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Chemical modification of alginic acid by ultrasonic irradiation

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Abstract: Chemical modification of alginic acid has been done by ultrasonic irradiation to obtain its methylated, ethylated and isopropylated derivatives. The influence of ultrasonic frequency and power on esterification process of alginic acid has been investigated. Alginate derivatives have been characterized by degree of esterification (DE) and IR-FT spectroscopy. It has been found that 45 kHz ultrasonic frequency accelerated modification process as reduced the reaction time from 16 hours to 2 hours. The obtained results showed that ultrasound irradiation increased the reaction efficiency in methanol and depended on the ratio of the M/G.

Keywords: ultrasound-assisted synthesis, alginic acid, methyl alginate, ethyl alginate, i-propyl alginate

Introduction

Alginic acid (ALG) is a major constituent of the cell wall of brown algae, which is obtained by alkaline extraction [1]. It is an anionic heteropolysaccharide consisted of β -D-mannuronic acid (M) in conformation ${}^{1}C_{4}$ and its α -L-guluronic acid residues (G) in ${}^{4}C_{1}$ conformation. Macromolecule of alginic acid is linear connected by 1 \rightarrow 4 glycoside bonds. Three types of structure blocks can be distinguished in its main polymer chain: MMMM-block, only composed of mannuronic residues (mannuronate), the other homopolymeric block GGGG-units consisted of guluronic residues (guluronate) and the third mixed block (gulurono-mannuronate), formed by alternating sequence of guluronic and mannuronic residues - GMGM-block (Figure 1). Therefore, the structure of alginic acid can be presented in the following sequence:

 $M-G-M-(M-M)_n-M-G-(M-M)_q-M-G-(G-G)_p-G.$



Figure 1. Chemical block structure of alginic acid

13 Corresponding author: denev57@abv.bg DOI: 10.1515/asn-2016-0002 It has been known that the properties of alginic acid depend on the ratio between these three structural blocks, as well as its degree of polymerization [2]. Each of these blocks has its own conformation and functional properties.

Alginic acid is a food additive, registered under E 400. The free alginic acid and its calcium salt do not dissolve in water. Water-soluble are its ammonium, alkali metal and magnesium salts (E401 - E403) and its salts with organic bases [3].

It is well known that alginate salts are poor emulsifiers. They are mainly used as stabilizers because improve consistency and increase the viscosity of the product. Their use as a powerful thickening agent is explained by their ability to absorb water [3-5].

Alginic acid and its salts find application in food industry for production of various desserts, low-sugar marmalade, gel products and jams, ice cream and dairy products as well [3].

Due to the abundance of algae in water bodies, there is a large amount of alginate material presented in nature. The annual industrial alginate production is approximately 30.000 metric tons, and is estimated to comprise less than 10% of the biosynthesized alginate material. Therefore there is significant additional potential to design sustainable biomaterials based on alginates [6].

The combination of chemical and biochemical methods reveals new opportunities for design of modified derivatives of alginic acid by controlling the monomeric unit sequences, reacting the available functional group with variation of the position and number of substituents in alginate main backbone. Moreover, the incorporation of new functional group alter significantly the properties of obtained alginate derivatives such as solubility, hydrophobicity, specific affinity for proteins, and many others [7].

Over the past two decades in organic synthesis began application of ultrasound and microwave-assisted irradiation as approach to green synthesis and as means to enhance the conversion process in chemical reactions. Ultrasonic irradiation accelerates the reactions in liquid media by cavitation [8]. This process is observed in liquids subjected to abrupt changes in hydraulic pressure. Under the influence of ultrasonic waves in a liquid acoustic environment zones of elevated values in pressure appear, which very quickly changes to a highly reduced pressure. Depending on the nature of the liquid, its elasticity, viscosity, temperature and other factors, the ultrasound generating gas microbubbles which produce very high pressures and temperatures. This unique energy provides potential applications - in metallurgy, mechanical engineering, chemistry, medicine, etc.

Therefore, the aim of the current study was to investigate the esterification process of alginic acid by application of ultrasonic irradiation.

Materials and methods

In this study, two different type of sodium alginate were used as initial substance for esterification process:

ALG-1 from brown algae (SIGMA-ALDRICH, 99 % puriss, France) with moisture content ≤ 10 %, ash content ≤ 3 % and pH = 2.5 – 3.2, ratio between β -D-mannuronic acid and α -L-guluronic acid residues: 1.55 ; and ALG-2 Manugel with M/G ratio 0.66 All others reagents and solvents (methanol, ethanol, *i*-propanol, H₂SO₄) were of analytical grade.

