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Vibrational circular dichroism of carbohydrate films formed from aqueous solutions

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Abstract—Vibrational circular dichroism (VCD) spectra in the entire 2000–900 cm^{-1} region have been recorded, for the first time, for films of carbohydrates prepared from aqueous solutions. Eight different carbohydrates, α-D-glucopyranosyl-(1→4)-D-glucose, cyclomaltohexaose, α-D-glucopyranosyl α-D-glucopyranoside, β-D-glucopyranosyl-(1→6)-D-glucose, β-D-glucopyranosyl-(1→4)-D-glucose, D-glucose, and both enantiomers of 6-deoxygalactose and of allose, were investigated. The VCD spectra obtained for films are found to be identical to the corresponding spectra obtained for aqueous solutions of carbohydrates. These measurements demonstrate several advantages of significant importance. The strong infrared absorption of water has prevented, in the past, the pursuit for routine applications of VCD in determining the structures of carbohydrates in aqueous solutions. This limitation is not present for film studies because water solvent is removed in the process of preparing the films. Also, strong infrared absorption of water at 1650 cm^{-1} requires the use of very short-pathlength (6 μm) cells for measurements on aqueous solutions. This requirement and concomitant inconveniences (such as laborious assembling of a demountable liquid cell or purchasing an expensive variable pathlength liquid cell) have been eliminated for film measurements. The removal of interfering water absorption in film studies resulted in higher light throughput and better signal-to-noise ratios for VCD measurements. Another point of significance is that the amount of carbohydrate sample required for VCD measurements on films is approximately one to two orders of magnitude smaller than that required for corresponding VCD measurements on aqueous solutions. Since carbohydrate samples can now be studied as films, VCD spectroscopy becomes much more broadly applicable for carbohydrates than previously believed. The present work, in combination with other film measurements in our laboratory, indicate that VCD studies on films can be used more generally, providing a convenient and powerful approach for probing structural information for biologically important compounds.

Keywords: Vibrational spectra; Circular dichroism; Carbohydrates; Saccharides; Films; Aqueous solutions

1. Introduction

Carbohydrates are easily identified as one of the most important classes of chemical compounds, because they are responsible not only for sustenance of life but also for various chemical processes, such as those in chiral chromatographic separations. Furthermore, carbohydrates play an important role in modern industrial society, such as in the manufacture of drugs. As the function of a given carbohydrate depends to a large extent on its structure, a large amount of research work is focused on developing methods that can reveal the structure of carbohydrates. Chiroptical spectroscopic methods have been explored since the very early days because chirality is an important aspect of carbohydrate chemistry. In the older literature on carbohydrate structural chemistry, the use of optical rotation has found widespread applications.[1] Although electronic circular dichroism (ECD), another chiroptical method, has become a routine method[2] in biochemical laboratories, its applications to carbohydrates[3] have been limited due to the absence of easily accessible electronic transitions in the visible/ultraviolet region. Special instrumentation extending into the vacuum ultraviolet is necessary for studying carbohydrates using ECD, and some such studies have been reported[3] for carbohydrates in

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solution. Nevertheless ECD measurements for film or solid-state samples are known to contain artifacts.[4a] Specially designed instruments[4b] that nullify the artifacts are necessary for solid-state ECD measurements, and such instruments are not accessible to many researchers.

In light of the difficulties encountered for ECD studies on carbohydrates, the emergence of mid-infrared vibrational circular dichroism (VCD)[5] and vibrational Raman optical activity (VROA)[6] in the early seventies was greeted with great enthusiasm, as reflected by rapid growth[7] in these areas. VCD is a differential absorption of left and right circularly polarized infrared light originating from molecular vibrations. Typical VCD magnitudes are approximately four to five orders of magnitude smaller than those of vibrational absorption. Carbohydrates were considered[8] as difficult samples for VCD measurements, because the flexible nature of carbohydrates yield smaller VCD signals compared to the signals obtained for molecules with rigid structures. In recent years, improved VCD instrumentation has overcome the problems in measuring the weak VCD signals associated with carbohydrates.[9] As these improvements took place, it soon became apparent that the strong infrared absorption of water at 1650 cm^{-1} overwhelms the sample absorption bands and prevents VCD measurements on carbohydrates in aqueous media. As a consequence, most VCD studies on carbohydrates were conducted[8,9] in dimethyl sulfoxide (Me_2SO) solvent, which is not quite native to biological processes. VCD measurements on carbohydrates in Me_2SO were restricted to the 1600–1150 cm^{-1} region, as the strong absorption of solvent at ~1000 cm^{-1} prevented VCD measurements below ~1150 cm^{-1}. Since water does not interfere in Raman spectra, VROA spectroscopy, on the other hand, was found to be useful for characterizing carbohydrates, and several applications of VROA for carbohydrates have been reported by Barron and coworkers.[10]

