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Research Article

# Microwave-Assisted Synthesis of Methyl Esters of Alginic Acids as Potential Drug Carrier

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**ABSTRACT**

Eco-friendly synthesis of methyl esters of two alginic acids with different ratio between M-block and G-blocks were performed by microwave irradiation. The alginates methyl esters have been characterized by degree of esterification (DE), HPLC-SEC, IR-FT and NMR spectroscopy and thermal analysis (TGA/DTA). The influence of reaction time on DE of alginic acids esters were investigated and compared with conventional and ultrasound–assisted synthesis. The highest DE (45 %) of methylated alginic acid (M/G ratio 61/39) was obtained by microwave-assisted synthesis (MAS) for 5 min. In addition, MAS accelerate esterification process as significantly reduced the reaction time from 60 min to 5 minutes. The microwave-assisted esterification did not degrade the alginic acid polymer chains as the coefficient of polydispersity remained constant 1.09 for alginic acid (M/G ratio 61/39) and 1.17 for alginic acid (M/G ratio 37/63), respectively. The ability of complexation of methyl esters of alginic acids with $Ca^{2+}$ ions was investigated and the promising results were obtained for future design of modified alginate-based micro particles as potential drug carriers.

**Keywords:** microwave-assisted esterification, alginic acids methyl esters, FT-IR, NMR spectroscopy, TGA, alginate esters microparticles

## INTRODUCTION

Microspheres containing biodegradable polymers are ideal for the applications in oral delivery systems[1]. Alginates are natural polysaccharides that are non-toxic, biocompatible and relatively inexpensive material that find significant application in food technology, biotechnology and pharmacy as matrix for encapsulating agents[2-8]. Alginic acid is linear binary copolymers consist of 1→4-linked M and G residues arranged with homopolymeric regions of α-L-guluronic acid residues (G-blocks) and a homopolymeric region of β-D-mannuronic acid sequences (M-blocks) interspersed by regions in which the two groups coexist in a strictly alternating sequence (MG-blocks)[10,11]. Alginic acids, its carboxylic salts and the esters are biopolymers which show interesting features such as biocompatibility, biodegradability, viscosifying and the ability of gelation with multivalent cations[1,2,9,12]. Substitution of alginate at the carboxyl group to form an ester is one potential approach to the preparation of derivatives with changed physicochemical properties[12]. In previous papers, we described the ultrasound-assisted synthesis (UAS) and physicochemical properties of hydrophobically modified alginate-ester derivatives in which methyl, ethyl, isopropyl residues were grafted onto the polysaccharide backbone via ester groups[3,13]. The application of ultrasound irradiation showed great efficiency and significant reducing in reaction time[13,14]. Many researches applied other promising green technology for successful modification of alginates and other polysaccharides – microwave irradiation[15-18]. The 'C–C' backbone of the preformed polymer being relatively non polar, remains unaffected by the microwave radiation, thus the structural integrity of the backbone remains intact, leading to a superior product[17]. To the best of our knowledge, there is no report in literature concerning the synthesis of methyl esters of alginic acid by microwave-assisted irradiation. Therefore, the objective of the present study was to synthesize and characterize methyl esters of alginic acids with different M/G ration under microwave-assisted irradiation and then to investigate the possibility of the preparation of calcium methyl alginate microparticles.

## MATERIALS AND METHODS

All reagents were of analytical grade and were used without further purification. Two types alginic acids with different content of mannuronic and guluronic acids were used in the esterification process. Alginic acid (ALG-1) with M-block 61 %, G-block 39 % and M/G ratio 1.55 was purchased from Sigma Aldrich, No. A-05550, 99 % puriss, France, while ALG-2 Manugel ® DMB (CAS 9005-38-3, Germany) with M/G ratio 0.66 and 63% guluronic acid (as specified by the manufacturer) were used. Before esterification process, alginic acids were washed with acidic 70 % ethanol to remove impurities, and then treated with 95 % ethanol to neutral pH values. The sample was dried at 40 °C in vacuum oven for 12 h, as previously described[13].

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*Conventional and ultrasound-assisted synthesis of methyl esters of alginic acids*

Conventional and ultrasound-assisted synthesis of methyl esters of alginic acids was performed under reflux at and in the ultrasonic bath VWR USC 100 TH, power 30 W) under constant ultrasonic frequency 45 kHz and temperature set at 40 °C as described[3,13].

