c) \times 100\%$, where A_c is the absorbance of control and A_s is the absorbance of sample.

2.4.2. Antiviral activity

Antiviral activities of AX were carried out using: HeLa cell/Coxsackie-virus B3 (CVB3); MDCK cell/influenza-A-virus Hong Kong/68; GMK cell/Herpes simplex virus type 1 (HSV-1); and DMSO as solvent. The tested doses were in the range 0.2–50 μg cm^{-3} (for cytotoxicity test) and 0.4–50 μg cm^{-3} (for zpE test).

Cytotoxicity test: This test was carried out in order to determine 50% cytotoxicity dosage and maximum tolerated dosage of substances in HeLa, MDCK, and GMK cell monolayers according to a reported method (Schmidtke, Schnittler, Jahn, Dahse, & Stelzner,

2001). The average value was set to 100%. The 50% cytotoxicity concentration (CC_{50}) was determined by means of linear interpolation using MS Excel® 2003.

zpE-inhibition test: Replication of viruses leads to a total destruction of host cells, which is considered to be a strong cytopathic effect (zpE). This test was performed as per reported method (Schmidtke et al., 2001). During the test, viruses were added to the treated and non-treated cell layers and kept for the specific time. The survived cells were fixed and dyed with crystal violet/formalin, and detected photometrically.

The antiviral activity was calculated from the optical density taking blank as 100%. The antiviral effect was categorized as: Category-0: no activity; Category-1: inhibition of the virus-induced zpE at non-cytotoxic concentration in at least 2 dilution steps: 20–50% (weak); and Category-2: inhibition of the virus-induced zpE at non-cytotoxic concentration in at least 2 dilution steps: >50% (good). A positive control was applied to every plate.

2.5. Drug release studies

2.5.1. Preparation of tablets

Diclofenac sodium (DS) and caffeine were used as model drugs. The isolated arabinoxylan and the drug (1:2) were mixed by geometric dilution. The tablets were prepared by direct compression on a rotary tablet press, fitted with 9 mm flat punches to give an average weight of 400 ± 11 mg with thickness 3.06 ± 0.09 mm. The hardness of the tablets was 31.8–36.8 N with friability 0.99–1.22%.

2.5.2. Preparation of dissolution media

Buffers were prepared according to the United States Pharmacopeia 30. Tablets were evaluated in five dissolution media including HCl buffer (pH 1.2), acetate buffer (pH 4.5), phosphate buffer (pH 6.8), phosphate buffer (pH 7.4) and distilled water.

2.5.3. Procedure

The drug release study was carried out in the appropriate dissolution medium (900 cm^3) using USP paddle dissolution apparatus II at 37 ± 0.1 °C and 50 rpm. Samples (2 cm^3 each) were withdrawn at preset intervals, filtered, suitably diluted and assayed spectrophotometrically, using UV-1700 double beam spectrophotometer (Schimadzu, Japan), at 276 nm (DS) and 273 nm (caffeine). An equal volume of the dissolution medium was replaced immediately after the removal of the sample solution. Amount of drug released was expressed as percent of the total loaded drug.

2.5.4. Stability studies

The tablets (20) were kept at 40 ± 1 °C and 70% ERH for six months in plastic securitainers and analyzed for drug release after two months intervals.

2.5.5. Mathematical and statistical analysis

Drug release data were analyzed using various models including zero order (Eq. (2)), first order (Eq. (3)) (Gibaldi & Feldman, 1967), Higuchi equation (Eq. (4)) (Higuchi, 1961, 1963) and Hixson–Crowell cube root law (Eq. (5)) (Hixson & Crowell, 1931).

$$M = k_0 t \tag{2}$$

where k_0 is the zero order release constant and M is the amount of drug released in time t.

$$\ln M = -k_1 t + \ln M_0 \tag{3}$$

where k_1 is the first order release constant, M is the remaining amount of drug in the tablet after time t and M_0 is the initial amount of drug in the tablet.

$$M = k_H t^{1/2} \tag{4}$$

where M is the amount of drug released in time t, and k_H is the Higuchi release constant.

$$M_0{}^{1/3} - M^{1/3} = -k_{HC} t \tag{5}$$

where M is the amount of drug released in time t, k_{HC} is the Hixson–Crowell release constant and M_0 is the initial amount of the drug in the tablet.

