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Phosphorylated polysaccharides. 2. Synthesis and properties of phosphorylated dextran

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ABSTRACT

New results regarding the synthesis and characterization of water-soluble phosphorylated dextran (PDex) are presented. The phosphorylation was made by reaction of dextran with phosphorous acid in molten urea. The structure of phosphorylated product was investigated by elemental analysis, by titration of acidic groups and by FTIR and NMR spectroscopy. It was found that phosphorylated dextran prepared in these conditions is a monobasic dextran phosphate with (H)P(O)(OH) groups. The degree of polymerization of PDex was found comparable to the parent dextran, suggesting that polysaccharide chain was not degraded during phosphorylation. The polyelectrolyte behavior was studied by potentiometric and conductometric titration and by viscometric measurements.

1. Introduction

Dextran is a family of neutral polysaccharides produced by bacterial enzymes, consisting of main chains from α-(1→6) linked D-glucose units with different ratios of linkages and branches, depending on the bacterial strains. The chemical structure of dextran includes α-(1→6) linkages that can vary from 97 to 50% of total glucosidic bonds. The branching is made in position α-(1→2), α-(1→3) and/or α-(1→4) (Heinze, Liebert, Heublein, & Hornig, 2006; Robyt, 1987). The hydroxyl groups in dextran are preferred for most derivatization. The C-2 hydroxyl groups appear to be the first to react (Allene, 1966) because the hydroxyl groups of C-6 are concerned in α-1, 6 linkages. Until now various dextran derivatives were obtained, such as: esters of aliphatic or aromatic organic acids, of inorganic acids such as nitric, sulfuric, and phosphoric (Allene, 1966; Gekko, 1981, chap. 29; Heinze et al., 2006; Larsen, 1990; Robyt, 1987; Whistler & Towle, 1969). The references regarding the synthesis of phosphorylated derivatives are presented in Table 1.

Phosphorylated derivatives of dextran were characterized by elemental analysis, potentiometric titration, ^{1}H, ^{31}P, and ^{13}C NMR spectroscopy and high-performance exclusion chromatography (Sacco, Klett-Zygmunt, & Dellacherie, 1988). Until now no mention was made about their water solubility or polyelectrolyte behavior, though these aspects are important in most of the applications.

In this article we report original results on the synthesis, characterization and properties of water-soluble phosphorylated dextran, including polyelectrolyte behavior. These results are a part of an extensive research regarding the phosphorylation of dissimilar polysaccharides and the properties of the phosphorylated polysaccharides the results of which will be the subject of subsequent articles.

2. Experimental

2.1. Materials

The parent dextran ($M_r \sim 200.000$, *Leuconostoc mesenteroides*) was purchased from Fluka, Switzerland. The other reagents were phosphorous acid 99% from Sigma–Aldrich, Germany, urea 99.5% from Carl Roth, Germany, and methanol from Chimopar, Romania. All chemicals were used without further purification.

2.2. Methods

Phosphorylation of dextran was performed by reaction of dextran with phosphorous acid (Inagaki, Nakamura, Asai, & Katsuura, 1976; Suflet, Chitanu, & Popa, 2006). In a 250 mL, four-necked flask equipped with nitrogen inlet, condenser, thermometer, and stirrer, 64 g (1.067 moles) urea was introduced and heated at 140 °C under flushing with N_2. 10 g (0.061 moles) dextran (Dex) and 51 g (0.622 moles) phosphorous acid were added portionwise alternatively to the molten urea. The reaction was allowed to proceed at 150 °C for different times. The reaction mixture was cooled, dis-

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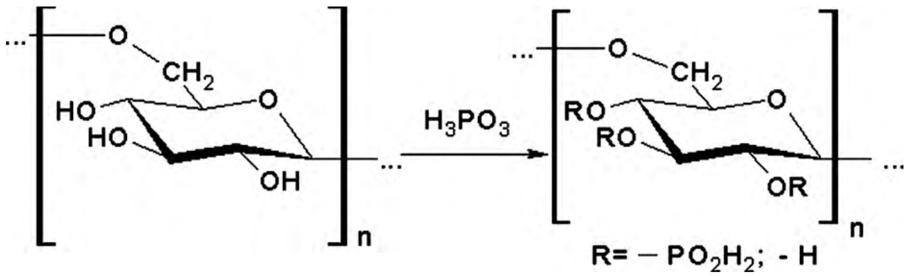


Scheme 1. Reaction of dextran with phosphorous acid.

solved in 1 N aqueous NaOH and precipitated in methanol. The precipitation was repeated several times. The final product was dried under reduced pressure, at 25 °C, over phosphorous pentoxide. The phosphorylated dextran (PDex) is a white, water-soluble powder. It was purified by diafiltration in a Millipore cell with PM10 membrane up to the diafiltrate conductivity was lower than 5 μS/cm. Then the polymer was recovered from aqueous solution by freeze-drying.

