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Journal of Chromatography B, 850 (2007) 488–492
JOURNAL OF CHROMATOGRAPHY B
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Determination of alginate copolymer in pharmaceutical formulations by micellar electrokinetic chromatography

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Received 15 May 2006; accepted 18 December 2006
Available online 30 December 2006

Abstract

A micellar electrokinetic chromatography method was developed for the determination and quantification of sodium alginate. The alginate peak migrated in the very short time of 1.33 min and calibrated easily though the polydisperse properties of alginates. The minimum detection limit (LOD) of the method was calculated as 0.393 mg/ml. This analysis method was successfully applied to the alginate quantification in an antacid pharmaceutical formulation. Precise and reproducible analysis results were obtained, with liquid formulations injected directly without any pre-separation process.

Keywords: Capillary electrophoresis; MEKC; Alginate; Antacid

1. Introduction

Alginates are the polysaccharides obtained from marine brown algae. Alginic acid is a linear, 1,4-linked copolymer of β-D-mannuronic acid (M unit) and its C5 epimer, α-L-guluronic acid (G unit), as seen in Fig. 1. The G and M units are joined together in homopolymeric and heteropolymeric sequentially alternating blocks [1]. Alginates and alginic acid are widely used in food industry, biotechnology, and medicine, because of their gel-forming capacity. The physical properties of alginates depend on the seaweed source of origin, the season of harvest, and the part of algae from which the alginate is extracted. Because of the complexity and the high molecular weight of the alginate polymer, analysis methods for this agent have been lacking, whereas the growing interest in the application of alginates for biomedical and pharmaceutical purposes requires sensitive methods for monitoring the quantity of alginate in quality control processes.

In fact, polysaccharide concentrations cannot be measured easily. General methodology depends on the colorimetric or chromatographic analysis of uronic acids released from an enzymatic depolymerization of polysaccharides. Colorimetric methods are non-specific between various uronic acids. Chromatographic methods are generally based on the prediction of the polysaccharide content from the mean uronic acid concentrations. There is no reported study on GC or HPLC for the total alginate separation. Desille et al. reported alginate analysis in biological fluids by high-performance anion-exchange chromatography [2]. Rourke et al. analyzed alginate content in lean ground pork by a cation exchange ion-exclusion HPLC [3]. Both studies were based on the analysis of uronic acids of alginate copolymer. Kennedy and Bradshaw reported a colorimetric method based on precipitation of alginate extracted from plant with a poly cation [4]. Horn et al. introduced a multivariate calibration method in order to relate near infra-red spectral data to monitor the alginate content of a plant stipe during biodegradation [5]. The method revealed some dependence on material type, reflecting differences in polyphenol content.

In recent years, capillary electrophoresis (CE) has gained continuous attention for the analysis of mono, oligo, and polysaccharides [6]. The advantages of the capillary electrophoretic methods are the considerable diminutions in the sample preparation and analysis times, as well as in the reagent consumption. CE is particularly suitable in the analysis of complex matrices, owing to its higher resolving power. Two studies have been reported by now on the alginate analysis by CE.

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doi:10.1016/j.jchromb.2006.12.025

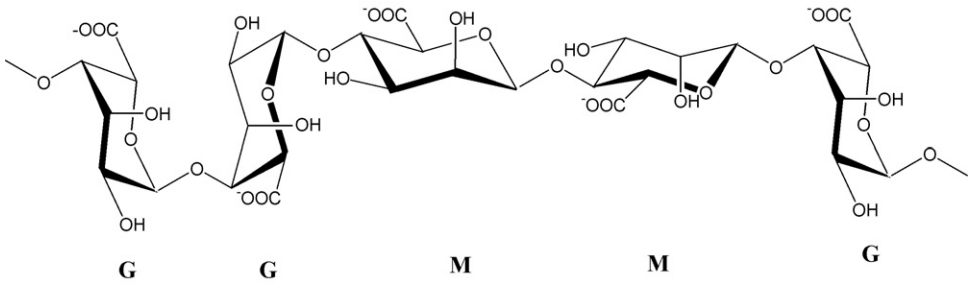

Fig. 1. The chemical structure of alginate.

Stefansson analyzed alginate samples according to molecular weight and chemical composition [7]. In the former case, the polydispersity of alginic acids is analyzed using linear polyacrylamide polymer solutions as sieving media, which provides the separation of alginate fractions. In the later case, a CZE method was applied using dynamic modification of alginate with calcium metal ion, but an unresolved peak with a double shoulder prevented total alginate calibration. Moore et al. reported a successful analysis of total alginic acid content in antacid formulations by capillary electrophoresis. The basis of the method is the pre-treatment of the formulation to remove most of the other ingredients, followed by the well-known borate complexation methodology for quantitative determination of total alginate [8].

