

Potentiometric titration of polyuronic acids*

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Using ion-exchange technique it was possible to prepare clear dilute solutions of polyuronic acids which, to the present, were considered insoluble or sparingly soluble materials. Potentiometric titrations of polymannuronic, polyguluronic, pectic, and polymethacrylic acid with potassium and calcium hydroxide were investigated. The titration of *clear* solutions of polyuronic acids with alkali hydroxides can be well expressed by the extended Henderson and Hasselbalch equation up to the degree of dissociation $\alpha = 0.80$ ($n = 1.6-1.7$). Neutralization of the studied polyacids with calcium hydroxide proceeds in the manner similar to that of weak monobasic acids ($n = 1$) which is due to the suppression of electrostatic effects of carboxylate group caused by Ca^{2+} ions. Small deviations were found only when pectic and polyguluronic acids were titrated with calcium hydroxide in which case the neutralization was accompanied by partial coagulation of corresponding calcium polyuronates. Apparent dissociation constants found for polyuronic acids are compared with dissociation constants of the corresponding monouronic acids.

Potentiometric titration of synthetic as well as naturally occurring polyacids has been studied by a great number of authors [1-13, 24]. The course of the potentiometric titration of polyacids with hydroxide can be expressed by the empiric equation

$$\text{pH} = \text{p}K_a - n \log [(1 - \alpha)/\alpha],$$

which represents an extended form of the Henderson and Hasselbalch equation [3]. K_a is the apparent dissociation constant, α is the degree of dissociation, and n is an empiric coefficient involving the effect of the neighbouring dissociated groups upon the dissociation of the acidic group. The parameters K_a and n depend upon the linear density of the electric charge of a macromolecule, upon the concentration of polyacid in the solution, upon the concentration and the type of the additional simple electrolytes, *etc.*

In the range of lower pH values the polyuronic acids exist in the form of gel. The potentiometric titration with alkali hydroxide carried out in such a system takes an anomalous course. For instance, *Haug* [6] studying the dissociation of alginic acids found the parameter n to be lower than 1 ($n = 0.34-0.70$). With increasing ionic strength n increased towards 1, while the normal behaviour of polyacids is that n is higher than 1 at low ionic strength and decreases towards 1 when the ionic strength increases. This peculiar behaviour of alginic acids is according to the author [6] due to the fact that

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a part of the titration takes place in a two-phase system where, particularly at a low ionic strength, the Donnan equilibrium causes a considerable difference in pH of the solution compared to the pH in the gel. According to Cordes [14] the alginic acid, when titrated with an alkali hydroxide, behaves in the range of $\alpha = 0.0-0.5$ as a monocarboxylic acid whereas in the range of $\alpha = 0.5-1.0$ it behaves as a polyacid with the value of $n = 3.5$. Similarly, Brunelle [15] found that the course of the titration curves of alginic and pectic acid in the acidic pH range differed. This depended on the question whether the polyuronic acid was titrated directly with an alkali hydroxide or its alkali salt was back-titrated with hydrochloric acid. An unusual course of the titration of the alginic and pectic acid was observed also when the titration was carried out in the presence of 1 M-KCl [2].

It was shown in our previous paper [16] that by the ion-exchange technique dilute solutions of polyuronic acids may be prepared without causing their coagulation into a gel-like form. The purpose of the present investigation was to ascertain whether the course of potentiometric titration of the *clear solutions* of polyuronic acids is comparable to that of other polyacids, *i.e.* whether the course of the whole potentiometric titration can be expressed in terms of the extended Henderson and Hasselbalch equation at $n > 1$.

Polyuronates (polymannuronate, polyguluronate, and polygalacturonate) depending upon their chemical composition, show in exchange reactions a different selectivity towards cations (see [17, 18]). Taking this phenomenon into consideration we compare the pK_a values of various polyuronic acids. These values are compared with the pK values of the corresponding monouronic acids as their basic structural units.

Experimental

Chemicals

Polyuronic acids were prepared from pectic acid and from sodium alginate *I* and *II* with a high content of L-guluronic or D-mannuronic acid, respectively. Alginates and pectic acid were prepared as described previously [18]; the preparations were identical with those used in [16]. In the following, the terms *polyguluronic acid* and *polymannuronic acid* are used for the preparations of alginic acid *I* and *II*, respectively.

