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Raman Spectroscopy as a Tool for Analysing Dye Distribution in Alginate Hydrogels

Abstract

Hydrogels are cross-linked three-dimensional macromolecular networks that contain a large fraction of water within their structure. One of the most important properties of alginate hydrogels, leading to their broad versatility, is their ability for controlled uptake, release and retention of molecules. This ability, in turn, is due to specific interactions of the macromolecular network with the diffusing or retained molecule. Raman spectroscopy has been employed to characterise the diffusion properties of solutes in hydrogels. Besides their application in the food sector, they are used in many biomedical, pharmaceutical and technical areas; for example, as natural tissues or drug carriers. In the latter case, controlled release of drugs from a wound dressing is of particular interest – or ion exchange between the drug and the structure of the dressing. Raman active vibrations were used to show the areas responsible for the penetration of the model azo-dyes (based on non-genotoxic benzidine analogs) within Ca-alginate/carboxymethylcellulose Medisorb A wound dressing. In this case, the intensity of the stretching bands was used to obtain the concentration profiles of the model dye in alginate/carboxymethylcellulose gel (Medisorb A).

Key words: alginate/carboxymethylcellulose Medisorb A wound dressing, Raman spectroscopy.

composed of homopolymeric regions of M and G, called respectively M- and G-blocks, interspersed within the regions of alternating structure.

The physico-chemical properties of alginate have been found to be greatly affected by the M/G ratio as well as by the structure of the alternating zones. The kinetic of the gel formation is usually very fast and the resulting gels are strong enough to be suitable for many industrial and biomedical applications. Another worth-while approach related to the in situ gel properties of alginate is represented by its possible application in wound dressing because of the presence of calcium ions in the exudates. The polymer matrix can be loaded with antibiotics or it can be mixed with other natural polymers, such as chitosan or gelatine, producing physical gels. Alginate could be chemically modified into low molecular weight oligomers that would be then cross-linked with a biodegradable spacer to form biodegradable hydrogels [1].

The surface of alginate gel matrix was modified by means of macromolecules, which are able to establish ionic interactions with alginate carboxylate ions, thus forming a shell around the alginate gel systems that, in turn, become more resistant and suitable for numerous applications.

Figure 2 presents schematic representation of Ca^{2+} - alginate disintegration [2].

Polyguluronate units in alginate molecules form a chelated structure with calcium ions, called an "egg-box" junction, with interstices, in which the cations may pack and be coordinated. The junctions between the chains formed in this way are kinetically stable toward dissociation, while polymannuronate units show the normal polyelectrolyte characteristics of cation binding [2].

In this respect, chitosan, chitosan derivatives, carboxymethylcellulose and poly-L-lysine were used as typical modifying agents. By mixing methylcellulose and

Introduction

Alginate is a well-known polysaccharide (Figure 1), widely used due to its gelling properties in aqueous solutions related to the interactions between carboxylate moieties and bivalent counter ions, such as calcium, lead and copper. Alginate exhibits a backbone of (1,4) linked β -D-mannuronic acid (M) and α -L-guluronic acid (G) residues of widely varying composition and sequence. This polymer can be regarded as a true block copolymer

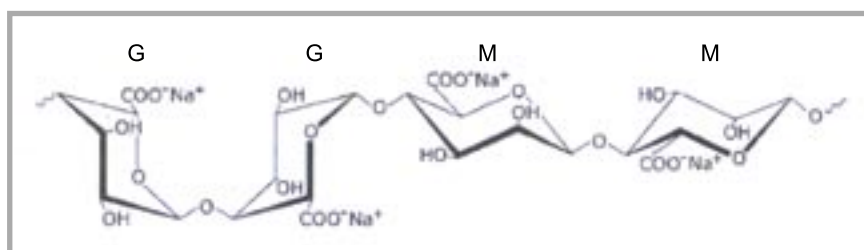


Figure 1. Schematic of structure of a GGMM segment of the sodium alginate molecule. G = guluronic acid residue; M = mannuronic acid residue.

alginate, a new interesting pH and temperature dependent drug delivery system was also obtained. At room temperature, the polymeric solutions can be mixed and loaded with the drug, but the system obtained turns into a gel upon entering the human body as a result of the increase in temperature, and also due to the thermogelling properties of methylcellulose [1].

