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An investigation of the structure of periodate-oxidised dextran

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ABSTRACT

The aldo-enol transition in periodate-oxidised dextrans has been studied by UV absorption spectroscopy and electrophoretic light-scattering. Absorption peaks at 267, 240, and 290 nm are attributed to aldehyde, enol, and enolate ion, respectively. The electrophoretic mobility of periodate-oxidised dextran appears to be proportional to the absorption at 290 nm, and the pH dependence of the ratio of the peaks at 240 and 290 nm follows a standard titration curve. These facts are in accord with the formation of enol and enolate ions.

INTRODUCTION

The absence of UV and IR absorption for aldehyde groups in periodate-oxidised polysaccharides is usually attributed to the formation of hydrated hemiacetal and gem-diol groups[1]. However, for periodate-oxidised dextrans[2], the UV spectra depend on the pH of the solution, and only within a narrow range (4–5.2) is the absorption for aldehyde groups absent. Thus, at pH < 4 and > 5.2, there were peaks at 267 and 240 nm, respectively (Fig. 1). The peak at 267 nm is characteristic of aldehyde groups and that at 240 nm is assigned tentatively to an enol group. Likewise, the IR spectra of periodate-oxidised dextrans contain a typical aldehyde peak at 1740 cm^{-1} at pH < 4 and peaks at 1740 and 1620 cm^{-1} at pH > 5.2, with the latter assigned to the enol. These optical properties of periodate-oxidised dextrans do not correspond to any known structure. Arguments in favour of the enol form have been suggested[2] although, for 1,5-dicarbonyl compounds, such forms have not been reported.

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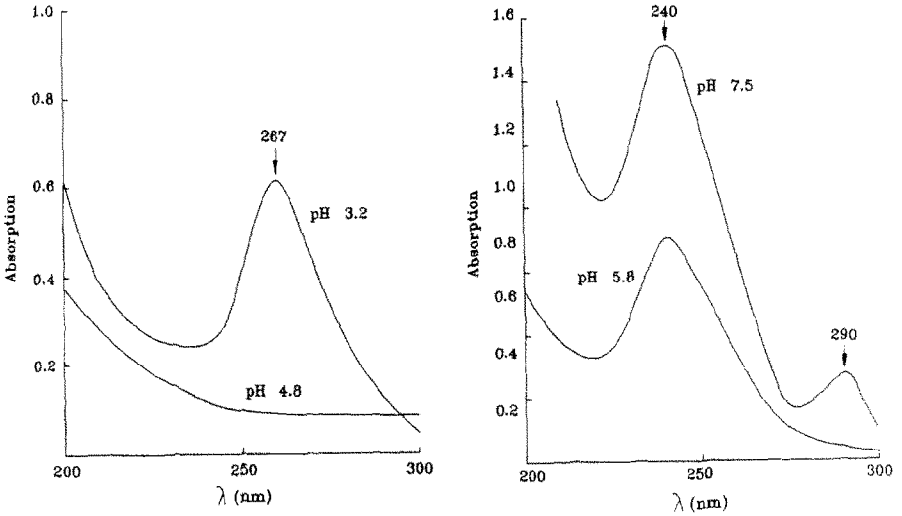


Fig. 1. UV spectra of periodate-oxidised dextrans as a function of pH.

We now report a more detailed study of this pH-dependent aldo → enol tautomerism. If an enol is formed at pH 5.2 then, at a higher pH, an enolate ion should be present. At pH > 7, a peak at 290 nm was observed and the shift (50 nm) from that at pH 5.2 is typical for an enol–enolate system[3]. In order to verify this interpretation, effects on the UV spectra and electrophoretic mobility[4] in the pH range 3–8 were studied.

