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**STUDY CONCERNING THE PHYSICAL
AND CHEMICAL DESCRIPTION
OF MAGNESIUM ALGINATE OBTAINED
FROM THE BROWN
ALGA CYSTOSEIRA BARBATA**

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INTRODUCTION

Alginates are quite abundant in nature since they occur both as a structural component in marine brown algae, comprising up to 40% of the dry matter, and as capsular polysaccharides in soil bacteria. Alginic acid was isolated for the first time by Stanford in 1880. The separation and purification methods have evolved with time because the applicability domains of alginates is an essential condition for the quality of these products.

The industrial applications of alginates are connected with their ability to retain water, and their gelling, viscosifying, and stabilizing properties. The biotechnological applications, on the other hand, are based either on specific biological effects of the alginate molecule itself or on its unique, gentle, and almost temperature-independent sol/gel transition in the presence of cations (e.g. Ca^{+2} , Mg^{+2}), which makes alginate highly suitable as an immobilization matrix for li-

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ving cells. Most magnesium ions isolated from brown algae come from the alginic acid fraction.

Alginates then have to be regarded as a binary copolymer composed of α -L-guluronic and β -D-mannuronic residues in varying proportions and sequences along the chain (STOKKE et al. 1991).

In the Phaeophyceae, alginates are found in cell walls in a crystalline arrangement parallel to the cellulose microfibrils and also in the intercellular matrix (KLOAREG, QUATRANO 1988, TRUUS et al. 2001). The quantity and distribution of the residues determines the properties of the alginates, such as gelling or immunologic properties, and antibacterial action. Considering these properties, we have separated a mixture of alginates by a specific method from the brown alga *Cystoseira barbata* (BALABAN et al. 2004). The product has been analyzed from a physicochemical perspective (BALABAN et al. 2004). The brown alga used was *Cystoseira barbata* and was collected in the Vama Veche area, on the coast of the Black Sea, in the autumn of 2003.

The anti-bacterial action of raw alginates was tested and it showed to be remarkable (BALABAN, BADEA 2004). Consequently, this study presents in brief a method of obtaining magnesium alginate as well as the physicochemical description of the alginate-type structure. The study is focused on three issues: determining the concentration of certain metals, confirming the structure of the alginate type through IR spectral analysis, determining the ratio of fixation of magnesium on the alginate.

MATERIAL AND METHODS

Brown algae were collected in the Vama Veche area on the Black Sea coast in the autumn of 2004. The phases for obtaining magnesium alginate were identical to those of obtaining raw alginates, only the method of precipitation differed. The phases were:

- treatment by boiling of the dried vegetal material with a diluted solution of formaldehyde;
- demineralization by boiling through successive cleansing treatments with aqueous solutions of hydrochloric acid in various concentrations;
- filtering and cleansing the vegetal material with distilled water;
- extraction with aqueous solution of sodium carbonate (pH 9-11);
- precipitation with ethylic alcohol in the presence of magnesium.

The immunomodulatory action of magnesium alginate has been tested and it has proved to be remarkable (BALABAN, SAMARINEANU 2005).

The magnesium alginate has been analyzed from a physicochemical perspective and the ratio of fixation of magnesium on the alginate has been determined.

1. Metal concentration analysis

In order to determine metal concentration, groups of three samples of both types of alginates were used. The samples were obtained in accordance with the above procedures. In order to determine the concentration of metals, the solutions were prepared as follows: the samples of the magnesium alginate and raw alginate have been dissolved in 10% hydrochloric acid solution. For this purpose, de-ionized water was used. The alginate concentrations were 1 mg L^{-1} . For the determination of metal concentrations, an AA6200 flame atomic spectrometer provided by Shimadzu Company was used. The calibration of spectrometer was done using standard solutions of each metal obtained from spectral pure substances. The concentrations of Mg, Fe, Zn, Cd, Mn and Cr were determined. All results are expressed as mean \pm standard deviation from each of the three samples of alginates.