Cotton and viscose gauze, although still much in use for acute wounds, has largely been replaced for the treatment of chronic wounds by modern wound dressings made from alginate and – more recently – carboxymethylcellulose (NACMC fibre, Medisorb A, Hydrofibers, Aquacel, ConvaTec). Wound dressings made from alginates have proved to have important advantages over gauze products in that upon hydration- ion exchange takes place resulting in the formation of a gel over the wound site. This offers advantages to the patient and the carer in that the alginate dressing can be removed directly or by irrigation without damage to the newly forming tissue. NaCMC fibres also gel in contact with wound exudate, but offer the carer additional advantages for fluid management. Ideally, in the care of chronic and exuding wounds, a material in contact with the wound should have high fluid absorbency and retention for long wear times and should not disrupt or damage newly formed tissue when removed. Sodium carboxymethylcellulose (NaCMC) fulfils these criteria, forming a soft gel or viscous solution with wound fluid. This gel-forming property observed for NaCMC has led to its use for wound care, in gel formulations and in hydrocolloid dressings that contain NaCMC in an insoluble matrix.

Fibrous dressings made from alginate combine the properties of fluid absorbency and gel formation and some also retain their integrity so that they can be readily removed. A combination of the soft gel properties of NaCMC and the greater structural integrity of needled alginate dressing would yield products with improved fluid handling and which would be easier to handle and remove from the wound [3].

A very recent paper deals with a new composite material based on alginate, gelatine and borax. This new matrix was obtained by the oxidation of alginate with periodate to give a dialdehyde capable of reacting with gelatine in the presence of borax. The matrix showed the haemo-

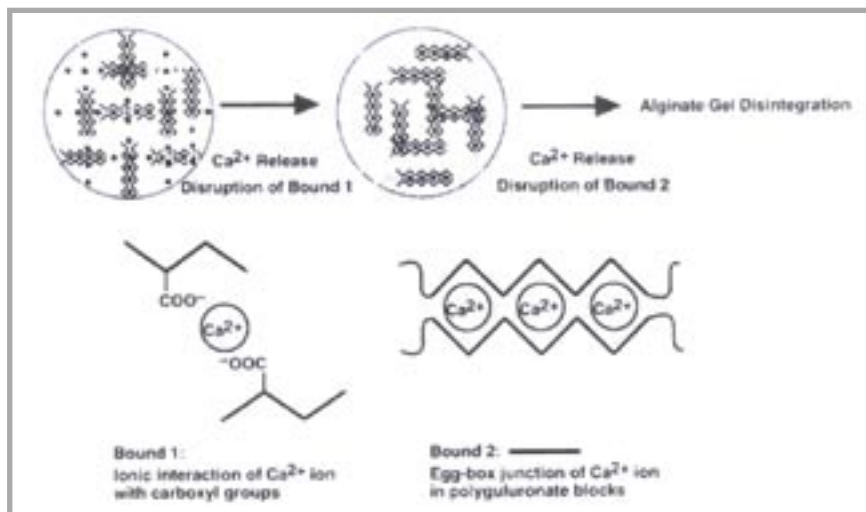


Figure 2. Schematic representation of Ca^{2+} - alginate disintegration [2].

static effect of gelatin, the wound healing features of alginate and the antiseptic property of borax, making it potential wound dressing material [1].

Although this paper concentrates on alginate hydrogels, some possible uses of alginate fibres should also be mentioned. Alginate fibres with an addition of silica nanoparticles were made, in order to obtain the best sorption or strength properties [4, 5]. Alginate-chitosan fibres are produced and used for medical purposes [6, 7]. Alginate-keratin fibrils are also characterised by better sorption properties than those of alginate fibrils [8, 9].

FT Raman spectroscopy is of importance for studying molecular structures. The width and intensity of spectral bands, as well as the position of peaks are all sensitive to environmental changes and the conformations of the macromolecule at a molecular level. In interpenetrating polymer networks, there are intermolecular interactions [10].

Raman spectroscopy has also been exploited to characterise the diffusion properties of solutes in hydrogels. Raman active vibrations were used as a kind in-

trinsic probes of the solute concentration in gel. The Raman technique effectively characterises the transport properties of solutes in gels, and this characterisation is required for developing several technical applications of gels, such as their use as materials for controlled release of drugs. The Raman spectroscopy approach presents several advantages. Raman active vibrational modes become intrinsic probes for the determination of solute tracer distribution. The small diameter of the laser beam allows a fine spatial resolution. Raman spectra can be obtained without any major interference from water bands due to its resistance to infrared (IR) spectroscopy. The reason for this is that water has a weak Raman signal, which is a critical advantage for diffusion studies in hydrogels [11].