2.2.1. *Analysis and characterization of phosphorylated dextran*

Elemental analysis of phosphorus was performed by Kjeldahl mineralization method combined with spectrophotometric assessment at 740 nm after adding of ammonium molybdate. Elemental analysis of nitrogen was performed using Kjeldahl method. FTIR spectra were recorded on KBr pellet using a Vertex 70 Bruker spectrometer. NMR spectra were recorded in D_2O using a Bruker Avance 400.13 MHz instrument. The molar mass of PDex was estimated by size exclusion chromatography using a Waters instrument equipped with two Ultrahydrogel columns (250 and 2000, 7.8 mm × 300 mm) and a refractive index detector. The eluent was aqueous 0.1 M $NaNO_3$ solution with 3% acetonitrile. The flow rate was 0.8 mL/min and the feed was 150 or 50 μL of 1.0 or 3.0 mg/mL polymer solution in aqueous 0.1 M $NaNO_3$. Poly(acrylic acid) samples from Waters were used as standard. The decomposition under dynamic conditions of heating has been investigated with a Paulik-Paulik-Erdey MOM-Budapest instrument on 50 mg samples, at 12 °C/min, in air.

Polyelectrolyte behavior was studied by potentiometric and conductometric titration and by viscometric measurements. Potentiometric titration was performed using a Metrohm all purposes 716 DMS Titrino apparatus equipped with a 6.0203.100 combined electrode. Monovalent bases LiOH and KOH were used for titration without or with added low molecular salt. Conductometric titrations were performed with same bases, with no added salt, using a 712 Metrohm conductometer with 6.0908.110 cell. The potentiometric and conductometric titrations were made on the acid form of PDex. As the phosphorylated dextran was obtained in form of Na salt, the free acid form was prepared by passing diluted solutions of Na salt through an ion exchange column filled with a sulfonic Dowex 50W×8, 20–50 mesh resin. Viscometric measurements were performed on diluted solutions of Na salt of PDex at 25 ± 0.1 °C, using an Ubbelohde viscometer with 0a capillary (K = 0.005319).

Table 1
Methods of synthesis of phosphorylated dextran.

Phosphoryiation agent	Ref.
Polyphosphoric acid/tributylamine (TBA)/dimethyl formamide (DMF)	Heinze et al. (2006), Sacco, Klett-Zygmunt, Vigneron, and Dellacherie (1990) and Spaltro and Aronson (1992)
H_3PO_4/faty acids esters	Suzuki, Suzuki, and Matsumoto (1975)
H_3PO_4/palmitic acid/tertiary amines	Heinze et al. (2006) and Suzuki, Suzuki, and Mikami (1977)
H_3PO_4/urea (the reaction results in crosslinked dextran)	Makarova et al. (1978)
H_3PO_4/formamide	Sato et al. (2004)

Table 2
Degree of substitution of phosphorylated dextran samples.

Sample code	Reaction time (h)	Dex/urea/ H_3PO_3	DS_P	DS_T
$PDex_2$	2	1/6/5	1.18	1.30
$PDex_5$	5	1/6/5	1.78	1.90

3. Results and discussion

3.1. *Reaction of dextran with phosphorous acid*

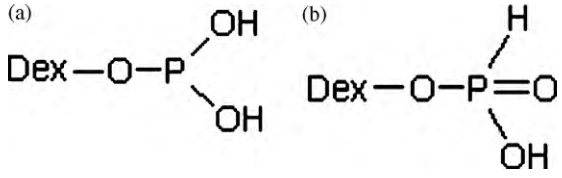
The degree of substitution (DS) of phosphorylated samples was evaluated by phosphorus elemental analysis (DS_P) and by potentiometric or conductometric titration (DS_T) according to the methods already verified (Suflet et al., 2006). The results are presented in Table 2. It can be noticed that the DS_P and DS_T are close. Taking into account the use of molten urea as reaction medium, nitrogen analyses were also performed in order of checking the purity of the reacted polymer. All samples showed a very low N content indicating a slight remanence of urea, even after diafiltration procedure.

The reaction of dextran with phosphorous acid is expected to proceed predominantly at the hydroxyl group of the C-2 atom (Heinze et al., 2006) according to Scheme 1.

It is known that the derivatives containing mobile hydrogen atoms directly attached to phosphor atom are in tautomeric equilibrium (Kabachnik & Mastryakova, 1953). Accordingly PDex could present the tautomeric forms given in Scheme 2. In order to check which one of these structures is preferred, FTIR spectra were registered and potentiometric/conductometric titrations were performed. The characteristic bands of $PDex_2$ and $PDex_5$ phosphorylated samples are summarized in Table 3 compared to the parent dextran (Purama, Goswami, Khan, & Goyal, 2009; Hesse, Meier, & Zeeh, 1984).

In Figs. 1 and 2 are shown the potentiometric and conductometric titration curves of PDex samples with monovalent bases (0.0838 or 0.0755 N KOH and 0.0947 N LiOH).

One inflexion point is observed on the potentiometric curve obtained by titration of $PDex_2$ with DS = 1.3 (Fig. 1), which can be



Scheme 2. Tautomeric forms of phosphorylated dextran.

Table 3
Characteristic FTIR bands and their attribution by dextran and phosphorylated dextran samples.

