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Microwave-promoted hydrolysis of plant seed gums on alumina support

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Abstract—Using a catalytic amount of potassium persulfate (1.48×10^{-4} M), eight different seed gums were fully hydrolyzed on alumina support under microwave irradiation. The hydrolysis time varied between 1.33 and 2.33 min depending upon the seed gum structure. The used solid support could be easily separated from the hydrolyzates and recycled. However, under microwave field in an aqueous medium, the same amount of persulfate was unable to hydrolyze the seed gums. Solid-supported microwave hydrolysis has been compared with the microwave-enhanced aqueous hydrolysis (using $K_2S_2O_8$ or 0.1 N H_2SO_4) and also with the conventional hydrolysis procedures.

Keywords: Seed gums; Microwave irradiation; Potassium persulfate; Alumina

1. Introduction

Constituent monosaccharides of any polysaccharide material are detected and isolated by its complete hydrolysis, followed by chromatographic separation of the resulting sugars. Polysaccharides require hydrolysis under the mildest conditions[1] to avoid any possible degradation of the sugars. Conventionally, complete hydrolysis of a polysaccharide to its constituent monosaccharides involves strong acids like trifluoroacetic acid[2] or sufficiently prolonged hydrolysis time.[3]

As microwave (mw) irradiation[4–8] is emerging as an efficient source of thermal energy, and constitutes a very original procedure for the heating of materials, in ways different from the classical ones. Microwaves can heat reactants selectively, directly, and without thermal inertia and heat exchange with the medium.[9] Several reports on hydrolysis reactions under microwave irradiation are available. Starch,[10] sucrose,[11] colominic acid,[12] polymide 6,[13] proteins,[14] and chitosan[15] have been hydrolyzed with acids in aqueous media under microwave irradiation. Effect of inorganic salts on the hydrolysis of starch[16] and chitosan[15] was also studied. Our group has reported[17] microwave-promoted hydrolysis of the seed gums in an aqueous medium under mild, acidic conditions. In the present study, we for the first time report on the microwave-accelerated complete hydrolysis of the seed gums on an alumina support using a catalytic amount of potassium persulfate. Eight different seed gums like *Ipomoea quamoclit* (IQ),[17] *Cassia abbreviata* (CA),[18] *Cassia javanica* (CJ),[18] *Cassia reticulata* (CR),[18] *Ipomoea dasyperma* (ID),[19] *Ipomoea hedracea* (IH),[20] *Ipomoea campanulata* (IC),[21] and *Cyamopsis tetragonolobus* (guar gum; GG)[22] were used in the study.

2. Results and discussion

The complete hydrolysis of *Cassia marginata* on alumina support using potassium persulfate was studied under conditions of microwave irradiation. Hydrolysis of the seed gum was monitored at different exposure times and persulfate concentrations at 100% microwave power (Tables 1 and 2). Minimum persulfate concentration

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Table 1. Hydrolysis of CM gum on Al_2O_3 support under 100% microwave power at different exposure times with fixed persulfate concentration[a]

Sample no.	Solid-supported microwave hydrolysis						Hydrolysis with 2 N H_2SO_4 for 48 h
	Without HQ			With HQ (20 mg)			
	Exposure (s)	pH after exposure	Cu_2O[b] (mg)	Exposure (s)	pH after exposure	Cu_2O[b] (mg)	Cu_2O[b] (mg)
1	20	6	—	20	6	—	68
2	40	4	—	40	4	—	
3	60	2	50	60	3	—	
4	80	2	65	80	3	—	
5	100	2	65	100	3	—	

[a] Hydrolysis of the CM gum (80 mg) at Al_2O_3 (200 mg) under 100% microwave power at different exposure time with 1.48×10^{-4} M $K_2S_2O_8$.
[b] Weight of Cu_2O obtained on gravimetric estimation of the hydrolyzates.