The study presented in this paper aimed at observing the process of dye desorption from a dyed Medisorb A wound dressing, triggered by the adding of Ringer's solution. The process of desorption – dye distribution in the wound dressing – was traced through the changes of the intensity of Raman stretching bands.

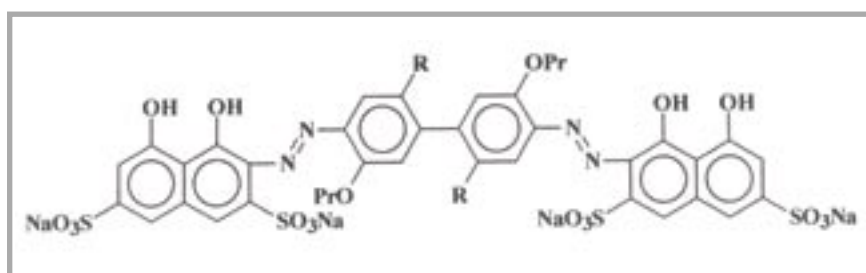


Figure 3. Model direct azo dye 3 ($R=Me$).

Experimental

Ca-alginate/carboxymethylcellulose Medisorb A wound dressings were studied (TZMO SA, Toruń). For comparison, the Raman spectre of Aquacel active wound dressing (hydrogel made from carboxymethylcellulose) was also shown. Model azo-dye, based on non-genotoxic benzidine analogs (3F), was selected as a water soluble, drug- synthesized model at the North Carolina State University, Raleigh NC [12] (Figure 3).

A blank Medisorb A wound dressing was suspended in 20 ml of 0.5% 3F dye solution for 45 minutes. The suspension was stirred for 24 h at 37 °C and then dried in air. Studies of 3F dye release from Medisorb A wound dressing were conducted in Ringer's solution (8.6 g NaCl, 0.3 g KCl, 0.48 g CaCl₂ in 1 litre of deionised water) at 37 °C and repeated several times (24 h, 48 h, 72 h, etc.). Aliquots of 20 ml samples were collected and replenished to maintain 250 ml. The concentration of 3F dye in the samples was measured spectroscopically (the results of UV-Vis examinations are not given in this paper).

The Raman spectra of the samples of dyed wound dressings (3F05) and of wound dressings exposed to Ringer's solution (3F05I, 3F05II, 3F05III) were recorded on an FTS 6000 spectrometer equipped with a MAGNA-IR 860, NICOLET, with FT RAMAN accessory. Dye and Medisorb A wound dressing was irradiated with a 1064 nm line of the T10-8S Nd spectra-physics model: YAG laser and scattered radiation were collected at 180°, using 4 cm⁻¹ resolution.

Results and discussion

Modern wound dressings are made from alginate (Hydrofibers, Aquacel, ConvaTec), and more recently fibres have been made from carboxymethylated cellulose (NaCMC fibre). Wound dressings made from alginate have proved to have important advantages over gauze products regarding hydration and ion exchange properties, resulting in the formation of a gel over the wound site. Sodium carboxymethylcellulose (NaCMC) forms a soft gel or viscous solution with wound fluid. This gel-forming property observed for NaCMC has led to its use in wound care, in gel formulations and in hydrocolloid dressings that contain NaCMC in an insoluble matrix. Fibrous dressings made from alginate combine the properties of

Table 1. Raman frequencies and vibrations of Medisorb A and Aquacel.

Raman frequencies, cm ⁻¹	Vibrational modes
3000 – 3750	OH stretching (ν_{OH})
2800 – 3000	CH stretching (ν_{CH})
1417	CH ₂ deformation $\delta(CH_2)$, $\delta(HCC)$, $\delta(HCO)$, $\delta(COH)$
1374	CH ₂ deformation $\delta(CH_2)$, $\delta(HCC)$, $\delta(HCO)$, $\delta(COH)$
1311	$\omega(CH_2)$, $\delta(HCC)$, $\delta(HCO)$, $\delta(COH)$
1239	COC stretching modes, $\nu(COC)$, $\delta(HCC)$, $\delta(HCO)$, $\delta(COH)$
1092	COH stretching modes, $\nu(COC)$, glycosidic, ring breathing, symmetric
904	$\delta(C_1H\beta)$
354	$\delta(CCC)$, $\delta(CO)$, $\delta(CCO)$, ring deformation
349	$\delta(CCC)$, $\delta(CO)$, $\delta(CCO)$, ring deformation

