Notes to the Editor

Studies on chitin: 7. I.r. spectroscopic determination of degree of deacetylation

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(Received 1 November 1977)

INTRODUCTION

Chitin, poly(*N*-acetyl-D-glucosamine), has become a polysaccharide of considerable interest because of its abundance and unique properties. Chitosan, which is derived from chitin by treating with alkali, has been reported to be a promising polymer not only in the chemical field but also in medical and industrial areas[1]. However, little attention has been paid to the extent of deacetylation of these polysaccharides, and to their congeners with intermediate degrees of deacetylation between them.

In view of the increasing interest in these polysaccharides, we have prepared two series of partly deacetylated chitin samples by homogeneous and heterogeneous hydrolyses, and compared their properties[2–5]. They were found to be different from each other in properties such as solubility[2,3], metal ion collection ability[4], etc, depending on the differences in the degree of deacetylation and the hydrolysis mode. The results pointed out that the degree of deacetylation influenced the properties of the chitin congeners quite delicately, and it is therefore necessary to determine the degree of deacetylation to characterize the samples fully.

In the course of the study, two procedures were established for estimation of the degree of deacetylation of the partly deacetylated chitin samples: one was the acidic hydrolysis of the acetamide group and subsequent titration of the liberated acetic acid[6]; the other is acidimetry of the amino group[3]. However these procedures are relatively complex and time consuming, though they have good accuracy. We then attempted to seek a rapid estimation technique of the degree of deacetylation.

The present paper treats a rapid assay system of the acetamide group content in a series of partly deacetylated chitin samples by i.r. spectroscopy.

EXPERIMENTAL

Chitin was isolated from shrimp shells by Hackman's method[7]. Two series of partly deacetylated chitin samples were prepared by treating chitin with NaOH under the homogeneous and heterogeneous conditions[3,5].

The degrees of deacetylation of the polysaccharides obtained above were determined by the slightly modified Elek and Harte method[8]

I.r. measurement was carried out as follows. A sample (3 mg) of the polysaccharide sample (200 mesh) was mechanically blended with 300 mg of KBr (200 mesh) for 10 min. The KBr disc obtained from a 200 mg aliquot of the mixed powder was desiccated for 12 h at 60°C under reduced pressure and then its i.r. spectrum was recorded with a Jasco Model IR–G i.r. spectrophotometer.

The intensity of maxima of the i.r. absorption bands was determined by the baseline method.

RESULTS AND DISCUSSION

Figure 1 shows an i.r. spectrum of chitin with a degree of deacetylation of 15%, together with the baselines used in this work. The absorption bands at 1655, 1550 and 1310 cm^{-1}, which are characteristic of chitin, have been reported to be the amide I, II, and III bands, respectively[9]. The sharp band at 1378 cm^{-1} has been assigned to the CH_3 symmetrical deformation mode. All these bands are known to become very weak in the i.r. spectrum of chitosan. The peak intensity of these bands in the i.r. spectra of a series of partly deacetylated chitin samples prepared by the heterogeneous hydrolysis was then evaluated using the baselines illustrated in *Figure 1*. The absorbance was plotted against the chemically-determined degree of deacetylation in order to make a correct selection of which band to use. Although a linear relationship was obtained for each plot of these bands, that of the amide II band at 1550 cm^{-1} was found to be appropriate for this purpose because of its steeper slope and the relatively smaller scatter. Thus the determination of the acetamide group content of partly deacetylated chitin samples was conducted by measurement of the i.r. absorption maximum at 1550 cm^{-1}.

The Beer–Lambert curves of the peak absorbance of the amide II band at 1550 cm^{-1} are shown in *Figure 2*. The lines A and B in *Figure 2* were

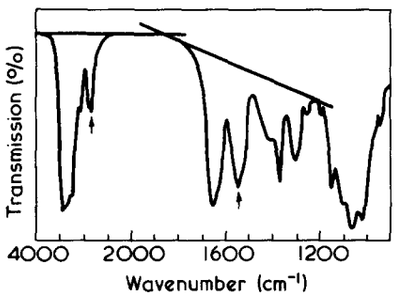

Figure 1 I.r. spectrum of chitin with 15% of deacetylation and baselines for determination of the peak absorbance

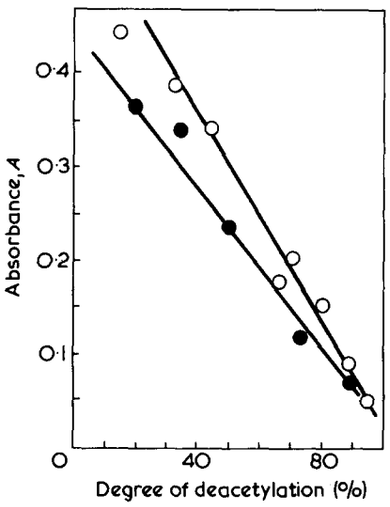

Figure 2 Beer–Lambert curves derived from the peak absorbance of the amide II band at 1550 cm^{-1} in the i.r. spectra of two series of partly deacetylated chitin samples: A, prepared by the heterogeneous and B, homogeneous hydrolyses

