Acta Chemica Scandinavica A 34 (1980) 749–754

A Low-Frequency Raman Study (20–400 cm^{-1}) of Aqueous Agarose and κ-Carrageenan Gels. A Contribution to the Study of the Structure of Water

O. FAURSKOV NIELSEN, P.-A. LUND and F. M. NICOLAISEN

University of Copenhagen, Chemical Laboratory V, The H. C. Ørsted Institute, Universitetsparken 5, DK-2100 Copenhagen, Denmark

Low frequency vibrations (20–400 cm^{-1}) of aqueous agarose and κ-carrageenan gels are studied by construction of a quantity $R(\bar{\nu})$ from the depolarized Raman scattering. The $R(\bar{\nu})$-curves of the gels are within experimental error identical to the $R(\bar{\nu})$-curve of pure water. The conclusion is that the local water structure in a gel is identical to the structure of liquid water and the amount of water directly influenced by the skeleton of the gel-forming substance is very small.

In some recent papers,[1-5] we described the so-called $R(\bar{\nu})$-technique in studying low-frequency Raman spectra (~20–400 cm^{-1}). The name "Rayleigh-wing" is normally used for this part of the spectrum. Our interest in the $R(\bar{\nu})$-technique was created from attempts to correlate far-infrared-(FIR)-absorption to depolarized Rayleigh-wing scattering for molecular liquids.[1]

The origin of the Rayleigh-wing scattering is not well understood. A serious problem is, that the intensity is very low in comparison to the intensity of the central Rayleigh-line. Many different transformations of the scattered light have been tried in order to solve this problem.[6-11]

A direct study of FIR-absorption spectra of aqueous solutions at frequencies below 200 cm^{-1} is extremely difficult due to the very high absorption coefficient of water in the FIR-region. Reversely, the $R(\bar{\nu})$-technique appeared to be very useful in our study of water[3] and of aqueous solutions of biologically important molecules.[4,5] The $R(\bar{\nu})$-spectra of water and the aqueous solutions showed bands below 300 cm^{-1} and special attention was focussed on a water band exhibiting a maximum at ~180 cm^{-1}. This band is assigned to an external water vibration and was, for this reason, expected to be sensitive to the "water structure". In connection with a ^{13}C NMR study of agarose[12] at this laboratory, we found that an investigation of gels by $R(\bar{\nu})$-technique might be valuable. The water structure in gels has been the subject of much discussion,[13-15] and the $R(\bar{\nu})$-curves might be expected to be sensitive to interactions between water and gel by either spectral frequency or intensity changes as compared to the $R(\bar{\nu})$-curve of pure water. In this contribution, we shall report investigations on two different aqueous polysaccharide gels by $R(\bar{\nu})$-technique and discuss the information obtained on water structure from these spectra.

RESULTS

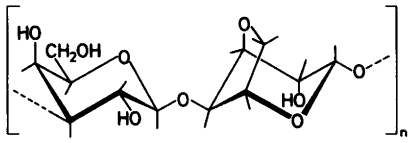
The gels investigated in this paper were agarose/water and κ-carrageenan/water. The repeating units for these are ahown in Fig. 1, and the polymer contains several hundred units. Raman spectra were recorded at room temperature from 20–400 cm^{-1} of 1, 2 and 4% aqueous gels. The agarose gels were all opaque resulting in an enormous scattering of the central-line. The κ-carrageenan gel was particularly chosen, because this gel yields a transparent aqueous gel,[13] well suited for light scattering.

The $R(\bar{\nu})$-curves were constructed from the intensity in the Stoke's side $[I(\bar{\nu})]$ of the Raman spectrum:

$$R(\bar{\nu}) \propto \bar{\nu}[1-\exp(-h\bar{\nu}(kT)^{-1}c)]I(\bar{\nu})$$

0302-4377/80/100749-06$02.50

I



II

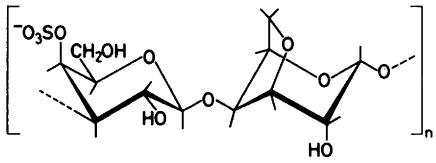


Fig. 1. Repeating structural units of agarose (I) and κ-carrageenan (II).