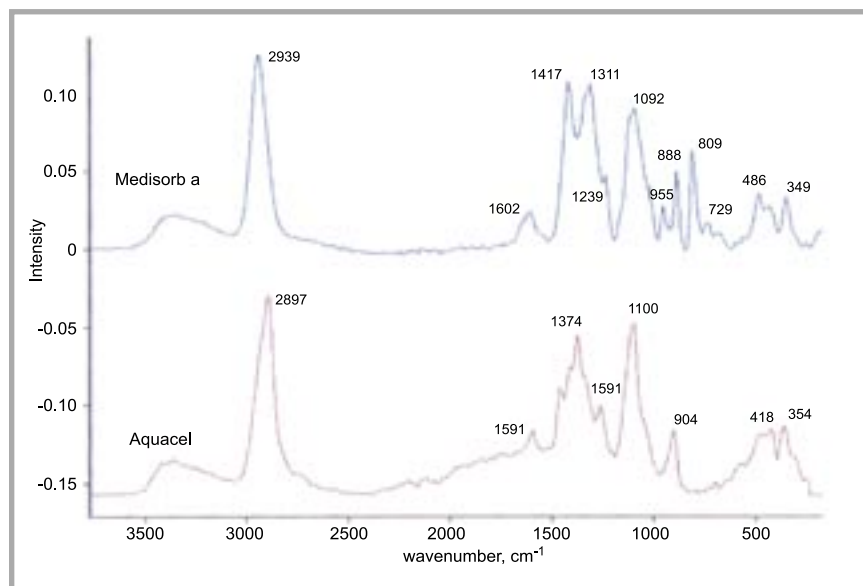


Figure 4. Raman spectrum of Medisorb A and Aquacel.

fluid absorbency and gel formation.

Table 1 and Figure 4 show the main bands in the FTR spectrum of Medisorb A and Aquacel (hydrogel made from carboxymethylcellulose) [11].

The two kinds of active wound dressings, Medisorb A and Aquacel, were compared in Table 1 in order to emphasise their spectral differences, which result from the very structure of both dressings. Both are based on carboxymethylcellulose, but Medisorb A also contains Ca-alginate and is the alginate hydrogel examined in this study.

As determined by other studies [13], alginate can, with a higher cross-linking degree, significantly reduce its swelling in the presence of the solvent, resulting generally in a reduction in the permeability of different solutes. As a consequence, the release of embodied drugs in alginate matrices will be delayed, allowing these systems to be used in controlled drug release. Drug-loaded alginate film can be applied in the pharmaceutical industry.

The active ingredient can also be dissolved or dispersed within these films [13]. Drug strength has to be selected carefully and known, whereas the drug itself has to be released repeatedly. Gelatin, because of its low intensity and high brittleness, is rarely used alone, but must be modified (cross-linking, grafting, blending). Blending is an effective and convenient method to improve the performance of polymer materials.

In this study, 3F dye was selected as the model compound. Its aim was to observe the process of dye desorption in dyed Medisorb A wound dressing, triggered by the adding of Ringer's solution. The release of a model drug (3F azo-dye) in Medisorb A wound dressing was studied as the ratio of the alginate and carboxymethylcellulose used, the concentration of 3F azo dye, the pH of Ringer's solution and ionic strength of the solution released, the thickness of the drug-loaded films and the cross-linking time with Ca²⁺, etc. Hopefully, these films can be used for localised drug delivery in both

in-vivo and in-vitro environments [13]. In Figure 5, the characteristic bands of azo dye groups ($1300 - 1400 \text{ cm}^{-1}$) and naphthalene groups (about 1600 cm^{-1}) of 3F dye were observed. An indistinct band at 1511 cm^{-1} on the 3FSPr spectrum can also be distinguished, which illustrates the adsorption of 3F dye spray in Medisorb A wound dressing. The characteristic band at 1511 cm^{-1} in the 3F05I spectrum was also observed.

In the spectrum of the 3F dye, at approximately 1511 cm^{-1} , a distinct band is visible, which does not disappear during the ion exchange process caused by Ringer's solution (3F05I). This band can also be observed during the sprinkling of Medisorb A with a solution of 3F dye (3FSpr).