*Microwave-assisted synthesis of methylated alginic acids*

The esterification of alginic acids was performed in an Erlenmeyer flask connected with reflux in microwave oven (Daewoo KOR, microwave output power 700 W and 2450 MHz frequency) for 15, 30, 60, 180 and 300 s. Five grams alginic acids (ALG-1 or ALG-2, respectively) were reacted with 2 mol/L $H_2SO_4$/anhydrous methyl alcohol (in molar ratio 1:10) by above mention conditions. At the end of certain reaction time the product was separated by filtration and the resulting methyl esters of alginic acids were washed several times with 70 % ethanol and then then twice with 95 % ethanol to neutral pH values. The synthesized methylated alginates were dried at 40 °C under vacuum.

*Homogeneity and molecular weight*

Number average molecular weight (Mn) and weight average molecular weight (Mw) of alginic acids and their methyl esters were determined by high performance size-exclusion chromatography (HPLC-SEC). SEC was performed in aqueous 0.1 M $NaNO_3$ solution as mobile phase at 30 °C, with the flow rate of 0.8 mL/min, employing a HPLC chromatograph ELITE LaChrome (VWR Hitachi, Japan) equipped with a column Shodex OH-pack 806 M (ID 8 mm, and length 300 mm), (Shodex Co., Tokyo, Japan) and a RI detector (VWR Hitachi Chromaster, 5450, Japan). The column was maintained at 30.0 ± 0.1 °C. All samples (3 mg/mL in phosphate buffer (pH = 6.4) were passed through a 0.45 μm syringe filter, PTFE45/25 mm (Isolab, Germany) before injection and 20 μL aliquot were injected for each run. The standard curve was established using different pullulans with known molecular weight (P-5, P-10, P-20, P-50, P-100, P-200, P-400, P-800). Polydispersity index (I = Mw/Mn) were obtained as characteristic of the polymers.

*Characterization of methylated alginic acids*.

The degree of esterification (DE) of methylated alginic acids was determined by titration method described in Food Chemical Codex from pectin analysis[19].

*FT-IR spectroscopy*

FT-IR spectra of alginic acids and their esters were recorded on a Nicolet FT-IR Avatar (Termo Science, USA) spectrometer in KBr pellets in the range 4 000–400 $cm^{-1}$ at resolution 4 $cm^{-1}$ as the absorption was reported in wavenumbers ($cm^{-1}$).

*NMR spectroscopy*

The $^{1}H$ and $^{13}C$ NMR spectra of alginic acids and their esters were recorded on a Brucker AVIII 500M spectrometer using polymer samples in form of sodium salts dissolved in $D_2O$ at a concentration of 40 mg/mL. All chemical shifts were given relative to a tetramethylsilane (TMS) as internal standard (D = 99.96 %).

*Differential thermal analysis* (TGA)

Alginic acids and their methyl ester were dried in a desiccator with $P_4O_{10}$ as drying agent. Their thermo-physical properties were investigated by differential thermal analysis –thermogravimetric analysis (DTA-TG) using LABSY TM Sevo (Setaram, France). The DTA- TG curves were obtained during heating of the samples with a heating rate of 5 °C/min from 10 °C to 400 °C under nitrogen atmosphere. All the analyses were performed in triplicate and the results were presented as mean values.

*Methyl alginate microspheres formation*

Methylated alginic acids solution (1 %) was prepared in phosphate buffer (pH = 6.4) followed by heating at 60 ºC, and stirring the mixture with laboratory homogenizing device Polytron ®PT45-80 (Kinematika, Switzerland) with technical characteristics – 1600 W, max 250.$s^{-1}$. The obtained suspension was rested for 20 min to remove the gaseous bubbles. The resulting liquid solutions were transferred into a syringe and were added as drops into calcium chloride solution (2 %), previously cool down at 4 ºC. Calcium chloride solution cross-linked the used carbohydrate polymer because $Ca^{2+}$ such as preferentially bind to polymer of L-guluronic acid of alginate that resulted in building of calcium alginate gel[5,20]. The resulting alginate microparticles were washed with distilled water. The dimeter of resulting particles was measured using a microscope system (microscope Olympus SC30, Philippines, equipped with a USB camera connected to a personal computer) were used. The drop sizes were observed at x100.

## RESULTS AND DISCUSSION

There are two available functional group types on the alginate backbone that may be used for derivatization - COOH and –OH groups. Selective reaction of charged carboxylate ($COO^{-}$) over neutral hydroxyl (-OH) groups is advantaged, since the former is a stronger nucleophile. The difference in nucleophilicity can be used to selectively react the carboxylate groups with substrates that can undergo $S_N2$ substitution[12]. Alginic acids with different M/G ration were esterified with acidic methanol to obtain methyl esters of alginic acid. Effect of reaction parameters such as reaction time, monomers ratio in alginate polymer under microwave power were investigated. The methylation of ALG-1 was carried out as illustrated (Fig. 1).