In the quest for finding ways to measure the VCD of carbohydrates in aqueous media, studies in our laboratory demonstrated that using very high concentrations (up to 4 M in some cases) and short-pathlength (6 μm) liquid cells, one can measure[11] VCD for aqueous carbohydrate solutions. Strong absorption bands for carbohydrates were found in the 1200–900 cm^{-1} region, so the initial measurements[11] were conducted in this region. Since the absorption bands for carbohydrates in the ~1600–1200 cm^{-1} region (where vibrational modes, such as –C–H bending, CH_2 wag, and –O–H in-plane bending appear) are weaker than those in the ~1200–900 cm^{-1} region (where C–O stretching bands appear), even higher concentrations than those already used for ~1200–900 cm^{-1} region were required for measurements in the ~1600–1200 cm^{-1} region. For this reason the previous aqueous solution investigation[11] focused in the ~1200–900 cm^{-1} region, a region which is not accessible in Me_2SO solvent. Despite this advance, the fact that a large amount of carbohydrate sample was required for these studies, which is a serious issue for those working in the biochemical arena, has dampened the enthusiasm for VCD studies on carbohydrates.

In the present study we report a significantly improved and simplified procedure for measuring the VCD spectra of carbohydrates, where the amount of sample required for VCD measurements has been reduced by one to two orders of magnitude. Specifically, using a newly modified instrument[12] that uses a dual polarization modulation method,[13] we show that by depositing aqueous solution samples of carbohydrates as films, VCD spectra obtained for films are identical to those obtained for aqueous solutions. Spectra obtained in this manner for carbohydrate films required up to two orders of magnitude smaller amounts of carbohydrate samples than those in the previous[11] aqueous solution study. Furthermore, removal of interfering water absorption resulted in higher light throughput and better signal-to-noise ratios. Additionally we present data demonstrating that the VCD spectra for carbohydrate films can be obtained in the entire 2000–900 cm^{-1} region by controlling the concentration or volume of parent aqueous solution used to prepare the films. Numerous new future directions for VCD studies can result from the current development.

2. Results and discussion

The absorption and VCD spectra of α-D-glucopyranosyl-(1→4)-D-glucose (maltose, Fig. 1), cyclomaltohexaose (α-cyclodextrin, Fig. 2), α-D-glucopyranosyl

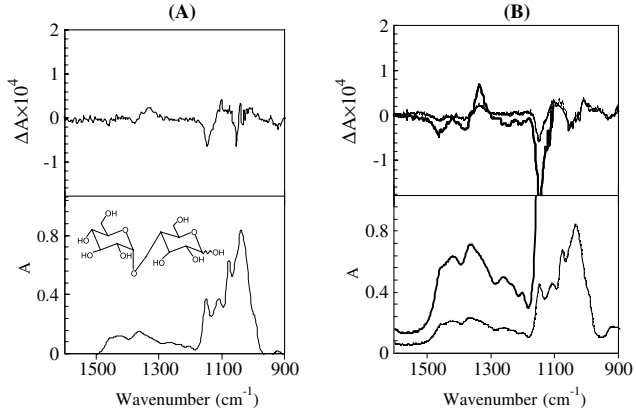

Figure 1. Vibrational absorption (bottom panels) and circular dichroism (top panels) of aqueous solution (left panels, A) and film (right panels, B) samples of α-D-glucopyranosyl-(1→4)-D-glucose (maltose). The spectra obtained with 45° rotation of the film around the light beam axis are shown by dotted line. See Table 1 for concentrations used. The spectra obtained for the film prepared from a higher-concentration (0.093 M) parent solution are shown as thick traces in the ~1600–1100 cm^{-1} region.

α-D-glucopyranoside (α,α-trehalose, Fig. 3), β-D-glucopyranosyl-(1→6)-D-glucose (gentiobiose, Fig. 4), β-D-glucopyranosyl-(1→4)-D-glucose (cellobiose, Fig. 5), and D-glucose (Fig. 6) are displayed in Figures 1–6. In each case absorption and VCD bands obtained from solution studies (left panels in the figures), are compared to those obtained for film samples (right panels in the figures).

