ISOLATION AND CARBOXYMETHYLATION OF ISPAGHULA SEED HUSK GEL POLYSACCHARIDE

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Introduction

- Ispaghula (*Plantago ovata*) seed husk has long history of use as a dietary fiber supplement to promote the regulation of large bowel function[1].
- It is used in folk medicines as demulcent, emollient and laxative.
- In recent studies it has been shown to lower the blood cholesterol level[2].
- The gel isolated from husk of *Plantago ovata* seeds is used to produce controlled drug delivery devices[3,4].

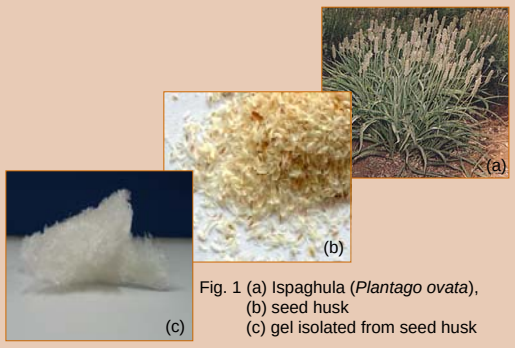


Fig. 1 (a) Ispaghula (*Plantago ovata*), (b) seed husk (c) gel isolated from seed husk

Purpose of study

Main objective is to get water soluble and water swellable arabinoxylans with anionic function by carboxymethylation reaction for pharmaceutical applications.

Isolation

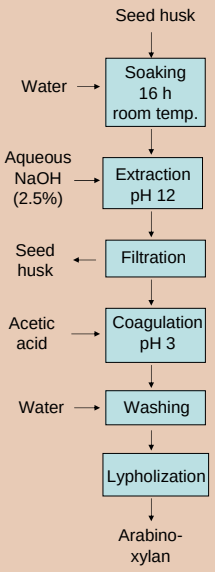


Fig. 2 Flow Chart for isolation of gel polysaccharide from seed husk.

Properties of polysaccharide

- The polysaccharide gel shows a pH depending water solubility and has high swelling ability in water. It has water retention value of about 8100%.
- The Polymer is thermally stable up to ~ 250°C
- Solubility
  -soluble in DMSO at 80°C
  -soluble in aqueous NaOH (2.5%)

Structure of polysaccharide

- It is mainly arabinoxylan with 74.8% xylose, 23.3% arabinose, 0.8% rhamnose, and 1.2% galactose.
- 40.4% carbon and 6.5% hydrogen were found in the elemental analysis. The absence of nitrogen indicates the absence of protein.
- With GPC analysis revealed: M_w 364470 g/mol, M_n 12800 g/mol and DP~ 2760.
- Capillary viscometry at c= 0.01 g/cm^3 in 2.45% aqueous NaOH at 20°C[η]= 115.67 cm^3/g.

Structural analysis of arabinoxylan after ultrasonic degradation

Ultrasonic degradation had been carried out with aqueous arabinoxylan suspension, samples were drawn out after short intervals, centrifuged for 40 min at 4000 rpm to remove titanium particles, filtrated and lypholized.
The dried degraded samples were subjected to GPC and NMR studies.

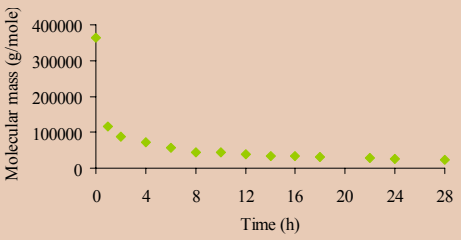


Fig. 3 Decrease of molar mass of Ispaghula seed husk gel Polysaccharide during treatment with ultrasound in water at 24-27°C with pulse amplitude 75% of 450 watts.

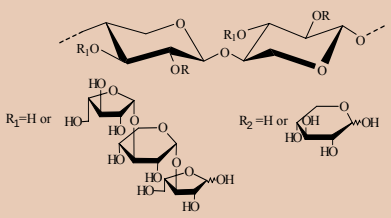


Fig. 4 Proposed structure of arabinoxylan from Ispaghula seed husk gel polysaccharide.