At the same time, there were no new characteristic absorption bands of dye-loaded films, which permits one to conclude that there were no obvious other chemical reactions between the dye and the matrix. A significant result was that 3F dye did not lose its activity in drug-loaded wound dressing.

Medisorb A wound dressings (3F05, 3F05I, 3F05II) with different 3F dye concentration were studied in the same release solution. The 3F dye loading was determined by Raman intensity of peaks (Table 2).

Table 2 shows the relative reduction in intensity for the 1511 cm^{-1} band of the original sample (3F05) when Ringer's solution was applied for 24 h (3F05I), 48 h (3F05II) and 72 h (3F05III). This reduction is evidence of dye desorption in the wound dressing.

The results show that the amount of 3F dye in Medisorb A during the release experiment decreased. The 1511 cm^{-1} band is associated with the permeation of 3F dye through the Medisorb A gel. It is possible that the pore size and their connectivity in gels are responsible for the permeation of 3F dye through the Medisorb A gel.

Table 2. Release studies of 3F dye from Medisorb A wound dressing.

Sample	$(I_{1511} - I_{1700}) / (I_{1564} - I_{1700})$
3F05	0.791
3F05I	0.785
3F05II	0.769
3F05III	0.680

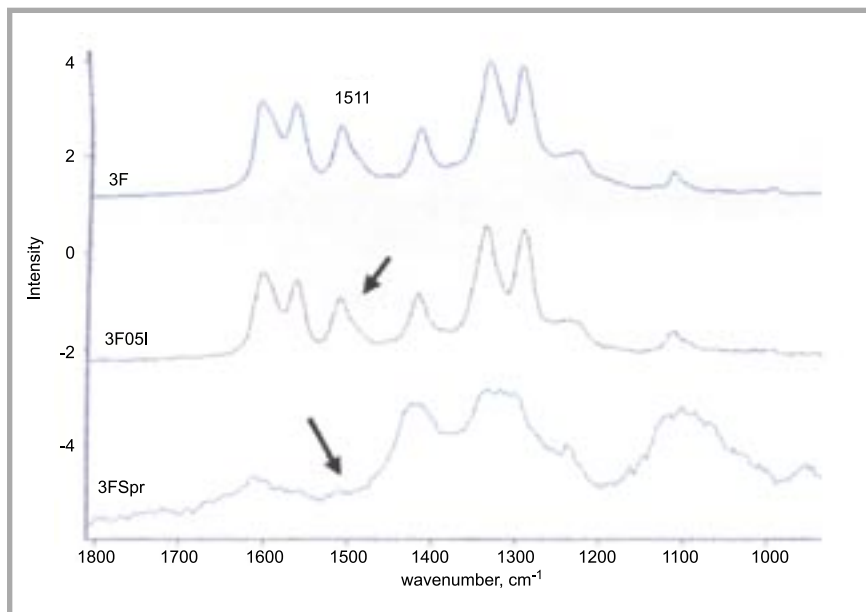


Figure 5. Raman spectrum of release: 3F azo-dye, 3F05I Medisorb A after the release studies and 3FSpr Medisorb A after the release process of 3F dye spray.

