Research Article

Journal of RAMAN SPECTROSCOPY

Received: 3 July 2009 Accepted: 26 August 2009 Published online in Wiley Interscience: 23 October 2009

(www.interscience.wiley.com) DOI 10.1002/jrs.2517

Characterization of sodium alginate and its block fractions by surface-enhanced Raman spectroscopy

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The surface-enhanced Raman scattering (SERS) of sodium alginates and their hetero- and homopolymeric fractions obtained from four seaweeds of the Chilean coast was studied. Alginic acid is a copolymer of β-D-mannuronic acid (M) and α-L guluronic acid (G), linked 1 → 4, forming two homopolymeric fractions (MM and GG) and a heteropolymeric fraction (MG). The SERS spectra were registered on silver colloid with the 632.8 nm line of a He–Ne laser. The SERS spectra of sodium alginate and the polyguluronate fraction present various carboxylate bands which are probably due to the coexistence of different molecular conformations. SERS allows to differentiate the hetero- and homopolymeric fractions of alginic acid by characteristic bands. In the fingerprint region, all the poly-D-mannuronate samples present a band around 946 cm^{-1} assigned to C–O stretching, and C–C–H and C–O–H deformation vibrations, a band at 863 cm^{-1} assigned to deformation vibration of β-C_1–H group, and one at 799–788 cm^{-1} due to the contributions of various vibration modes. Poly-L-guluronate spectra show three characteristic bands, at 928–913 cm^{-1} assigned to symmetric stretching vibration of C–O–C group, at 890–889 cm^{-1} due to C–C–H, skeletal C–C, and C–O vibrations, and at 797 cm^{-1} assigned to α C_1–H deformation vibration. The heteropolymeric fractions present two characteristic bands in the region with the more important one being an intense band at 730 cm^{-1} due to ring breathing vibration mode.

Keywords: alginates; poly-D-mannuronates; poly-L-guluronates; surface-enhanced Raman spectroscopy

Introduction

Alginic acid is the major structural polysaccharide present in all brown seaweeds (Phaeophyta); it is a linear copolymer of β-D-mannopyranuronic acid (M) and α-L-gulopyranuronic acid (L) linked 1 → 4 that are arranged in homopolymeric and heteropolymeric blocks.[1,2] The content of the uronic acids and their distribution in blocks vary with species and tissues types, and no relation between M/G values and block composition in alginic acids was found.[3–5] Vibrational spectroscopy has been applied for the characterization of alginic acid samples and block fractions, and it was found that homopolymannuronic and homopolyguluronic acids fraction presented characteristic bands in FT-IR spectra.[6–8] According to Pereira *et al*.[9] alginates can be identified by Raman spectroscopy, and Salomonsen *et al*.[10] reported the application of IR, Raman, and NIR spectroscopies and chemometrics for the determination of M/G ratio in alginic acid. Recently, the use of salts of alginic acid as model compounds in the analysis by Raman spectroscopy of biofilm matrix has been reported.[11] However, the usefulness of Raman and IR spectroscopies may be limited by two factors: the high fluorescence of biological systems can mask the vibrational signals and low concentration of the samples result in poor spectra. Therefore, the use of vibrational spectroscopy amplified by metal surfaces, could remedy these shortcomings, as it works with low concentrations of analytes and the intrinsic fluorescence of the samples is quenched by the effect of metal nanoparticles.[12–14] Besides, the use of metal colloids allows measurements in aqueous media, which could help conformational studies of these macromolecules. Surface-enhanced Raman spectroscopy (SERS) is a powerful analytical tool in determining the chemical information on complex molecules.[14] The electromagnetic field surrounding all the nanoparticles allows the amplification of Raman signals of molecules and could lead to an enhancement of the total vibrational signal approximately 10^6 fold.[14] Fleischmann *et al*.[15] made the first step in Raman amplification by surface adsorbed molecules of pyridine in a silver electrode through an electrochemical process. Since then, SERS has been applied extensively in biomolecules, and a significant number of studies based on the detection of proteins, amino acids, peptides, and other types of biomolecules, at concentrations as low as 10^{-12} to 10^{-14} M have been reported.[16–23] Recently, Schmid *et al*.[24] studied by tip-enhanced Raman spectroscopy of alginates samples mixed with Ag colloids.

In this work we present, the application of SERS for the characterization of sodium alginates and their hetero- and homopolymeric fractions from four seaweeds of the Chilean coast.