Conventional esterification.

Before esterification process, alginic acid was dried at 40 °C in vacuum oven for 12 h. The dried alginic acid were esterified by $2M H_2SO_4$ /anhydrous alcohol (in ratio 1:10) at 40 °C under periodically stirring.

Ultrasound-assisted esterification process.

The esterification process was performed by above mention conditions but in the ultrasonic bath (VWR USC 100 TH, power 30 W) under constant ultrasonic frequency at 45 kHz and temperature set at 40 °C.

The new-synthesized methylated alginates obtained after the both esterification methods were washed three times with 70 % ethanol and then twice with 95 % ethanol until the neutral reaction.

The degree of esterification (DE) was determined by titration [9], as correction in molecular weight for ethoxilated and proposilated groups were done, respectively.

IR-FT spectra of newly synthesized alginic acid esters were recorded on a Nicolet FT-IR Avatar Nicolet, (Termo Science, USA) spectrometer using KBr pellets and absorption was reported in wavenumbers (cm⁻¹).

Results and discussion

Alginic acid is hydrocolloids that find wide application in food technologies. In the recent year its use in molecular gastronomy constantly increases. As a drawback in its functional properties can be regarded the fact that alginic acid is water insoluble, but its alkali metal and ammonium salts were easy soluble. The reason for the insolubility of alginic acid can be explained with formation of intermolecular hydrogen bonds between the free carboxyl groups of uronic acids. The idea for esterification of alginic acid with methyl alcohol was connected with design of backbone to resemble the the main chain of pectin. In nature, macromolecules of pectin are with degree of esterification in range from 63 to 75 % that makes them easily soluble in water and can be used for the formation of gels with different texture and gel strength.

In our previous studies we were obtained esters of alginic acid as the esterification reaction was conducted in heterogeneous media by Fisher's method [1].

In the current research, the modification was performed by application of new accelerated method – ultrasound-assited irradiation. Depending on the nature of the liquid, its elasticity, viscosity, temperature and other factors, the ultrasound generates gas micro bubbles which implosively disrupted. As a result, cavitation bubbles are appeared in extremely high pressures about hundreds of atmospheres and temperatures in the order of thousands of degrees Kelvin. Because of these processes, the acceleration of reaction was observed (Fig. 2).



Figure 2. Esterification of alginic acid ($R = -CH_3, -C_2H_5, -C_3H_7$)

The results from esterification of alginic acid (ALG-1) with different alcohols were presented in Table 1.

Time, min	MAA^1	EAA^2	IPAK ³				
	DE,%						
Ultrasonic irradiation							
5	8.6 ± 1.2	4.6 ± 0.8	2.6 ± 0.8				
15	21.4 ± 2.1	8.6±1.3	5.6 ± 1.1				
30	45.5 ± 2.2	12.3 ± 1.8	9.6 ± 2.0				
60	55.3 ± 3.5	14.2 ± 3.1	10.5 ± 3.0				
120	65.9 ± 4.8	16.3 ± 3.8	12.2 ± 2.9				
Conventional extraction							
1200	63.0±3.6	15.1 ±3.6	11.3 ± 3.1				
¹ MAA – methylated alginic acid							
$^{2}\Sigma$ A A sthulston d alginia agaid							

Table 1. Esterification of alginic acid (AA-1) with different alcohols

[•]EAA – ethylated alginic acid

³IPAA – i-propylated alginic acid

The results showed that the degree of esterification depended on the type of alcohols. The esterification reaction most readily takes place with methanol (MAA), and most slowly with i-propanol (IAA) Comparing these results with conventional esterification showed that sonication at 45 kHz decreased the reaction time almost ten times at equal other conditions (Table 1).

Alginic acid is heteropolysacchride with complex structure. The arrangement of G and M blocks along the main chain determines the chemical and physical properties of alginic acid [10]. M/G ratio can be defined by complete hydrolysis and further separation [11], by IR-FT spectroscopy by relationship between the experimental absorbance A_{1320}/A_{1290} : for M-unit v =1320 cm⁻¹ and for G-unit v=1290 cm⁻¹ [12] or by ¹H and ¹³C NMR spectroscopy [13].