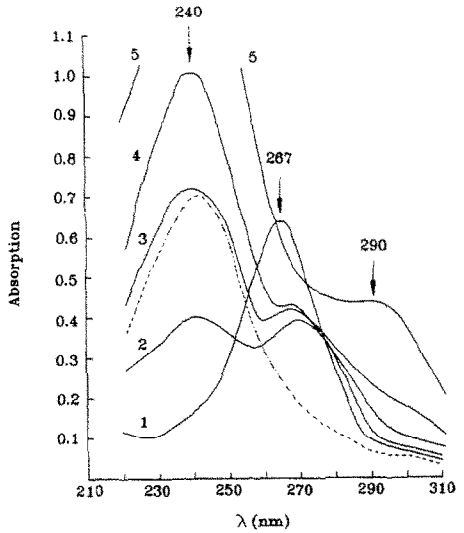


Fig. 2. UV spectra of periodate-oxidised dextran (60% oxidation, mol wt 60×10^3) as a function of time after a change in pH from 3 to 7. 1, zero; 2, after 2 h; 3, after 4 h; 4, after 6 h; 5, after 24 h; then after readjustment of the pH from 7 to 3 and 3-fold dilution (------).

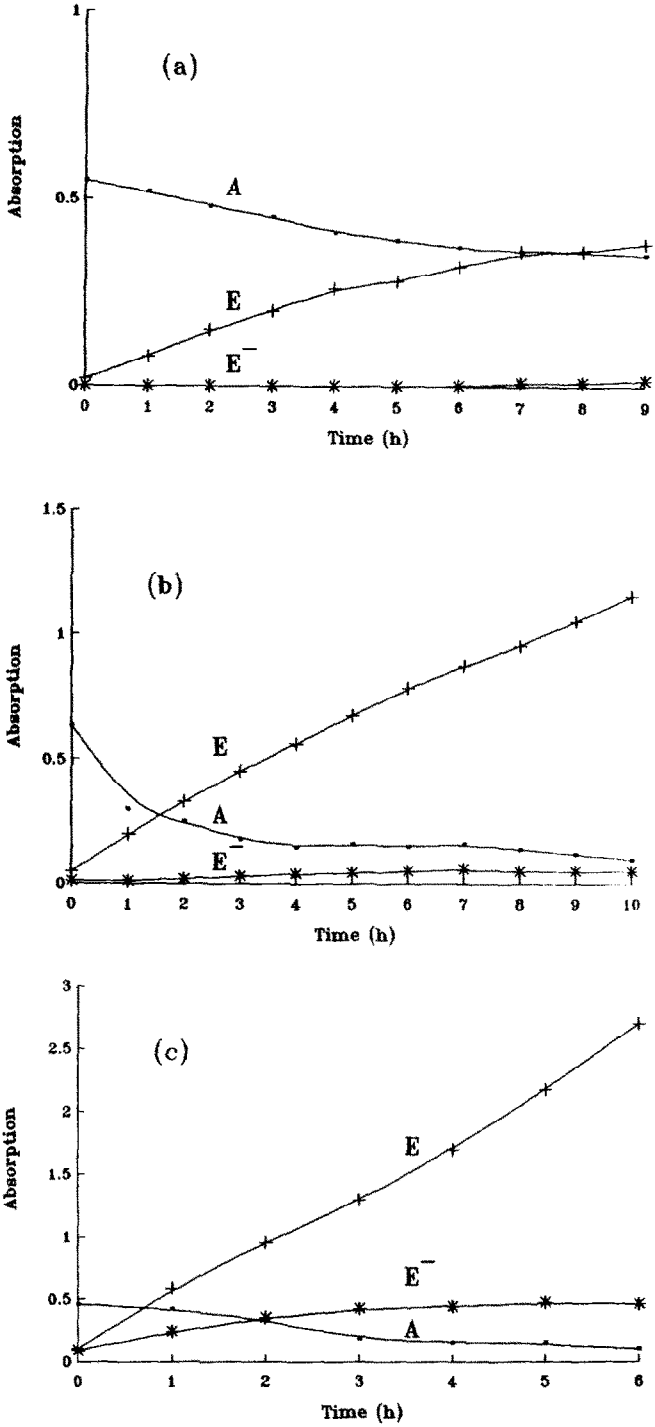


Fig. 3. UV absorption as a function of time for the periodate-oxidised dextran (see Fig. 2) after adjustment of the pH from 3 to (a) 6.5, (b) 7.0, (c) 7.7 at 267 (A), 240 (E), and 290 nm (E^-).