The concentrations of parent solutions and the volume of solution used to deposit the films were varied to give optimal absorption (between 0.5 and 1) in the ~1200–900 cm^{-1} region. The volumes of aqueous carbohydrate solutions deposited are 150 μL for allose and fucose and 200 μL for the rest of carbohydrates studied. Table 1 summarizes the concentrations of parent solutions used and the peak absorbances obtained for both solution and film studies. It should be noted that even with the high concentrations used for solution studies on α-cyclodextrin and cellobiose, peak absorption intensities obtained in solution spectra are lower than those in the corresponding film spectra. For example, while actual peak absorption intensity for α-cyclodextrin is 0.35 for the solution sample, its peak absorption intensity is 0.70 for film sample. Similarly, while the peak absorption intensity for cellobiose is 0.28 for the solution sample, the corresponding intensity is 0.58 for the film sample. This difference is not apparent in the figures because all the intensities displayed for solution-based spectra are scaled in order to match the peak intensities of the film-based spectra. This scaling has been performed in order to provide a straightforward comparison of the spectra obtained for the solution and film samples.

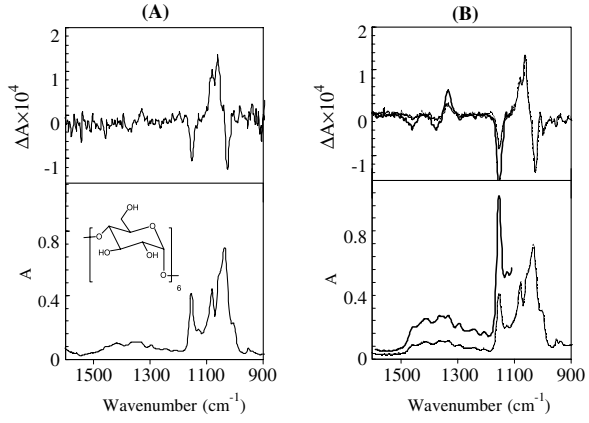

Figure 2. Vibrational absorption (bottom panels) and circular dichroism (top panels) of aqueous solution (left panels, A) and film (right panels, B) samples of cyclomaltohexaose (α-cyclodextrin). The spectra obtained with 45° rotation of the film around the light beam axis are shown by dotted line. See Table 1 for concentrations used. The spectra obtained for the film prepared from a higher-concentration (0.035 M) parent solution are shown as thick traces in the ~1600–1100 cm^{-1} region.

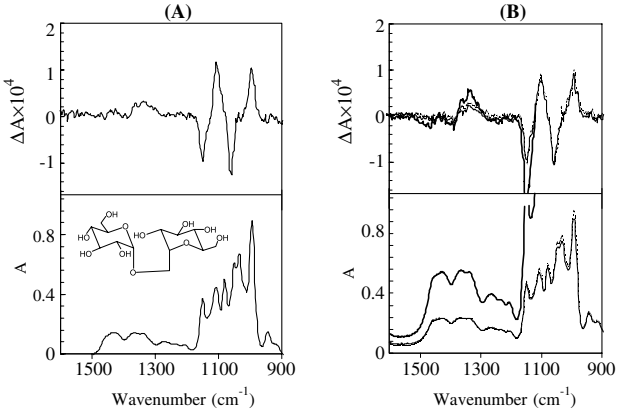

Figure 3. Vibrational absorption (bottom panels) and circular dichroism (top panels) of aqueous solution (left panels, A) and film (right panels, B) samples of α-D-glucopyranosyl α-D-glucopyranoside (α,α-trehalose). The spectra obtained with 45° rotation of the film around the light beam axis are shown by dotted line. See Table 1 for concentrations used. The spectra obtained for the film prepared from a higher-concentration (0.10 M) parent solution are shown as thick traces in the ~1600–1100 cm^{-1} region. The peak absorption intensity of band at ~1148 cm^{-1}, and of the corresponding VCD, for the film prepared from high concentration solution are off-scale and are therefore not fully shown.

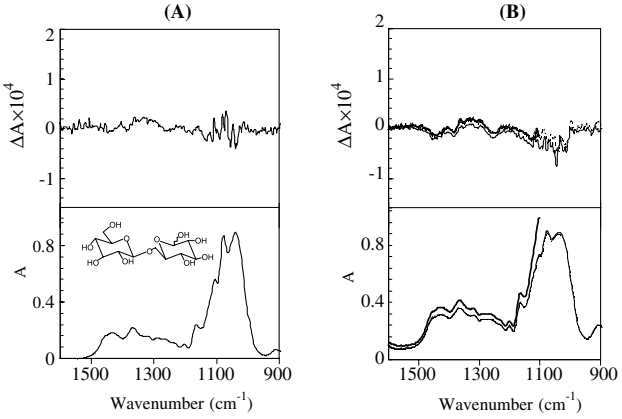

Figure 4. Vibrational absorption (bottom panels) and circular dichroism (top panels) of aqueous solution (left panels, A) and film (right panels, B) samples of β-D-glucopyranosyl-(1→6)-D-glucose (gentiobiose). The spectra obtained with 45° rotation of the film around the light beam axis are shown by dotted line. See Table 1 for concentrations used. The spectra obtained for the film prepared from a higher-concentration (0.065 M) parent solution are shown as thick traces in the ~1600–1100 cm^{-1} region.