*Effect of reaction time*

The degree of esterification of alginic acid (ALG-1) varies depending on the time of microwave irradiation. The effect of reaction time on methylation reaction was firstly investigated by changing time interval from 15 to 300 s keeping other parameters constant. The results were summarized in Table 1 and Figure 2.

It was observed that the DE increased with increasing reaction time until 300 s, but the yield slightly decreased (80 %). We did not perform reaction more than 300 s, because the reaction media changed its color to faintly yellow that was indication for possible degradation after this time of esterification under the above mentioned conditions. Methyl alginate esters were soluble in polar aprotic solvents such as DMSO, DMF, they were insoluble

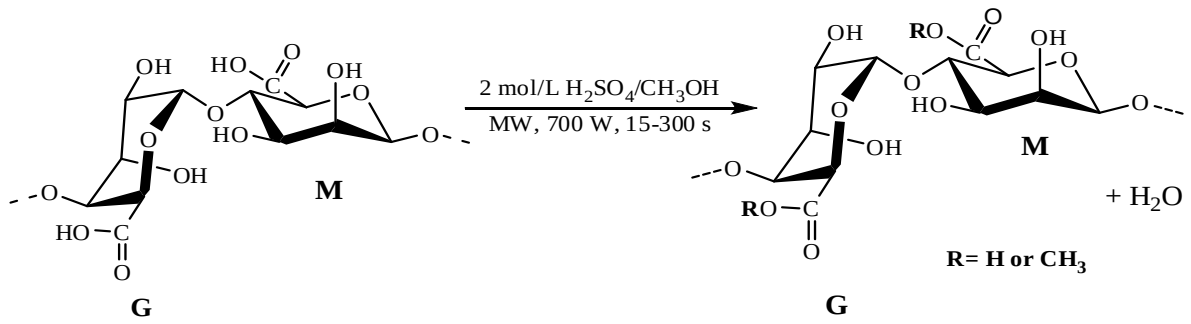
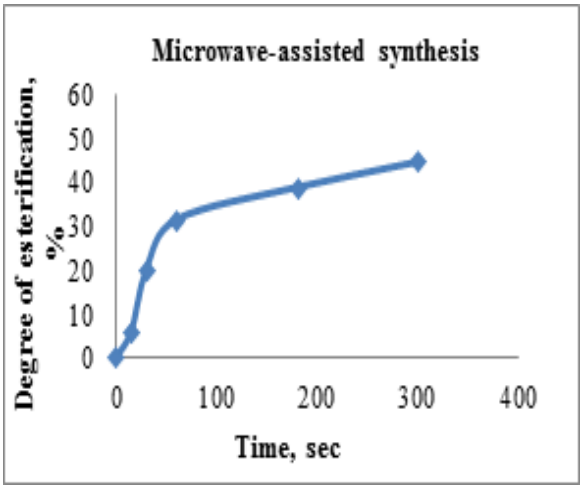
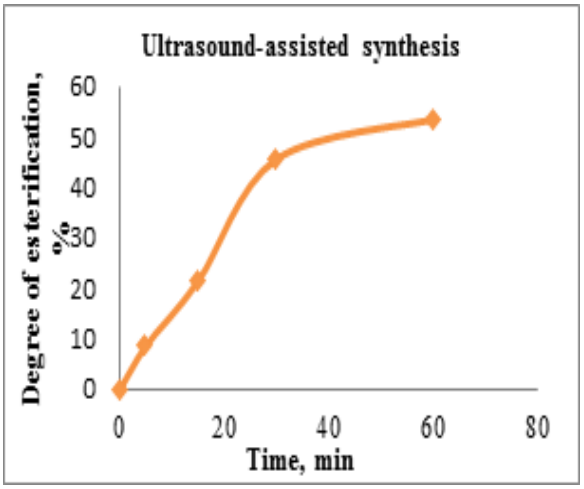



Figure 1: General reaction scheme for microwave-assisted esterification of alginates with acidic methanol

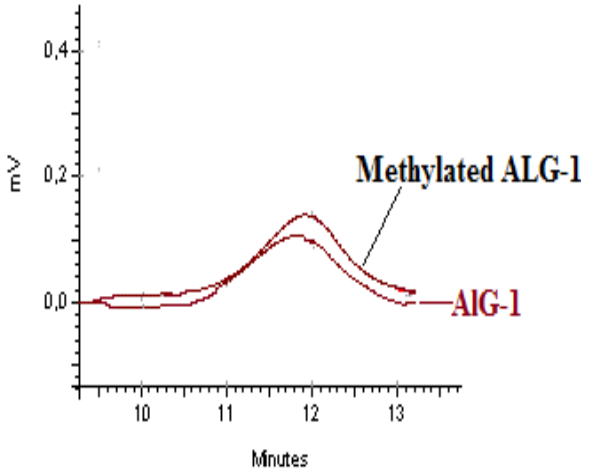



2 (a)