$\bar{\nu}$ is the Raman shift in cm^{-1}, h is Planck's and k is Boltzmann's constant, T is the absolute temperature and c is the velocity of light. $R(\bar{\nu})$ is to be considered as an absorption corresponding to selection rules, valid for scattering. In Fig. 2 the Raman spectrum ($I(\bar{\nu})$-curve) at different gains together with the $R(\bar{\nu})$-curve of a 1 % κ-carrageenan are shown. The spike in Fig. 2 at 112 cm^{-1} is most probably due to a dust particle. No special precaution was taken to prevent dust, because spikes are effectively removed by a running mean smoothing procedure[17] which also removes most of the "noise" in the $R(\bar{\nu})$-curve. Fig. 3A shows the smoothed curve corresponding to the $R(\bar{\nu})$-curve in Fig. 2. Smoothed $R(\bar{\nu})$-curves of 2 % and 4 % κ-carrageenan gels are shown in Figs. 3B and 3C, respectively. In Fig. 4 are shown smoothed $R(\bar{\nu})$-curves for 1, 2 and 4% aqueous agarose gels.

The $R(\bar{\nu})$-curves shown appear qualitatively identical, but changes in the absolute intensity may take place. However, the intensity of different spectra are not directly comparable mainly because the background scattering is very different from one sample to another. An internal water vibration might be used as a reference band, but the frequencies of these vibrations are high (>1600 cm^{-1}) and it is inconvenient to use a reference band separated to far in frequency. The problem was solved by adding CD_3CN (99 % D, from Merck, Darmstadt) as an internal standard. The $R(\bar{\nu})$-curve of pure CD_3CN is shown in Fig. 5A. The CCN-bending vibration is observed at ~350 cm^{-1}, which is somewhat lower than the corresponding band in CH_3CN at ~380 cm^{-1}. The band in liquid CD_3CN with a maximum at 62 cm^{-1} is, in analogy with our results for other molecular liquids,[1] most probably due to a libration around an axis perpendicular to the top-axis. In

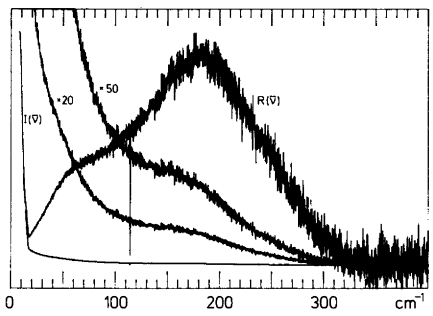


Fig. 2. Raman spectrum (I($\bar{\nu}$)) of a 1 % carrageenan water gel and the $R(\bar{\nu})$-curve constructed from these experimental data (see text).

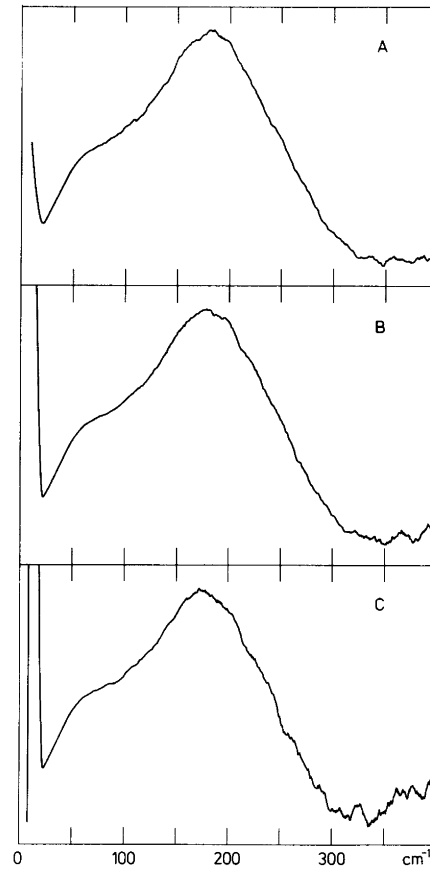


Fig. 3. Smoothed $R(\bar{\nu})$-curves of κ-carrageenan water gels. A, B and C show 1, 2 and 4 %, respectively.