Dissolution profiles were compared by difference (f_1) and similarity (f_2) factors calculated by reported method (Moore & Flanner, 1996). These factors are defined by Eqs. (6) and (7).

$$f_1 = \frac{\sum_{t=1}^{n} |R_t - T_t|}{\sum_{t=1}^{n} |R_t|} \times 100 \tag{6}$$

$$f_2 = 50 \times \log \left[\left[1 + \left(\frac{1}{n} \right) \sum_{t=1}^{n} |R_t - T_t| \right]^{2}{}^{-0.5} \times 100 \right] \tag{7}$$

where R_t and T_t are the dissolution values of the reference batch and the test batch respectively at time t. The summation is done over all time points n. For two curves to be similar, f_1 should be close to zero and f_2 close to 100. Generally, f_1 value in the range 0–15 and f_2 greater than 50 are sufficient to establish the equivalence of two dissolution profiles.

2.5.6. Mechanism of drug release

A number of models have been put forward to explain the release mechanism of drugs from swellable systems but none of them adequately explains the release mechanism for all types of systems (Siepmann & Siepmann, 2008). An empirical relationship called Power Law (Eq. (8)) is generally used to describe the Fickian, non-Fickian and case-II transport mechanisms of drug release from a polymer matrix (Korsmeyer, Gurny, Doelker, Buri, & Peppas, 1983; Peppas, 1985; Ritger & Peppas, 1987a, 1987b).

$$\ln \frac{M_t}{M_\infty} = \ln k_p + n \ln t \tag{8}$$

where M_t/M_∞ is the fraction of drug released in time t, k_p is the Power Law constant characteristic of the drug-matrix system and n is the diffusion exponent. The value of n identifies different mechanisms for drug release. For different geometries the limits of n are different (Ritger & Peppas, 1987a, 1987b). For cylindrical systems (tablets), for which $n = 0.45$, the drug releases by Fickian diffusion, for $n = 0.89$ it is case-II transport (swelling-controlled mechanism) and for $0.45 < n < 0.89$ the mechanism is anomalous transport, i.e., the release is governed by swelling and diffusion phenomena (Siepmann & Peppas, 2001). For best model selection, a modified Akaike Information Criterion called Model Selection Criterion (MSC) was used (Eq. (9)) (Scientist Handbook, 1995).

$$\mathrm{MSC} = \ln \left[\frac{\sum_{i=1}^{n} w_i (Y_{\mathrm{obs}_i} - \bar{Y}_{\mathrm{obs}})^2}{\sum_{i=1}^{n} w_i (Y_{\mathrm{obs}_i} - Y_{\mathrm{cal}_i})^2} \right] - \frac{2p}{n} \tag{9}$$

where Y_{obs_i} and Y_{cal_i} are the observed and calculated value of ith data point respectively, $\bar{Y}_{\mathrm{obs}}$ the mean of observed data points, w_i the optional weight factor, n the number of data points and p the number of parameters. MSC is independent of the scaling of data points and the model with largest MSC value is considered to be the most appropriate. All the calculations were performed by the use of MS Excel® 2003.

3. Results and discussion

Arabinoxylan was economically separated, because of elimination of organic solvents, from dry ispaghula seeds in good yield (30% of the dry seed weight). The approximate cost was US$ 3.00/kg.

Table 1
GPC parameters for two fractions of the isolated arabinoxylan.

Parameters	Fraction I	Fraction II
M_n (g mol^{-1})	6.94×10^6	4.43×10^3
M_w (g mol^{-1})	7.15×10^6	5.41×10^3
M_z (g mol^{-1})	7.36×10^6	6.90×10^3
M_p (g mol^{-1})	7.27×10^6	3.26×10^3
V_p (cm^3)	4.34	7.81

The dried material had a light brown color with 8–10% weight loss on drying and a high swelling index (48.17), which indicates that the arabinoxylan possesses a good water holding capacity. The isolated arabinoxylan was characterized by CHNS, FT-IR, thermal, sugar analysis, GPC and AFM analysis and found to be similar to that reported previously (Laidlaw & Purcival, 1949, 1950) with slight variations in mass and sugar content due to variation in source. The results of GPC and release data are given in Tables 1–3 and discussed as follows.