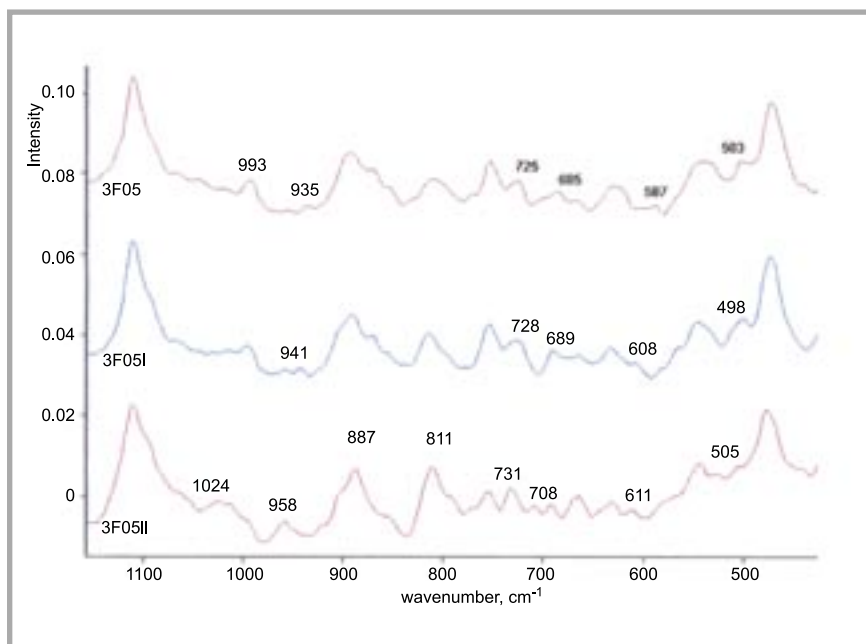


Figure 6. Raman spectrum of: 3F05, 3F05I and 3F05II Medisorb A after the release studies.

Calcium-alginate gel disintegrates through the ion exchange of calcium ions chelated with the carboxylate anions in the alginate and sodium ions from, for example, Ringer's solution. Due to the difference in stability of the complex between calcium ions and polyguluronate, or polymannuronate sequences in alginate toward dissociation, it is speculated that the sodium ion release from Ca^{2+} -alginate gel should be bimodal when gel is made from alginate with a certain ratio of polymannuronate and polyguluronate units [2]. Alginate disintegration occurs

in the "egg-box" structure. As Ca^{2+} ions are being released, electrostatic repulsion between carboxylate anions enhances the swelling of alginate gels and eventually facilitates their erosion. This electrostatic repulsion forces dissociation into soluble alginate molecules.

From other studies [14] it is known that different types of alginate present different proportions and/or different alternation patterns of guluronic and mannuronic units. The presence of these units can be identified from their characteristic bands:

while the guluronic units originate a band at approximately 1025 cm⁻¹, the mannuronic units originate a band at approximately 1100 cm⁻¹. Thus, the guluronic/mannuronic concentration ratio, characterizing a certain alginate sample, can be inferred from the relative intensity ratio of the 1025 cm⁻¹ and 1100 cm⁻¹ bands [14].

From the above, it is expected that the release of macromolecules from calcium-alginate gel could/can be controlled in an alginate disintegration-governed, pulsatile manner. It would be interesting to show dye desorption and other changes in the structure of an alginate dressing depending on the presence of guluronic and mannuronic units. Unfortunately, for this study, only commercial active dressings with diversified structures were available (the proportion of guluronic and mannuronic units is unknown).

Figure 6 shows the Raman spectra of alginate immersed for different times in Ringer's solution.

For the same source of alginate, peak shifts, differences in peak shapes and the appearance of new bands were observed as the sodium content increased. As new ions replace calcium ions and the atomic weight of the cation is changed, this creates a new environment around the carbonyl group; hence a peak shift would be expected. In the 1100–500 cm⁻¹ region a set of sharp peaks can be observed. Therefore, Raman spectra can give direct information on the ion exchange process. Only the major peak variations are described below. Some of the peaks at 894–885, 810–808 cm⁻¹ become broader, the peaks around 958, 725, 666 and 471 cm⁻¹ exhibit a large shift to higher wavenumbers (Figure 6).

The peak intensifies the change from pure calcium alginate Medisorb A (3F05) to sodium alginate Medisorb A (3F05II). This suggests a change in the binding within these blocks as sodium is introduced.

There is a new peak at 1024 cm⁻¹ in the 3F05II spectrum. Peaks relating to metal-oxygen-metal bonds are commonly observed in this region. This could, therefore, correspond to a partial bonding between sodium and oxygen atoms in the guluronic units of alginate. This is currently under further investigation using a wider range of cations [14].

Conclusions

In this study the model 3F azo-dye penetration in Ca-alginate/carboxymethylcellulose Medisorb A wound dressing was examined. In Figure 5 the characteristic band at 1511 cm⁻¹ in the 3F05I spectrum is presented. This peak indicates that new band positions were observed following dye adsorption on wound dressing.

The Raman spectra of alginate immersed for different times in Ringer's solution reveal peak shifts. Differences in peak shapes and the appearance of new bands are observed as the sodium content increases. Raman spectra give direct information on the exchange process. There are also new peaks appearing at 1024 cm⁻¹ in the 3F05II spectrum. This could, therefore, correspond to a partial bonding between sodium and oxygen atoms.



Acknowledgments

I would like to thank W. Biniś, PhD, for the FT Raman analysis.

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Received 15.11.2007 Reviewed 15.01.2008



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