EXPERIMENTAL

Dextrans (Fluka) of molecular weights ($\times 10^3$) 20, 40, 60, 70, 110, and 500 were used. Each dextran was oxidised[2] with sodium periodate. Oxidation was carried out in glass-stoppered flasks protected from light. A solution of the dextran (2.4 g) in water (50 mL) was treated with 0.2 M sodium metaperiodate (50 mL for 20% oxidation) for 24 h at room temperature at pH 4. After the excess of periodate had been destroyed with ethylene glycol, the solution was dialysed against running water for 24 h, then dialysed at pH 3 (acetate buffer) in order to remove products with molecular weights < 13000. Acetate ions were then removed by dialysis for a short time against water, and the solution was freeze-dried. To a solution of each

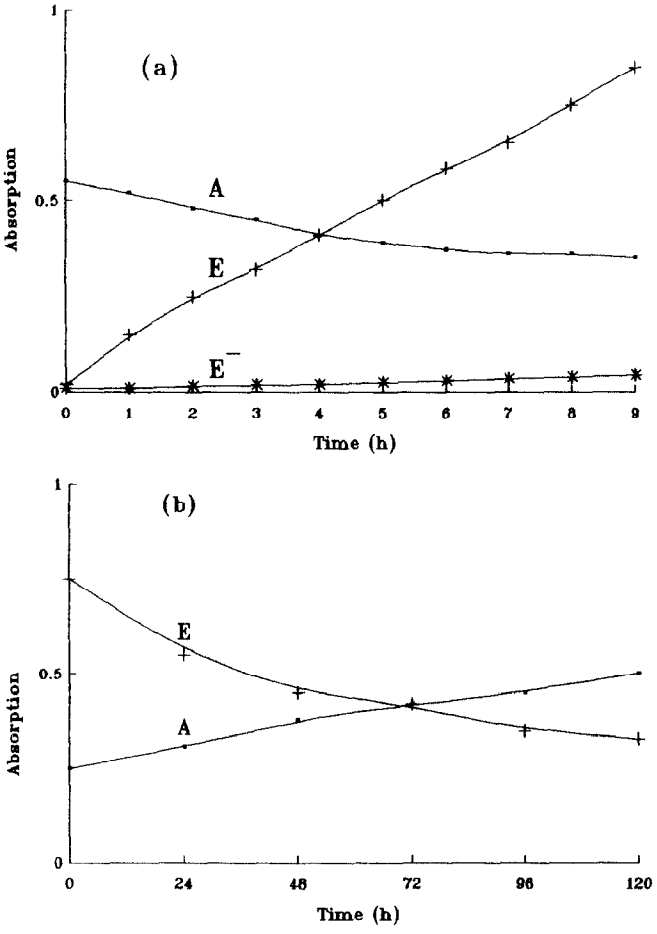


Fig. 4. UV absorption as a function of time for a periodate-oxidised dextran (40% oxidation, mol wt 60×10^3), after adjustment of the pH from 3 to (a) 7.5, and (b) back to 3 at 267 (A), 240 (E), and 290 nm (E^-).

periodate-oxidised dextran (6 mg) in water (1 mL) was added 0.01 M sodium phosphate buffer to give the desired pH in the range 6–9. UV spectra of the solutions were measured at intervals of 1 h or, in some experiments at high pH, at intervals of 10 min, with a Specord M-40 spectrophotometer. The aldehyde groups were determined by the iodine number[5], carboxyl groups by the method of Davidson[6], and enol by titration with bromine[7].

Electrophoretic mobilities were determined, with a Zeta Sizer II (Malvern Instruments) and an installation designed in St. Petersburg Nuclear Physics Institute, on solutions (10 mg/mL) in 30 mM sodium phosphate–citrate–borate buffer that contained 1% of NaCl, in order to obtain a conductivity of 2 mS/cm, and mM sodium azide for sterilisation. Each sample was centrifuged at 15000 rpm at 4°C for 1 h before measurements were made.