3.1. Elemental analysis

The elemental analysis indicated the absence of nitrogen and sulfur. The percentages of carbon and hydrogen were 40.38% and 5.93% respectively, which are similar to those found for arbinoxylan isolated from ispaghula husk (Saghir et al., 2008). However, these values are lower than those of natural polysaccharides (~45% C and ~6.1% H), which may be due to the presence of uronic acids in the material (Laidlaw & Purcival, 1949, 1950).

3.2. FT-IR spectra

The absorption bands in the FTIR spectrum of the gel were assigned by comparing with the literature values for similar materials (Kačuráková et al., 1999; Saghir et al., 2008; Unlu, Gunister, & Atici, 2009). The assignments of the observed absorption bands are: 3358 cm^{-1} (OH stretching as broad band), 2926 cm^{-1} (aliphatic saturated CH stretching), 1608 cm^{-1} (deformation due to absorbed H_2O), 1419 cm^{-1} (δ CH_2), 1377 cm^{-1} (δ CH), 1247 cm^{-1} (antisym. bridge oxygen δ), 1157 cm^{-1} (ν C–O–C, ν C–C, an evidence of arabinosyl side chain), 1053 cm^{-1} (ν C–C, ν C–O, δ C–O–H strongly influenced by branching), a sharp band at 896 cm^{-1} (β-glycosidic bond, antisym. out-of-plane δ), and 617, 528 cm^{-1} (polymer backbone). The bands at 1650 and 1550 cm^{-1} due to –NHCO– of protein units and a characteristic band of ferulic acid at 1520 cm^{-1} were absent, which confirms the absence of proteins and ferulic acid group in the isolated material.

Table 3
Difference factor (f_1) and similarity factor (f_2) analysis for various release profiles.

	pH 1.2	pH 4.5	pH 6.8	pH 7.4
Comparison of caffeine and DS release profiles with that in distilled water				
Caffeine (f_1)	4.58	15.52	15.12	14.26
Caffeine (f_2)	73.75	51.45	51.46	52.53
DS (f_1)	83.63	73.24	31.85	13.64
DS (f_2)	21.87	24.22	40.77	57.69

	Distilled water	pH 1.2	pH 4.5	pH 6.8	pH 7.4
Comparison of caffeine and DS release profiles with each other in the same medium					
f_1	20.81	87.68	82.15	54.33	38.87
f_2	48.57	15.61	14.60	24.02	31.66

3.3. Thermogravimetric analysis and differential scanning calorimetry

TGA of the dry arabinoxylan showed two stages of major decomposition. In the first stage the initial decomposition temperature (IDT) was 218 °C and final decomposition temperature (FDT) was 310 °C. The first stage of decomposition resulted in 41.64% weight loss with very small exothermic enthalpy change (Fig. 1a). This corresponds to a major breakdown of the polymer chain resulting in the formation of some reasonably high molecular mass volatiles. In the second stage, the IDT was 442 °C and FDT was 535 °C. The weight loss at the second stage was 21.95% accompanied by a large exothermic enthalpy change (Fig. 1a), which is due to complete degradation of the arabinoxylan resulting in evolution of gaseous molecules like CO_2, H_2O. Glass transition temperature (t_g) could not be observed in the experimental temperature range. The char yield was ~11% at 600 °C. The TGA also showed an earlier small endothermic weight loss up to 100 °C which appears due to loss of adsorbed water. The activation energies, 141 kJ mol^{-1} (first stage) and 251 kJ mol^{-1} (second stage) as calculated from Broido plots (Fig. 1b and c) indicate good thermal stability and slow decomposition rate of the material. The IPDT (308 °C) and ITS (0.47) values also indicate a good thermal stability and are comparable with those of other natural, commercial and modified polysaccharides (Zohuriaan & Shokrolahi, 2004).

Table 2
Fitness of release data to various mathematical models.