Table 2. Hydrolysis of CM gum on Al_2O_3 support with different persulfate concentrations and 100% microwave power and hydrolysis in an aqueous solution and 100% microwave power[a]

Sample no.	Solid-supported microwave hydrolysis			Aqueous microwave hydrolysis	
	$K_2S_2O_8$	pH after exposure	Cu_2O[b] (mg)	pH after exposure	Cu_2O[b] (mg)
1	9.25×10^{-6} M	7	—	4	—
2	1.85×10^{-5} M	6	—	3	—
3	3.7×10^{-5} M	4	30	2	—
4	7.39×10^{-5} M	2	40	2	—
5	11.09×10^{-5} M	2	55	2	—
6	1.48×10^{-4} M	2	66	2	—
7	1.85×10^{-4} M	2	66	2	2.01
8	2.22×10^{-4} M	2	66	2	8.85

[a] Hydrolysis of CM gum (80 mg) with different persulfate concentration at Al_2O_3 (200 mg); in 25 mL H_2O at 100% microwave power and 1.33 min exposure.
[b] Weight of Cu_2O obtained on gravimetric estimation of the hydrolyzates.

and time required for the complete hydrolysis of *C. marginata* at 100% microwave power were 1.48×10^{-4} M and 1.33 min, respectively. Using potassium persulfate (1.48×10^{-4} M) at 100% microwave power, all the seed gums under study could be fully hydrolyzed on alumina support between 1.33 and 2.33 min, and the results were found to be reproducible. Complete hydrolysis time for all the seed gums are recorded in Table 3. The use of the recovered alumina as a solid support in the hydrolysis experiments gave the same results as before (Table 3). Paper chromatography (solvent A) of the complete hydrolyzates of all the seed gums revealed the presence of galactose (R_f 0.15) and mannose (R_f 0.21). Constituent monosaccharides from the hydrolyzates (separated by column chromatography) were identified to be D-galactose and D-mannose by their melting points, co-chromatography with authentic samples and by preparation of derivatives:[21] D-Galactose, mp 163 °C, $[\alpha]_D^{30}$ +80 (water); D-galactose phenylhydrazone, mp 153 °C; D-mannose, mp 131 °C, $[\alpha]_D^{30}$ +14 (water); D-mannose phenylhydrazone, mp 198 °C.

No hydrolysis was observed when aqueous solutions of the seed gums were exposed to microwaves in the presence of 1.48×10^{-4} M persulfate, indicating that the reagents immobilized on the porous solid support have advantages over the conventional solution-phase reaction as shown by enhanced reaction rates, and higher yields than those reported in other solid-supported reactions.[7] In an aqueous medium, even with 2.22×10^{-4} M persulfate, only partial hydrolysis could be observed (Table 2).

When the same seed gums were hydrolyzed with 0.1 N sulfuric acid (under aqueous conditions) under 100% microwave power, complete hydrolysis was observed between 1.45 and 7.66 min (Table 2). All the seed gums[17–23] under study possess a linear chain of β-(1→4)-linked D-mannopyranosyl units to which D-galactopyranosyl side chains are attached through an α-(1→6) linkage. It is clear that with the increase in β linkages hydrolysis time increased as expected. Due to the peripheral position[17–23] of the α-linked galactopyranosyl units in the seed gums and the weaker nature of the α linkages compared to β, seed gums having higher mannose content (β linkages) had higher hydrolysis time. Hydrolysis of the seed gums by conventional method (2 N H_2SO_4, 48 h) also furnished the same monosaccharides. Identities and configurations of the monosaccharides were confirmed by co-chromatography with authentic samples and the preparation of derivatives.

In a control experiment, gravimetric estimation of a known mixture of galactose and mannose, before and after microwave exposure, gave the same results, revealing that they do not degrade under the microwave conditions (Table 4). Gravimetric estimation of monosaccharides in the complete hydrolyzates of the seed gums hydrolyzed by the conventional method and under microwaves gave same results, showing their

Table 3. Hydrolysis of CM gum on Al_2O_3 support with fixed persulfate concentration and 100% microwave power, with 0.1 N sulfuric acid and 100% microwave power, and with 2 N sulfuric acid and 100 °C[a]

Sample no.	Seed gum (80 mg)	Ratio Gal:Man	Solid-supported microwave hydrolysis						Microwave hydrolysis using 0.1 N H_2SO_4		Hydrolysis (2 N H_2SO_4, 48 h, 100 °C)
			On solid support (200 mg)			On recovered solid support (200 mg)					
			Expos. in (min)	Cu_2O (mg)	pH	Expos. (min)	Cu_2O[b] (mg)	pH	Expos. (min)	Cu_2O[b] (mg)	Cu_2O[b] (mg)
1	GG	1:2.00	1.33	65	2	0.33	64	2	1.66	67	68
2	CM	1:2.54	1.33	65	2	1.33	62	2	2.18	65	65
3	CR	1:1.78	1.33	64	2	1.33	64	2	1.50	67	67
4	CA	1:2.00	1.33	65	2	1.33	64	2	1.72	65	68
5	ID	1:6.01	2.33	66	2	2.33	65	2	7.66	68	68
6	IH	1:3.00	2.16	65	2	2.16	63	2	3.33	68	67
7	IC	1:1.50	1.33	65	2	1.33	64	2	1.45	67	67
8	IQ	1:2.00	1.33	65	2	1.33	65	2	1.66	66	64