Polyguluronic acid contained 89.5% of L-guluronic acid and 10.5% of D-mannuronic acid; the degree of polymerization, based on the determination of reducing end units (\overline{DP}_n), was 81. Polymannuronic acid contained 92% of D-mannuronic acid and 8% of L-guluronic acid; its degree of polymerization, determined viscometrically (\overline{DP}_n), was 4000. Pectic acid contained 91% of D-galacturonic acid together with some residual neutral saccharides; its degree of polymerization (\overline{DP}_n) was 91.

Sodium polymethacrylates *I* and *II* were obtained from the Research Institute of Synthetic Varnishes and Resins, Pardubice, and from the Central Research Institute of Sugar Industry, Kiev (USSR). The corresponding polymethacrylic acids were of 100% purity having the degree of polymerization \overline{DP}_n 390 (*I*) and 3200 (*II*), respectively. Carbonate-free 0.05 N-KOH was used throughout. Calcium hydroxide was a saturated solution of $\text{Ca}(\text{OH})_2$ of about 0.042 N. Other chemicals were of reagent grade. Boiled distilled water having the conductivity of 2.5×10^{-6} ohm $^{-1}$ cm $^{-1}$ was used.

Preparation of the dilute solutions of polyuronic and polymethacrylic acids

The insoluble pectic acid was first converted to its soluble potassium salt by neutralization with 0.05 N-KOH to the equivalence point of the potentiometric titration curve.

Slight turbidity was eliminated by centrifugation at 13,000 *g*; this produced a perfectly clear solution of potassium pectate.

Dilute solutions of potassium or sodium polyuronate containing 1.4–1.6 mg of the solute per 1 ml were prepared. The solution of sodium polymethacrylate contained roughly 1 mg/ml. The solutions were slowly percolated through a column of Dowex 50W × 4 in H⁺ form. Perfectly clear solutions of polyacids were obtained, excepting the solution of polyguluronic acid which was slightly turbid. Centrifugation at 13,000 *g* yielded, also in this case, a clear solution. Atomic absorption spectrometry showed that the exchange reaction on the column of ion exchanger was complete and that the solutions of polyacids contained H⁺ ions only; the approximate concentration of polyacids was 4–6 mequiv [–COOH]/l. Before the potentiometric titration the solutions were diluted to the concentration of 3.00 mequiv [–COOH]/l.

Analytical methods

Polyuronic and polymethacrylic acids were characterized using previously described methods [16, 18]. Atomic absorption spectrometry was carried out using Perkin–Elmer Atomic Absorption Spectrophotometer.

Potentiometric titrations were run at 21–23°C using a compensation pH-meter Radiometer equipped with a glass electrode and a calomel electrode (sat. KCl). Buffer solutions (Radiometer) of pH 1.68, 4.65, and 6.50 were used. The titrations were performed with clear solutions of polyacids in the *usual manner* without addition of further electrolyte *i.e.* by step-wise additions of small volumes of the solutions of potassium or calcium hydroxide in 3–4 min. intervals; a fast stabilization of the potential of the glass electrode was observed. The ionic strength and the concentration of the solution were not kept at constant level during the titration. As the volume of the consumed solution of the hydroxide was small compared to the volume of the titrated solution the decrease of concentration during the titration was only small (6–7%).

In order to calculate the degree of dissociation α the concentration of dissociated carboxyl groups was determined using the equation

$$[-\text{COO}^-] = [\text{B}] + [\text{H}^+] - [\text{OH}^-],$$

where [B] is the concentration of the added hydroxide in equivalents.

Results and discussion

When a very dilute solution of sodium (potassium) polyuronate is percolated through a column of a cation-exchange resin in H⁺ form, due to the slow gradual exchange of Na⁺ (K⁺) ions for H⁺ ions, no coagulation of polyuronic acids occurs. Using this procedure, it was possible to prepare clear solutions of the polymannuronic, polyguluronic, pectic, and polymethacrylic acid containing 4–6 mequiv [–COOH]/l. The solutions of polyuronic acids filtered easily through blue ribbon No. 589³, Schleicher–Schüll filter paper, no solute being retained. It is not known whether these solutions are really true solutions of macromolecules or whether the solutions contain small aggregates of macromolecules. The addition of an excess of hydrochloric acid to the solution of polyuronic acids caused immediately their irreversible gel formation.