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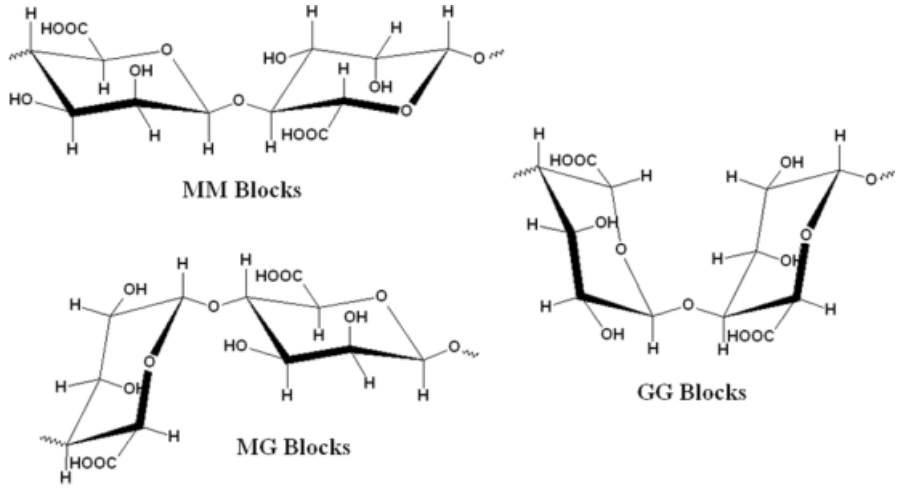

Figure 1. Chemical structures of hetero- and homopolymeric fractions of sodium alginate.

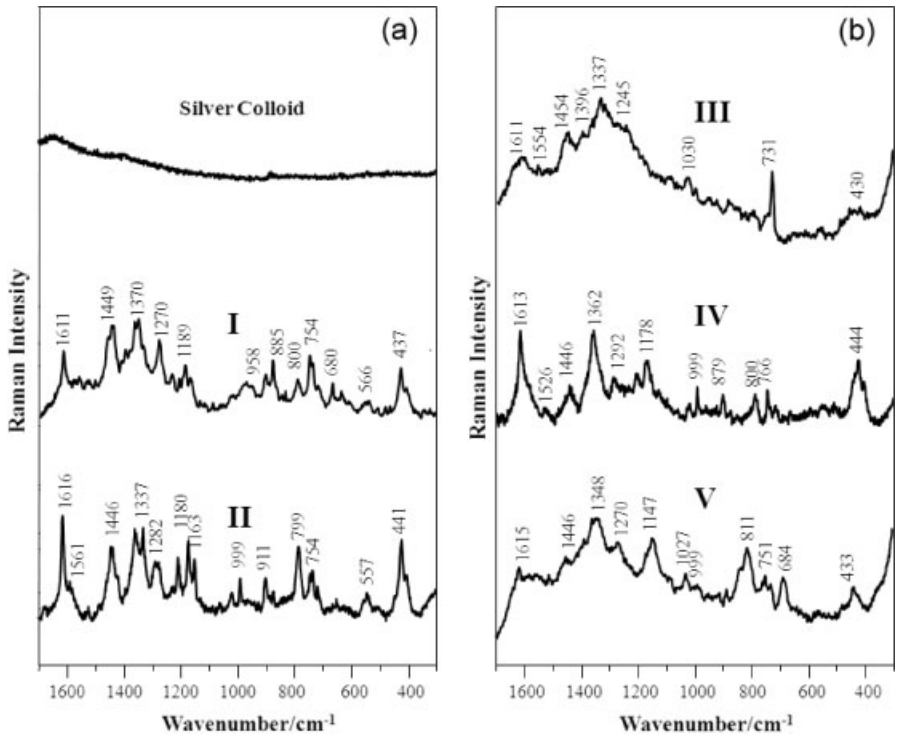

Figure 2. SERS spectra of (a) Ag colloid, sodium alginates from *L. vadosa,* collected in Puerto del Hambre in winter (I) and in Fuerte Bulnes, in spring (II); (b) alginates from *L. flavicans* (III), *L. trabeculata* (IV), and *D. ligulata* (V).