Sample	ν (cm^{-1})
Dex	3434 (OH); 2924 (CH, CH$_2$); 1652 (C=O); 1462–1280 (glucosidic units); 1162–998 (C–O, C–C); 913 (α-glycosidic bonds)
PDex$_2$	3426 (OH); 2938 (CH, CH$_2$); 2398 (P–H); 1663 (C=O); 1463–1342 (glucosidic units); 1226 (P=O); 1057 (P–OH); 1160–980 (C–O, C–C); 859 (P–O–C)
PDex$_5$	3428 (OH); 2937 (CH, CH$_2$); 2398–2366 (P–H); 1656 (C=O); 1461–1347 (glucosidic units); 1222 (P=O); 1160–1019 (C–O, C–C); 1055 (P–OH); 861 (P–O–C)

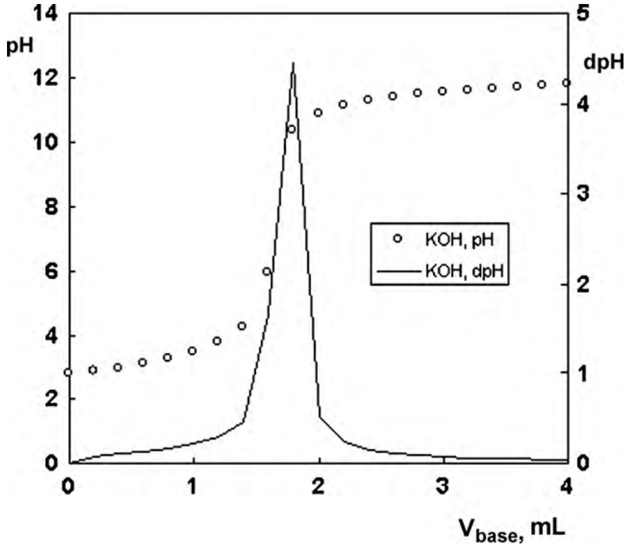


Fig. 1. Potentiometric titration of PDex$_2$ with KOH.

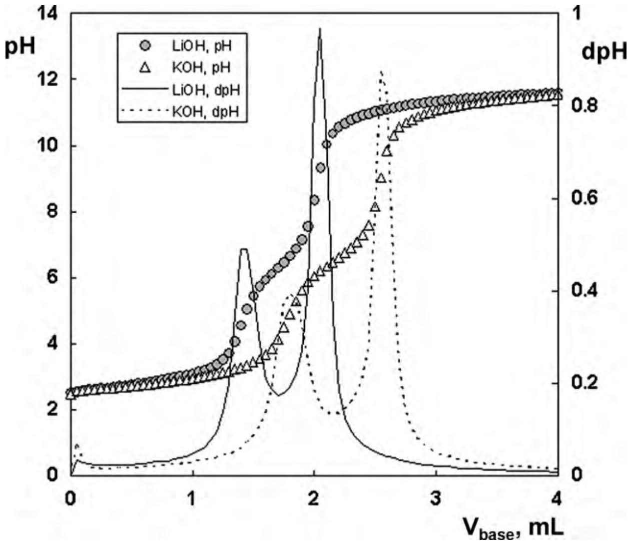


Fig. 2. Potentiometric titration of PDex$_5$ with LiOH and with KOH.

attributed to –H$_2$PO$_3$ groups with one acidity (P–OH). By titration of PDex$_5$ having DS 1.9 (Fig. 2) potentiometric curves present two inflection points. These two inflection points are not in the ratio of 1:2 corresponding to a dibasic acid behavior, as described in the case of dextran phosphate obtained by reaction of dextran with phosphoric acid (Sacco et al., 1988). These points can be attributed rather to the phosphoric groups with one P–OH group attached in different positions of the glucosidic unit. These data in association with FTIR spectra (band at 2398 cm^{-1}, corresponding to the P–H bond) support the structure depicted in Scheme 2b and allow classifying our phosphorylated dextran as a dextran phosphate, namely monobasic dextran phosphate. A determinant argument of this attribution can be obtained from the NMR spectra. NMR ^{1}H spectrum of PDex is presented in Fig. 3.

On ^{1}H NMR spectrum two signals at 5.989 and 7.559 ppm are observed which correspond to a doublet and the same case for 6.099 and 7.736 ppm. Their coupling constant J_{H-P} is equal to 640 Hz. That is characteristic of a P–H bond. These data confirm the structure 2b. Moreover from ^{31}P non-decoupled spectrum (Fig. 4a) we have two peaks (0.608 and 4.564 ppm) which converge at 2.623 ppm in the totally decoupled spectrum (Fig. 4b) exactly at the centre of the previous doublet.

The phosphorylation was performed in quite severe conditions when the destruction of the polysaccharide chain could be expected. The molar mass of phosphorylated dextran was estimated by size exclusion chromatography in aqueous salt solution.