RESULTS

The UV absorption curves shown in Fig. 2 for the product derived from the dextran with a mol wt 60×10^3 are typical. At pH 3, there is only the aldehyde peak at 267 nm. As the pH is increased, the enol peak at 240 nm appears; finally, the peak at 290 nm becomes visible together with that at 240 nm. At high pH, the enol absorption shifts to greater wavelengths as enolate ions are formed[3]; hence, the peak at 290 nm can be assigned to an enolate ion. Since the above three peaks overlap, identification of the forms of the individual absorptions is necessary for quantitative evaluation. The curve for the aldehyde group is that at pH 3. However, at pH > 9, rapid irreversible destruction of the periodate-oxidised dextran occurred and the curve for the peak at 290 nm could not be determined. If, after several hours at pH 7.5, the pH of the solution was reduced to 3, the peak at 290 nm disappeared immediately, whereas that at 240 nm was restored slowly (dashed line in Fig. 2.) so that the absorption curve for the enol could be obtained.

TABLE I

UV absorption and analytical data for a solution (1 mg/mL) of 40% periodate-oxidised dextrans as a function of time at pH 7.5

Time after dissolution (h)	UV spectra				Analysis		
	A [a] (240 nm)	C=C–OH groups [b] (ϵ 2400) [c]	A (267 nm)	CHO groups [b] (ϵ 31) [c]	CHO groups [b]	C=C–OH groups [b]	COOH groups [b]
0			0.18	87	88	0	0.8
2	0.10	0.6	0.14	67	86	0.8	0.8
4	0.18	1.1	0.13	63	86	1.1	0.8
6	0.28	1.7	0.12	58	84	1.9	0.9
8	0.33	2.1	0.11	53	84	2.3	0.9
10	0.37	2.3	0.10	48	82	2.5	1.0

[a] Absorbance. [b] Per 100 residues. [c] Determined in a separate experiment.

Fig. 3 shows a plot of the extinctions of the aldehyde, enol, and enolate groups versus time after increase of the pH from 3 to 6.5, 7.0, and 7.5. Fig. 4 shows the effect of reducing the pH from 7.5 to 3. The peak at 290 nm disappeared immediately; the peak at 240 nm reappeared first, followed by that at 267 nm. The extinction coefficients of the absorptions of the aldehyde and enol groups are quite different and a small proportion of enol contributes significantly to the absorption