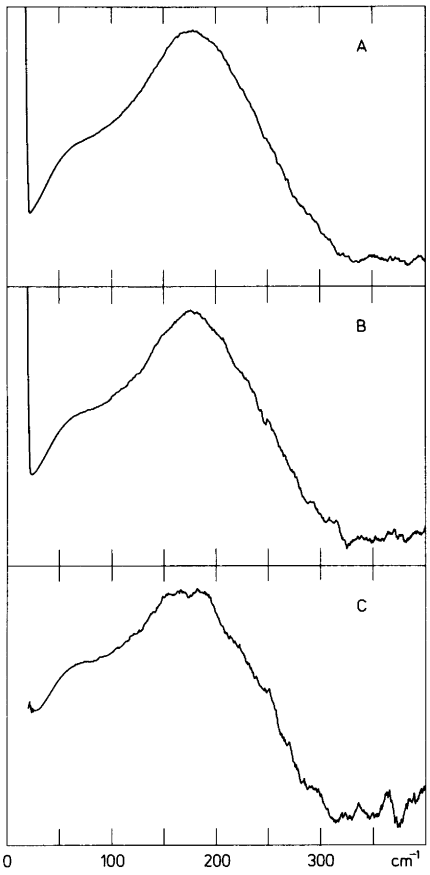


Fig. 4. Smoothed $R(\bar{\nu})$-curves of agarose–water gels. A, B and C show 1, 2 and 4 %, respectively.

the following, we shall call this band the (CD_3CN) librational band. The reasons for this choice of internal standard are: (*i*) the CCN-bending vibration is observed at 350 cm^{-1} where no water bands are found in the $R(\bar{\nu})$-curve (Fig. 5B), (*ii*) the CCN-bending mode is very intense in the Raman spectrum and consequently a low concentration of CD_3CN may be used, (*iii*) CD_3CN is soluble in water and finally (*iv*) the librational band at 62 cm^{-1} is rather weak compared to the CCN-bending mode at 350 cm^{-1}.

In Fig. 6A is shown the $R(\bar{\nu})$-curve of a 2 % standard solution of CD_3CN in water. Fig. 6B, C and D show spectra of three different κ-carrageenan gels (1, 2 and 4%), prepared from the standard solution. The two curves in each figure (6A–D) are recordings from each of the two separate preparations of the same concentration, described in the experimental section. Evidently, the preparations are very reproducible. Fig. 7 shows all $R(\bar{\nu})$-curves obtained from water and κ-carrageenan samples containing an internal standard, drawn on the same figure. All curves in Figs. 6 and 7 were normalized to the same intensity (ordinate) at 180 cm^{-1}.

DISCUSSION AND CONCLUSION

The $R(\bar{\nu})$-curves given in Figs. 3 and 4 are all very similar to the $R(\bar{\nu})$-curve of pure liquid water (Fig. 5B). Deviations in the low-frequency part of these $R(\bar{\nu})$-curves may be due to differences in the intensity of the central line. This is especially pronounced for the very opaque samples. Minor differences between the curves in Figs. 3 and 4 may also be observed above ~350 cm^{-1}. However, the signal is very weak, and we shall not pay more attention to this frequency region in the present paper. An inspection of Figs. 6 and 7 clearly demonstrates, that within experimental uncertainties, there seem to be no deviations in relative intensity of the CD_3CN band at ~350 cm^{-1} and the water bands in any of the $R(\bar{\nu})$-curves. In other words:

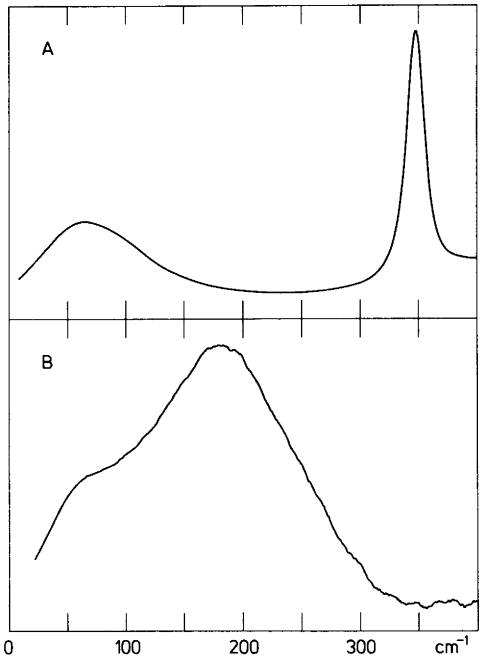


Fig. 5. The $R(\bar{\nu})$-curve of liquid CD_3CN (A) and liquid water (B).

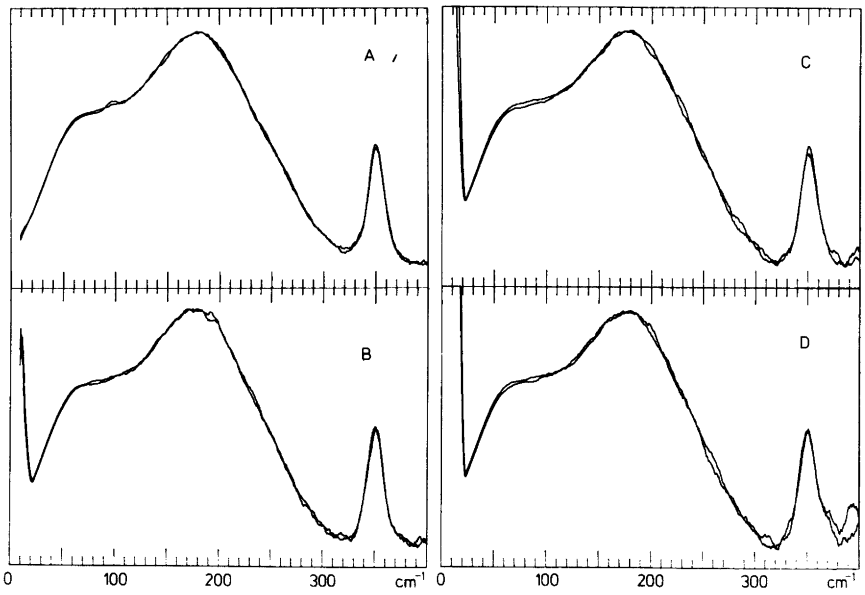


Fig. 6. $R(\bar{\nu})$-curves using 2 % CD_3CN as an internal standard. A: liquid water, B: 1 % κ-carrageenan, C: 2 % κ-carrageenan and D: 4 % κ-carrageenan. The two curves in a given figure are from two separate preparations.

these low-frequency external water vibrations are within experimental error identical for pure water and the 1, 2 and 4 % κ-carrageenan gels. In drawing these conclusions, it has been necessary to assume that the intensity of the CD_3CN-vibration used as internal standard is the same in different samples.

Previously, we have assumed, that the low-frequency spectrum reflects the water structure,[3] but it should here be emphasized, that the tetrahedral model in Ref. 3 is only tentative, and other models might yield similar isotopic shifts. However,

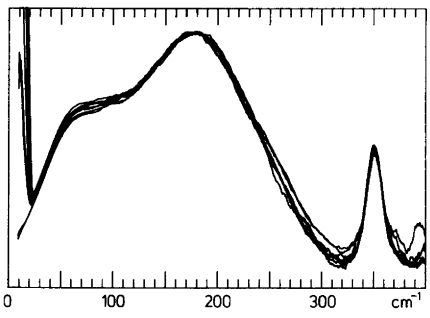


Fig. 7. All curves given in Figs. 6A – D are drawn on the same scale.

the similarity of the $R(\bar{\nu})$-curves for the gels (Figs. 3, 4, 6B – D and 7) and for water (Figs. 5B, 6A and 7) seems to indicate, that the environments around the major part of the water molecules are similar in pure water and in all the gel-solutions, but the water vibrations may be sensitive only to a local structural order. It is then impossible to conclude from our experiments whether there may be differences in longer range order between the different samples investigated. We could also say, that our $R(\bar{\nu})$-curves may be sensitive only to the smallest unit in the water clusters and therefore independent upon the real cluster size, which may differ from water to gel.