[a] Hydrolysis under 100% microwave power with 1.48×10^{-4} M $K_2S_2O_8$ at Al_2O_3 support; with 0.1 N H_2SO_4 under 100% microwave power and with 2 N H_2SO_4 at 100 °C for 48 h.
[b] Weight of Cu_2O obtained on gravimetric estimation of the hydrolyzates.

Table 4. Gravimetric estimation of the monosaccharides in the control experiment before and after microwave exposure

Sample no.	Galactose + mannose (mg)	Weight of Cu_2O (mg)	
		Before exposure to mw	After exposure to mw
1	80	64	64

hydrolysis to monosaccharides is complete under microwave conditions (Table 3).

Hydrolysis of the seed gums on the recovered alumina (obtained after extraction of the hydrolyzates) gave the same results (Table 3). Thus the solid support used can be easily recycled and thus minimizes the cost. No acid is initially required for the hydrolysis, rather it is produced during the course of the reaction and is consumed as the reaction proceeds.

Hydrolysis was not observed when a small amount of hydroquinone (HQ) was added to the reaction mixture. HQ is a radical quencher that is capable of combining any free radical present in the reaction mixture to give stable HQ radicals that cannot further propagate the radical mechanism. (The stability of the HQ radical results from delocalization of electronic charge density throughout the aromatic structure.) Quenching of the hydrolysis with HQ indicates the possibility of participation of free radicals in the hydrolysis.

However, as the reaction proceeds the pH of the reaction mixture drops to pH 2, suggesting acid formation during the reaction. It may be assumed that the formed acid is responsible for the glycosidic bond breaking, and an ionic mechanism similar to that of conventional hydrolysis is operative. However, it was observed that on addition of the hydroquinone to the reaction mixture, no hydrolysis takes place even though the pH was pH 2, indicating that under a microwave field, a mechanism other than ionic is operating. Additional work is required for a complete mechanistic understanding of the microwave-promoted hydrolysis process.

3. Experimental

A Kenstar (Model No. MOW 9811) domestic microwave oven with a frequency of 2450 MHz and a power output from 0 to 1200 W with continuous adjustment was used for all the experiments. Neutral alumina (BDH) was used as solid support. D-Galactose (Loba) and D-mannose (Loba) were used as authentic samples for co-chromatography with the hydrolyzates. All the solvents (E. Merck) used for chromatography were of AR grade. Solutions were concentrated at diminished pressure at 60–62 °C. Paper chromatography was carried out at room temperature with solvent systems[17] (A) 11:6:3 BuOH–2-PrOH–H_2O; (B) 10:4:3 EtOAc–pyridine–H_2O; (C) 2:1:2 EtOAc–pyridine–H_2O, with detection using aniline hydrogen phthalate. A Neukon 5700 Gas Chromatograph equipped with flame-ionization detector, at 190 °C with a Superleco S P 2380 column (3.0×0.53 mm) was used for GLC. The carrier gas was nitrogen. All the seeds were supplied by Himani Seed Stores, Dehradun, and identified by Botanical Survey of India, Allahabad, India.

3.1. Isolation of the seed gums

After exhaustive extraction[17] with petroleum ether (60–80 °C) and EtOH to defat and decolorize, respectively, the seeds (1 kg) were suspended in 1% aq AcOH overnight, mechanically stirred, and filtered. The filtrates (mucilage) were precipitated with 95% EtOH. Dissolution and reprecipitation of all of the seed gums were repeated six times to get pure seed gums. The crude gums were collected, washed with EtOH and dried.

While commercial sample of GG was used as such after purification.

3.2. Purification of the seed gums

The crude polysaccharides were purified[17] through barium complexing by preparing 2.5% (w/v) solutions of the gums by continuous stirring at 60 °C (12 h) and precipitating with satd $Ba(OH)_2$ solution. The complexes were separated by centrifugation and taken in 1 M HOAc, stirred (8 h), centrifuged, precipitated with EtOH, and were washed with 70%, 80%, 90%, and 95% EtOH. The samples were finally purified by dialysis and filtration through 0.45-μm membranes. The pure seed gums were non-reducing, white, fibrous materials.