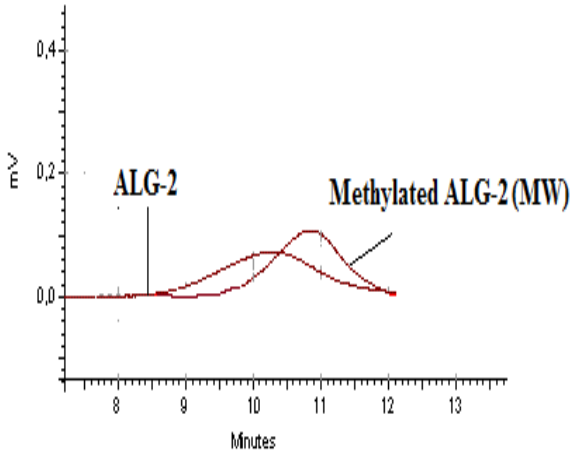



2 (b)

Figure 2: Methylation of alginic acids with M/G ratio 1.55 under a) microwave-assisted irradiation and b) ultrasound-assisted irradiation




3(a)




3(b)

Figure 3: SEC chromatograms of alginic acids and their methyl esters: a) alginic acid (M/G ratio 1.55) and its methyl ester; b) of alginic acid (M/G ratio 0.66) and its methyl esters

in methanol, ethanol, acetone and water. Our findings were in agreement with previous report for methylated alginic acids[12]. This to our knowledge is the first demonstration of successful substitution at the carboxyl group position to form alginate methyl esters under microwave irradiation. This method for synthesis demonstrated great efficiency as reducing the reaction time.

*Effect of structure of alginic acids and reaction conditions of esterification*

The microwave-assisted methylation of alginic acid was compared with other two methods for esterification (ultrasound-assisted and conventional synthesis at 40 ºC) (Fig. 2a and b and Table 2). Microwave-assisted synthesis demonstrated higher DE only for 5 min (300 s) (DE=44.7) (Fig. 2a) that was comparable with DE of methylated alginic acid (DE=45.5) under ultrasound irradiation for 30 min (Fig. 2b). The results presented in Table 2 showed that alginic acid containing more guluronic units was esterified more easily, than this with M-block (ALG-1). The possible

Table 1: Microwave-assisted methylation of alginic acids with different ratio of the structural units

| Product | | Time, s | Yield, % | DE, % |
|---|---|---|---|---|
| Methylated (M/G ratio 1.55) | ALG-1 | 15 | 88.9 | 6.0 |
| | | 30 | 85.7 | 20.1 |
| | | 60 | 81.6 | 31.5 |
| | | 180 | 85.0 | 38.9 |
| | | 300 | 80.4 | 44.7 |
| Methylated (M/G ratio 0.66) | ALG-2 | 60 | 80.6 | 33.3 |

DE: degree of esterification

Table 2: Synthesis of methylated alginic acids by different reaction conditions

| Alginic acid | Reaction conditions of methylation | | |
|---|---|---|---|
| | MAS, 60 s | UAS, 60 min | Conventional 20 h |
| ALG-1(M/G ratio 1.55) | DE=31.5 | DE=65.9 | DE=63.0 |
| ALG-2 (M/G ratio 0.66) | DE=33.3 | DE=82.9 | DE=86.8 |

MAS: microwave-assisted synthesis
UAS: ultrasound-assisted synthesis
DE: degree of esterification

explanation could be the proposed Smidsroed et al.[21] model for formation of intermolecular hydrogen bonds in algnic acids with dominating M-block, that block the access to the carboxyl group. The presented results (Table 2)

showed that synthesis of methyl esters of alginic acids can be successfully performed under microwave power, as the reaction time was reduced to 60 s. In comparison with conventional conditions for methylation the both green methods for synthesis (microwave and ultrasound irradiation) demonstrated significantly reducing the reaction time with promising results for the DE of methylated alginates. Our earlier report proved the efficiency for synthesis of methylated alginic acids as reducing the time up to 60 min[13]. Now, in the current study we demonstrated the efficiency for methylation of alginic acids under microwave power.