Experimental

The sodium alginates samples from *Lessonia vadosa*, collected in Puerto del Hambre in winter and in Fuerte Bulnes in spring, and from *Lessonia trabeculata, Lessonia flavicans*, and *Desmarestia ligulata* were used. The preparation of block fractions by partial hydrolysis of alginates and their characterizations by FT-IR and NMR spectroscopies have been reported.[6–8,25]

Stock solutions of sodium alginate, and sodium heteropolymeric and homopolymeric block fractions in nanopure water were prepared with a final concentration of 1 mg/ml.

Colloidal silver nanoparticles were obtained by the reduction of silver nitrate with trisodium citrate in an aqueous medium using the method published by Lee and Meisel.[26] Samples for SERS measurements were prepared by adding 10 µl of the stock solution to 1 ml of the silver colloid to give a final solution of 10 µg/ml. The solutions were incubated at 4 °C for 12 h to improve the interaction of macromolecules with nanoparticles.[27] An aliquot of the original non-activated colloid was placed on a glass slide with a shallow groove, and then the cover glass slide containing the dried activated Ag nanoparticles was placed on the groove with the side containing the dried nanoparticles facing downwards so that the suspension is placed in the groove.

Instrumentation

The micro-Raman scattering spectra were recorded with a Renishaw Raman RM1000 equipped with the 632.8 nm laser line, an

Table 1. Assignments of SERS spectra of sodium alginate from *L. vadosa* (samples I and II), *L. trabeculata* (III), *L. flavicans* (IV), and *Desmarestia ligulata* (V)

Wavenumber (cm^{-1})					
I	II	III	IV	V	Assignments
1611	1616	1611	1613	1615	ν_{asym} COO^-
1561	1561	1554			ν_{asym} COO^-
1524	1532		1526		ν_{asym} COO^-
1502					ν_{asym} COO^-
1449	1446	1454	1446	1446	ν_{sym} COO^-
1394		1396			δ C–H
1370	1360		1362		δ C–H
1329	1337	1337		1348	δ C–H
1270	1282		1292	1270	δ C–H
1206	1216	1245	1213		ν C–O
1189	1180		1178		ν C–O, δ C–O–H
1175	1163			1147	ν_{asym} C–O–C (glycosidic linkage)
	1030	1030	1031	1027	ν C–C, ν C–O
	999		999	999	δ COO^-
958					δ C–C–H, δ C–O–H
912	911				ν_{sym} C–O–C
885	887		879	879	ν C–C–H, δ C–O–H, ν_{sym} C–O–C (1,4 glycosidic link)
800	799		800	811	δ C–O–H, skeletal (ν C–C, ν C–O, δ C–C–H, δ C–C–O)
754	754		766	751	Ring breathing
731	731	731			Ring breathing
662	680			684	ν_{sym} C–O–C (glycosidic linkage), ν_{sym} skeletal
645	647				Ring deformation
566	557				δ C–C–C, δ C–O–C
437	441	430	444	433	δ C–C–C, δ C–O–C
417	422		422		δ C–C–C, δ C–O–C

Table 2. Characteristic bands in the SERS spectra of hetero- and homopolymeric blocks of sodium alginates

Wavenumber (cm^{-1})			
MM	GG	MG	Assignments
	1644–1642		ν_{asym} COO^-
1616–1598	1588	1545	ν_{asym} COO^-
		1368–1366	δ C–H
		1335–1332	δ C–H
1252	1290–1288	1274–1269	δ C–H
1211–1210	1212–1211		ν C–O
1044–1041		1152	ν_{asym} C–O–C (glycosidic linkage)
	1000–999		δ COO^-
947–946	943–938	957–943	δ C–C–H, δ C–O–H
	928–913	912	ν_{sym} C–O–C
	890–889		ν C–C–H, δ C–O–H, ν_{sym} C–O–C (1,4 glycosidic link)
863			δ C–H (β anomeric)
	797		δ C–H (α anomeric)
799–788			Ring breathing
753	752–751	730	Ring breathing
	559–557	558–553	δ C–C–C, δ C–O–C
	529–527		δ C–C–C, δ C–O–C, Ring deformation
	438–437		δ C–C–C, δ C–O–C

MM, poly-**D**-mannuronate; GG, poly-L-guluronate; MG, heteropolymeric fraction.

electrically refrigerated CCD camera, and a notch filter to eliminate the elastic scattering. The spectra shown here were obtained by using a 20× microscope objective. The output laser power at the samples was about 0.2 mW. Spectral resolution was 4 cm^{-1}.