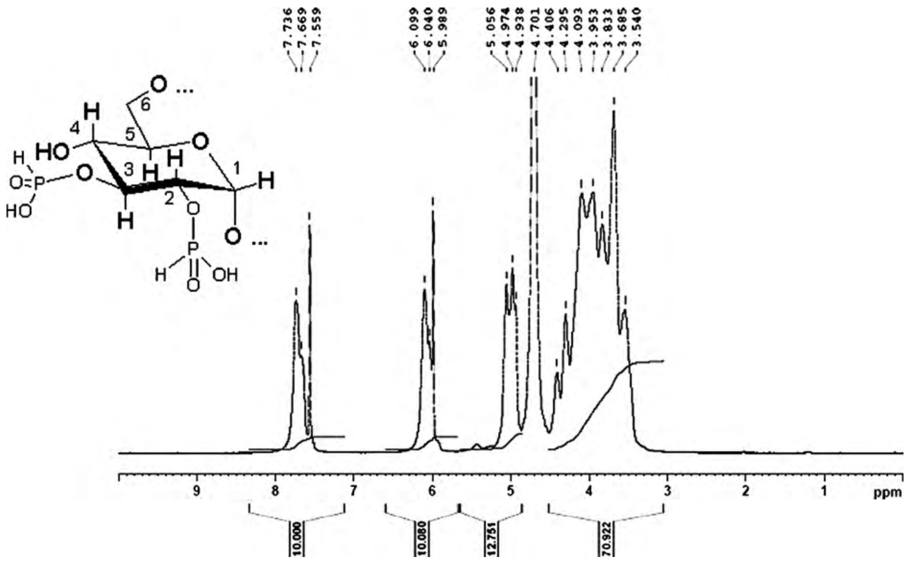


Fig. 3. ^{1}H NMR analysis of phosphorylated dextran PDex$_5$.

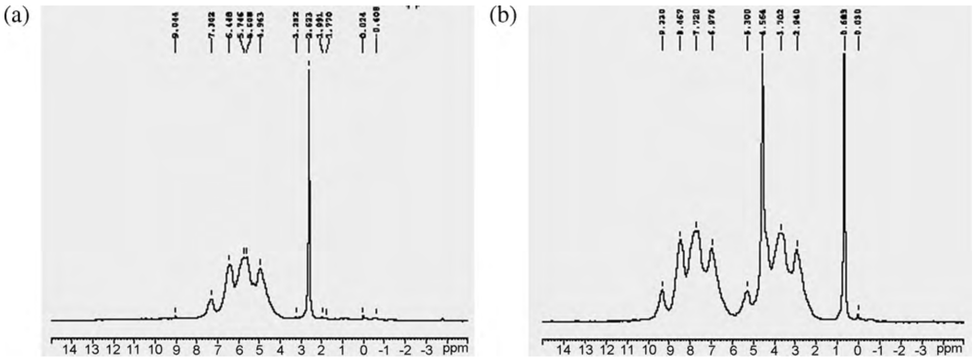


Fig. 4. ^{31}P NMR spectra of phosphorylated dextran: (a) non-decoupled and (b) decoupled.

Table 4
Molar mass and polymerization degree of the parent and phosphorylated dextran.

Sample	Molar mass	DP
Dex[a]	200,000	1230
PDex$_5$	324,000	1010

[a] Value given by the producer.

Table 5
Thermogravimetric parameters of parent and phosphorylated dextran.

Sample	Decomposition step	Characteristic temperatures			$\Delta m/m\%$
		T_i (°C)	T_m (°C)	T_f (°C)	
Dex	I	25	80	151	9
	II	248	291	385	64
	III	429	483	528	12
PDex$_5$	I	44	90	160	6
	II	248	278	308	18
	III	400	438	483	8

In Table 4 are presented the molar mass and the degree of polymerization (DP) of the parent and phosphorylated dextran. It can be observed that the degree of polymerization of PDex is of the same magnitude as of parent dextran, suggesting that polysaccharide chain was not broken during phosphorylation. This behavior could be related to the thermal behavior. In Fig. 5 are presented the TG and DTG curves of phosphorylated dextran compared to the parent dextran. It is observed a big difference between the amounts of residue at 500 °C, the PDex having higher amount of residue, as expectable.

In Table 5 are presented the typical parameters of TG curves. The thermal decomposition proceeds mainly in two steps: the first one has a high rate of decomposition while the second phase takes place more slowly. In the temperature range 25–151 °C, dextran and PDex exhibit weight loss of 9% and almost 6%, respectively, that can be attributed to the evaporation of adsorbed moisture. The second step at temperatures higher than 240 °C can be attributed to the oxidative decomposition of the polymers (Jain, Lal, & Bhatnagar, 1986). The total loss of dextran is 85% while PDex presents a total loss of 32%. This behavior could be explained by the possibility that during the decomposition some polymetaphosphoric acid is formed which inhibit the further decomposition (Granja, Barbosa, Pouysegu, De Jeso, & Baquey, 1999).

3.2. Polyelectrolyte behavior

3.2.1. Dissociation

Potentiometric titration. Phosphorylated samples in acid form were titrated with monovalent bases in presence or absence of added salt, at room temperature. The added salt was KCl, in concentration from 10^{-3} up to 1 M. In Fig. 6 are presented the potentiometric curves and their first derivates obtained by PDex$_5$ titration with monovalent bases (LiOH and KOH). The polymer concentration was $C_P = 5.00 \times 10^{-3}$ equi./L.