2. IR spectral analysis

The sample of magnesium alginate was characterized by IR spectroscopy. The spectrum samples were recorded with a Vector 22IR spectrometer with Fourier transformation type, using KBr pellets.

3. Ratio of fixation of magnesium on the alginate

Repeated separations of raw alginates showed that the separation efficiency of the raw alginates was of 35%. Repeated measurements of the concentration of Mg and raw alginate led to a value of $(56\ 250 \pm 2) \text{ mg kg}^{-1}$. These data provided a starting point for the determination of the ratio of fixation of magnesium on the alginate. To this end, a known quantity of Mg was added ($17\ 500 \text{ mg kg}^{-1}$) in the final phase of precipitation with alcohol of the magnesium alginate. The difference between the concentration of Mg in magnesium alginate and raw alginate is exactly the quantity of magnesium fixed on the alginate via the carboxyl and hydroxyl groupings. The ratio between the quantity of magnesium fixed on the alginate and the quantity of magnesium introduced represents the rate of fixation of magnesium on the alginate.

RESULTS AND DISCUSSIONS

1. Metal concentrations analysis

Results regarding the levels of concentrations of metals in the magnesium alginate versus raw alginate are presented in Table 1.

Table 1

Concentration of metals (mean \pm std deviation) in raw alginate and magnesium alginate

Element	Element contained in raw alginate mg kg ⁻¹	Element contained in AlgMg mg kg ⁻¹
Mg	56 250 \pm 2	70 312.5 \pm 31
Fe	94 \pm 2	82.8 \pm 1.3
Zn	41.8 \pm 1.2	40 \pm 1.1
Mn	29.8 \pm 1.2	22.9 \pm 1.6
Cr	< 0.05	< 0.05
Cd	< 0.06	< 0.06

Magnesium alginate contains the largest quantity of magnesium, which proves the existence of free carboxyl groupings which can interact with the metal ions. The hydroxyl groups on the polymer also play some role in ion binding.

The value of the concentration of Fe is smaller in magnesium alginate than in raw alginate and in fresh algae (CHIRILA et al. 2004). As regards Mn, in raw alginates the Mn concentration is the highest, in comparison with magnesium alginate and fresh algae. The lower value of the Mn concentration in magnesium alginate, it is probably due to the stronger affinity of Mg ions towards the carboxyl and hydroxyl groups in the alginate. In both types of alginates, the concentrations of Zn are approximately equal and smaller than in fresh algae (260 \pm 0.1) mg kg⁻¹. The values of the concentration of Cr and Cd are under the limit of detection of the equipment in both types of alginates. Similarly, in fresh algae the concentration of Cr and Cd is lower than that of Mg, Fe, Zn, and Mn. Cd is a toxic element, which is supposed to be carcinogenic.

The fact that Cd is found at very low concentration in both algae and especially in Mg alginate may imply that algae are environment-friendly raw material for the preparation of alginates.

2. IR spectral analysis

The structure of magnesium alginate is confirmed by IR spectra (Figure 1). The IR spectra of the three analyzed samples of magnesium alginates are almost identical.

In the region 1427–1641 cm⁻¹ there appeared the frequencies characteristic of –COO⁻, –COOH dimerised groups and that of C-H bond in the ring, as well as ν_{OH} associated and ν_{OH} unassociated, broad band in the region 3200–3600 cm⁻¹ having the maximal signal at 3477 cm⁻¹, $\nu_{\text{CO(COOH)}} \text{ asim} = 1641 \text{ cm}^{-1}$; $\nu_{\text{CO(COOH dimerised)}} = 1484 \text{ cm}^{-1}$, $\nu_{\text{CO asim}} = 1427.2 \text{ cm}^{-1}$; $\nu_{\text{C-O- (eteric)}} = 1031 \text{ cm}^{-1}$; $\nu_{\text{CH}} = 882 \text{ cm}^{-1}$