It is worth noting that, depending on the sample solution and its surface interaction with the IR-transparent BaF_2 plate, some sample solutions are more easily spread on the surface of a BaF_2 plate than others. For instance, α-cyclodextrin solution spreads more easily than that of allose. For solutions which spread easily, films formed were thinner than for those which did not spread easily. To verify the independence of film

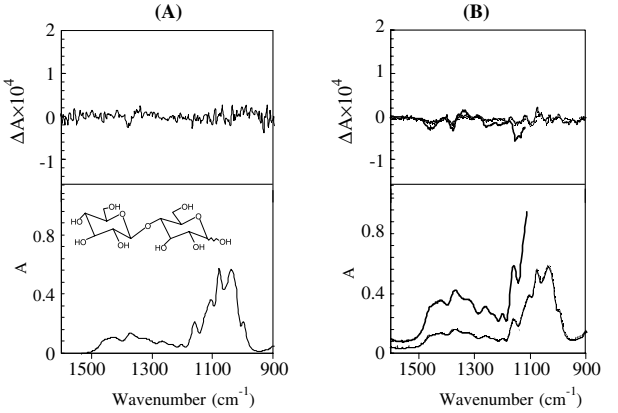

Figure 5. Vibrational absorption (bottom panels) and circular dichroism (top panels) of aqueous solution (left panels, A) and film (right panels, B) samples of β-D-glucopyranosyl-(1→4)-D-glucose (cellobiose). The spectra obtained with 45° rotation of the film around the light beam axis are shown by dotted line. See Table 1 for concentrations used. The spectra obtained for the film prepared from a higher-concentration (0.11 M) parent solution are shown as thick traces in the ~1600–1100 cm^{-1} region.

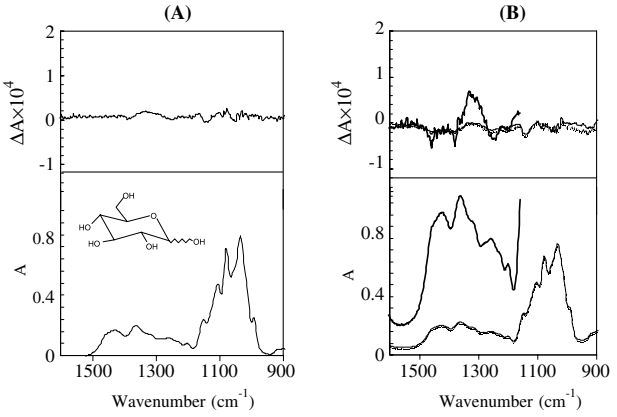

Figure 6. Vibrational absorption (bottom panels) and circular dichroism (top panels) of aqueous solution (left panels, A) and film (right panels, B) samples of D-glucose. The spectra obtained with 45° rotation of the film around the light beam axis are shown by the dotted line. See Table 1 for concentrations used. The spectra obtained for the film prepared from a higher-concentration (0.19 M) parent solution are shown as thick traces in the ~1600–1100 cm^{-1} region.

orientation, both absorption and VCD spectral measurements were repeated by rotating the film 45° around the light beam axis. Thus each film-based spectrum contains two traces. The full-line trace represents the film spectra prior to the 45° rotation, while the dashed line represents the spectra obtained upon 45° rotation. For all the absorption and VCD spectra reported here, the band positions, magnitudes, and signs were found to be unaffected upon 45° rotation of the film.

An alternate way to verify the reliability of VCD spectra of films is to examine the mirror-image nature of VCD signals expected for enantiomers of a given carbohydrate sample. The absorption and VCD spectra for film samples of D and L enantiomers of 6-deoxygalactose (fucose), and the D and L enantiomers of allose are displayed in Figures 7 and 8, respectively. The mirror-image VCD spectra obtained for enantiomers of fucose and allose confirm the reliability of VCD spectra for films. As an additional test, the VCD spectra for enantiomers of fucose film samples are also compared to those obtained for solution samples in Figure 7. The solution spectra are again in excellent agreement with those obtained for the corresponding films. For allose, we measured the VCD spectrum in solution for a single enantiomer D-allose, and this spectrum (Fig. 8) again matches well with the corresponding spectrum of D-allose film.