The only water molecules sensitive to the gel state are thus those bound directly to the polysaccharide network responsible for the gel, but the similarity between the $R(\bar{\nu})$-curves of the gels and pure water leads to the conclusion, that only a few percent of the water molecules may interact directly with the polysaccharide network.

From NMR experiments[14–16] it is shown that the magnetic relaxation of the water nuclei (^1H, ^2H and ^{17}O) are significantly affected by the presence of a gel-forming polysaccharide. Previously these results were interpreted as caused by changes in

the water structure, when the gel was formed[18,19]. More recently[14-16] the results are interpreted in terms of the existence of several "types" of water, differing in the degree of bonding to the polysaccharide. The estimated amount of bound water is very low (a few percent) in agreement with our conclusions above. However, the model used in the interpretation of the NMR-measurements is not necessarily correct. It is recently claimed,[20] that changes in the magnetic relaxation of the water nuclei in aqueous solutions of proteins are probably not caused by strong interactions between the water molecules and the protein, but "rather, it appears that the protein molecules influence the dynamics of the motion of the solvent water molecules in their neighbourhood in a manner that imposes on all the solvent molecules a correlation time for their orientational relaxation which equals that of the solute protein". In other words the macromolecule simply acts, as a whisk. The large change in mobility of the polymer when a gel is formed is, therefore, the main reason for the observed changes in magnetic relaxation times for the water nuclei.

By the present light scattering technique we measure preferably time events with characteristic times below 1 ps. At this time scale rotational and translational diffusion and the low-frequency movements of the polysaccharide skeleton are "frozen". A slower technique such as NMR averages over the above-mentioned movements and is therefore sensitive to movements of the polysaccharide skeleton. The significant changes in nuclear magnetic relaxation times observed for water in aqueous polysaccharide gels as determined by NMR-technique may therefore be fully in agreement with a nearly unchanged water structure as directly measured by the light scattering technique described here.

Low-frequency bands from some other molecules are rather easily detected by $R(\bar{\nu})$-technique in diluted solutions of water as we recently reported.[4,5] The insensitivity of the $R(\bar{\nu})$-curve to the presence of the gelling substance in an aqueous gel indicates that the $R(\bar{\nu})$-technique might be used in a direct study of chemical reactions or of diffusion of such molecules dissolved in gels.

EXPERIMENTAL

Chemicals and sample preparation. The agarose and the κ-carrageenan were commercial products from LITEX (P.O. Box 7, Glostrup, Denmark). The agarose (type LSA) is a purified extract from red seaweeds of the family Gelidiaceae. The κ-carrageenan (cation potassium) is an alkaline treated extract from red seaweeds of the order Gigartinales. More details concerning the chemicals are available from the manufacturer. The samples of agarose were prepared by heating the mixture of agarose and redistilled water in a 10 mm NMR-tube to *ca.* 95 °C for *ca.* $\frac{1}{2}$ h. The κ-carrageenan samples with CD_3CN as internal standard were prepared using a standard solvent consisting of 20 ml redistilled water and 400 μl CD_3CN (2 % (v/v) CD_3CN). The wanted amount of κ-carrageenan was transferred to a 10 mm NMR-tube prepared for vacuum sealing and degassed in high vacuum. Then 2 ml of the standard solution was distilled (*in vacuo*) to the NMR-tube, and the tube was sealed under high vacuum. The κ-carrageenan was brought into solution by heating the sealed tubes to *ca.* 95 °C for $\frac{1}{2}$ h. Samples with 0, 20, 40 and 80 mg carrageenan were prepared, corresponding to 0, 1, 2 and 4 % solutions (w/v). For each concentration, two samples were made and all samples were treated in an exactly identical manner.