*Molecular weight of alginic acids and their methyl esters*

In the current study work, SEC-RI analysis was used for the determination of the weight-average molecular weights (Mw), number-average molecular weights (Mn) and the polydispersity (Table 3). The molecular weights and distributions of methylated alginic acids synthesized by conventional, ultrasound and microwave irradiation were analyzed by SEC and then were compared. The elution curves were presented in Figure 3. The elution profile of methylated alginic acids with dominating M-block (ALG-1) resembled the elution profile of initial alginic acid. After esterification molecular weight of the resulting esters of alginic acid with higher G-block ALG-2 decrease in grafting percentage in comparison with alginic acid (Figure 3). This observation could be explained with rigidity of G-block in acid media[8,26]. The various average molecular weights and polydispersity index of methylated alginic acids was summarized in Table 3. It was observed the decrease in molecular weights of methyl esters of alginic acids synthesized in conventional and ultrasonic conditions with increase of reaction time. Methyl esters of alginic acid obtained after microwave-assisted synthesis did not differ significantly with the initial alginic acids. Therefore, microwave irradiation did not cause destruction of algininate polymeric chain. It is well known that alginates suffer a loss in molar mass in both strongly acidic[21]. The methylation in acid conditions of alginic acids with higher G-block content (ALG-2) under conventional and ultrasonic conditions lead to reducing of molecular weight more than 8 or 6 times and polydispersity index decrease from 1.17 to 1.12. Therefore, the significant degradation of polymer was observed. However, methylated alginic acids with higher M-block remained their polydispersity index constant 1.09. This could be explained with ribbon like structure of alginic acids[2].

*FT-IR spectra*

The successful esterification of alginic acids ALG-2 (M/G ratio 37:63) by microwave irradiation was confirmed by FT-IR spectroscopy. The obtained spectra methyl esters of ALG-2 was presented (Fig. 4) and the most important bands were assigned (Table 4). Stretching vibrations characteristics for C-H bond in methyl alginate were appeared at $\nu_{as}(CH_3)$ = 2954 $cm^{-1}$ and $\nu_s(CH_3)$ = 2863 $cm^{-1}$. 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 $CH_2$ and $CH_3$ groups. The presence of new band at 1743 $cm^{-1}$ due to the C=O stretching vibrations of carbonyl alkylated ester showed that the esterification process was completed successfully. Bands at 1247, 1180 and 1103 $cm^{-1}$ were assigned to C–C–H and O–C–H deformation, C–O stretching, and C–O and C–C stretching vibrations of pyranose rings, respectively; the band at 1031 $cm^{-1}$ was due to C–O stretching vibrations. The anomeric, region (950– 750 $cm^{-1}$) was the most discussed in alginate. The spectrum showed a band at 925 $cm^{-1}$ that stretching in pyranose ring, α-bond in polysaccharide chains. The band at 808 $cm^{-1}$ was characteristic of mannuronic acid residues, and 738 $cm^{-1}$ for guluronic acid residue, respectively. FT-IR spectra of methyl esters of alginic acids (ALG-1) DE=44 % showed characteristic bands that were in agreement with the results reported in the literature for alginate derivatives[10,22,23], especially methyl esters[3,12].

*$^1H$ and $^{13}C$ NMR spectra*

Grafting of methyl groups onto alginate backbone was also confirmed by NMR studies.

Alginic acid ($^1H$ NMR (500 MHz, $D_2O$) δ 4.62, 4.49, 4.31, 4.17, 4.00, 3.89, 3.72) and $^{13}C$ NMR (126 MHz, $D_2O$) δ 181.47, 179.86, 175.79, 175.59, 171.15, 168.40, 101.37, 100.51, 80.65, 79.55, 77.50, 77.16, 75.88, 71.69, 71.37, 70.55, 70.27, 69.27, 67.73, 67.35, 65.80, 64.78, 42.56, 39.56, 39.25. The $^1H$ spectra of initial alginic acid (ALG-1) possessed chemical shifts between 3.72 ppm and 4.62 ppm. The signal at 4.62 ppm corresponds to the H-1 of

Table 3: Average molecular weights for alginic acids and their methyl esters determined by HPLC-SEC

| Samples | Conditions | Retention time ($t_R$), min | Weight average molecular weight ($M_w$), kDa | Number average molecular weight ($M_n$), kDa | Polydispersity index, $M_w/M_n$ |
|---|---|---|---|---|---|
| ALG-1 (M/G ratio 1.55) | - | 11.83 | 261.06 | 239.66 | 1.09 |
| Methylated ALG-1 DE = 54.1 %; | 4 h 40 °C, conventional | 12.09 | 166.91 | 155.17 | 1.08 |
| Methylated ALG-1 DE = 46.6%; | 60 min, US | 11.90 | 231.76 | 213.47 | 1.09 |
| Methylated ALG-1 DE = 31.5%; | 60 s, MW | 11.88 | 239.78 | 220.65 | 1.09 |
| Methylated ALG-1 DE = 44,7%; | 300 s, MW | 11.99 | 199.88 | 184.88 | 1.08 |
| ALG2 (M/G ratio 0.66) | - | 10.25 | 383.41 | 326.37 | 1.17 |
| Methylated ALG-2 DE = 86.2% | 4 h 40 °C, conventional | 11.49 | 46.54 | 42.04 | 1.11 |
| Methylated ALG-2 DE = 60.1 % | 60 min, US | 11.32 | 62.14 | 55.68 | 1.12 |
| Methylated ALG-2 DE = 33.3 %, | 60 s, MW | 10.34 | 328.99 | 281.26 | 1.17 |