The micro SERS spectra were recorded with a Renishaw Raman RM1000 equipped with the 632.8 nm laser line, an electrically refrigerated CCD camera, and a notch filter to eliminate the elastic scattering. The output laser power at the sample was about 0.2 mW. Spectral resolution was 4 cm^{-1}. The spectral scanning conditions were chosen to avoid sample degradation.

Results and Discussion

Sodium alginate samples from the Chilean brown seaweeds *L. vadosa,* collected in Puerto del Hambre in winter (I) and in Fuerte Bulnes, in spring (II), and from *L. flavicans* (III), *L. trabeculata* (IV), and *D. ligulata* (V), and their heteropolymeric and homopolymeric fractions (Fig. 1) were analyzed by SERS. Figure 2 shows the SERS spectra and Table 1 summarizes the assignments of the SERS bands of sodium alginates. Assignments were conducted taking into consideration data published for the Raman spectra of alginic acids, pectins, and natural gums containing uronic acids. All the samples present the characteristic asymmetric and symmetric bands of carboxylate ion (COO^-) at 1616–1611 cm^{-1} and near 1446 cm^{-1}, respectively.[11,24,28,29] The SERS selection rules indicate that the most enhanced Raman signals should correspond to those chemical groups oriented perpendicular to the metal surface.[14] In the present case the COO^- moieties could be perpendicular to the surface. The coexistence of several conformations in these molecules guarantees that at least one or two COO^- groups interact with the surface.

In the region 1400–1270 cm^{-1}, three bands assigned to the C–H deformation vibration, and a band around 1200 cm^{-1} assigned to the C–O stretching vibration are shown.[11,28,30,31] The SERS spectra of alginate samples show several bands in the region between 1200–950 cm^{-1}; this region deals with C–OH deformation, C–C–H bending, C–O, and C–C stretching vibrations; every band may be due to contributions of two or more kinds of motions.[28,30,32] The region between 950 and 750 cm^{-1} is called the 'finger print' or the anomeric region; the spectra of alginate samples present a band near 800 cm^{-1} assigned to skeletal stretching and deformation modes, and another around 770–730 cm^{-1} due to ring breathing.[28,30] Below 700 cm^{-1}, four bands related to the deformation of pyranosyl rings and C–O–C vibration of glycosidic linkage are shown.[11,28–30] Alginate samples, I from *L. vadosa* and IV from *L. flavicans* present similar mannuronic to guluronic acid ratio (M/G 1.08 and 1.03, respectively) while samples II from *L. vadosa*, IV from *L. Trabeculata*, and V from *D. ligulata* are found to be enriched in guluronic acid (M/G 0.64, 0.43, and 0.77, respectively).[6–8,25] However, no correlation between M/G values and the presence of characteristic bands was found, but it is important to mention

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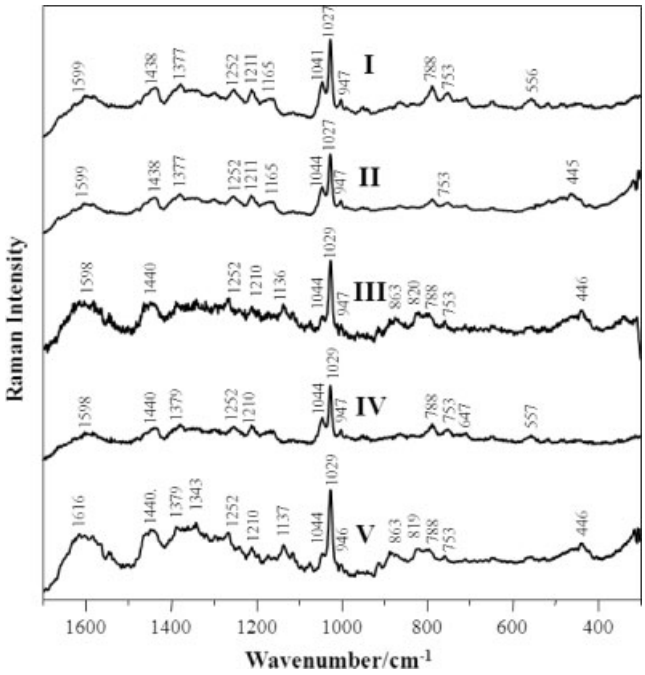

Figure 3. SERS spectra of sodium poly-mannuronate fractions of sodium alginate from *L. vadosa,* collected in Puerto del Hambre in winter (I) and in Fuerte Bulnes, in spring (II), *L. flavicans* (III), *L. trabeculata* (IV), and *Desmarestia ligulata* (V).