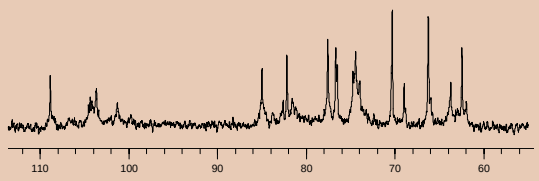


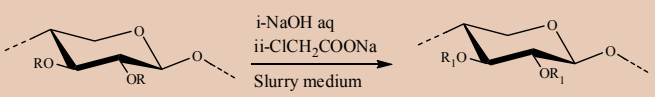
Fig. 5 ^{13}C NMR spectrum (in D_2O) of the polymer after ultrasonic degradation in water for 16 h. DP ~220.

Tab. 1 Comparison of ^{13}C NMR chemical shifts for glycosyl residues of arabinoxylan after 16 h ultrasonic degradation with literature[5] (in Italic).

Glycosyl residue	Assignment				
	C-1	C-2	C-3	C-4	C-5
L-Ara*f*	108.6	81.86	77.1	84.6	61.9
	109.0	*82.0*	*77.4*	*85.0*	*61.8*
→3)-D-Xyl*p*-β-(1→	104.0	74.25	83.41	68.44	65.7
	8	*74.0*	*84.2*	*68.5*	*65.7*
	104.5				
T-Xyl*p*	103.4	73.9	76.2	69.8	65.7
	103.8	*73.7*	*76.4*	*70.0*	*66.0*
→2[→4]-D-Xyl*p*-β-(1→	101.0	81.2	73.5	76.0	63.1
	101.3	*81.4*	*74.3*	*77.3*	*63.5*

Carboxymethylation of arabinoxylan

Carboxymethylation of arabinoxylan was carried out by common method for carboxymethylation of the polysaccharides like cellulose, starch or xylan[6].



(**1-20**)

R= H or sugar moity

R_1= H or Sugar moity or -CH_2COONa

Scheme 1. Schematic representation of carboxymethylation of arabinoxylan

Reaction Parameters studied with the order to get higher DS values
Molar ratio of AXU:NaOH:SCMA= 1:3:3<1:5:5< 1:10:10< 1:20:20
Slurry media= methanol<DMSO<**ethanol**<2-propanol
Temperature= 30°C< 45°C< 55°C
NaOH conc.= 45% <15%< 25%
Time= 5h< 10h< 20h

Results and Discussion

Most of the carboxymethylation reactions were carried out under heterogeneous conditions except for sample **20** (DS_{CM} 0.24), where DMSO was used as solvent and reaction was carried out in homogeneous phase.
The DS of the carboxymethyl groups was determined after hydrolytic chain degradation by HPLC[7] and ^{1}H NMR spectroscopy[8].
Change of reaction parameters had a distinct influence on the total DS and functinalization pattern of carboxymethyl arabinoxylan (CMAX).

Ethanol was found to be the best slurry medium for carboxymethylation of arabinoxylan because the higher DS values were obtained combined with an easy work up.
Products with lower DS values have higher substitution at *O*-2 while products with higher DS values had substitution on other positions also (*O*-3, *O*-5) resulting in comparative lower DS at *O*-2.

Tab. 2 Degree of substitution (DS) of carboxymethyl arabinoxylan (CMAX) obtained under different reaction conditions.