		Caffeine					Diclofenac sodium		
		Distilled water	pH 1.2	pH 4.5	pH 6.8	pH 7.4	Distilled water	pH 6.8	pH 7.4
Zero order	R^2	0.9832	0.9868	0.9806	0.9447	0.9466	0.9924	0.9135	0.9527
	$k_o \times 10^2$	7.34	7.10	7.26	7.12	6.89	6.48	3.32	4.52
	MSC	3.59	3.83	3.44	2.39	2.43	4.38	1.95	2.55
First order	R^2	0.9892	0.9825	0.9846	0.9905	0.9960	0.9908	0.9281	0.9712
	$k_1 \times 10^2$	−0.16	−0.16	−0.20	−0.18	−0.17	−0.11	−0.04	−0.07
	MSC	4.06	3.51	3.67	4.15	4.98	3.71	2.09	2.58
Higuchi	R^2	0.9862	0.9937	0.9948	0.9878	0.9921	0.9781	0.9558	0.9786
	$k_H \times 10^2$	284	276	283	282	273	249	131	176
	MSC	3.78	4.57	4.76	3.90	4.34	3.32	2.62	3.34
Hixson–Crowell	R^2	0.9833	0.9866	0.9804	0.9445	0.9462	0.9924	0.9135	0.9527
	$k_{HC} \times 10^2$	−2.50	−2.42	−2.48	−2.43	−2.35	−2.16	−1.11	−1.51
	MSC	3.59	3.81	3.43	2.39	2.42	3.63	1.95	2.17
Power Law	R^2	0.9872	0.9970	0.9880	0.9964	0.9971	0.9893	0.9692	0.9732
	$k_p \times 10^2$	1.22	1.85	3.46	2.45	2.55	0.57	1.06	1.48
	MSC	4.41	5.32	4.41	4.66	4.87	4.22	2.96	3.08
	n	0.60	0.55	0.46	0.52	0.51	0.69	0.53	0.52
% release in 900 min		76	79	84	80	80	64	38	50

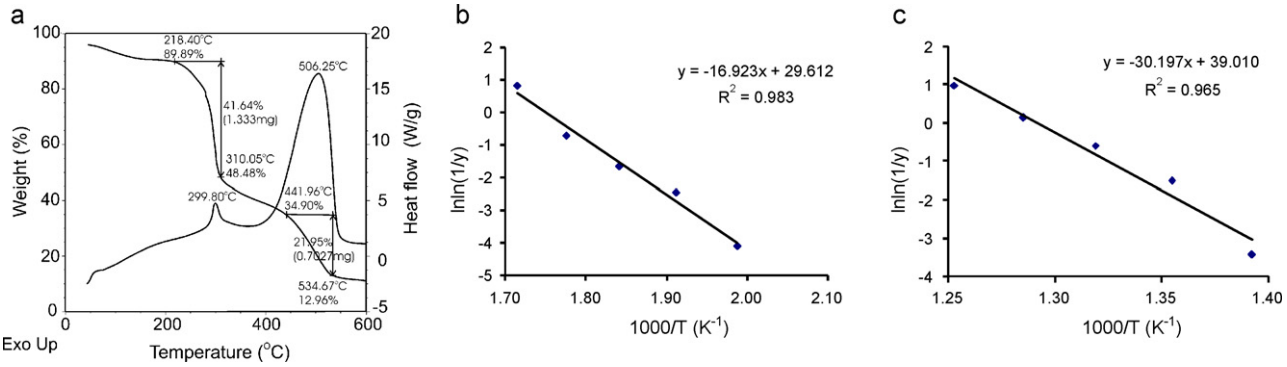

Fig. 1. (a) TGA and DSC scans of the isolated arabinoxylan. Calculated integral procedural decomposition temperature and comprehensive index of thermal stability are 308 °C and 0.47 respectively; (b) Broido plot of first stage of decomposition. Calculated activation energy is 141 kJ mol^{-1} and (c) Broido plot of second stage of decomposition. Calculated activation energy is 251 kJ mol^{-1}.

3.4. Gel permeation chromatography

GPC analysis (Table 1) showed two polymeric components in the isolated fraction with weight-average molar masses 7.15×10^6 g mol^{-1} (fraction I) and 5.41×10^3 g mol^{-1} (fraction II). This result suggests the presence of two distinct polysaccharides in the water-extracted gel of *P. ovata* as reported earlier (Laidlaw & Purcival, 1949, 1950). The mass of fraction I is in the characteristic range of the arabinoxylan of the hull-less barley (Saghir, 2009). For an ideal monodisperse polymer, the molar mass averages are equal, i.e. $M_n = M_w = M_z$. However, for a polydispersed system the relationship is $M_n < M_w < M_z$. In case of fraction I the values of M_n, M_w and M_z are not significantly different, whereas in case of fraction II these values are more scattered. Thus the fractions I and II with PDI values of 1.03 and 1.22, respectively, appear to be almost monodispersed polymers.

3.5. Microscopy

The AFM image (Fig. 2a) showed that the AX possessed a nanostructure in the form of chains with non-uniform surface topography and some variation in phase shifts. The SEM image (Fig. 2b) depicted a lamellar nature of the material with some deposits.