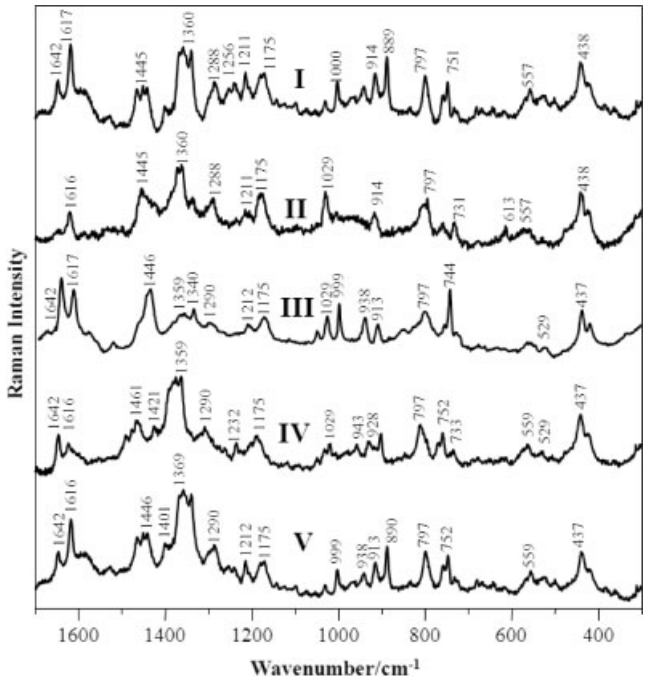

Figure 4. SERS spectra of sodium polyguluronate fractions of sodium alginate from *L. vadosa,* collected in Puerto del Hambre in winter (I) and in Fuerte Bulnes, in spring (II), *L. flavicans* (III), *L. trabeculata* (IV), and *Desmarestia ligulata* (V).

that in the spectrum of the sample IV which contains only 22% of polyguluronate,[8] the signal near 890 cm^{-1} is missing.

Table 2 presents the assignment of SERS spectral (Fig. 3) bands of homopolymannuronate fractions; it is noteworthy that the antisymmetric stretching vibration of the carboxylate group appears at 1616–1598 cm^{-1}. The shift to lower wavenumber in relation to the sodium alginate samples may indicate an interaction of the regular homopolymeric chain with the surface of the Ag colloid. The main peak in the SERS spectra of homopolymannuronate appears at 1029–1027 cm^{-1}; the same peak is present in the homopolyguluronate spectra (Fig. 4), but with lower intensity. Furthermore, this band is present in the FT-IR spectra of sodium alginate and its homopolymeric fractions.[8] In the fingerprint region, all the polymannuronate samples present at 947 cm^{-1} a band assigned to the C–O stretching vibration of uronic acids with contributions from C–C–H and C–O–H deformation,[8] and a band at 862 cm^{-1} assigned to deformation vibration of β-C_1–H group. According to Pereira *et al*.[9] the most prominent band in the Raman spectra of sodium alginate is the one at 950 cm^{-1} mainly due to O–H deformation vibration. This band, assigned to the C–O stretching vibration of carboxylate group, was previously found in the FT-IR spectra of sodium alginates and homopolymeric fractions from *L. flavicans* and *D. ligulata*.[8]

The homopolyguluronate fractions present (Table 2) in the SERS spectra (Fig. 4) a band at 1642 cm^{-1} which may be assigned to carboxylate vibrations; previously Synytsya *et al*.[28] reported in the Raman spectrum of sugar beet pectin two bands at 1633 and 1602 cm^{-1}. SERS selection rules indicate that the most enhanced Raman signals should correspond to those chemical groups oriented perpendicular to the metal surface. It can be proposed that in these fractions and in sodium alginate, the COO^- moieties are perpendicular to the surface of the Ag colloid. The coexistence of several conformations in these molecules guarantees that at least one or two COO^- groups interact with the surface. In the case of sodium alginate and the polyguluronate fraction we observe several carboxylate bands which are probably due to the coexistence of several molecular conformations. These conclusions are supported by the spectral signals belonging to the C–O stretching mode near 1175 and 1104 cm^{-1}; the second one is not observed in the case of polymannuronate fraction. Furthermore, the spectra show three characteristic bands at 928–913 cm^{-1} assigned to symmetrical stretching vibration of C–O–C group, at 890–889 cm^{-1} due to C–C–H, skeletal C–C, C–O–H, and symmetrical stretching vibration of the C–O–C of 1,4 glycosidic link, and at 797 cm^{-1} assigned to α C_1–H deformation mode.[11,28,30,33]