Visual inspection of VCD band intensities and positions indicates a very good agreement between the spectra obtained for aqueous solution and those for films. A quantitative consideration also indicates that the solution and film VCD spectra are essentially identical. There are some minor differences in band widths, which reflect more so in absorption than in VCD spectra. In general band widths are slightly larger for film samples than those for solution samples.

The most pronounced absorption and VCD bands are found in the 1200–900 cm^{-1} region, characteristic of C–O/C–C stretching modes. For instance, maltose (Fig. 1) is characterized by a negative VCD ($\Delta A/A = -1.8\times10^{-4}$ for solution, -1.4×10^{-4} for film) at

Table 1. Concentrations used and maximum peak absorbances obtained for solution and film samples

Carbohydrate	Aqueous solution studies		Film studies	
	Concentration (M)	Maximum peak absorbance[a]	Concentration[b] (M)	Maximum peak absorbance
Maltose	1.8	0.88	0.036	0.84
α-Cyclodextrin	0.14	0.35	0.016	0.7
α,α-Trehalose	1.9	0.76	0.039	0.9
Gentiobiose	2.7	1.04	0.044	0.9
Cellobiose	0.5	0.28	0.043	0.58
D-Glucose	3.8	0.79	0.078	0.76
D-Fucose	3.97	1.18	0.072	1.07
D-Allose	2.3	0.63	0.11	0.76

[a] These values are after subtracting the water solvent absorbance.
[b] This concentration refers to that of parent solution used for preparing the film.

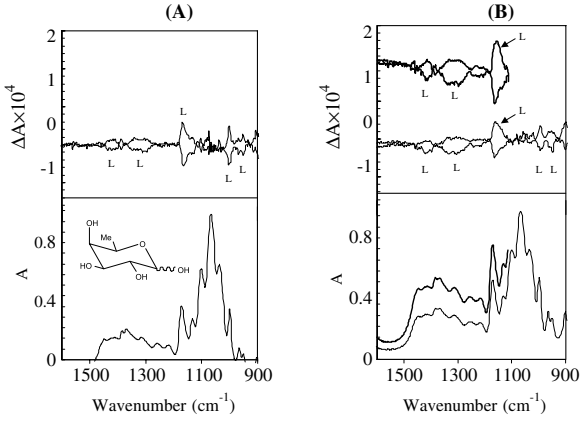

Figure 7. Vibrational absorption (bottom panels) and circular dichroism (top panels) of aqueous solution (left panels, A) and film (right panels, B) samples of both enantiomers of 6-deoxygalactose (fucose). The raw VCD spectra obtained for both the D and L enantiomers are shown for film samples. See Table 1 for concentrations used. The spectra obtained for the films prepared from a higher-concentration (0.14 M) parent solution are shown as thick traces (and offset from zero line for clarity) in the ~1600–1100 cm^{-1} region.

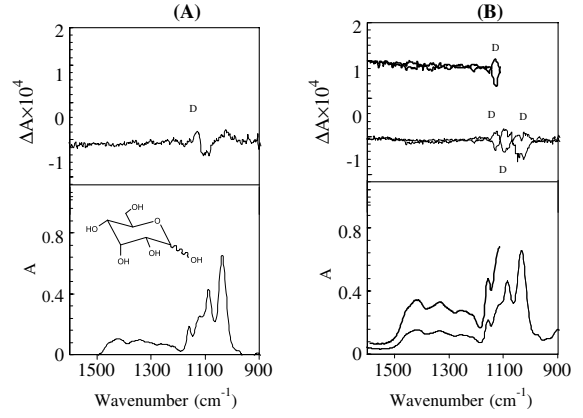

Figure 8. Vibrational absorption (bottom panels) and circular dichroism (top panels) of aqueous solution (left panels, A) and film (right panels, B) samples of enantiomers of allose. The raw VCD spectra obtained for both D and L enantiomers are shown for film samples. The VCD spectrum shown for the solution sample is that for D-allose. See Table 1 for concentrations used. The spectra obtained for the films prepared from a higher-concentration (0.18 M) parent solution are shown as thick traces (and offset from zero line for clarity) in the ~1600–1100 cm^{-1} region.