Neutralization of polyuronic acids with potassium hydroxide

An exact investigation of the dissociation of polyacids requires that the pH values, corresponding to different degrees of neutralization of polyacid, should be determined at a constant ionic strength and at a constant concentration of solutions. The determination of pH requires further that the time variation of potential of the indication electrode should be followed at every measurement. The object of the present work is the potentiometric titration of clear solutions of polyacids carried out in the *usual*, above described, manner. It was of interest, therefore, to find out to what extent could these titrations be evaluated using the extended Henderson and Hasselbalch equation. The measurements were performed with D-galacturonic acid, the dissociation constant of which is known in the literature.

The solution of D-galacturonic acid (3.00 mequiv $[-\text{COOH}]/\text{l}$) was neutralized with potassium hydroxide. The dependence of pH *vs.* $\log [(1 - \alpha)/\alpha]$ was a linear function up to the degree of dissociation $\alpha = 0.80$ (Fig. 1, curve 1). The determined values of $\text{p}K = 3.48 \pm 0.01$ and $n = 1.00 \pm 0.02$ (Table 1) are in a good agreement with the previously published data. *Speiser et al.* [1] found for D-galacturonic acid $\text{p}K = 3.42$ at 27°C ; according to *Karrer and Schwarzenbach* [19] $\text{p}K = 3.49$ at 23.6°C . The found value of $n = 1.00$ is in perfect agreement with the theoretical value of $n = 1$, corresponding to the titration of a weak monobasic acid.

The results of the potentiometric titrations of polyuronic acids with potassium hydroxide are summarized in Table 1 and pictured in Fig. 1 (curves 2, 3, and 4). All measurements were done with solutions of the concentration of 3.00 mequiv $[-\text{COOH}]/\text{l}$. The plot: pH *vs.* $\log [(1 - \alpha)/\alpha]$ is a linear function from the beginning of the neutralization up to the degree of dissociation $\alpha = 0.80$. At $\alpha = 0.90$ a certain shift to the lower pH values can be seen. The coefficient n for all polyuronic acids under investigation was greater than 1 ($n = 1.6 - 1.7$), which is in agreement with the usual course of the titration of weak polyacids. For comparison, Table 1 contains also the results of the titration of polymethacrylic acid with potassium hydroxide; $n \approx 2$ compares well with the reported value [3].

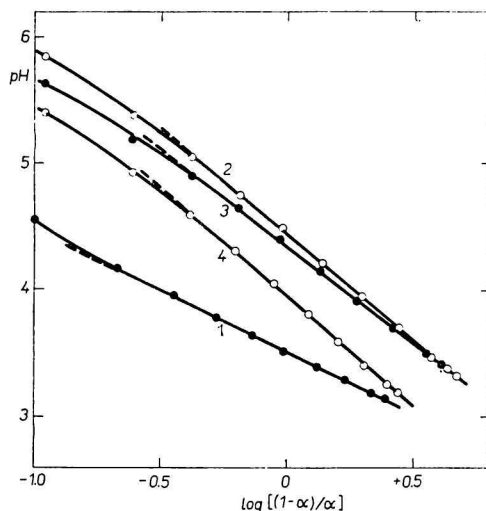


Fig. 1. Neutralization of polyuronic acids with potassium hydroxide.

α — degree of dissociation.

1. D-galacturonic acid; 2. pectic acid; 3. polyguluronic acid; 4. polymannuronic acid.

Table 1

Neutralization of polyacids with potassium hydroxide and calcium hydroxide
Apparent dissociation constant pK_a and the coefficient n

Acid	KOH		Ca(OH) ₂	
	pK_a	n	pK_a	n
D-Galacturonic	3.48 ± 0.01	1.00 ± 0.02	3.48 ± 0.01	1.01 ± 0.02
Pectic	4.41 ± 0.02	1.70 ± 0.02	3.81 ± 0.01	0.85 ± 0.01
Polyguluronic	4.32 ± 0.02	1.60 ± 0.07	3.76 ± 0.01	0.99 ± 0.01
Polymannuronic	3.91 ± 0.03	1.74	3.50 ± 0.03	1.00 ± 0.02
Polymethacrylic I	7.59	1.97	6.31	0.97
Polymethacrylic II	7.46	2.10	6.28	1.04

Speiser et al. [1] have studied the dissociation of pectinic acids with a degree of esterification of $E = 10-44\%$. In contrast to an unesterified pectic acid these polyacids were, as a result of partial esterification of carboxyl groups with methanol, water soluble. *Katchalsky* and *Spitnik* [3] having interpreted the results of the authors [1] showed that the function pH vs. $\log [(1 - \alpha)/\alpha]$ is linear at $n = 1.69$. Similarly, when dilute solutions of carboxymethylcellulose were titrated without additional electrolyte [10] as well as in titrations of oxycelluloses containing carboxyl groups [11] the n values were close to 1.7.