In all titrations the curves present two inflexion points as corresponding to the dissociation of two kinds of acidic groups (Fig. 7). As shown in Section 3.1 by means of FTIR and ^{1}H NMR spectra there is one type of acidic groups with different accessibilities, due to the position of the substituted group on the polysaccharide backbone (Fig. 2). The presence of salt in system has a slight influence on the polyion dissociation. In the limit of precision of the experiments, no selectivity between Li$^+$ and K$^+$ was observed. From potentiometric data the apparent dissociation constant, pK_a, have been calculated

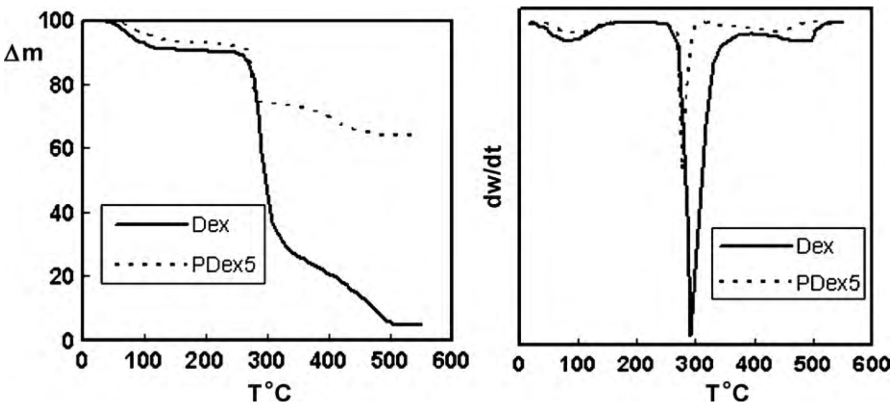


Fig. 5. Thermogravimetric curves of parent and phosphorylated dextran.

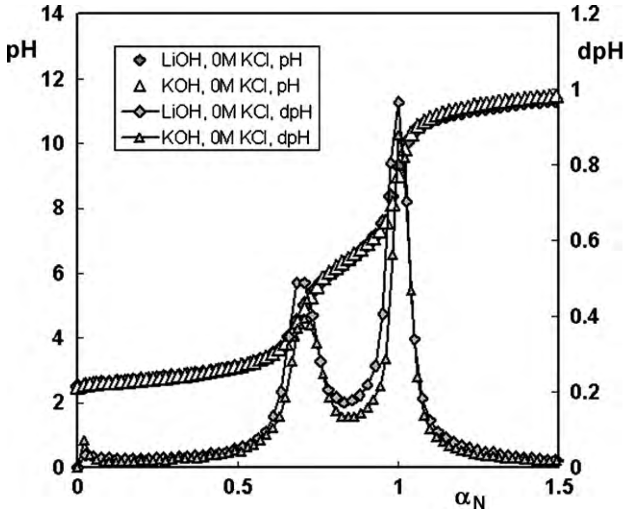


Fig. 6. Potentiometric titration of PDex$_5$ with LiOH or KOH without added salt.

using the Henderson–Hasselbalch relation:

$$pK_a = pH + \log\left[\frac{1-\alpha_T}{\alpha_T}\right] \qquad (1)$$

where pK_a is the apparent dissociation constant; $\alpha_T = \alpha_H + \alpha_N$ means the fraction of ionized groups, α_H is the initial degree of dissociation, and α_N is the degree of neutralization. The intrinsic dissociation constant pK_0 was estimated from extrapolation of p$K_a = f(\alpha_T)$ curves. The pK_0 values by titration with monovalent bases were found about 2.6 both in presence or absence of added salt.

Conductometric titration. Phosphorylated dextran in acid form was titrated with monovalent bases at room temperature. In Fig. 8 are presented the conductometric titration curves of PDex$_5$. The curves have the “V” shape, well-known for low molecular strong acids (Dautzenberg et al., 1994).

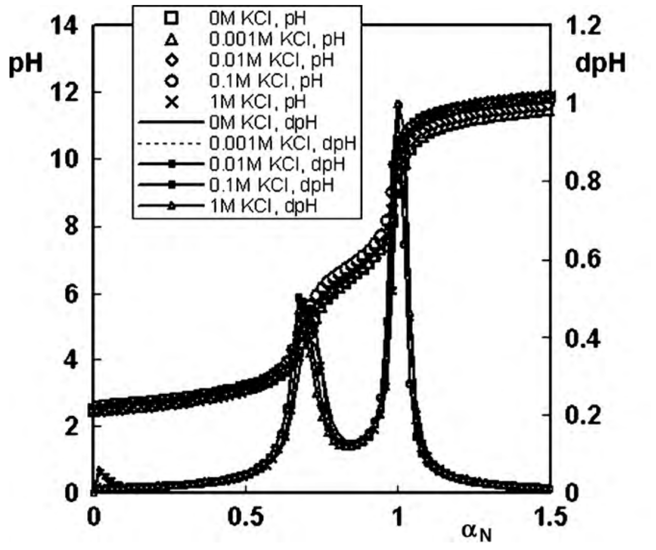


Fig. 7. Influence of added salt on the potentiometric titration of PDex$_5$ with KOH.

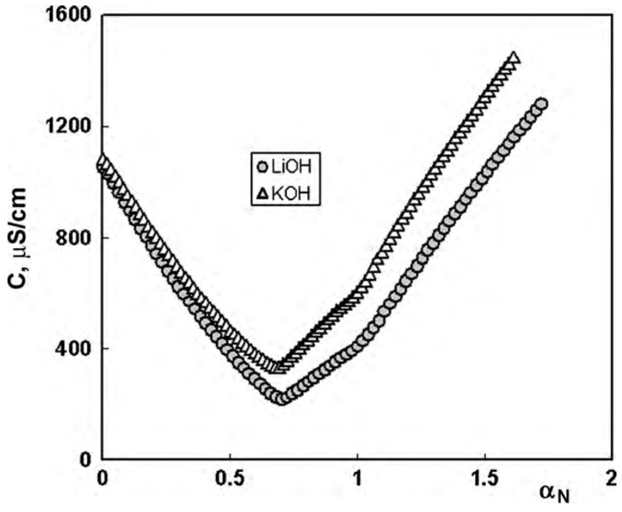


Fig. 8. Conductometric titration of PDex$_5$ with monovalent bases.