3.6. Interaction of arabinoxylan with the immune system and its toxicity study

3.6.1. Complement fixing activity

Complement is an important part of the innate immune system and it is closely linked to the adaptive immune system (Caroll & Prodeus, 1998) and as such it plays an important role in host defense, inflammation, or allergic reactions. This system can be activated by polysaccharides, certain immunoglobulins, viruses, fungi, bacteria, certain animal cells, or parasites. It was, therefore, considered relevant to determine the effect of AX on human complement. The complement-fixation test was designed to measure the concentration of the AX necessary to inhibit 50% of the haemolysis (ICH_{50}). A plot between concentrations of AX and percent haemolysis shows (Fig. 4) that the percent haemolysis caused by AX and PMII is same at 250 μg cm^{-3}. Below this concentration AX exhibits lower haemolysis in comparison with PMII. This means AX is safer than PMII at lower concentrations. From the plot of concentration versus percent inhibition of haemolysis (Fig. 3) the ICH_{50} value was estimated to be ~29 μg cm^{-3}.

3.6.2. Antiviral activity

The antiviral activity of AX was determined by the use of a non-enveloped single-stranded positive sense RNA virus enterovirus Coxsackievirus B3 (CVB3), an enveloped double-stranded DNA virus herpes simplex virus type I (HSV-1), and an enveloped single negative stranded RNA virus (influenza A). The CC_{50} values of AX fro HeLa, MDCK and GMK cells were found to be >50 μg cm^{-3} and it did not exhibit any antiviral activity. Thus these results indicate that AX is non-toxic and possesses no antiviral activity against the test viruses.

3.7. Drug release studies

The release of the drug from a swellable matrix starts with the penetration of water into the matrix. The water hydrates the polymer which swells forming a gel like structure, dissolves the drug, bring about relaxation of the polymer chains and the drug diffuses out of the matrix. Drug release is greatly dependent upon solubility of drugs in the dissolution media. In the present study two model drugs, caffeine and DS, were chosen. Caffeine, being soluble over a wider range of pH, is considered to be a good model drug for evalu-

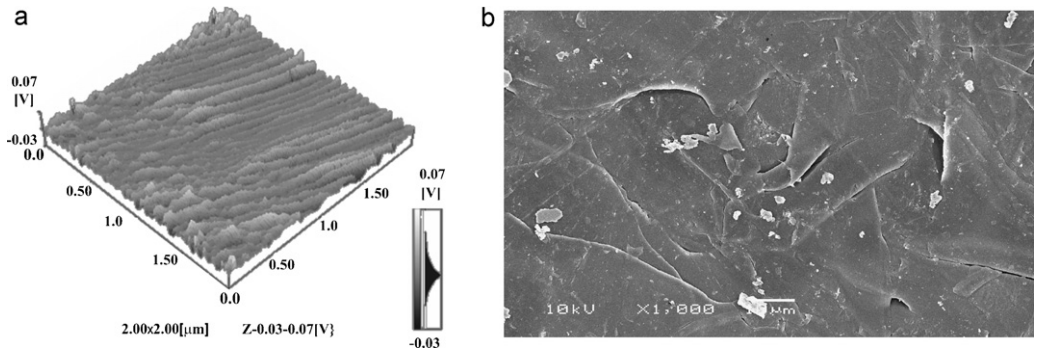

Fig. 2. Microscopic images of the isolated arabinoxylan. (a) AFM and (b) SEM .