Some scientific publications suggested that acetylation of alginic acid is preferred in mannuronic residues to guluronic units [14]. To check this statement we chose to esterified two types of alginic acids ALG-1 with M/G ratio 1.55 and ALG-2 with M/G ratio 0.66, respectively. The content of guluronic and manuronic acid residues were defined from IR-FT spectra by method described from Filippov [12].



Figure 3. Methylation of alginic acids with different molar ratio of structural units under ultrasound-assisted irradiation

From the results obtained was found that the alginic acid containing more L-guluronic acid residues in its backbone was esterified rapidly (Fig. 3). This tendency was reported in our previous investigation on alginic acid esters, but without application of ultrasonic influence [15].



Figure 4. Model of mannuronic acid residues with intermolecular hydrogen bonds suggested by *Smidsrod et al.* [16]

In the same study [17] it was presented that the hydrolysis of the alginates at pH > 2 was faster than that of the neutral polysaccharides under the same conditions. Thus it was shown that the reaction was catalyzed by undissociated carboxyl groups connected with intramolecular hydrogen bonds (Fig. 4). Obviously, this hypothesis requires the presence of carboxyl group next to the glycosidic linkage and the effect strictly depends on the sequence of monosaccharide units and the configuration of the C4 and C5. It was established that the hydrolysis rate degreased in the following order: MM > GM > MG > GG.

Based on the model proposed by Smidsrod et al. (Fig. 4) the preferred esterification of guluronic acid residues in alginic acid can be explained (Fig. 2).

Alginic acid and its esters were characterized by IR-FT spectroscopy. The data were presented in Table 2 and the assignment of bands was compared with reported in literature values for polysaccharides and alginates [19-24]. IR-FT spectra showed some characteristic bands. The broad band in range between 3400 cm⁻¹ and 3490 cm⁻¹ characteristic of the OH stretching vibration were shown.

Wavenumbe	Experimental IR band, cm ⁻¹				
r, cm ⁻¹ [citat-19 - 24]	ALG	MA A	EAA	IPA A	Assignment
3200-3600	3446	3502	3446	3421	$v_{O-H}(OH)$; H-bonds
2850-2904	-	2956	-	-	$v_{C-H}^{as}(CH_3)$
2933-2981	2929	2929	2929	2929	$v_{\text{C-H}}^{s}(\text{CH}_2)$
1720-1740	1740	1747	1740	1740	C=O stretch
1634-1664	1635	1635	1635	1635	Absorption of water, C=O
1416-1430	1418	1444	1418	1418	$\delta_{\text{C-H}}^{s}(\text{CH}_2), \text{C=O},$

Table 2. Assignment of IR-FT spectra of alginic acid and its esters

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				1373	COO
1350-1380	1356	1375	1373		$\delta_{C-H}(CH_3)$
1000–1290	1244	1246	1244	1244	v^{as} (C-O- C) and C-OH
1015-1060	1032	1033	1032	1034	v _{C-0} (C-O)
925 - 930	927	925	927	927	Stretching in pyranose ring,α-bond in polysaccharide chains
808 or 822	808	816	809	809	M block
787 or 887	879	875	877	877	G block

Stretching vibrations characteristics for C-H bond in methyl alginate were appeared at $v_{as}(CH_3) = 2960$ cm⁻¹ and $v_s(CH_3) = 2870$ cm⁻¹, while for i-propylated esters at 1365 cm⁻¹ and 1375 cm⁻¹. The observed in our study bands in IR-FT spectra (1361 cm⁻¹) were consistent with previously reported data in the literature [18]. All new synthesized alginic acid esters were characterized with decreasing the brands at 3446 cm⁻¹, due to the stretching vibrations of O–H from carboxyl groups. Moreover, the increase in width of the brands at 2960 cm⁻¹ and 2930 cm⁻¹ was assigned to C–H stretching and asymmetric deformations in CH₂ and CH₃ groups. The presence of new band at 1747 cm⁻¹ due to the C=O stretching vibrations of carbonyl alkylated ester showed that the esterification process was completed successfully.

Conclusion

Ultrasonic influence accelerates esterification process, by increasing the degree of esterification of the alginic acid esters. It has been found that ultrasound-assisted synthesis performed at 45 kHz reduced reaction time from 20 hours to 2 hours, in contrast to the esterification carried out under conventional conditions of heating.

The degree of esterification depends both on the type of alcohol and on the ratio of mannuronic and guluronic units in alginic acid backbone. FT-IR spectra showed that the carboxyl groups of alginic acid were esterified successfully.

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