Molar ratio AXU:SMCA:NaOH	Slurry medium	Time h	NaOH aq.(%)	Sample number	DS_{HPLC}	DS ^{1}H NMR		
						ΣDS	*O*-2	*O*-3*
1:5:5	Methanol	5	15	**1**	0.14	0.13	0.10	0.03
1:7:7	Methanol	5	15	**2**	0.33	0.31	0.22	0.09
1:10:10	Methanol	5	15	**3**	0.43	0.37	0.24	0.13
1:20:20	Methanol	5	15	**4**	0.48	0.38	0.20	0.18
1:20:20	Methanol	5	25	**5**	0.61	0.50	0.31	0.19
1:3:3	Methanol	5	45	**6**	0.08	0.07	0.05	0.02
1:5:5	Methanol	5	45	**7**	0.17	0.15	0.09	0.06
1:5:5	Methanol	5	25	**8**	0.27	0.23	0.14	0.09
1:3:3	Methanol	5	25	**9**	0.12	0.10	0.06	0.04
1:3:3	Methanol	10	25	**10**	0.16	0.14	0.11	0.03
1:3:3	Ethanol	10	25	**11**	1.72	1.12	0.52	0.60
1:3:3	Ethanol	20	25	**12**	1.81	1.33	0.59	0.74
1:3:3	Ethanol	5	25	**13**	1.18	0.91	0.42	0.49
1:3:3	Ethanol	5	15	**14**	1.07	0.74	0.35	0.39
1:3:3	Ethanol	5	15	**15**[a]	0.73	0.51	0.25	0.26
1:3:3	Ethanol	5	15	**16**[b]	0.26	0.20	0.12	0.08
1:5:5	Ethanol	5	15	**17**	1.37	1.14	0.45	0.69
1:10:10	Ethanol	5	15	**18**	1.47	1.34	0.54	0.80
1:3:3	2-Propanol	5	25	**19**	1.33	1.23	0.44	0.79
1:3:3	DMSO	5	25	**20**	0.24	0.20	0.12	0.08

[a] reaction at 45°C, [b] at 30°C, * *O*-3 representing substitution at all other expected positions except position 2

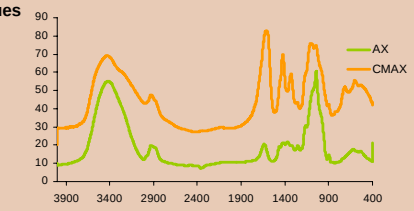


Fig. 6 FTIR spectra of arabinoxylan (AX) and carboxymethyl arabinoxylan (CMAX) sample **13**, DS=1.18

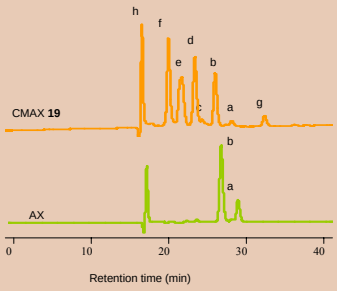


Fig. 7 HPL chromatograms of arabinoxylan (AX) and carboxymethyl arabinoxylan (CMAX **19**) after complete chain degradation with $HClO_4$.
a = arabinose, b = xylose, c= mono-*O*-CMA, d= mono-*O*-CMX, e= di-*O*-CMAX, f = tri-*O*-CMAX, g = glycolic acid, h= inorganic salt

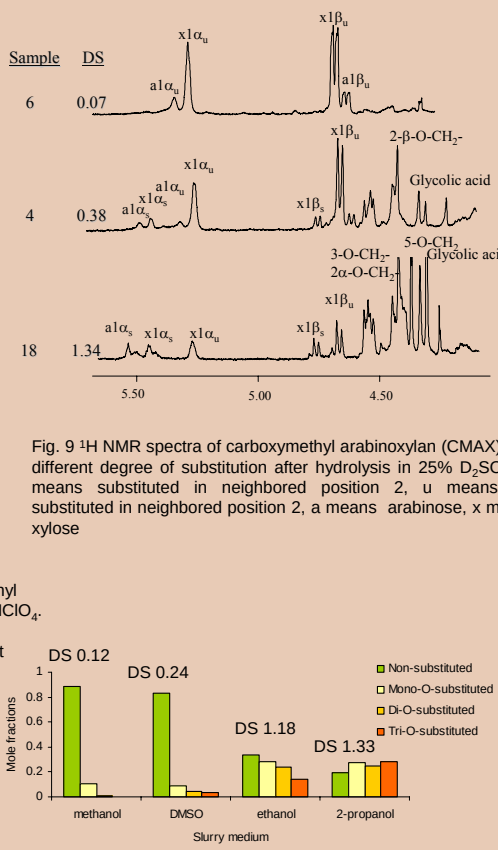
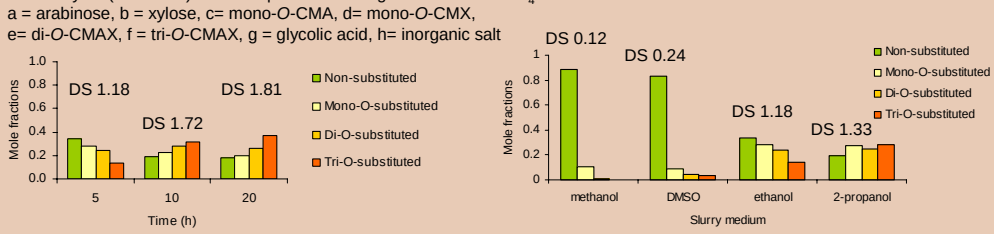