~1148 cm^{-1} and a negative VCD ($\Delta A/A = -0.6 \times 10^{-4}$ for solution, -0.5×10^{-4} for film) at ~1053 cm^{-1}. Similarly, α-cyclodextrin (Fig. 2) shows a negative VCD signal ($\Delta A/A = -2.5 \times 10^{-4}$ for solution, -2.2×10^{-4} for film) at ~1149 cm^{-1}, a positive VCD signal ($\Delta A/A = +2.4 \times 10^{-4}$ for solution, $+1.8 \times 10^{-4}$ for film) at ~1080 cm^{-1}, a positive VCD signal ($\Delta A/A = +2.8 \times 10^{-4}$ for solution, $+2.6 \times 10^{-4}$ for film) at ~1060 cm^{-1} and a negative VCD signal ($\Delta A/A = -2.6 \times 10^{-4}$ for solution, -2.3×10^{-4} for film) at ~1027 cm^{-1}. Further, α,α-trehalose (Fig. 4) is characterized by a negative VCD ($\Delta A/A = -2.9 \times 10^{-4}$ for solution, -2.1×10^{-4} for film) at ~1148 cm^{-1}, a positive VCD ($\Delta A/A = +2.6 \times 10^{-4}$ for solution, $+1.5 \times 10^{-4}$ for film) at ~1105 cm^{-1}, a negative VCD ($\Delta A/A = -2.6 \times 10^{-4}$ for solution, -2.1×10^{-4} for film) at ~1057 cm^{-1}, and a positive VCD ($\Delta A/A = +1.1 \times 10^{-4}$ for solution, $+1.0 \times 10^{-4}$ for film) at ~995 cm^{-1}. The most pronounced VCD signals exhibited by D-fucose are a negative VCD ($\Delta A/A = -2.7 \times 10^{-4}$ for solution, -1.7×10^{-4} for film) at ~1164 cm^{-1}, a positive VCD ($\Delta A/A = +2.0 \times 10^{-4}$ for solution, $+1.3 \times 10^{-4}$ for film) at ~1001 cm^{-1} and a positive VCD ($\Delta A/A = +2.0 \times 10^{-4}$ for solution, $+8.8 \times 10^{-5}$ for film) at ~964 cm^{-1}. For D-allose, the dominant signals are a positive VCD ($\Delta A/A = +1.2 \times 10^{-4}$ for solution, $+1.0 \times 10^{-4}$ for film) at ~1126 cm^{-1}, a negative VCD ($\Delta A/A = -6.6 \times 10^{-5}$ for solution, -7.3×10^{-5} for film) at ~1093 cm^{-1}, and a positive VCD ($\Delta A/A = -5.9 \times 10^{-5}$ for solution, -4.1×10^{-5} for film) at ~1026 cm^{-1}. Overall, the $\Delta A/A$ magnitudes observed for film samples are slightly smaller than those for the solution samples, which may be partly because of larger bandwidths in the spectra of films. The $\Delta A/A$ values were determined from the peak intensities, while areas, or widths, of the bands were not taken into consideration. But the above-noted differences are not large enough to cause any concern. For D-glucose, gentiobiose, and cellobiose no prominent VCD signals (such as those seen for maltose, α-cyclodextrin, and α,α-trehalose) are seen in the considered region. All these data present evidence for satisfactory quantitative agreement between the solution and film VCD spectra.

The fact that the amounts of samples needed for obtaining sufficient absorption, and hence VCD signal, in film studies are much lower than those needed for obtaining solution-based spectra is a significant advantage associated with film studies. For example, to obtain nearly the same absorbance in solution and film studies on maltose, solution studies used a concentration of 1.8 M, while the film study used a parent solution concentration of only 0.036 M. This translates into approximately a 50-fold smaller amount of sample required for film studies than that in solution studies. However it should be noted that this advantage varies from sample to sample and depends on the surface tension of the solution on the BaF_2 window. If the solution spreads more easily, as in the case of α-cyclodextrin, a thinner and wider film is formed. As a consequence, a parent solution of higher concentration or more volume is needed to prepare the film to obtain a given absorption. On the other hand, for solutions that do not spread easily, thicker films are formed in a smaller region of the window, yielding higher absorbance. Inspite of the varying thicknesses for different sample films, we did not notice any significant differences between solution and film

VCD spectra. We also did not notice any artifacts in the film VCD spectra. This favorable observation for VCD spectra on films is attributed to the longer wavelengths of incident light in the infrared region.