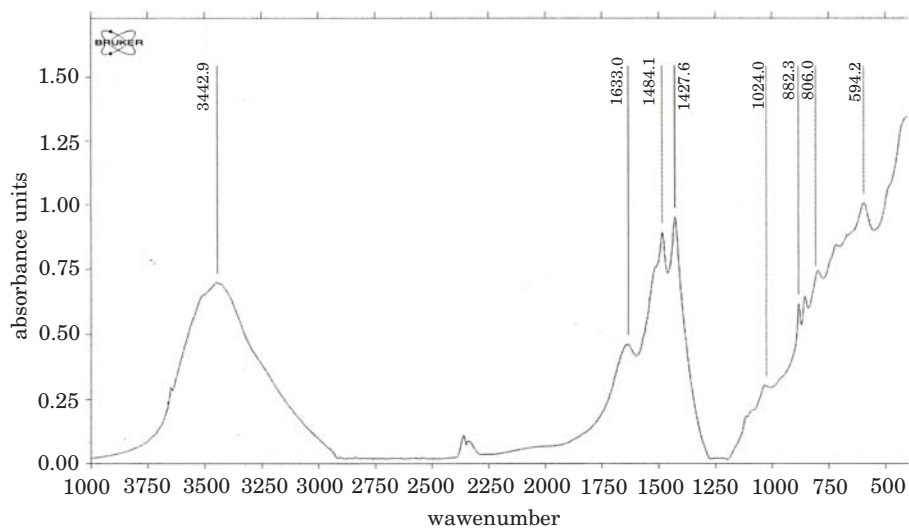


Figure 1. IR spectrum of magnesium alginate

3. Ratio of fixation of magnesium on the alginate

The capacity of fixation of magnesium on alginate is presented in Table 2. As a result of the separation process through precipitation with alcohol in the presence of magnesium, approximately 80% of the quantity of added magnesium was fixed by the alginate. The difference between the values of concentration of magnesium in the two types of alginates represents the quantity of magnesium fixed on the alginate via the carboxyl and hydroxyl groupings.

Table 2

Ratio of fixation of magnesium on alginate (mean \pm std deviation)

Mg contained in raw alginate mg kg ⁻¹	Quantity of Mg added mg kg ⁻¹	Mg contained in AlgMg mg kg ⁻¹	Mg fixed on AlgMg mg kg ⁻¹	Ratio of fixation %
56 250 \pm 2	17 500	70 312.5 \pm 3.1	14 062 \pm 1	80.35

CONCLUSIONS

1. Among the metals, magnesium is found at the highest concentration in the magnesium alginate sample.
2. The value of concentration of Fe is lower in magnesium alginate than in raw alginate and fresh algae.
3. The value of concentration of Mn is higher in raw alginate as compared with magnesium alginate and fresh algae.
4. In both types of alginates, the concentrations of Zn are approximately equal and lower than in fresh algae.
5. In both types of alginates, the levels of concentration of Cr and Cd are under the limit of detection of the equipment.
6. IR spectral analysis confirmed the alginate-type structure.
7. The ratio of fixation of magnesium on alginate is of approximately 80% of the quantity of magnesium added.
8. The results of the determinations show that the toxic metal concentrations in *Cystoseira barbata* and the two types of alginates are not higher on the Romanian Black Sea coast and algae may be an environment-friendly raw material for the preparation of magnesium alginate.

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Key words: *Cystoseira barbata*, magnesium alginate.

Abstract

The study examines the physical and chemical description of magnesium alginate obtained from the brown alga *Cystoseira barbata*, existing in the coastal area of the Black Sea. We have separated a magnesium alginate by a specific method, which involves the precipitation of the alginate with an alcohol solution of magnesium chloride. The study is focused on three directions: determining the concentration of certain metals, confirming the structure of the alginate type through IR spectral analysis and the examination of the ratio of fixation of magnesium on the alginate. Results confirmed the alginate type structure as well as the presence of certain metals, among which Fe, Cd, Mn, which are to be found in the alga. The presence of Mg is to be noticed, which was fixed on the alginate during the process of obtaining the alginates. The magnesium was fixed in the ratio approximately 80% from the added magnesium quantity in the phase of precipitation of the magnesium alginate.