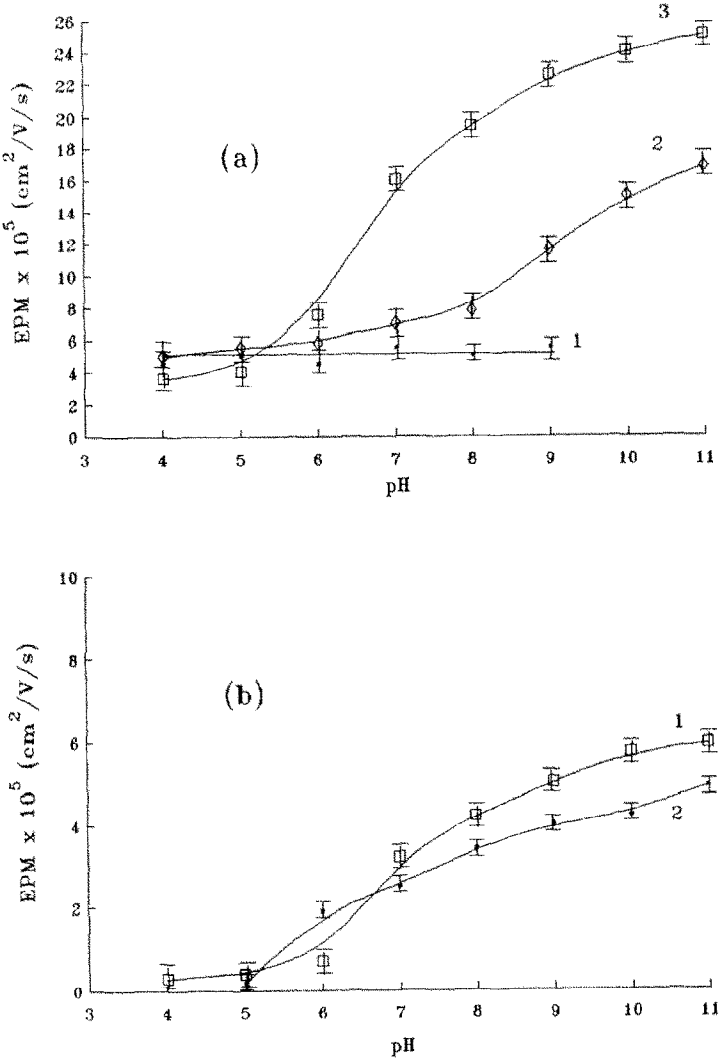


Fig. 5. Electrophoretic mobility (EPM) of periodate-oxidised dextrans as a function of pH: (a) 1, dextran (mol wt 500×10^3); 2, after 4% oxidation; 3, after 40% oxidation; (b) 1, 4% oxidised dextran of mol wt 20×10^3; 2, 4% oxidised dextran of mol wt 60×10^3.

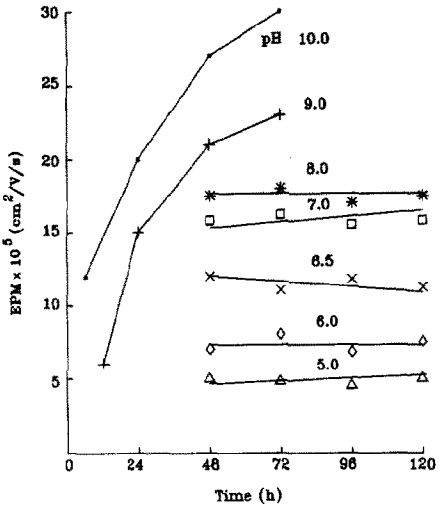


Fig. 6. Stability of the electrophoretic mobility (EPM) of periodate-oxidised dextran (mol wt 60×10^3, 40% oxidation) as a function of pH.

(see Table I). The extinction coefficient for the aldehyde has a standard value (31), whereas that (2400) for the enol was 5 times less than normal.

In order to confirm the assignment of the peak at 290 nm to enolate ions, the charge on the periodate-oxidised dextrans was investigated by the electrophoretic light-scattering method. Fig. 5a shows that the electrophoretic mobilities of dextran and periodate-oxidised dextran at pH 4–5 are similar. This mobility depends on molecular weight. As the pH is increased, only the mobility of the periodate-

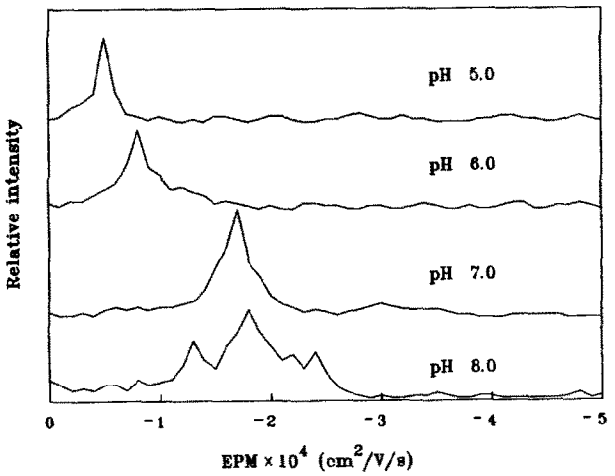


Fig. 7. Electrophoretic light-scattering spectra of periodate-oxidised dextrans as a function of the pH of the solution.