In order to measure VCD in the ~1600–1200 cm^{-1} region with a sufficient signal-to-noise ratio, the intensities of absorption bands in this region are to be increased. For solution-based studies, a solution of higher concentration would be needed for this purpose, but the restrictions imposed by the solubility of some carbohydrates limit the concentrations that one can use. Considering α-cyclodextrin as an example for obtaining the VCD spectra in aqueous solutions, a concentration of 0.14 M has been used. This is a nearly saturated solution because the solubility[14] for α-cyclodextrin in water is 0.15 M. Thus there is not much freedom to increase the concentration of α-cyclodextrin for solution study, and to enhance the weak absorption bands in the ~1600–1200 cm^{-1} region. This restriction is removed from film studies because a parent solution of 0.016 M was sufficient for preparing films with higher absorption in the ~1200–900 cm^{-1} region and a parent solution of higher concentration can be used to enhance the absorption of weak bands in the ~1600–1200 cm^{-1} region. To demonstrate the ability to increase the absorption of films and measure VCD signals in the ~1600–1200 cm^{-1} region, an additional parent solution concentration of 0.035 M has been used for an α-cyclodextrin sample (see Fig. 2). The thick line traces in Figure 2 represent the spectra of film prepared from higher-concentration solution. The VCD features in the ~1600–1200 cm^{-1} region are now clearly seen.

For all of the samples studied, the 1600–1200 cm^{-1} region has been remeasured by preparing films from higher-concentration parent solution and are displayed in Figures 1–8 as thick traces. The concentrations of parent solutions used for preparing films were 0.093 M for maltose, 0.035 M for α-cyclodextrin, 0.10 M for α,α-trehalose, 0.065 M for gentiobiose, 0.11 M for cellobiose, 0.19 M for glucose, 0.14 M for fucose, and 0.18 M for allose. The VCD spectra of carbohydrates connected via α-linkage [α-(1→4) for maltose and α-cyclodextrin and α-(1→1) for α,α-trehalose] have revealed the existence of three distinct bands, two negative and one positive, in the 1600–1200 cm^{-1} region. In the case of maltose, the first negative VCD band ($\Delta A/A = -9.1 \times 10^{-5}$) occurs at ~1459 cm^{-1}, the second negative VCD band ($\Delta A/A = -4.7 \times 10^{-5}$) occurs at ~1375 cm^{-1}, and the positive VCD band ($\Delta A/A = +1.1 \times 10^{-4}$) occurs at ~1333 cm^{-1}. The pronounced peaks characteristic of α-cyclodextrin are a negative VCD ($\Delta A/A = -2.2 \times 10^{-4}$) at ~1458 cm^{-1}, a negative VCD ($\Delta A/A = -1.8 \times 10^{-4}$) at ~1374 cm^{-1}, and the positive VCD band ($\Delta A/A = +1.9 \times 10^{-4}$) at ~1332 cm^{-1}. The peaks exhibited by α,α-trehalose are a negative VCD ($\Delta A/A = -7.4 \times 10^{-5}$) at ~1465 cm^{-1}, a negative VCD ($\Delta A/A = -7.3 \times 10^{-5}$) at ~1386 cm^{-1}, and the positive VCD band ($\Delta A/A = +9.4 \times 10^{-5}$) at ~1340 cm^{-1}. The three bands found in the 1600–1200 cm^{-1} region, for maltose, α-cyclodextrin and α,α-trehalose, are also found for other α-linked oligosaccharides (not shown here), but they are not as prominent for β-linked oligosaccharides (gentiobiose and cellobiose). The film prepared from higher-concentration parent solution of D-glucose exhibits a weak negative VCD band at ~1458 cm^{-1}, a weak negative VCD band at ~1379 cm^{-1}, a positive VCD band ($\Delta A/A = +7.6 \times 10^{-5}$) at ~1329 cm^{-1}, and a negative VCD band ($\Delta A/A = -5.3 \times 10^{-5}$) at ~1241 cm^{-1}. VCD bands of D-fucose film, obtained from higher-concentration parent solution, are a positive VCD ($\Delta A/A = +6.1 \times 10^{-5}$) at ~1413 cm^{-1} and a positive VCD ($\Delta A/A = +7.2 \times 10^{-5}$) at ~1313 cm^{-1}.

The spectra obtained for films of maltopentaose, maltohexaose, and maltoheptaose are similar (not shown here) to those of maltose (Fig. 1). However, the VCD spectrum of cyclomaltohexaose (α-cyclodextrin, Fig. 2) shows strong VCD bands at 1080, 1060, and 1027 cm^{-1} that are not seen for noncyclic maltose or its higher oligomers. Enhancement in the intensity of VROA couplet at around 915 cm^{-1} in cyclodextrin, compared to that in maltose, and its higher oligomers, was attributed[15] to the constrained conformation of cyclodextrin.