The heteropolymeric blocks (Table 2) present in the SERS spectra (Fig. 5) three wide and weak bands, at 1368–1366, 1335–1332 cm^{-1}, and at 1274–1269 cm^{-1} assigned to deformation vibrations of C–H and a prominent band at 730 cm^{-1} attributed to 'ring breathing' vibration.[28] This band with the same wavenumber has been also observed in *N*-acetyl-**D**-glucopyranosylamine and **D**-glucopyranose and assigned to the glycosidic ring vibration.[34] It is noteworthy that in the SERS spectrum of sodium alginate (Table 1, Fig. 2) from *D. ligulata*, which contained very low amount of MG fraction (3.5%), the latter band is shifted up to 751 cm^{-1}; in the case of *L. vadosa* from Puerto del Hambre, and *L. trabeculata* alginates, which contained 16.2 and 14.2% of heteropolymeric fraction, respectively, the alginate spectra present the corresponding band at 730 cm^{-1}.

Figure 6 shows the Raman spectra of the hetero- and homopolymeric fractions of the sodium alginate from *L. vadosa* collected in Puerto del Hambre. The polymannuronate fraction (MM), polyguluronate fraction (GG), and heteropolymeric fraction (M/G) present the characteristic asymmetric and symmetric vibrations of carboxylate group near 1602, and 1413 cm^{-1}, respectively. The three

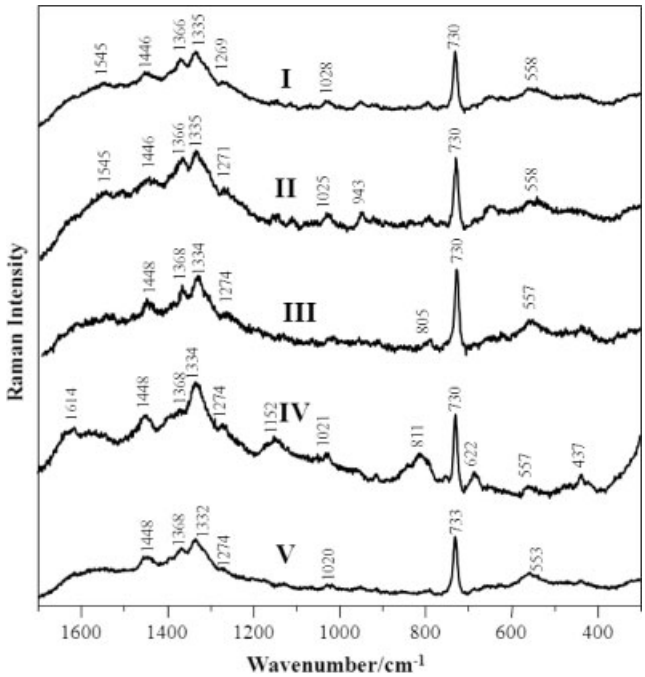

Figure 5. SERS spectra of sodium salts of heteropolymeric fractions of sodium alginate from *L. vadosa,* collected in Puerto del Hambre in winter (I) and in Fuerte Bulnes, in spring (II), *L. flavicans* (III), *L. trabeculata* (IV), and *Desmarestia ligulata* (V).