Table 4: Characteristic bands of methylated alginic acids found in the FT-IR spectrum

| Bands, $cm^{-1}$ | Assignment |
|---|---|
| 3304 | Hydrogen bonded O–H stretching vibrations |
| 2954 | C–H stretching vibrations |
| 1743 | Ester C=O stretching vibrations |
| 1247 | Ester C=O stretching |
| 1416 | Symmetric stretching vibration of the carboxylate group |
| 808 | M-block |
| 738 | G-Block |

guluronic units, whereas the H-5 of guluronic units in GG block appears at 4.31 ppm, and the large signal between 4.49 and 4.7 ppm belongs to H-1 of mannuronic units and H-5 of guluronic units in GM moieties. Six major signals at 101.3 (C-1), 71.69 ppm (C-2), 72.29 ppm (C-3), 79.55 ppm (C-4), 77.16 ppm (C-5) and 175.79 ppm (C-6), confirmed presence of mannuronic acid residue. The chemical shifts characteristics for guluronic acid residue were observed: 100.51 ppm (C-1), 65.80 ppm (C-2), 70.27 ppm (C-3), 80.65 (C-4), 67.73 (C-5), 175.79 (C-6). The results were close to previously described for alginic acids from different sources[10,11,22]. Methylated alginic acid. $^1$H NMR (500 MHz, $D_2O$) δ 4.63, 4.48, 4.29, 4.18, 4.08, 4.02, 3.95, 3.90, 3.74, 3.58, 3.41, 3.35 ppm and $^{13}$C NMR (126 MHz, $D_2O$) δ 175.83, 175.62, 171.15, 168.40 (G-6), 101.36 (M-1), 99.94, 80.73 (G-3), 77.11, 75.90, 71.73 (G-4), 70.57, 69.33, 66.98 (G-5), 65.12 (G-2), 48.83, 42.56, 39.27 ppm. The signals for O–$CH_3$ protons appeared in region of chemical shifts 3.35 – 3.58 ($^1$H NMR) and carbon atoms from esters groups were observed at δ 175.83, 175.62, 171.15, 168.40 ($^{13}$C NMR). In $^{13}$C NMR spectra, the signals at 69.33 and 48.83 ppm were assigned to methyl groups from carboxyl groups of uronic residues. The peak at around 176 ppm was assigned to the carbon atoms of the carboxylic groups, which come from alginate[15,16]. Similar findings have been reported for polysaccharides esters[14] and derivatives of alginic acids[12,16].

*Thermal analysis of alginate polymers*

The TGA and DTA curves of the investigated alginic acids and their methyl esters were shown in Figure 5 and Figure 6. The pure alginic acid (Figure 5) exhibited two-stage degradation behavior. The first mass loss of the commercial alginic acid of 7.2 % was observed in temperature between 60.7 - 138 °C. The first weight loss of methylated alginic acid of 5.6 % was accompanied with two endothermic peaks with maxima at 69.3 ˚C and 114.3 ˚C, respectively. These endothermic processes were probably due to dehydration. The similar observations were reported for alginate-graft-poly(N-isopropylacrylamide)[16]. The maximum decomposition rate at 69.3 °C, was assigned to the elimination of free water adsorbed to the hydrophilic polymer[15,17]. The differential thermogravimetric curve of methylated alginic acids showed three main degradation steps (Figure 6). The degradation of the alginic acid polymer macromolecules began at 190 °C with mass loss 55.2 %. Destruction of methylated alginic acids was observed after 242.3 °C with weight loss 53.5 %, which also reveals that copolymer is more thermal stable than alginate backbone. These decomposition was ascribed to a complex process including dehydration of the saccharide rings, depolymerization with the formation of water, $CO_2$ as reported in previous studies[15-17]. Similar observation was described for other alginate derivatives on lauryl grafted alginate and sodium alginate-graft-poly (N, N-dimethylacrylamide obtained by microwave synthesis[16]. All these observations revealed the higher thermal stability of synthesized methyl ester of alginic acid for potential application in food processing and pharmaceutical preparations.