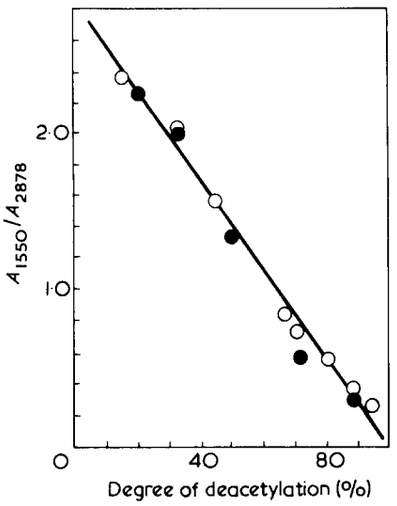

Figure 3 A calibration line obtained by plotting the ratio of the absorbance of the band at 1550 cm^{-1} to that of the band at 2878 cm^{-1} (A_{1550}/A_{2878}) against the degree of deacetylation. (●), i.r. data of the samples made by the homogeneous hydrolysis; (○), those of the samples obtained by the heterogeneous hydrolysis

derived from the i.r. data obtained from the two series of partly deacetylated chitin samples prepared by the heterogeneous and homogeneous hydrolyses, respectively. The interesting comparison is that the slopes of the lines A and B are appreciably different from each other. We have already reported that the structures of the chitin congeners would be different from each other, depending on the hydrolysis mode[5]. The difference in the slopes of the Beer–Lambert lines A and B in *Figure 2* may reflect sensitively the difference of the structures of the chitin congeners.

Very similar phenomena have been reported to be observed in the estimation studies of the acetyl content of cellulose acetate[10]. The Beer–Lambert curves for the intensity of the C=O stretching band were known to give different slopes depending on the difference in acetylation procedures. O'Connor suggested that the difference in slopes might be a clue to the position or site of the chemical modification[10]. This suggestion is quite similar to our view about the structural difference of the chitin congeners.

Figure 2 shows considerable scatter probably due to some experimental errors including that in weighing, as chitin and its congeners are very hygroscopic. In order to correct the errors, the absorbance of the amide II band at 1550 cm^{-1} was divided by that of C–H band at 2878 cm^{-1} which was previously confirmed not to be affected by the deacetylation.

Figure 3 depicts the plot of the ratio of the absorbance of the amide II band at 1550 cm^{-1} to that of the band at 2878 cm^{-1} against the degree of deacetylation. As can be seen from *Figure 3*, the correction technique gave data which showed considerable decrease in scatter of the points about the calibration line. More attractive is the fact that the plot resulted in a single linear relationship for both partly deacetylated chitin samples made by the homogeneous and heterogeneous deacetylation. This single relationship appeared to correspond well to that of the cellulose acetate case, which made the determination of acetyl content in any cellulose acetates accurate[11].

The results obtained above indicate that with this calibration technique, the degree of deacetylation of any partly deacetylated chitin samples can be estimated rapidly with satisfactory precision and accuracy regardless of the deacetylation mode.

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Determination of the solubility parameter of o-hydroxypropyl cellulose

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(Received 15 August 1977; revised 20 October 1977)

INTRODUCTION

A polymer is normally soluble[2] only in liquids whose solubility parameter values fall within the range $\delta_p \pm 3.0$ Hildebrands, the solubility decreasing with an increase in the difference between δ_p and δ_L. *o*-Hydroxypropyl cellulose with an *MS* of approximately 4.0 exhibits solubility[3] in liquids over a much wider range of δ values, from water ($\delta = 23.4$) to piperidine ($\delta = 8.7$) and having widely differing hydrogen bonding abilities and polarities, e.g. water, dimethylformamide and chloroform.

In view of the wide solubility spectrum of this polymer the value of δ_p has been determined, using the maximum limiting viscosity number (*LVN*) method[2]. In addition δ_p has been calculated directly using Small's technique[4], and has been determined by a novel method based on surface tension measurements[1].

EXPERIMENTAL

The *o*-hydroxypropyl cellulose used was an unfractionated commercial sample (Klucel MF produced by Hercules Inc,) with a nominal *MS* value of 4.0. The solvents were either Analar grade or purified by distillation prior to use. Viscosities were measured at 25.0° ± 0.05°C, and the surface tension measurements were carried out using a Du Noüy balance.