The 2000–1600 cm^{-1} region is not shown in the spectra presented here because the unsubstituted carbohydrates do not exhibit fundamental absorption bands in this region. When it becomes necessary to investigate substituted carbohydrates that exhibit absorption bands in this region, films prepared from aqueous solutions do not pose any additional limitations. Recent studies in our laboratory indicated that good quality VCD spectra can be obtained in the 2000–1600 cm^{-1} region for protein films.[12]

The fact that the measured VCD spectra are identical for solution and film samples indicates that the molecular structure in films is not significantly different from that in concentrated solutions, at least as 'seen' by VCD. This observation is significant for VCD spectroscopy because in the past VCD researchers had to avoid the chemical/biological problems that involved water solutions or substitute either D_2O or unnatural organic solvents for water. But now the measurements on aqueous solutions can be undertaken by using films that are conveniently prepared from aqueous solutions.

Based on our experience thus far, we believe that VCD studies on carbohydrate films is a general approach applicable for any carbohydrate solution that can form a film (without microcrystalline deposits) upon solvent evaporation. Thus a wide variety of problems in carbohydrate chemistry can now be tackled using VCD spectroscopy that was not possible before.

3. Experimental

The carbohydrates used in this study were obtained from Sigma Chemical Co. and were used as received. In the case of allose, fucose, and glucose, the solutions were allowed to equilibrate (allowing for mutarotation) for at least 24 h. The choice of carbohydrates studied was made based on the availability of aqueous solution-based VCD spectra for comparison, and also on the variety of glycosidic linkages.

3.1. Spectral measurements on films

In order to prepare the film samples, a drop-cast method was used, where a certain amount of parent aqueous solution was deposited on a 2.5-cm diameter BaF_2 window and allowed to dry at room temperature for approximately 2–3 h in a fume hood, which provided a constant airflow over the sample.

The film VCD measurements were obtained on a modified ChiralIR (Bomem-Biotools, Canada) instrument[12] using the double polarization modulation method,[13] a ZnSe beam splitter, a BaF_2 polarizer, an optical filter (transmitting below 2000 cm^{-1}) and a 2×2 mm HgCdTe detector. Both PEMs used in this instrument contained ZnSe optical elements that did not have antireflection coating. As a consequence, the throughput of our instrument is only 50%, and better signal-to-noise ratios than those presented here can be obtained, in principle, by using antireflection coated ZnSe optical elements. VCD spectra were recorded at 4-cm^{-1} resolution, with PEM settings of 1214 and 1400 cm^{-1} for the first and second PEM, respectively. The spectral collection times varied from 1 to 3 h, depending on the sample. Spectra for trehalose and cyclodextrin were recorded for 1 h (as these samples are known[11] to exhibit larger signals). Those for allose and fucose were recorded for 2 h, and those for the rest of the samples were recorded for 3 h. Baseline corrections were performed by subtracting the VCD spectrum of a blank BaF_2 window, obtained under the same conditions as the sample spectrum. For the absorption spectra of films, neither the solvent absorbance subtraction nor a background subtraction was needed. To verify the independence of film orientation, both absorption and VCD spectral measurements were repeated by rotating the film 45° around the light beam axis.

A point that needs to be clarified is the fact the concentration in a given film is undefined because of solvent evaporation during film formation. The concentrations we refer to for film studies are those of parent solutions used to make the films.

3.2. Spectral measurements on aqueous solutions

The VCD measurements for carbohydrates in water solutions were obtained in our laboratory several years ago as described previously[11] using a ChiralIR instrument with a single PEM. The aqueous solution spectra presented here for maltose, cyclodextrin, trehalose, glucose, cellobiose, and gentiobiose are same as those reported earlier,[11] but those for fucose and allose have not been reported before. For solution measurements, a 6-μm pathlength cell was used, and the absorption and VCD spectra of water solvent were subtracted from their respective parent spectra.

4. Conclusions

The present work demonstrates that VCD spectra for carbohydrates in aqueous solutions can be obtained in the entire 2000–900 cm^{-1} region by depositing the aqueous solutions as films. VCD measurements on films are advantageous over solution-state measurements because the amount of carbohydrate sample required for the VCD film study is approximately one to two orders of magnitude smaller that that for the corresponding aqueous solution studies. The VCD spectra obtained for films derived from aqueous solutions are found to be identical to those obtained for aqueous solutions. The spectral regions that are not easily accessible in aqueous solution studies can be accessed using films, because the water interference has been removed in film studies, which also results in higher throughput and better signal to noise. These observations open up many new applications for VCD in carbohydrate chemistry that could not be attempted before.

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