*Ca-methyl alginate microparticles formation*

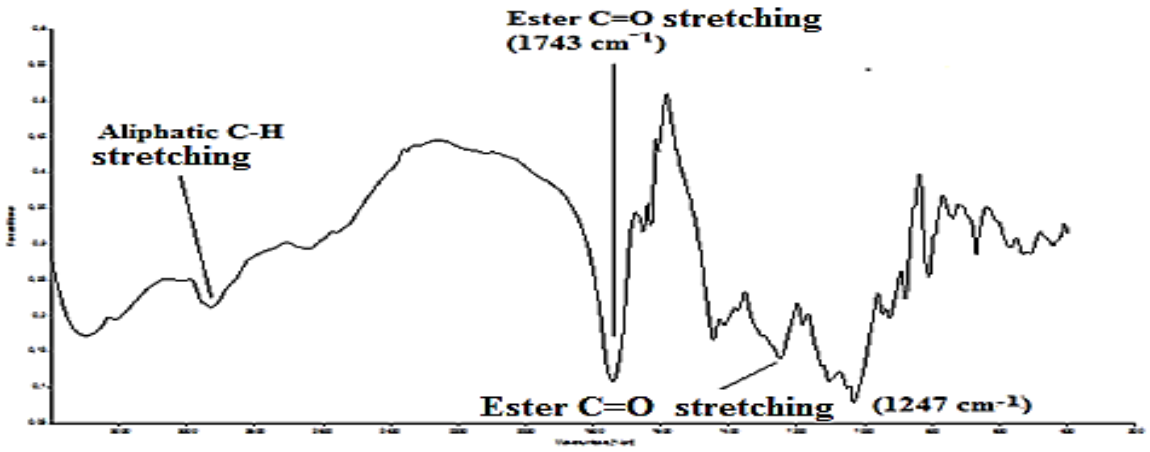



Figure 4: FT-IR spectrum of methylated alginic acid by microwave irradiation for 300 s