From conductometric data the transport coefficients of the monovalent counterions (f^{M^+}) and the equivalent conductance of polyanion λ_p was calculated with relations (2):

$$\begin{aligned} f^{M^+} &= \frac{\sigma_{K^+} - \sigma_{Li^+}}{10^{-3}C_P(\lambda_{K^+} - \lambda_{Li^+})} \\ \lambda_p &= \frac{\sigma_{Li^+} - 10^{-3}C_P f^{M^+}\lambda_{Li^+}}{10^{-3}C_P f^{M^+}} \\ f^{H^+} &= \frac{\sigma_i}{10^{-3}C_P\lambda_{H^+}} \end{aligned} \qquad (2)$$

where σ_{Li^+} and σ_{K^+} (μS), are the conductivities at $\alpha_N = 1$ by neutralization with KOH or LiOH; σ_i is the conductivity of polyelectrolyte solution at the beginning of titration; C_P is the polymer concentration (equi./L), and λ_{H^+}, λ_{Li^+}, λ_{K^+}, and λ_p (cm^2 Ω^{-1} equi.$^{-1}$) are the equivalent conductivities of H$^+$, Li$^+$, K$^+$ ions and of polyion, respectively (Manning, 1975). It is admitted that λ_p and f are not dependent on the counterion of the titrating base. f is an interaction parameter, related to the ionization degree of polyion. f is defined as the ratio of the self-diffusion coefficients of the counterion in polyelectrolyte solution and in pure solvent, respectively (Dautzenberg et al., 1994; Manning, 1972). In Table 6 are presented the transport coefficients of the monovalent counterions and the polyion equivalent conductivity calculated from conductometric data, at complete neutralization.

The values α_{H^+} and f_{H^+} calculated from potentiometric and conductometric data, respectively, are comparable. The values are comparable to those of synthetic macroions with large size (Chitanu, Rinaudo, Milas, Desbrieres, & Carpov, 1999; Dautzenberg et al., 1994).

3.2.2. Viscometric behavior

The viscometric measurements were performed on PDex$_5$ sample without or with added salt in concentration from 5×10^{-4} up to 1 M. Fig. 9 shows the dependence $\eta_{sp}/C_P = f(C_P)$ of PDex$_5$ in the absence or in the presence of low molecular added salt (KCl).

The phosphorylated sample exhibits typical polyelectrolyte behavior. With no added salt, the reduced viscosity decreases exponentially with the increase of polymer concentration because in much diluted solutions the charges induce a fully stretched conformation of macroions. As the polymer concentration increases the

Table 6
f parameters and polyion equivalent conductivity of phosphorylated dextran PDex$_5$.

f_{M^+}	f_{H^+}	α_{H^+}	λ_p
0.516	0.396	0.427	54.70

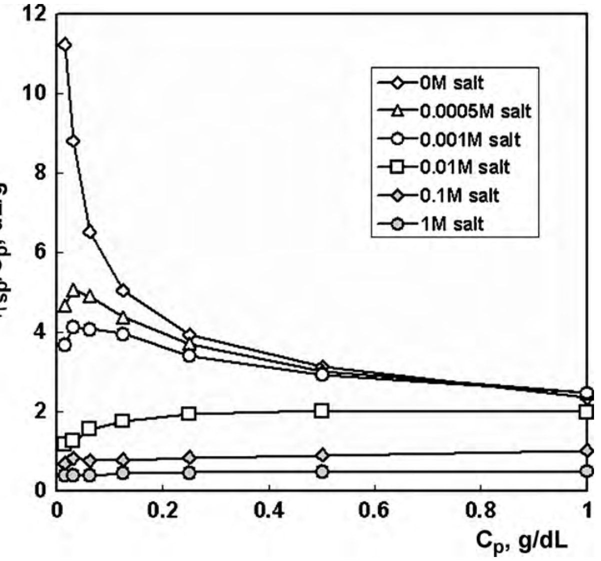


Fig. 9. η_{sp}/C_P dependence on polymer concentration at various ionic strengths.

partial auto-screening of charges appears resulting in the decrease of the viscosity. By increasing the amount of added salt the electrostatic repulsions are gradually screened, so that at high salt concentration the macroion behaves as neutral polymer: the viscosity increases linearly with the polymer concentration (Cohen & Priel, 1989; Cohen, Priel, & Rabin, 1988; Mandel, 1987). The Fuoss relation (3) was used to evaluate the intrinsic viscosity of PDex_5, as usually by polyelectrolytes.

$$\frac{\eta_{sp}}{C_P} = \frac{A}{1 + B \times C_p^{1/2}} \quad \text{or} \quad \frac{C_P}{\eta_{sp}} = \frac{1}{A} + \left(\frac{B}{A}\right) C_P^{1/2} \tag{3}$$

where A is the intrinsic viscosity and B is a constant (Fuoss & Cathers, 1949). In Fig. 10 is shown the Fuoss plot of PDex_5 at various concentrations of added salt and at polymer concentration higher than that corresponding to the peaks in Fig. 9.