The obtained results demonstrated that the course of potentiometric titrations of *clear solutions* of polyuronic acids with an alkali hydroxide is similar to that of other polyacids containing carboxyl groups. The titration curves can be well expressed using the extended Henderson and Hasselbalch equation up to the degree of dissociation of $\alpha = 0.80$. The anomalous course of the potentiometric titration of polyuronic acids found by other workers can be explained by the presence of solid polyuronic acids in the titrated samples.

Neutralization of polyuronic acids with calcium hydroxide

The addition of a simple neutral salt (*e.g.* NaCl, KNO₃) to a solution of weak polyacids reduces the electrostatic interactions of its dissociated anionic groups. If the concentration of the salt in the solution increases both the pK_a value and the coefficient n decrease. Polyvalent cations are particularly efficient in this respect. According to [3] the addition of a small quantity of CaCl₂ to a solution of polymethacrylic acid half neutralized with an alkali hydroxide resulted in a noticeable decrease of pH together with the precipitation of the polymer. *Gregor et al.* [5] observed a considerable decrease of pH when polyacrylic acid was potentiometrically titrated with an alkali hydroxide in the presence of small quantities of Cu²⁺ ions. In this case the plot of pH vs. $\log [(1 - \alpha)/\alpha]$ was not a linear function in contrast to the titration realized without the addition of Cu²⁺ ions. *Gustafson* and *Lirio* [9] studied the interaction of polyvalent metal ions with the cross-linked polymethacrylic acid. When the titration was carried out in 1 M solution of NaNO₃ and in the presence of these cations, the course of the above-mentioned function was linear and, at the same time, a decrease of pK_a and n was observed; in the presence of Cu²⁺ ions n was approximately equal to 1 (see also [20, 21]). Similarly, the titration of chondroitin sulfuric acids with alkali hydroxides performed in solutions containing CaCl₂ gave lower pH values as compared to the ones obtained when the titration was

carried out in the absence of Ca^{2+} ions [7]. These interesting observations prompted us to study the titration of polyuronic acids also with calcium hydroxide.

The solutions of polyuronic acids and of polymethacrylic acid having the concentration of 3.00 mequiv $[-\text{COOH}]/\text{l}$ were titrated with a clear solution of calcium hydroxide. In the case of polymannuronic acid and polymethacrylic acid the solutions remained clear. On the other hand the titration of polyguluronic acid and pectic acid was accompanied by a partial coagulation of corresponding calcium polyuronates in about 50% quantity of their total concentration (for more details see [16]).

Fig. 2 shows the titration of D-galacturonic acid and of pectic acid with potassium and calcium hydroxide. The titration of the monomeric D-galacturonic acid with these hydroxides yields perfectly identical curves (curve 1, Table 1). This is in a good agreement with our previous results according to which in a dilute solution of calcium galacturonate no formation of an undissociated complex occurs [18, 22]. The titration of pectic acid (*polygalacturonic acid*) with calcium hydroxide (curve 2) is accompanied by a considerable decrease of pH compared to the titration with potassium hydroxide (curve 3). The titration of pectic acid with calcium hydroxide is similar to the titration of D-galacturonic acid with an alkali hydroxide. The course of the titration curves 1, 2, and 3 is, in the range of $\text{pH} > 8$, virtually identical; this is the reason why the points of the pH values of the individual measurements are omitted in this graph. The titrations of other polyuronic acids proceed in a similar manner.

The dependence of pH vs. $\log [(1 - \alpha)/\alpha]$ is pictured in Fig. 3; the respective pK_a and n values are given in Table 1. The course of the potentiometric titration of polymannuronic (curve 1) and polymethacrylic (curve 3) acid, in which case the neutralization

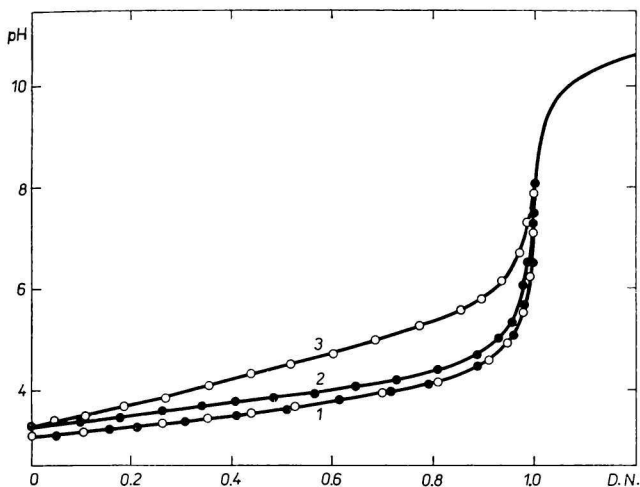


Fig. 2. Potentiometric titration of D-galacturonic and pectic acid with potassium hydroxide and calcium hydroxide.