Instrumental. The Raman spectra (~20–400 cm^{-1}) were recorded at room temperature on a Coderg PH1 spectrometer using 90°-scattering in a horizontal scattering plane and a spectral slit width of 4 cm^{-1}. Depolarized scattering (I_{VH}) was measured. The green line (514.5 nm) of a Spectra-Physics 165 Ar^+-laser was used as the exciting source (power ~400 mW). The detector was a cooled PM-tube (EMI 9558). The number of counts per step in frequency (8.4 step per cm^{-1}) was directly sampled and fed to an RC-4000 computer. The cylindrical high quality NMR-tubes mentioned above were used as cells. Some gel samples were opaque giving rise to a very intense central line and high "background" throughout the frequency region studied. A simple averaged intensity was found for each spectrum over a region of 12 cm^{-1} around the lowest intensity in the spectrum. This average value was then simply subtracted from each intensity value in the Raman spectrum [$I(\bar{\nu})$]. All further calculations were performed on the RC-4000 computer.

Acknowledgements. The authors wish to express their gratitude to the Danish Natural Science Research Council for partially financing the Raman equipment and to Dr. D. Christensen and Prof. E. Praestgaard for helpful discussions.

REFERENCES

1. Lund, P.-A., Nielsen, O. F. and Praestgaard, E. *Chem. Phys. 28* (1978) 167.

2. Nielsen, O. F., Christensen, D. H., Lund, P.-A. and Praestgaard, E. In *Proc. 6th Int. Conf. Raman Spectrosc. Vol. 2*, Heyden, London 1978, p. 208.
3. Nielsen, O. F. *Chem. Phys. Lett. 60* (1979) 515.
4. Nielsen, O. F. *Chem. Phys. Lett. 66* (1979) 350.
5. Nielsen, O. F., Lund, P.-A. and Praestgaard, E. *J. Raman Spectrosc. 9* (1980) 286.
6. Dardy, H., Volterra, V. and Litovitz, T. A. a. *Faraday Symp. Chem. Soc. 6* (1972) 71; b. *J. Chem. Phys. 59* (1973) 4491.
7. Bucaro, J. A. and Litovitz, T. A. *J. Chem. Phys. 55* (1971) 3585.
8. Wang, C. H. and Wright, R. B. a *J. Chem. Phys. 55* (1971) 1617; b. *Ibid. 55* (1971) 3300.
9. Perrot, M., Devaure, J. and Lascombe, J. *Mol. Phys. 36* (1978) 921.
10. Mazzacurati, V., Nardone, M. and Signorelli, G. a. *J. Chem. Phys. 66* (1977) 5380; b. *Mol. Phys. 38* (1979) 1379.
11. Brooker, M. H. and Perrot, M. *J. Mol. Struct. 60* (1980) 317.
12. Nicolaisen, F. M., Meyland, I. and Schaumburg, K. a. *Acta Chem. Scand. B 34* (1980) 103; b. *Ibid. B 34* (1980) 579.
13. Rees, D. A. *Adv. Carbohydr. Chem. Biochem. 24* (1969) 267 and references therein.
14. Ablett, S., Lillford, P. J., Baghdadi, S. M. A. and Derbyshire, W. *J. Colloid Interface Sci. 67* (1978) 355.
15. Ablett, S., Lillford, P. J., Bagdadi, S. M. A. and Derbyshire, W. *Am. Chem. Soc. Symp. Ser. 34* (1976) 344.
16. Woessner, D. E., Snowden, B. S., Jr. and Chiu, Y.-C. *J. Colloid Interface Sci. 34* (1970) 283.
17. Savitzky, A. and Golay, M. J. E. *Anal. Chem. 36* (1964) 1627.
18. Hechter, O., Wittstruck, T., McNiven, N. and Lester, G. *Proc. Natl. Acad. Sci. U.S.A. 46* (1960) 783.
19. Hazlewood, C. F., Nichols, B. L. and Chamberlain. N. F. *Nature 222* (1969) 747.
20. Koenig, S. H., Hallenga, K. and Shporer, M. *Proc. Natl. Acad. Sci. U.S.A. 72* (1975) 2667.

Received May 9, 1980.