Alginate based raft-forming formulations have been marketed word-wide for over 30 years under various brand names. Recently, alginate based formulations were widely reviewed by Mandel et al. [9]. These formulations are used for the symptomatic treatment of heartburn and oesophagitis, and appear to act by a unique mechanism, which differs from that of traditional antacids. In different commercial samples, even if the same alginate content appears on the labels, different characteristics will obtain with different alginate sources. Preliminary studies, with borate complexation and CE, carried in our laboratory for the alginate determination in a commercial antacid prevented, by a double-shouldered peak, an acceptable calibration for this alginate. The fact that Moore et al. obtain a single alginate peak by this method, whereas we obtain a double-headed peak, establishes that, depending on the origin, the relative proportion of the uronic acids determines the complex forming ability of polysaccharide. Accordingly, the current study aimed at the development of a micelle electrokinetic method that makes possible the determination of the total alginate content and that can be used in antacid formulations.

2. Materials and methods

2.1. Materials

Sodium alginate (Protanal®), from FMC Biopolymer (Brussels, Belgium), was obtained from Deva Holding Inc. (Istanbul, Turkey). Sodium dodecylsulfate and Tris were purchased from Merck (Darmstadt, Germany). Gastril Forte Suspension (a newly developing antacid formulation) was obtained from Deva Holding Inc. (Istanbul, Turkey). The liquid formulation contains, in each 10 ml, 1200 mg of calcium carbonate, 140 mg of magnesium carbonate, and 300 mg of sodium alginate, together with protective and flavoring agents.

2.2. Apparatus and operating conditions

Separations were performed with an Agilent capillary electrophoresis system equipped with a diode-array detector. The data processing was carried out with the Agilent ChemStation software. The wavelength was set at 195 nm. The separation was performed at 28 kV. Injections were made at 30 mbar for 4 s. The temparature was set at 30 °C.

The fused silica capillaries used for separation experiments were 75 μm i.d. and were obtained from Polymicro Technologies (Phoenix, AZ, USA). The total length of the capillary was 35 cm and the length to the detector was 27 cm. The fused silica capillary was conditioned prior to use by rinsing with 1 mol/l NaOH for 30 min, water for 10 min, and buffer with 10 min. The capillary was washed with buffer solution for 1 min between the runs. All solutions were prepared with deionized water purified in an Elgacan C114 (Elga, England) filtration system.

2.3. Preparation of solutions

For the alginate analysis in the pharmaceutical suspensions, the aliquots taken from the well-shaken suspension were weighed between 1.5 and 2.5 g in a 10 ml volumetric flask and were diluted to the volume with water. After sonification for 10 min, the suspension was filtered from 0.2 μm micro filter and directly injected.

Calibration solutions were prepared in the placebo, a formulation that contains all ingredients except for the alginate. Appropriate volumes from the stock solution of standard sodium alginate were added to the placebo solution in a 10 ml of volumetric flask and the mixture was diluted to the volume with water. A similar procedure to that of the prepared sample solution was followed before injections.

3. Results and discussion

Since the constituents of the alginate copolymer, mannuronic acid and guluronic acid, are acidic monomers having pK_a values 3.2 and 3.6, respectively [5], we tested first the electrophoretic behavior of alginate in CZE conditions. For this purpose, citrate buffer was used at three different pH values, *i.e.*, 3, 5, and

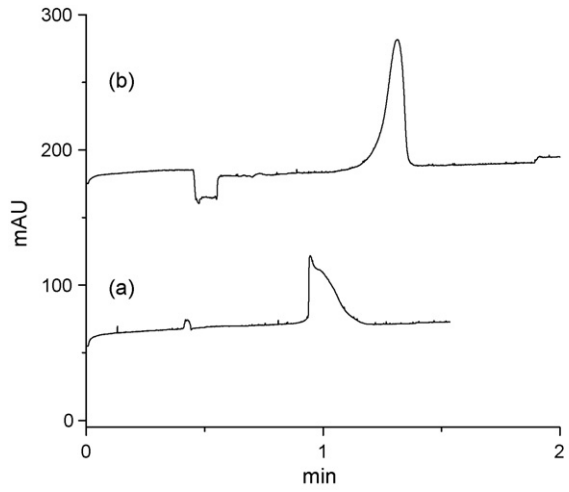

Fig. 2. The electropherogram of standard sodium alginate sample (6 mg/ml) (a) in 10 mmol/l borate buffer at pH 9 and (b) in 50 mmol/l SDS, 5 mmol/l Tris buffer at pH 10.5. The applied voltage for both conditions is 28 kV. The current is 130 and 150 μA for (a and b), respectively.