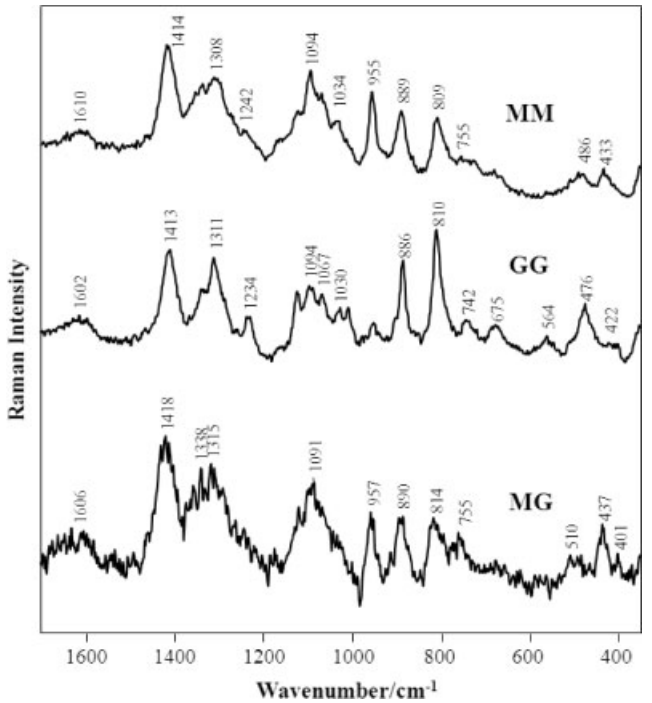

Figure 6. Raman spectra of hetero- and homopolymeric fractions of sodium alginate from *L. vadosa,* collected in Puerto del Hambre in winter (I).

spectra present bands at 1310, 1090, 955, 890, and 810 cm^{-1}, already assigned in the corresponding SERS spectra. The Raman spectra present lesser bands than SERS spectra and it can be seen that no characteristic bands could be clearly proposed to differentiate the hetero- and homopolymeric fractions of sodium alginates. According to the literature,[10,33] the intensity of four bands in the region 1412–708 cm^{-1} increased with increasing M/G ratio while the intensity of four bands in the region 1313–806 cm^{-1} decreased with increasing M/G ratio. It can be pointed out that the spectra were taken with a 1064 nm laser line. We could not find those bands, probably assigned to polymannuronic and polyguluronic acids, respectively, in the Raman spectra of the block fractions of sodium alginate. Finally, in relation to Raman spectra, the SERS spectra show the amplified bands corresponding to $-COO^-$ groups (around 1616–1502 cm^{-1}), and characteristic bands, at 1368–1366 cm^{-1} and 1335–1332 cm^{-1} assigned to deformation vibration of C–H, and the bands at 750–730 cm^{-1} attributed to 'ring breathing' vibrations.

Conclusions

The use of SERS is particularly advantageous due to fluorescence quenching and enhanced detection of certain functional groups. SERS spectra show several vibrational bands that could be used for the identification of sodium alginates and their hetero- and homopolymeric fractions.

From Figs 2–5, and Tables 1 and 2, it can be concluded that different COO^- vibrations are due to the chemical environment of the three molecular systems, which are homopolymannuronate, homopolyguluronate, and heteropolymeric fractions. SERS spectroscopy allows to differentiate the hetero- and homopolymeric fractions of alginic acid by characteristic bands, the most important ones being for poly-D-mannuronate the β glycosidic linkage deformation vibration at 863 cm^{-1}, for poly-L-guluronate the band associated to α glycosidic linkage at 797 cm^{-1}, and for the heteropolymeric fraction the intense band at 730 cm^{-1}.

Acknowledgements

Financial support of DICYT, Universidad de Santiago de Chile is gratefully acknowledged. N. P. Chandía and I. O. Osorio-Román thank FONDECYT for postdoctoral grants, and D. Leal and S. Torres thank CONICYT for doctoral fellowships.

References

[1] T. Painter, Algal polysaccharides, in *The Polysaccharides*, vol. 2 (Ed.: G. O. Aspinall), Academic Press: Orlando, **1983**, pp 196.
[2] H. Grasdalen, *Carbohydr. Res.* **1983**, *118*, 255.
[3] B. Matsuhiro, D. E. Zambrano, *Bol. Soc. Chil. Quím.* **1989**, *34*, 21.
[4] M. Venegas, B. Matsuhiro, M. E. Edding, *Bot. Mar.* **1993**, *36*, 47.
[5] B. Larsen, D. M. S. A. Salem, M. A. E. Sallam, M. M. Misheikey, A. I. Beltagy, *Carbohydr. Res.*. **2003**, *338*, 2325.
[6] N. P. Chandía, B. Matsuhiro, A. E. Vásquez, *Carbohydr. Polym.* **2001**, *46*, 81.
[7] N. P. Chandía, B. Matsuhiro, E. Mejías, A. Moenne, *J. Appl. Phycol.* **2004**, *6*, 127.
[8] D. Leal, B. Matsuhiro, M. Rossi, F. Caruso, *Carbohydr. Res.* **2008**, *343*, 308.
[9] L. Pereira, A. Sousa, H. Coelho, A. M. Amado, P. J. A. Ribeiro-Claro, *Biomol. Eng.* **2003**, *20*, 223.
[10] T. Salomonsen, H. M. Jensen, D. Stenbæk, S. B. Engelsen, *Carbohydr. Polym.* **2008**, *72*, 730.
[11] N. P. Ivleva, M. Wagner, H. Horn, R. Niessner, C. Haisch, *Anal. Bioanal. Chem.* **2009**, *393*, 197.
[12] M. Moskovits, *Rev. Mod. Phys.* **1985**, *57*, 783.
[13] S. Link, M. A. El-Sayed, *J. Phys. Chem. B* **1999**, *103*, 8410.
[14] R. Aroca, *Surface-Enhanced Vibrational Spectroscopy*, Wiley: Chichester, **2006**.