Fig. 9 ^{1}H NMR spectra of carboxymethyl arabinoxylan (CMAX) with different degree of substitution after hydrolysis in 25% D_2SO_4. s means substituted in neighbored position 2, u means un-substituted in neighbored position 2, a means arabinose, x means xylose



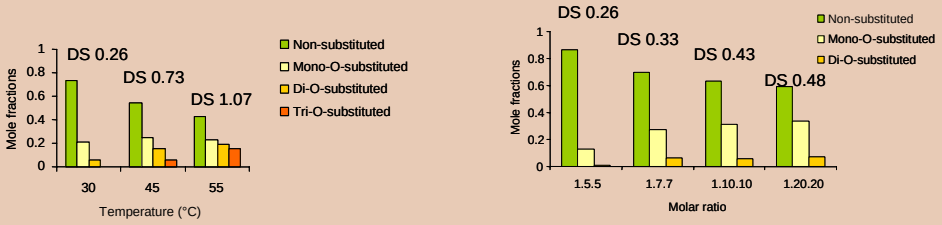


Fig. 8 Effect of change in reaction parameters on the total DS and mole fractions of CMAX. Reactions are carried out by keeping one parameter variable and other constant. (see Table 2.).

Conclusion

- Arabinoxylan gel with DP 2760 was isolated from Ispaghula seed husk by alkali -extraction with a yield of 45% of the weight of husk.
- Ultrasonic degradation of arabinoxylan for 28 h yielded a product with DP 177.
- HPLC analysis showed the presence of 74.8% xylose and 23.2% arabinose, along with 0.8% rhamnose and 1.2% galactose relative to total sugar content.
- Polymer was thermally stable up to 250°C.
- Carboxymethylation was carried out under different reaction conditions and maximum DS was found 1.81.
- A distinct influence of the slurry medium on the DS and functionalization patterns was found. The DS increases in the order 2-propanol>ethanol>DMSO>methanol while keeping all other parameters constant.
- The highest DS could be realized applying ethanol as slurry medium, 25% aqueous NaOH, a reaction temperature of 55°C within 20 h, and higher molar ratio of SMCA and NaOH to anhydro sugar unit.
- CMAX with tri-*O*-substituted mole fraction indicated presence of three free OH groups in branched pentose sugars.
- The products with higher DS values were found with higher tri-*O*-substituted mole fraction as compared to mono-*O*-substituted and di-*O*-substituted mole fractions.
- Carboxymethyl arabinoxylan is water soluble starting at DS of 0.33.
- The rheological properties of water soluble carboxymethyl arabinoxylan will be studied regarding their application as thickening- and surface active agents for pharmaceutical applications.
- Comparative studies of arabinoxylan and water swelling carboxymethyl arabinoxylan as controlled drug delivery devices will be carried out.

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Acknowledgements

The fellowship for Shazia Saghir by Higher Education Commission (HEC) of Pakistan is gratefully acknowledged.
The authors wish to thank Dr. B. Saake (Hamburg, Germany) for sugar analysis.

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