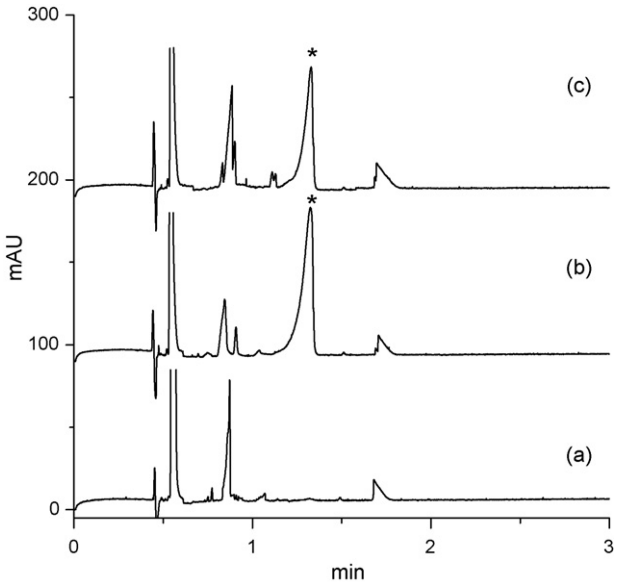

Fig. 3. The electropherograms of placebo (a); placebo spiked with alginate (b); and Gastril Forte formulation (c). Symbol (*) shows the alginate peak. The separation conditions are 50 mmol/l SDS, 5 mmol/l Tris, pH 10.5. Voltage 28 kV. The current is 150 μA. The total length of the capillary was 35 cm and the length to the detector was 27 cm. The capillary i.d. is 75 μm. UV detection was at 195 nm.

6.8. Even at pH 6.8, the alginate peak appeared at the migration time of electroosmotic flow marker (negative water peak). The pH of the running electrolyte was increased to 8.5 with the use of Tris buffer, but the alginate peak did not gain a noticeable electrophoretic mobility. When the borate buffer was used at pH range between 8 and 10, the alginate peak appeared after the electroosmotic flow marker due to the well-known complexation effect of borate buffer with polysaccharides. Fig. 2a shows the peak of the standard alginate solution in borate buffer. As seen from the electropherogram, the peak has two shoulders. Different borate concentrations were tested to improve the peak shape, but did not yield one well-shaped alginate peak.

In order to apply MECK to alginate analysis, the most commonly used surfactant SDS was chosen and its concentration was changed between 15 and 50 mmol/l in Tris buffer. In that concentration range, one symmetrical peak was obtained for standard alginate samples (Fig. 2b). As mentioned above, in plain Tris buffer at pH 8.5, the alginate peak comes with the electroosmotic flow, whereas in a pH 8.5 medium with added SDS the alginate peak arrives after the electroosmotic flow peak. This clearly shows that SDS and the alginate interact. Bu et al. [10] have reported an interaction between unmodified alginate and SDS at high surfactant concentrations. Since the interaction is not electrostatic, but hydrophobic-hydrophobic interaction, it can be theoretically expected that the SDS carbon chain and the alginate copolymer chain will show this interaction.

The pH range of the buffer was changed between 8.5 and 10.5. The 50 mmol/l SDS at pH 10.5 were chosen as optimal SDS concentration according to peak shape and reproducibility of peak migration time and area. Even with the broad peak shape expected for polymeric compounds, the reproducibility of migration time and peak area for seven successive injections was found as 0.48 and 2.30%, respectively. The very satisfactory precision values suggest that MEKC can be an applicable method for the quantification of alginate in pharmaceutical formulations.

The pharmaceutical formulations were prepared as described in Section 2.3, and injected directly. Peak identification was performed by spiking the samples with the standard compound and by UV spectral analysis. The placebo did not give any peak at the alginate peak area. Although the placebo contained considerable matrix, the peak heights and shapes were not different from those in aqueous solutions. Fig. 3 shows the elecropherogram of the placebo (a), the placebo spiked with alginate (b), and the Gastril Forte formulation (c).

4. Validation of the method

4.1. Linearity range and calibration curve

The linearity for sodium alginate was checked using six levels of concentrations added to the placebo solution. The linearity

Table 1
Calibration data, detection limits and quantification limits

Compound	Regression equation	Correlation coefficient	LOD (mg/ml)	LOQ (mg/ml)
Alginate	$0.6119x - 0.03770$	0.99933	0.393	1.31

ranges were determined to be 2–12 mg/ml. The peak symetry did not change with concentration.

4.2. Limit of detection

The limit of detection (LOD) was obtained as the concentrations of the sodium alginate that caused a peak with a height three times the baseline noise level.

Regression equation, correlation coefficient, LOD and LOQ values are given in Table 1.