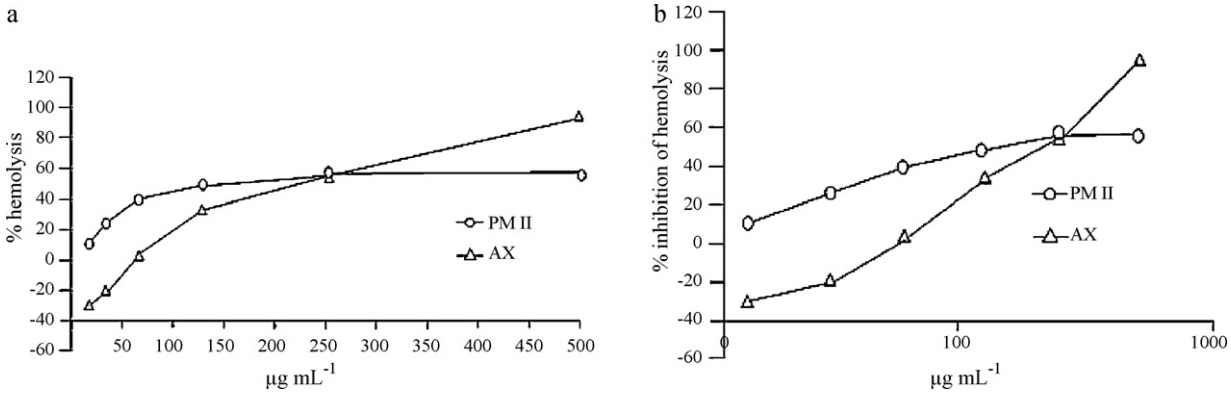

Fig. 3. Toxicity analysis. (a) Plot between concentration of the test materials and percent haemolysis caused by them. (b) Plot between log concentration of the test materials and percent inhibition of haemolysis caused by them.

ating a new polymer intended for sustained release. DS was chosen as a standard for pH-dependent release product.

Caffeine showed almost similar release profiles in all the media under study (Fig. 4a). On the other hand DS, being insoluble at pH 1.2 and pH 4.5, did not show any marked release in these media. The results are shown in Fig. 4b. The release data were fitted into Eqs. (2)–(5) and (8), and coefficients of determination, R^2 values, calculated from their plots indicate a good fitness in all the applied models (Table 2). Dissolution profiles in the media were compared with that in distilled water by difference (f_1) and similarity (f_2) factors. The f_1 and f_2 values did not show any significant differences in the release profiles of caffeine in all the media (Table 3). Diclofenac sodium release profiles also did not show a significant difference in pH 7.4 medium and distilled water (Table 3). This represents pH-independent release profiles of the drugs from the arabinoxylan matrix. However, in pH 6.8 medium, the release profile of DS is different from that in distilled water (Table 3); this can be attributed to the very slow release behavior of DS in media with pH less than 7 due to its low solubility.

From the slope and intercept of the plot of $\ln(M_t/M_\infty)$ vs $\ln t$ (Fig. 4c), the diffusion exponent n and polymer–drug characteristic constant k_p were calculated; the results are given in Table 2. In case of caffeine, the value of n varies from 0.46 to 0.60 in different media suggesting that the release pattern follows a simple diffusion or anomalous transport mechanism. In distilled water, the mechanism is of anomalous transport type, where rate of diffusion and rate of polymer-swelling are comparable and the release appears to be controlled by both the phenomena. However, in going from pH 1.2 to pH 7.4 the value of n decreased to approximately 0.45, which corresponds to a shift in mechanism from anomalous transport to simple Fickian diffusion where the rate of diffusion is much less than that of the polymer-swelling. For DS, the case was similar to that of caffeine in distilled water, pH 6.8 and 7.4 media, however, at pH 1.2 and 1.4 the mathematical models were not applied as very small amount of drug was released. First order and Higuchi's equation showed a good fit to the experimental data, which means that the release of drugs from the matrix decreased with time. Zero order and Hixson–Crowell models gave relatively a poor fit because drug release was not constant over the entire period. MSC analysis showed that Power Law is more versatile in explaining the rate and mechanism of drug release (Table 2). Release profiles of caffeine and DS in the same dissolution media were different from each other (Table 3). Release of DS was very slow as compared to that of caffeine (Table 2). This suggests that the arabinoxylan holds DS better than caffeine. This can be attributed possibly to hydrogen bonding between diclofenac and the arabinoxylan.

In another experiment release profiles of diclofenac sodium were compared with those of Voltral® SR-100 tablets (Novartis, Pakistan), a commercial standard preparation, under identical conditions in distilled water and in media having pH 1.2 and pH 7.4. It was found that the arabinoxylan afforded a more sustained release than the standard (Fig. 5). In 660 min the release of DS from the arabinoxylan was 59% and 50% and from Voltral® it was 83% and 73% in distilled water and pH 7.4 media, respectively. The f_1 values were: 40.35 (for distilled water) and 38.90 (for pH 7.4 medium), and the f_2 values were: 29.58 (for distilled water) and 32.64 (for pH 7.4 medium). At pH 1.2 both the tablets showed negligible release due to lower solubility of DS at this pH.