[15] M. Fleischmann, P. J. Hendra, A. J. Mc Quillan, *Phys. Chem. Lett.* **1974**, *26*, 163.
[16] G. W. Litman, J. P. Rast, M. J. Shamblott, R. N. Haire, M. Hulst, W. Roess, R. T. Litman, K. R. Hinds-Frey, C. T. Zilch, *Mol. Biol. Evol.* **1993**, *10*, 60.
[17] J. D. Driskell, K. M. Kwarta, R. J. Lipert, M. D. Porter, J. D. Neill, J. F. Ridpath, *Anal. Chem.* **2005**, *77*, 6147.
[18] E. Podstawka, E. Y. Ozaki, L. M. Proniewicz, *Appl. Spectrosc.*. **2004**, *58*, 570.
[19] K. K. Jain, *Clin. Chem.* **2007**, *53*, 2002.
[20] L. Sun, K.-B. Sung, *Nano Lett.* **2007**, *7*, 351.
[21] G. P. Naja, S. Bouvrette, S. Hrapovic, J. H. T. Luong, *Analyst* **2007**, *132*, 679.
[22] Z. Ming-Liang, Y. Chang-Qing, P. Kui-Qing, W. Ning-Bew, Y. Meng-Su, Z. Rui-Qin, L. Shuit-Tong, *Appl. Phys. Lett.* **2008**, *92*, 21.
[23] H. Xie, E. J. Becraft, R. H. Baughman, A. B. Dalton, G. R. Dieckmann, *J. Peptide Sci.* **2008**, *14*, 139.
[24] T. Schmid, A. Messmer, B.-S. Yeo, W. Zhang, R. Zenobi, *Anal. Bioanal. Chem.* **2008**, *391*, 1907.
[25] B. Matsuhiro, S. E. Torres, J. Guerrero, *J. Chil. Chem. Soc.* **2007**, *52*, 1095.
[26] P. C. Lee, D. Meisel, *J. Phys. Chem.* **1982**, *86*, 3391.
[27] A. E. Aliaga, I. Osorio-Román, P. Leyton, C. Garrido, J. Cárcamo, C. Caniulef, F. Célis, G. Díaz, E. Clavijo, J. S. Gómez-Jeria, M. M. Campos-Vallette, *J. Raman Spectrosc.* **2009**, *40*, 164.
[28] A. Synytsya, J. Čopíková, P. Matějka, V. Machovič, *Carbohydr. Polym.* **2003**, *54*, 97.
[29] C. F. Séné, M. C. McCann, R. M. Wilson, R. Gunter, *Plant Physiol.* **1994**, *106*, 1623.
[30] H. G. M. Edwards, M. J. Falk, M. G. Sibley, J. Alvarez-Benedi, F. Rull, *Spectrochim. Acta Part A* **1998**, *54*, 903.
[31] M. Mathlouthi, J. L. Koenig, *Adv. Carbohydr. Chem.* **1986**, *44*, 7.
[32] M. Sekkal, V. Doincq, P. Legrand, J. P. Huvenne, *J. Mol. Struct.* **1995**, *349*, 349.
[33] A. Pielesz, M. K. K. Bak, *Int. J. Biol. Macromol.* **2008**, *43*, 438.
[34] R. M. Jarvis, R. Goodacre, *Anal. Chem.* **2004**, *76*, 40.