4.3. Precision of method and quantification of alginate in pharmaceutical formulation

The precision of the method was calculated as the coefficient of variation (CV) of six analysis results of the pharmaceutical formulation.

Six samples of pharmaceutical formulation were prepared as described in Section 2.3 and injected directly to the capillary column. The relative standard deviation of the six results was found as 2.19%.

The 95% confidence limit for the mean value of six results was found as 304.20 ± 7.54 mg/10 ml. The producer's claim for alginate content is 300 mg/10 ml (acceptable range 285–315 mg/10 ml).

4.4. Recoveries

Accuracy was evaluated by performing recovery experiments. The pharmaceutical suspensions spiked with sodium alginate at three concentration levels (49, 98, and 146% of the normal alginate concentration in the suspension) were analyzed. The solutions were replicated three times each, and the amounts determined were compared to the theoretical amounts. The mean recovery was 99.13% and the relative standard deviation was 2.89%, which is adequate for the validation of accuracy of the method. Concentrations and recoveries are given in Table 2.

4.5. Analysis of pharmaceuticals

The CE method was applied on three different pharmaceutical formulations. The liquid formulations were prepared as indicated in Section 2.3. The pharmaceutical extracts were injected into CE column after filtration from 0.2 μm micro filter. The alginate peaks were obtained for all formulations as seen from electropherograms in Fig. 4. The other compounds in the pharmaceutical preparations, such as sodium bicarbonate, calcium carbonate, magnesium carbonate, methyl paraben, propyl paraben, sodium saccharin, peppermint oil, chocolate flavor, benzyl alcohol, mannitol, colloidal silica, did not interfere with the alginate. All extracts were spiked with alginate standard in three different concentration level and recovery values as seen from Table 3 are very acceptable. The intermediate precision between the migration times of alginate peaks for four

Table 2
Recovery values for sodium alginate

Number of sample	Amount added (mg)	Percent increase (%)	Recovery (%)
1	2.01	49	95.60
2	2.01	49	95.79
3	2.01	49	95.32
4	4.01	98	100.8
5	4.01	98	100.1
6	4.01	98	99.01
7	6.00	146	100.9
8	6.00	146	102.1
9	6.00	146	102.6

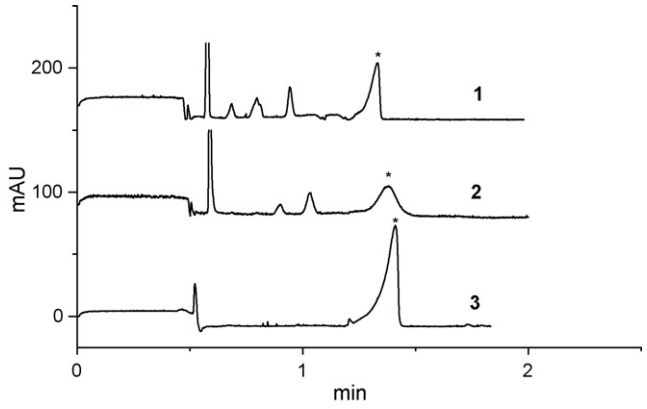

Fig. 4. The electropherograms of three different pharmaceutical formulations. 1, 2, and 3 refer to brands 1, 2, and 3 given in Table 3. Electrophoretic conditions are the same as in Fig. 3 and symbol (*) shows the alginate peak.

Table 3
Recovery values of alginate for commercial pharmaceuticals

Commercial pharmaceutical	Labeled amount of alginate	Other ingredients	Recovery (%)
Brand 1 (liquid)	500 mg sodium alginate/10 ml	Sodium bicarbonate (267 mg), calcium carbonate (160 mg), methyl paraben, propyl paraben, sodium saccharin, peppermint oil	97, 108, 104
Brand 2 (liquid)	300 mg sodium alginate/10 ml	Calcium carbonate (1200 mg), magnesium carbonate (140 mg), sodium saccharin, peppermint oil, chocolate flavor, benzyl alcohol, propyl paraben	95, 101, 103
Brand 3 (powder)	225 mg sodium alginate, 87.5 mg magnesium alginate per sack (0.65 g powder)	Mannitol, colloidal silica	89, 98, 96

different pharmaceutical extracts obtained in different days is 2.88%.

5. Conclusion

The MEKC method described was found to be applicable to the determination of the alginate content in the antacid pharmaceutical preparations. Successful determination of alginate and accurate results were obtained in a short period of time without any time-consuming purification step except centrifugation. The sample matrix did not interfere with the analysis. Micelle formation contributed both to increased resolution and to increased solubility of the ingredients in the solution, and consequently well-resolved peaks were obtained by direct injection. The method is simple, fast, inexpensive, and precise. Thereby, it promises to be applicable to the quality control of alginate content in antacid formulations.

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