3.3. Hydrolysis using $K_2S_2O_8$ under microwave irradiation on alumina support

An aqueous solution of the pure *C. marginata* (CM) seed gum (80 mg, 2 mL) was mixed well with a calculated amount of $K_2S_2O_8$ and Al_2O_3 (200 mg), and was exposed to 100% microwave power for different exposure times to monitor the complete hydrolysis. Water content in the slurry was maintained throughout the exposure by adding the required amount of water to it. After exposure, the reaction mixture was extracted with 5 mL of distilled water, neutralized with $BaCO_3$, and filtered. The filtrate was concentrated and examined by paper chromatography in solvent systems A, B, and C to detect the monosaccharides. The same experiments were performed with hydroquinone (20 mg).

All the other seed gums were also hydrolyzed as described above, using 1.48×10^{-4} M $K_2S_2O_8$ under 100% microwave power.

Recovery of alumina from the hydrolyzates was made by filtration. Recovered alumina was washed well with water, dried, and reused in the hydrolysis experiments. Gravimetric estimation of a known mixture of D-galactose/D-mannose (control) and of those obtained from all the seed gums before and after microwave exposure was also made, and the yields of the Cu_2O thus obtained were compared with the Cu_2O obtained by the gravimetric estimation of the reducing sugars in the same volume of the respective conventional hydrolyzates (hydrolysis with 2 N H_2SO_4 for 48 h).

3.4. Hydrolysis using $K_2S_2O_8$ under microwave irradiation in an aq medium

The seed gums (80 mg in 25 mL H_2O) were exposed to 100% microwave power for 1.33 min, with a calculated amount of $K_2S_2O_8$. The hydrolyzates were concentrated and examined by paper chromatography in solvent systems A, B, and C to detect the monosaccharides. Gravimetric estimation of the monosaccharides was also performed on the hydrolyzates.

3.5. Hydrolysis using H_2SO_4 under microwave irradiation in an aq medium

Seed gums (80 mg) in H_2SO_4 (25 mL, 0.1 N) were exposed to 100% microwave power for different exposure times. The hydrolyzates were neutralized with $BaCO_3$ and filtered, and the reducing sugars in the hydrolyzates were estimated by Fehling's method as described above.

3.6. Hydrolysis by conventional method

The seed gums (80 mg) were hydrolyzed with H_2SO_4 (25 mL, 2 N, 48 h) at 100 °C. The hydrolyzates were neutralized with $BaCO_3$ and were filtered. Quantitative determinations of the reducing monosaccharides were performed on the filtrates.

3.7. Identification of the monosaccharides

The seed gums (1 g each) were fully hydrolyzed on the solid support (2.5 g), using 1.85×10^{-3} M $K_2S_2O_8$ under microwave irradiation, and the resulting hydrolyzates were dissolved in 1:1 MeOH–H_2O and adsorbed on cellulose columns separately. Separation of the constituent sugars was done in each case using 5:1:4 BuOH–EtOH–H_2O as a solvent. Identities of the monosaccharides[17] were confirmed by co-chromatography with authentic samples and preparations of derivatives.

3.8. Quantitative estimation of the monosaccharides

The ratios of the constituent monosaccharides in the seed gums were determined[2] by GLC. The complete hydrolyzates of the seed gums (both microwave hydrolyzed and conventionally hydrolyzed) were evaporated, and the residues were reduced separately with $NaBH_4$. The resulting products were acetylated with 1:1 pyridine–Ac_2O (1 h at 100 °C). The alditol acetates thus obtained were in each case analyzed by GLC[2] (Table 5).

3.9. Stability of monosaccharides under microwave conditions

To ensure the stability of the constituent monosaccharides under the microwaves conditions, a control experiment was performed in which an equimolar mixture (40 mg each) of the galactose and mannose (supported on 200 mg Al_2O_3), mixed well with 1.48×10^{-4} M $K_2S_2O_8$, was exposed to 100% microwave power for 3 min. The reaction mixture was extracted with 5 mL

Table 5. Quantitative estimation of the monosaccharides in the hydrolyzates obtained by solid-supported microwave hydrolysis and hydrolysis with 2 N H_2SO_4 for 48 h at 100 °C

Sample no.	Seed gum	Solid-supported microwave hydrolysis	Hydrolysis with 2 N H_2SO_4 for 48 h at 100 °C
1	GG	1:2.00	1:2.01
2	CM	1:2.54	1:2.55
3	CR	1:1.78	1:1.76
4	CA	1:2.00	1:2.00
5	ID	1:6.01	1:6.00
6	IH	1:3.00	1:3.02
7	IC	1:1.50	1:1.51
8	IQ	1:2.00	1:2.00

of distilled water. The extract was boiled with excess of Fehling's solution, and the precipitated Cu_2O was washed well with water, dried, and weighed. D-Galactose and D-mannose were also estimated similarly in a separate reaction mixture with identical composition but without exposing it to the microwave field, and the yields of Cu_2O in the two were compared.