The release profiles of caffeine and DS from the arabinoxylan matrix after storage for a period of two, four and six months, at 40 °C and 75% ERH, were quite similar to the initial profiles. For example, comparison of the initial profile to the six months profile, the f_1 values were: 14.48 and 12.35, and the f_2 values were: 63.30 and 66.79 in distilled water and pH 7.4 medium, respectively for

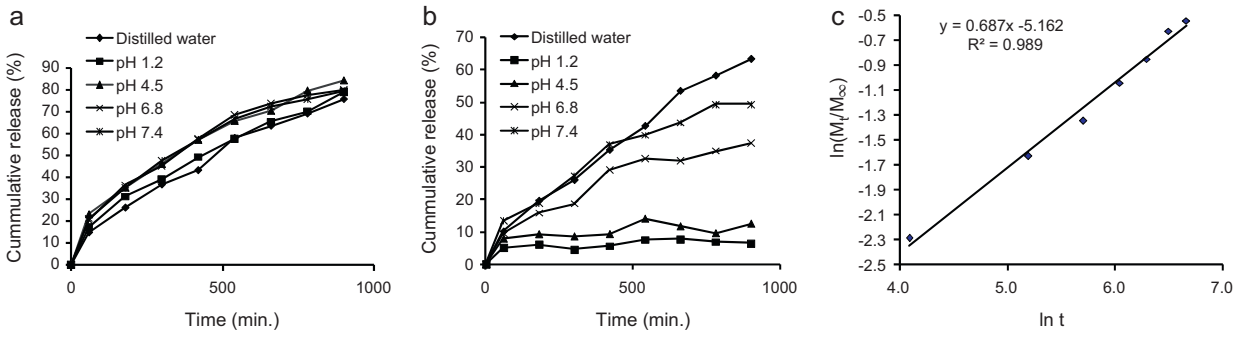

Fig. 4. Release profiles of drugs from the arabinoxylan matrix in different dissolution media at 37 °C: (a) caffeine; (b) DS and (c) representative Power Law plot for the release of DS from arabinoxylan matrix in distilled water at 37 °C.

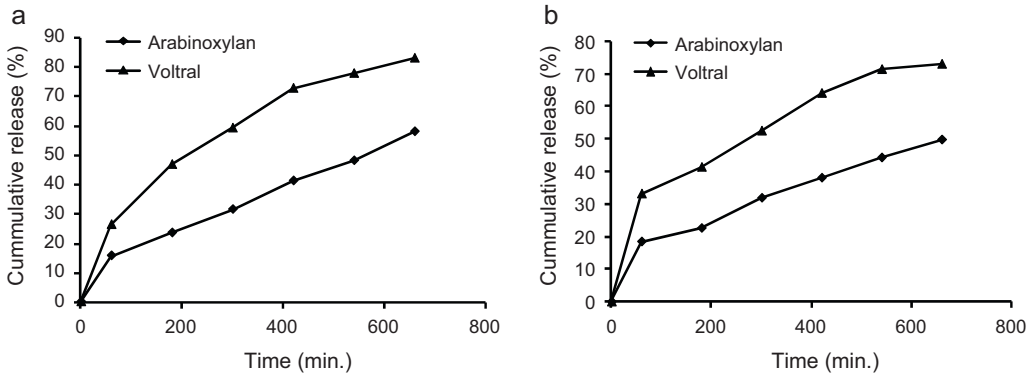

Fig. 5. Comparative release of DS from arabinoxylan matrix and Voltral® at 37 °C: (a) distilled water and (b) pH 7.4.

DS, while for caffeine these were: 10.57 and 9.36, and 63.26 and 63.48, respectively. This clearly demonstrates that the preparations remain stable for at least six months at 40 °C and 75% ERH.

4. Conclusion

Hot water-extracted arabinoxylan has emerged as a potential candidate for controlled drug delivery matrix. It can provide sustained release of drugs largely depending upon their solubilities and drug-polymer interactions. Similar release profiles of caffeine in different media suggested a pH independent release from the arabinoxylan. However, release of DS was greatly influenced by its solubility. The mechanism of drug release from direct-compressed tablets was found to be controlled by diffusion and anomalous transport. Power Law explained the rate and mechanism of drug release from the arabinoxylan more satisfactorily. After comparison with a commercial DS standard the use of arabinoxylan as the sustained release matrix appears to have better prospects in terms of efficacy, safety and cost.

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