Oscillographic Polarography of Inorganic Polyphosphates and Certain Polymeric Substances Having the Character of Polyanions Isolated from Biological Material

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Conditions were established for oscillopolarographic observation of inorganic polyphosphates and other polymeric substances, such as dextransulphate, hyaluronate, heparin and chondroitinsulphate. Studies on the relationship of the depth of indentation to the molecular weight of polyphosphates have shown that the main factor responsible for the origin and depth of the indentation is the degree of condensation of the polyphosphate chain. The electrode process responsible for the producing of the indentation is of capacity character.

In recent times, some polymeric substances of biological importance, such as certain di- and tripeptides [1], polyglutamic acid [2], nucleic acids [3—5] and proteins [6, 7] have been studied with the aid of A. C. oscillographic polarography.

In this paper the oscillopolarographic activity of polyphosphates, dextransulphate, heparin, hyaluronate and chondroitinsulphate is described.

Experimental and Results

The registration of the oscillographic curves $dE/dt = f(E)$ was carried out on a P 576 Polaroscope. The recording of the first curves was performed and studies on vibrating electrode were carried out on a universal oscillopolarograph designed and constructed in the electronic laboratory of our Institute [8]. A summary of the substances used in these experiments is given in Table 1.

Beginning with polyphosphate P_6 all higher polyphosphates yield indentations on the oscillographic curves in the following supporting electrolytes: sulphuric acid (Fig. 2), formic acid, acetic acid, citric acid (Fig. 1), trichloracetic acid and perchloric acid. Also in the medium of pH 3.8 ammonium formate and pH 5 to pH 7 sodium acetate, indentations arise (the concentration of the supporting electrolytes always being 1 M). In the medium of sodium acetate, however, turbidity appears in higher concentrations of polyphosphates, the amount of which increases at the same weight concentration with the molecular weight of the polyphosphate rising approximately up to P_{16}.

Organic acids are the most expedient medium for oscillopolarographic observation of polyphosphates. Thus, for instance, in the medium of 1 M citric acid an indentation of P_{230} can already be observed at a concentration of 10 γ/ml. In neutral (NaCl, KCl) and alkali media (NH_4OH + NH_4Cl, NaOH), polyphosphates do not produce any inden-

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Table 1

Chemicals used: a list of the polyphosphate and other polymeric substances

Name	Abbreviation	M	Manufacturer
Polyphosphate	P_{90}	8 900	Dr. U. P. Strauss, U. S. A.
Polyphosphate	P_{114}	11 400	Dr. U. P. Strauss, U. S. A.
Polyphosphate	P_{145}	14 500	Dr. U. P. Strauss, U. S. A.
Polyphosphate	P_{190}	18 900	Dr. U. P. Strauss, U. S. A.
Trimetaphosphate	M_3	306	Dr. E. Karl Kroupa,
Tripolyphosphate	P_3	366	Monsanto Chemical Co., U. S. A.
Tetrametaphosphate	M_4	408	Monsanto Chemical Co., U. S. A.
Tetrapolyphosphate	P_4	468	Monsanto Chemical Co., U. S. A.
Polyphosphate	P_{12}	1 284	Monsanto Chemical Co., U. S. A.
Polyphosphate	P_{230}	23 520	Monsanto Chemical Co., U. S. A.
Polyphosphate	P_{16}	1 630	Prof. E. Thilo, Berlin
Calgon S	P_{12}	1 284	Messrs Albright and
Calgon	P_{25}		Wilson, London, U. K.
Hexametaphosphate	P_6	660	Messrs Chemical Judex Co., U. K.
Dextransulphate	B_{13}		Dr. Ricketts, U. K.
Dextransulphate	D_5		Dr. Ricketts, U. K.
Hyaluronate			Dr. Ch. Singh, India
Heparine			Connaught Lab. Univ. Toronto, Canada
Chondroitinsulphate			General Biochem. Inc., Ohio, U. S. A.

Note: All condensed phosphates were in the form of sodium salts and were of synthetic origin. The given molecular weights of the higher polyphosphates represent mean values.

tations. Upon acid hydrolysis of polyphosphates (1 N-H_2SO_4, 100 °C, 10′) the indentations produced by polyphosphates disappear altogether. Mono-, di-, tri-, and tetraphosphates do not produce any indentation even at higher concentrations.

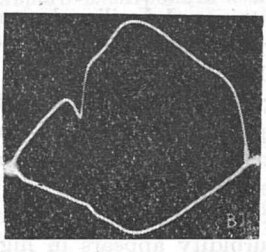

Fig. 1. Curve $dE/dt = f(E)$ of P_{25}, conc. 200 γ/ml in 1 M citric acid medium. Vibrating electrode.

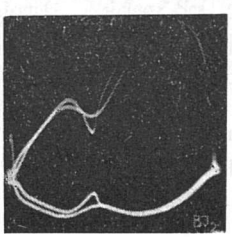

Fig. 2. Comparative measurement: P_{114}, conc. 3.3 mg/ml, supporting electrolyte 1 N-H_2SO_4.

For this reason we followed up the relationship of the depth of indentation to molecular weight of the polyphosphate at equi-weight quantities of different polyphosphates. In Fig. 3, the relationship of the depth of indentation (distance of peak measured from potential axis) to the molecular weight of the polyphosphates is plotted. As supporting electrolyte, 1 M citric acid was used. All polyphosphates were in 100 γ/ml concentration. This concentration was chosen for the reason that on the calibration curve for polyphosphates it lay in the region of a steep rise of the curve. As is to be seen from Fig. 3, no

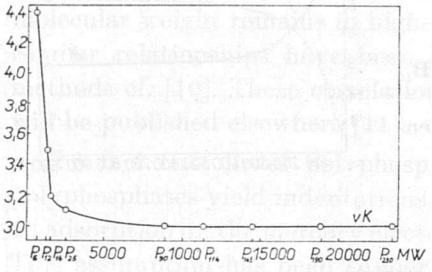

Fig. 3. Relationship of depth of indentation to molecular weight of polyphosphates at equi-weight quantities (100 γ/ml). Electrolyte: 1 M citric acid.
P_x = 100 γ/ml in 1 M-$C_3H_4(OH)(COOH)_3$.

indentation is produced until polyphosphate P_6 is used. With the molecular weight increasing the indentations are deepening up to P_{25}, with higher polyphosphates the indentations remains at the same level.