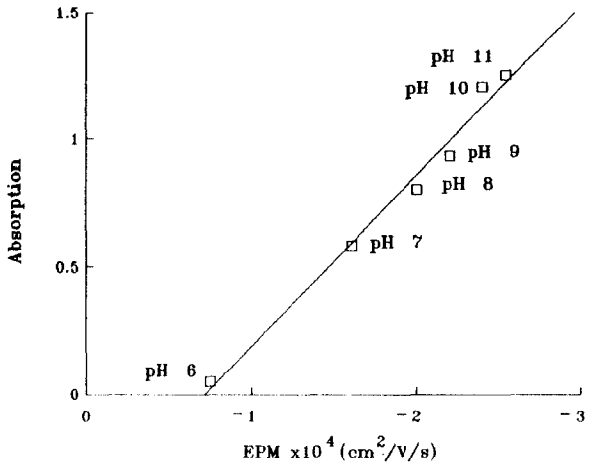


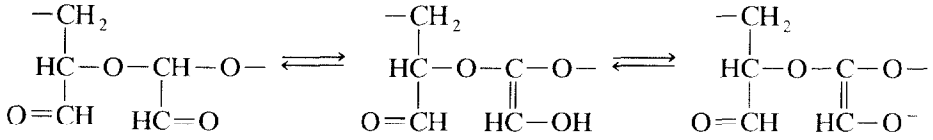
Fig. 8. UV absorption at 290 nm vs. electrophoretic mobility (EPM) for a periodate-oxidised dextran (mol wt 60×10^3, 40% oxidation) as a function of pH.

oxidised dextran increases in a manner that is roughly proportional to the extent of oxidation, and the effect of variation of molecular weight is negligible (Fig. 5b).

The electrophoretic mobility was practically independent of time at pH 5–8 (Fig. 6). At pH > 9, the mobility of extensively periodate-oxidised dextrans (> 20%) increased sharply and then became constant. For less-extensively oxidised dextrans (4–10%), there was no increase in mobility. That decomposition of the polysaccharides occurs at pH < 8 and results in the appearance of extra charges accords with the shape of scattered-light spectra. Fig. 7 shows that, at low pH, the sample was homogeneous with respect to charge, but that the distribution became broad at high pH. Fig. 8 shows a plot of the electrophoretic mobility against absorption at 290 nm as a function of pH.

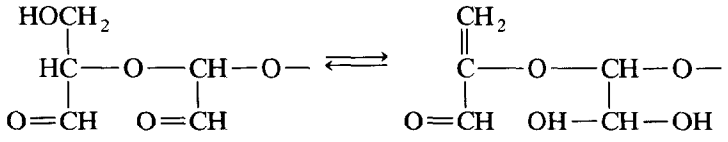
DISCUSSION

The optical properties of periodate-oxidised dextrans[2] suggested that, at alkaline pH, aldo-enol tautomerism occurred by the usual mechanism (**1** ⇌ **2**).



1 **2** **3**

At sufficiently high pH, the enol group loses a proton to give the enolate ion (**3**). However, a molecule of water could be lost[3] from the oxidised terminal residue (**4** → **5**), with formation of an unsaturated aldehyde that also absorbs at ~ 240 nm.



4 5

In spite of the relatively small proportion of end groups (2–4%), the aldehyde absorption could be significant due to the formation of conjugated bonds [the initial dextrans have 96–98% of (1 → 6) linkages]. The destruction processes also could yield products that absorb in this region. Thus, UV absorption at 240 nm does not prove that an enol is formed. In the absence of conjugation, the presence of two aldehyde groups in periodate-oxidised dextrans could promote enolisation by hydrogen bonding between the enol and aldehyde groups. Further, the two oxygen atoms attached to C-1 could promote the transfer of hydrogen (**1** → **2**) to give the enol.