4. Conclusions

Seed gums can be efficiently hydrolyzed to their constituent monosaccharides under microwave irradiation in a very short time on an alumina support using a catalytic amount (1.48×10^{-4} M) of potassium persulfate. Under a microwave field in an aqueous medium, the same persulfate amount could not hydrolyze the seed gums. The present method of hydrolysis does not involve the use of acid, and the used solid support can be easily recovered for recycling. Complete hydrolysis time for the seed gums was found dependent on the types of linkages present in the polysaccharides.

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References

1. Bouveng, H. O.; Lindberg, B. *Adv. Carbohydr. Chem.* **1960**, *15*, 53–89.
2. Kapoor, V. P.; Chanzy, H.; Taravel, F. R. *Carbohydr. Polym.* **1995**, *27*, 229–233.
3. Singh, V.; Mishra, U. C.; Khare, G. C.; Gupta, P. C. *Carbohydr. Polym.* **1997**, *33*, 203–205.
4. Galema, S. A. *Chem. Soc. Rev.* **1997**, *26*, 233–238.
5. Deshayes, S.; Liagre, M.; Loupy, A.; Luche, J.-L.; Petit, A. *Tetrahedron* **1999**, *55*, 10851–10870.
6. Loupy, A.; Petit, A.; Hamelin, J.; Texier-Boullet, F.; Jacquault, P.; Mathé, D. *Synthesis* **1988**, *9*, 1213–1234.
7. Varma, R. S. *Green Chem.* **1999**, *1*, 43–45.
8. Kappe, C. O. *Angew. Chem.* **2004**, *43*, 6250–6284.
9. Wiesbrock, F.; Hoogenboom, R.; Schubert, U. S. *Macromol. Rapid Commun.* **2004**, *25*, 1739–1764.
10. Xia, L.; Li, K.; Cao, G.; Xi, Z. *Huaxue Schiji* **2000**, *41*, 352–355.
11. Plazl, I.; Leskovsek, S.; Koloini, T. *Chem. Eng. J.* **1995**, *59*, 253–257 (Amsterdam).
12. Cheng, M.-C.; Wang, K.-T.; Inoue, S.; Inoue, Y.; Khoo, K.-H.; Wu1, S.-H. *Anal. Biochem.* **1999**, *267*, 287–293.
13. Klun, U.; Kržan, A. *Polymer* **2000**, *41*, 4361–4365.
14. Zhong, H.; Marcus, S. L.; Li, L. *J. Am. Soc. Mass Spectrom.* **2005**, *16*, 471–481.
15. Xing, R.; Liu, S.; Yu, H.; Guo, Z.; Wang, P.; Li, C.; Li, Z.; Li, P. *Carbohydr. Res.* **2005**, *340*, 2150–2153.
16. Li, K.; Xia, L.; Li, J.; Pang, J.; Cao, G.; Xi, Z. *Carbohydr. Res.* **2001**, *331*, 9–12.
17. Singh, V.; Sethi, R.; Tiwari, A.; Srivastava, V.; Sanghi, R. *Carbohydr. Polym.* **2003**, *54*, 523–525.
18. Singh, V.; Tiwari, A.; Shukla, P.; Singh, S. P.; Sanghi, R. *React. Funct. Polym.*, in press.
19. Singh, V.; Pandey, M.; Srivastava, A.; Srivastava V. *Fitoterapia*, in press.
20. Singh, V.; Tiwari, A.; Tripathi, D. N.; Sanghi, R. *Biomacromolecules* **2005**, *6*, 453–456.
21. Singh, V.; Pandey, M.; Srivastava, A.; Srivastava, V. *Fitoterapia* **2003**, *74*, 40–44.
22. Whistler, R. L. In *The Encyclopedia of Polymer Science and Technology*; John Wiley & Sons: New York, 1969; Vol. 11, pp 1–416.
23. Srivastava, M.; Kapoor, V. P. *Chem. Biodiv.* **2005**, *2*, 295–317.