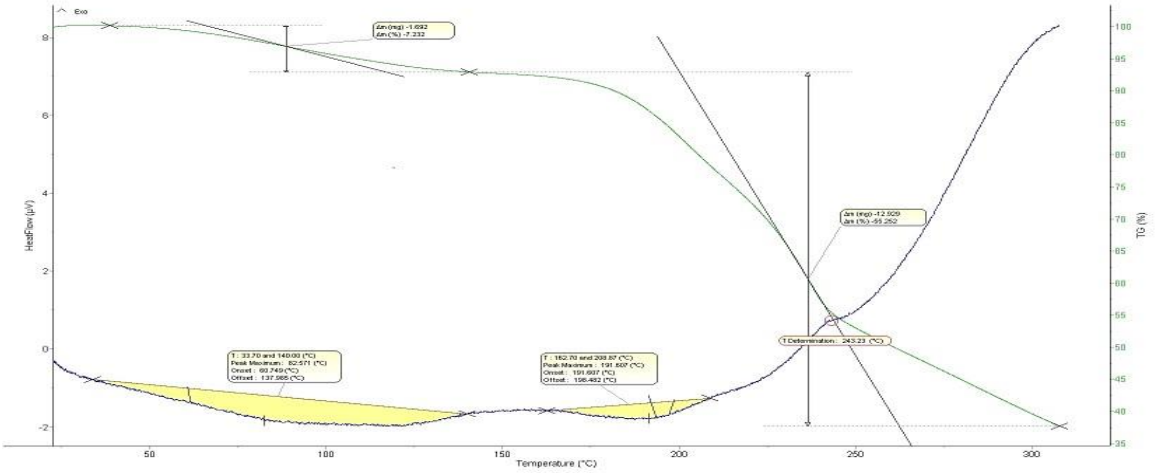


Figure 5: TGA/DTA analyses of alginic acid

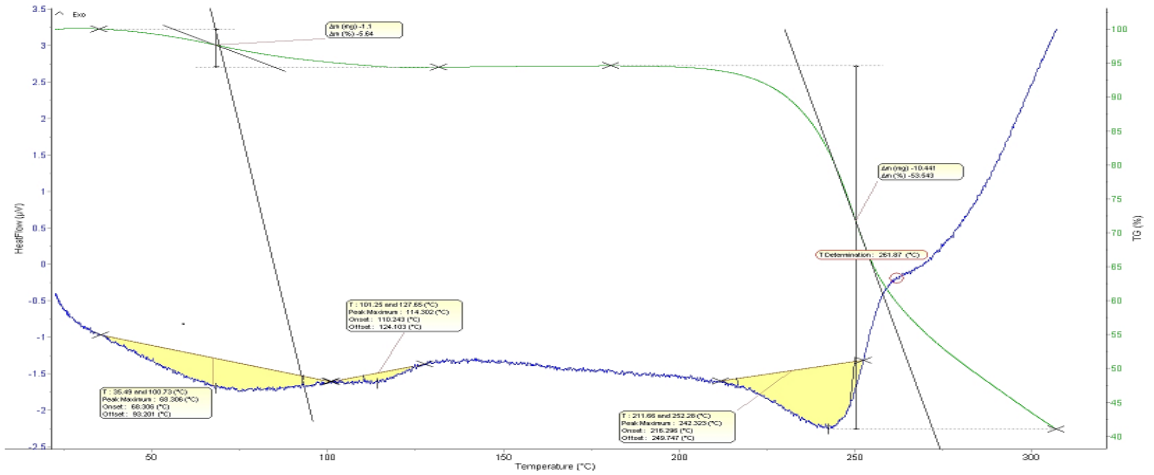
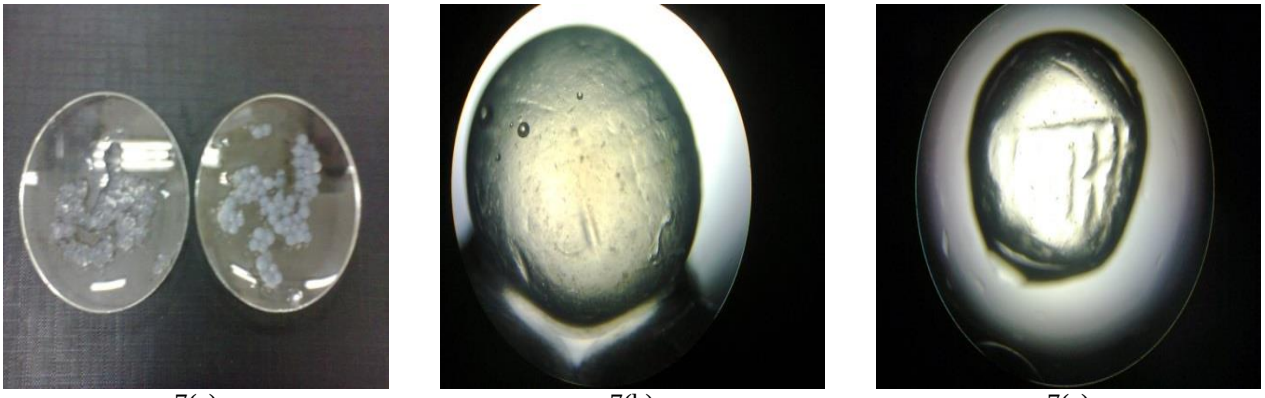


Figure 6: TGA/DTA analyses of methylated alginic acid

There many reports for encapsulation of sodium alginate and different alginic derivatives[1,2,4,5,20,23,25,28]. Until know the ability of methyl esters of alginic acids with different M/G ratio to interact with $Ca^{2+}$ were not investigated. Therefore, the object of our study was to investigate the microparticles formation of methylated alginic acid with calcium ions. The effect of the mannuronic/guluronic acid content on shape of the formed microparticles, prepared with methylated different types of alginates, was investigated. The methyl esters of alginic acids formed Ca-alginate particles (Figure 7). The mean particle sizes of the microparticles were also determined. The resulting Ca-methyl esters alginate particles had a particle size ranging between 500-800 μm. The methylated alginate gel



7(a) 7(b) 7(c)

Figure 7: Methylated alginate microparticles; Optical microscope image of methylated alginate microcapsules b) methylated ALG-2 and c) methylated ALG-1, (magnification x100) Olympus microscope. Mean diameter 500-650 μm.

microparticles prepared with dominant guluronic acid content (M/G ratio 37:63) methylated alginate (ALG-2) ALG-2 (showed better spherical shape. This could be explained with the ability of guluronic block to form egg-box model[26]. It was observed that the alginate methyl esters microparticles were stable at pH 7-7.5. The stability in this pH range was also observed for another alginate grafted polymers[16]. Therefore, the formed methylated alginate microparticles were evaluated as possible candidate for design of new pharmaceutical products.

## CONCLUSION

The series of methylated alginic acids were synthesized by microwave-assisted irradiation. The highest degree of esterification (44.4 %) was obtained for 300 s under microwave power. The application of microwave in synthesis of methyl esters of alginic acids demonstrated significantly reducing of time for modification to 2-5 min in comparison to ultrasound and conventional esterification. The synthesized methyl esters of alginic acids formed microcapsules with $Ca^{2+}$ but more detailed analysis should be done. Therefore, the methylated alginic acids showed promising results for future production of drug delivery microparticles.

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