The sequence **1** → **2** → **3** explains the major kinetic features of the system studied. As the pH is increased, the formation of enolate ions is promoted. The data in Figs. 3 and 4a illustrate this tendency. However, it may be that, after restoration of the pH to 3 from 7.5 (Fig. 4b), the enol does not disappear. The reverse reaction is slow and it is possible that, at low pH, a metastable state is observed both initially and after re-acidification from pH 7.5. The data in Table I show that only a small proportion of the aldehyde is converted into enol. However, the magnitude of the peak at 267 nm varies significantly, which reveals the existence of other reactions, most probably the formation of hydrated hemiacetal and/or gem-diol groups[1]. If the final pH is < 6, there is no increase in the peak at 240 nm, but that at 267 nm gradually disappears. The restoration of the aldehyde peak after re-acidification is connected with dehydration rather than with enol → aldehyde transformation. The accuracy of data plotted in Figs. 3 and 4a is insufficient for unambiguous determination of the rate constants. Nevertheless, it is possible to check quantitatively whether the assignment of the peaks at 240 and 290 nm to enol and enolate ion, respectively, accords with the observed pH dependence of their magnitudes. Since the rate of enol dissociation is rapid, the relation between the absorptions of the enol and enolate ion should be governed only by pH. If [E] and $[E^-]$ are the concentrations of the enol and the enolate ion, respectively, then

$$[E^-]/[E] = K/[H^+],$$

where K is the dissociation constant. There is no reason for K to be markedly dependent on pH; hence, the ratio (R) of the absorptions at 290 and 240 nm should be nearly proportional to 10^{pH}, i.e.,

$$\log R = pH - pK + \log A,$$

where $pK = -\log K$ and A is the ratio of the extinction coefficients for enolate ion and enol. As shown in Fig. 9, where log R is plotted against pH, this proportionality is fulfilled. Since the parameter A is unknown, the pK of the enol

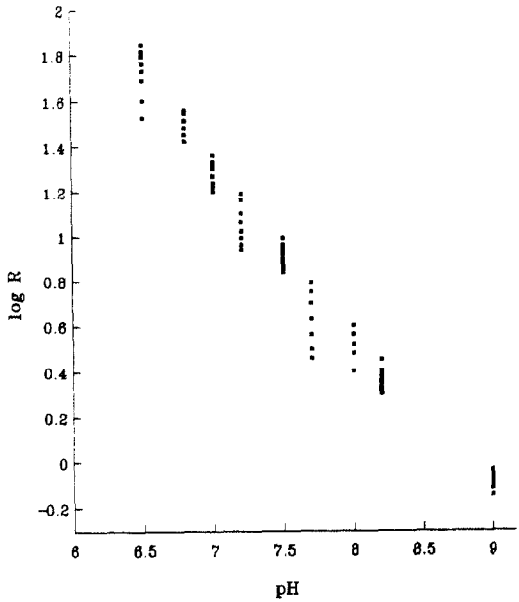


Fig. 9. The ratio (log R) of the absorptions at 240 (E) and 290 nm (E^-) as a function of pH for periodate-oxidised dextran (mol wt 60×10^3, both 40 and 60% oxidation).

dissociation cannot be determined exactly. If it is assumed that the extinction coefficients for the absorptions of the enol and enolate ion are of the same order, i.e., A is ~ 1, then the pK will be ~ 7.5.

Electrophoretic light-scattering experiments support the above interpretation. Thus, at pH > 5, negatively charged groups appear on periodate-oxidised dextrans. These charges do not arise by oxidation of aldehyde to carboxyl groups, since[8] the pK for chelated 1,3-dicarbonyl compounds is 8–11 and that for carboxylic acids is 1–5. Thus, the electrophoretic behavior of periodate-oxidised dextrans (Fig. 5a, curve 3) is consistent with the formation of enolate ions. The distribution of electrophoretic mobility at pH > 8 is wide (Fig. 7). Since the mobility of a uniformly charged polymer is practically independent of its molecular weight[9], this effect could be caused by charged end groups created during decomposition of the polysaccharide. Therefore, the data have to be interpreted with caution. At pH < 8, no such problem exists and the electrophoretic mobility can be considered as proportional to the concentration of charged groups. Fig. 8 shows that the magnitude of the peak at 290 nm appears to be related linearly to the electrophoretic mobility. Thus, it is concluded that the absorption at 290 nm is due to enolate ions and that no other charged groups are present in significant proportion.

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