D. N. — degree of neutralization.

○ titration with potassium hydroxide; ● titration with calcium hydroxide.

1. titration of D-galacturonic acid; 2. and 3. titration of pectic acid.

is not accompanied by a coagulation of their calcium salts, is similar to the titration of a weak monobasic acid with an alkali hydroxide; the coefficient n is, within the range of experimental errors, equal to 1. The dependence of pH *vs.* $\log [(1 - \alpha)/\alpha]$ is a linear function up to the degree of dissociation $\alpha = 0.80$. At $\alpha = 0.90$ only small deviations of pH were observed. The values of ΔpH corresponding to the neutralization of poly-

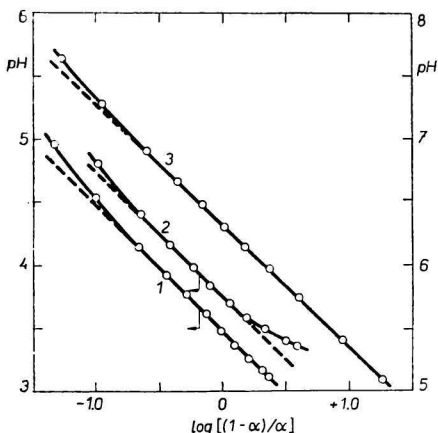


Fig. 3. Neutralization of polyuronic acids and polymethacrylic acid with calcium hydroxide.

α — degree of dissociation.

1. polymannuronic acid; 2. polyguluronic acid; 3. polymethacrylic acid.

mannuronic and polymethacrylic acid are $+0.03$ and $+0.06$, respectively. In the case of $\alpha = 0.95$ these deviations are $+0.13$ and $+0.09$ for polymannuronic and polymethacrylic acid, respectively. The titration of polymethacrylic acid (curve 3) proceeds exactly according to the Henderson and Hasselbalch equation.

The titration of pectic acid with calcium hydroxide has a linear course which is similar to the titration of polymannuronic acid, except for the value of n which is smaller than 1 ($n = 0.85$). (In Fig. 3 this titration curve is not included as it is overlapping the curve 2 and would so make the drawing unclear.) The titration of polyguluronic acid can be described by the above-mentioned linear function with the coefficient $n \doteq 1$ only in a narrower range of α (curve 2). A deviation of pH is observed also in the initial stage of the titration. The course of the two last titrations, which is to a certain extent anomalous, can be explained by a partial coagulation of calcium salts of these polyuronic acids during the neutralization with calcium hydroxide.

There are relatively strong bonds between the Ca^{2+} ions and the carboxyl groups of polyuronic acids and of polymethacrylic acid. This is demonstrated by the low activity coefficients $\gamma_{\text{Ca}^{2+}}$ determined in dilute solutions of calcium salts of pectic, polyguluronic, and polymethacrylic acid: $\gamma_{\text{Ca}^{2+}} = 0.06, 0.08, \text{ and } 0.07$, respectively. These measurements were carried out in solutions of $1.2\text{--}3.0$ mequiv $[-\text{COOCa}_{1/2}]/l$ [16]. As a result of this interaction the electrostatic effects of the neighbouring carboxylate groups upon the dissociation of the remaining $-\text{COOH}$ groups are markedly suppressed. The interaction of Ca^{2+} ions with the carboxylate groups of calcium polymannuronate is, as shown by the value $\gamma_{\text{Ca}^{2+}} = 0.28$, markedly weaker than in the case of other polyuronates (see [16]). Following the course of the neutralization of polymannuronic acid with calcium hydroxide it is obvious that this interaction suffices to make the titration follow the same course as it normally occurs in the case of a weak monobasic acid with the coefficient $n = 1$.