The intrinsic viscosity of polyelectrolyte solutions decreases with increase of the ionic strength (added salt concentration) as Pals and Hermans evidenced in 1952 (Dautzenberg et al., 1994; Malovikova, Milas, Rinaudo, & Borsali, 1993, chap. 24).

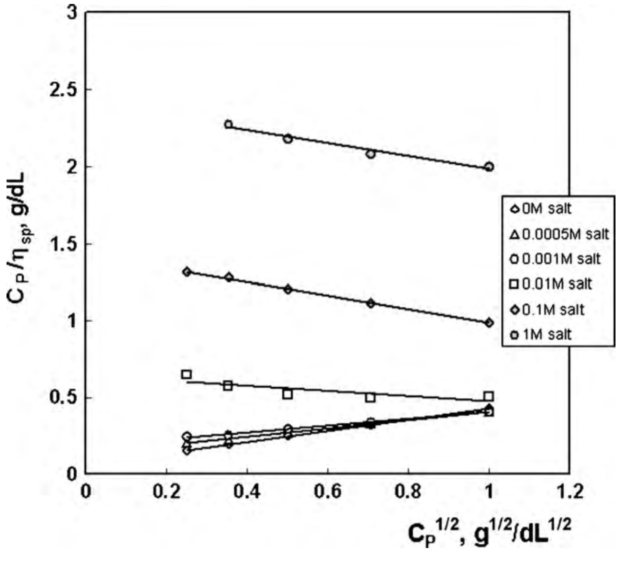


Fig. 10. C_P/η_{sp} dependence on the $C_P^{1/2}$ of phosphorylated dextran PDex_5 at various concentrations of added salt.

$$[\eta]_{C_S} = [\eta]_\infty + SC_S^{-1/2} \tag{4}$$

where C_s is the added salt concentration (mol/L), $[\eta]_\infty$ is the intrinsic viscosity (g/dL) extrapolated at infinite ionic strength, and the slope S is related to the chain stiffness and expansion. From the curves of Fig. 10 the intrinsic viscosity at different concentrations of added salt was determined and plotted as function of $C_s^{-1/2}$, according to relation (4), in order to get an idea on the flexibility of PDex_5. The intrinsic viscosity at infinite salt concentration $[\eta]_\infty = 0.15$ was obtained by extrapolation which is considered as the intrinsic viscosity in θ conditions. The stiffness parameter S is 0.139.

4. Conclusion

Phosphorylated dextran was synthesized by reaction of dextran with phosphorous acid in molten urea. Fully water-soluble derivatives were obtained with degree of substitution up to 2, depending on the reaction time. The degree of substitution was evaluated by P elemental analysis and by potentiometric and conductometric titration. The values obtained by these methods were in good accordance so that the potentiometric titration could be proposed as an easier and convenient method of DS estimation. FTIR spectra, titration, and NMR spectra confirmed that the reaction between dextran and H_3PO_3 takes place with formation of dextran monobasic phosphate Dex–O–P(H)(O)OH. The thermogravimetric analysis evidenced the stability of samples up to 250 °C allowing the application of phosphorylated dextran at higher temperature. The polyelectrolyte behavior was investigated by potentiometric and conductometric titrations with monovalent bases in presence or absence of low molecular added salt and by viscometric measurements. The potentiometric curves display two inflexion points corresponding to the dissociation of two kinds of acid groups, attributed to phosphoric groups with one P–OH bond attached in different positions of the glucosidic units. The addition of low molecular salt has a slight influence on the polyion dissociation. PDex exhibits typical behavior of polyelectrolyte. The intrinsic viscosity of PDex was calculated by using Fuoss relation then the intrinsic viscosity to infinite salt concentration and the stiffness parameter was obtained, evidencing a higher flexibility which can be due to the branched structure of dextran or/and to the α-(1→6) linkage of D-glucose units.

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References

Allene, J. (1966). Dextran. In H. F. Mark, N. G. Gaylord, & N. M. Bikales (Eds.), *Encyclopedia in polymer science and engineering technology* (pp. 805–824). New York: John Wiley & Sons.

Chitanu, G. C., Rinaudo, M., Milas, M., Desbrieres, J., & Carpov, A. (1999). Behavior of nonalternating maleic acid copolymers in aqueous solution. *Langmuir*, *15*, 4150–4156.

Cohen, J., & Priel, Z. (1989). Viscosity of diluted polyelectrolyte solution: Concentration, dependence on sodium chloride, magnesium sulfate, and lanthanum nitrate. *Macromolecules*, *22*, 2356–2358.

Cohen, J., Priel, Z., & Rabin, Y. (1988). Viscosity of diluted polyelectrolytes solution. *Journal of Chemical Physics*, *88*, 7111–7116.

Dautzenberg, H., Jaeger, W., Koetz, J., Philipp, B., Seidel, Ch., & Stscherbina, D. (1994). *Polyelectrolytes. Formation, characterization and application*. Munich: Hansel Publishers. (p. 145).

Fuoss, R. M., & Cathers, G. I. (1949). Polyelectrolytes III. Viscosities of η-butyl bromide addition compounds of 4-vinylpyridine-styrene copolymers in nitromethane-dioxane mixtures. *Journal of Polymer Science*, *4*, 97–120.

Gekko, K. (1981). Solution properties of dextran and its ionic derivatives. In D. A. Brant (Ed.), *Solution properties of polysaccharides. ACS symposium series* (pp. 415–438). Washington, D.C.: American Chemical Society.

Granja, P. L., Barbosa, M. A., Pouysegu, L., De Jeso, B., & Baquey, C. (1999). Cellulose phosphates as biomaterials. In R. M. Ottenbrite (Ed.), *Frontiers in biomedical polymer applications* (pp. 195–225). Lancaster, P.A., USA: Technomic Press.

Heinze, T., Liebert, T., Heublein, B., & Hornig, S. (2006). Functional polymers base on dextran. *Advance in Polymer Science*, *205*, 199–291.

Hesse, M., Meier, H., & Zeeh, B. (1984). *Spektroskopische Methoden in der Organischen Chemie*. New York: Georg Thieme Verlag Stuttgart. (p. 50).

Inagaki, N., Nakamura, S., Asai, H., & Katsuura, K. (1976). Phosphorylation of cellulose with phosphorous acid and thermal degradation of the product. *Journal of Applied Polymer Science*, *20*(10), 2829–2836.

Jain, R. K., Lal, K., & Bhatnagar, H. L. (1986). Thermal studies on C-6 substituted cellulose and its subsequent phosphorylated products in air. *Thermochimica Acta*, *97*, 99–114.

Kabachnik, M. I., & Mastryakova, T. A. (1953). Theory of tautomeric equilibrium communication 3. The question of pseudomerism, structure and properties of dialkyl thiophosphites. *Russian Chemical Bulletin*, *2*(1), 145–157.

Larsen, C. (1990). Preparation and pharmacological properties of dextran carrier. In C. Larsen (Ed.), *Dextran prodrugs. Physicochemical and chemical aspects in relation to in vivo properties*. Denmark: Villadens & Christensen (p. 22).

Makarova, S. B., Aptova, T. A., Litvak, Zh. M., & Raldugina, T. F. (1978). Ion exchanger. *U.S.S.R. Patent No. 597 682*.

Malovikova, A., Milas, M., Rinaudo, M., & Borsali, R. (1993). Viscometric behavior of sodium polygalacturonate in the presence of low salt content. In K. S. Schmitz (Ed.), *Macro-ion characterization, ACS symposium series* (pp. 315–321). Washington, D.C.: American Chemical Society.

Mandel, M. (1987). Polyelectrolytes. In J. I. Kroschwitz (Ed.), *Encyclopedia of polymer science and engineering* (2nd ed., Vol. 11, pp. 739–829). New York: John Wiley and Sons.

Manning, G. S. (1972). Polyelectrolytes. *Annual Review of Physical Chemistry*, *23*, 117–140.

Manning, G. S. (1975). Limiting law for the conductance of the rod model of a salt-free polyelectrolyte solution. *The Journal of Physical Chemistry*, *79*, 262–266.

Pals, D. T. F., & Hermans, J. J. (1952). Sodium salts of pectin and of carboxy methyl cellulose in aqueous sodium chloride. *Recueil des Travaux Chimiques des Pays-Bas*, *71*, 433–457.

Purama, R. K., Goswami, P., Khan, A. T., & Goyal, A. (2009). Structural analysis and properties of dextran produced by *Leuconostoc mesenteroides* NRRL B-640. *Carbohydrate Polymers*, *76*, 30–35.

Robyt, J. F. (1987). Dextran. In J. I. Kroschwitz (Ed.), *Encyclopedia of polymer science and technology* (2nd ed., Vol. 3, pp. 752–767). New York: John Wiley & Sons.

Sacco, D., Klett-Zygmunt, D., & Dellacherie, E. (1988). A re-investigation of the phosphorylation of dextran with phosphoric acid: Evidence for the formation of different types of phosphate moieties. *Carbohydrate Research*, *184*, 193–202.

Sacco, D., Klett-Zygmunt, D., Vigneron, C., & Dellacherie, E. (1990). Covalent fixation of hemoglobin to dextran phosphates decreases its oxygen affinity. *Biochimica et Biophysica Acta*, *1041*, 279–284.

Sato, T., Nishimura-Uemura, J., Shimosato, T., Kawai, Y., Kitazawa, H., & Saito, T. (2004). Dextran from *Leuconostoc mesenteroides* augments immunostimulatory effects by the introduction of phosphate groups. *Journal of Food Protection*, *67*(*8*), 1719–1724.

Spaltro, S. M., & Aronson, M. P. (1992). Phosphorylated polyhydroxy compounds for tartar control. *European Patent No. 512 599*.

Suflet, D. M., Chitanu, G. C., & Popa, V. I. (2006). Phosphorylation of polysaccharides. New results on synthesis and characterization of phosphorylated cellulose. *Reactive and Functional Polymers*, *66*(11), 1240–1249.

Suzuki, S., Suzuki, M., & Matsumoto, T. (1975). Polysaccharides bonded with phosphoric acid and fatty acid esters. *Japanese Patent No. 50 054 685*.

Suzuki, S., Suzuki, M., & Mikami, T. (1977). Esters of polysaccharides with phosphoric acid and palmitric acid. *Japanese Patent No. 52 028 583*.

Whistler, R. L., & Towle, G. A. (1969). Preparation and characterization of polysaccharide phosphates. *Archives of Biochemistry and Biophysics*, *135*, 396–401.