The preliminary experiments have shown that the dissociation constant K_a determined by titration of the above-mentioned polyacids with calcium hydroxide depends upon the concentration of the titrated solution. Thus, for instance, at the concentration of polymannuronic acid 5.9 mequiv $[-COOH]/l$ the value of pK_a was 3.38, whereas at the concentration of 3.00 mequiv $[-COOH]/l$ $pK_a = 3.50$; in both cases $n = 1.00$. A similar decrease of pK_a with the increasing concentration was observed when pectic acid was titrated. Based on these results it can be concluded that, despite the suppression of the electrostatic effects of carboxylate groups resulting in $n = 1$, the observed K concerning the titration of polyacids with calcium hydroxide is actually an *apparent* dissociation constant K_a . Since the potentiometric titrations described here were in most cases carried out only at one concentration of polyacids, it was not possible to interpret these results with the aim to obtain the true dissociation constant related to the isolated carboxyl groups in the molecule of polyuronic acid.

On the basis of the apparent dissociation constants pK_a (Table 1) polymannuronic acid appears to be a stronger acid than the polyguluronic and pectic ones. The same can be concluded from the results obtained with both hydroxides. The pK_a values of the polyguluronic and pectic acid are rather close. Their salts (polyguluronates and pectates) show a similar high selectivity in exchange reactions of bivalent and monovalent cations. On the contrary, polymannuronates, the salts of the stronger polymannuronic acid, show a lower selectivity in the exchange of cations [17, 18]. Let us now compare the apparent dissociation constants (pK_a) of polyuronic acids with the dissociation constants (pK) of the corresponding monouronic acids. The pK values of D-mannuronic, L-guluronic, and D-galacturonic acids are 3.38, 3.65 [23], and 3.42 or 3.48, respectively. The similarity in the difference in the strength of D-mannuronic acid and L-guluronic acid appears to remain unchanged when one compares the strength of the corresponding polyacids. Haug [6] came to the same conclusion carrying out his measurements on suspensions of alginic acids containing different proportions of D-mannuronic and L-guluronic acid. According to the dissociation constant of D-galacturonic acid one would expect, however, the value of pK_a of pectic acid to be lower than that experimentally determined.

References

1. Speiser, R., Hills, C. H., and Eddy, C. R., *J. Phys. Chem.* **49**, 328 (1945).
2. Saric, S. P. and Schofield, R. K., *Proc. Roy. Soc. (London)* **A185**, 431 (1946).
3. Katchalsky, A. and Spitnik, P., *J. Polym. Sci.* **2**, 432 (1947).
4. Kagawa, I. and Gregor, H. P., *J. Polym. Sci.* **23**, 477 (1957).
5. Gregor, H. P., Luttinger, L. B., and Loebel, E. M., *J. Phys. Chem.* **59**, 34 (1955).
6. Haug, A., *Acta Chem. Scand.* **15**, 950 (1961).
7. Mathews, M. B., *Biochem. Biophys. Acta* **48**, 402 (1961).
8. Bak, K., *Acta Chem. Scand.* **16**, 229 (1962).
9. Gustafson, R. L. and Lirio, J. A., *J. Phys. Chem.* **69**, 2849 (1965).
10. Chowdhury, F. H. and Neale, S. M., *J. Polym. Sci. A*, **1**, 2881 (1963).
11. Chowdhury, F. H. and Neale, S. M., *J. Polym. Sci. A*, **1**, 2893 (1963).
12. Mandel, M., *Eur. Polym. J.* **6**, 807 (1970).
13. Mandel, M., *J. Polym. Sci. A-2*, **8**, 1841 (1970).
14. Cordes, J., *PhD. Thesis*. University Kiel, 1968.
15. Brunelle, T. E., *PhD. Thesis*. University of Minnesota, 1968.
16. Kohn, R. and Larsen, B., *Acta Chem. Scand.* **26**, 2455 (1972).

17. Smidsrod, O. and Haug, A., *Acta Chem. Scand.* **22**, 1989 (1968).
18. Kohn, R., Furda, I., Haug, A., and Smidsrød, O., *Acta Chem. Scand.* **22**, 3098 (1968).
19. Karrer, P. and Schwarzenbach, G., *Helv. Chim. Acta* **17**, 58 (1934).
20. Howe, P. G. and Kitchener, J. A., *J. Chem. Soc.* **1955**, 2143.
21. Mandel, I. M. and Leyte, J. C., *J. Polym. Sci. A*, **2**, 2883 (1964).
22. Kohn, R., *Carbohydr. Res.* **20**, 351 (1971).
23. Haug, A. and Larsen, B., *Acta Chem. Scand.* **15**, 1395 (1961).
24. Rinaudo, M. and Daune, M., *J. Chim. Phys.* **64**, 1761 (1967).

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