We have found that from among other polymeric substances of polyanion character oscillopolarographic activity is displayed by dextransulphate (Fig. 4), heparin (Fig. 5), hyaluronate (Fig. 6) and chondroitinsulphate. Whilst polyphosphates and dextransul-

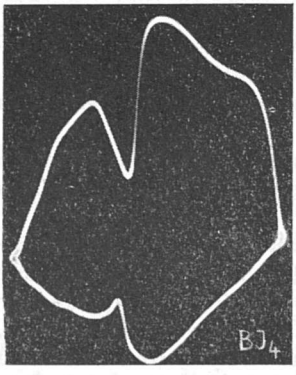

Fig. 4. Curve $dE/dt = f(E)$ of dextransulphate B_{13}, conc. 1 mg/ml in 1 M citric acid medium.

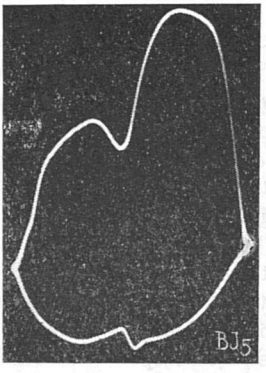

Fig. 5. Curve $dE/dt = f(E)$ of heparin, conc. 1 mg/ml in 1 M-HCOOH.

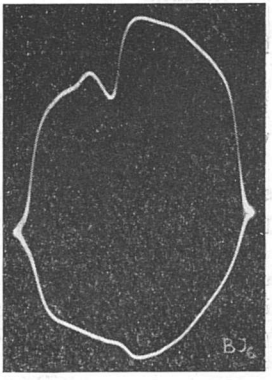

Fig. 6. Curve $dE/dt = f(E)$ of hyaluronate, conc. 1 mg/ml in 1 M-KOH.

phates produce very sharp indentations also at low concentrations of 20—100 γ/ml, the other substances require higher concentrations, i. e. 200—2000 γ/ml, in order to produce an indentation. The indentations are rather blunt.

The calibration curve for dextransulphate in 1 M citric acid medium is plotted in Fig. 7. From this graph it is evident that the region of the steep rise of the curve ends

with a concentration of 300 γ/ml. Concentrations exceeding this limit no longer produce deepening of the indentation.

The most important results of the oscillopolarographic activity of all the substances followed by us are summarized in Table 2.

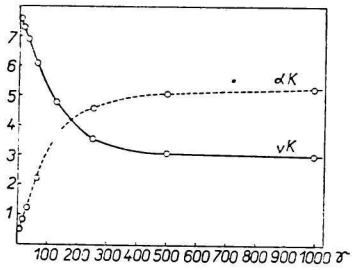

Fig. 7. Calibration curve for dextransulphate B_{13} in 1 M citric acid medium.
vK = depth of indentation — distance from potential axis.

Table 2

Q_k values in 1 M electrolytes

Substances conc. 1 mg/ml	Sulphuric acid	Citric acid	Sodium hydroxide
Polyphosphate	0.43	0.32	—
Dextransulphate B_{13}	0.56	0.38	0.26
Dextransulphate D_5	0.58	0.45	0.31
Hyaluronate	—	—	0.38
Heparin	—	0.40	—
Chondroitinsulphate	—	0.35	—

For elucidating the kind of electrode process responsible for the origin of indentations we used the following known criteria [9]:

1. Effect of concentration of substance. Neither high concentrations of these polymeric substances cause cleaving of the oscillographic curve in the spot of indentation. This phenomenon speaks for capacity process.

2. Comparative measuring. Comparative measurements between the curve for the supporting electrolyte and that for the electrolyte with the substance added are recorded in Fig. 2. The supporting electrolyte was 1 N-H_2SO_4, the indentation was produced by polyphosphate P_{114} in 3.3 mg/ml concentration. In the region of the positive potentials, the curve is somewhat lowered in comparison with pure electrolyte, whilst at more negative potentials its elevation is clearly to be seen.

3. First curve. On the first curves and likewise also on the resultant curves obtained on the vibrating electrode (Fig. 1) the indentations produced by these substances are equally well developed as on normal curves. This is proof that the indentations are not due to an artefact.

Remark: It is not practically possible to evaluate the relationship of the depth of indentation on temperature, since hydrolytic spliting of the polyphosphate chains takes place in acid medium at higher temperatures. After acid hydrolysis of dextransulphate, heparine and hyaluronate the indentations of these substances also completely disappeared.

Discussion

The results of our experiments show that in acid media polyphosphates, beginning with P_6, produce indentations on the curves $dE/dt = f(E)$ and that with the increasing molecular weight of polyphosphates the depth of the indentation in the series P_6 to P_{25} grows. Upon reaching the condensation degree of P_{25} the curve for the relationship of the depth of indentation to the molecular weight remains in higher polyphosphates already at the same level. Similar relationships have been studied by many investigators with other methods cf. [10]. These correlations are rather wide to be discussed here and will be published elsewhere [11].

The fact that lower polyphosphates up to P_6 are inactive, whilst higher polyphosphates yield indentations, can be explained in such a manner that for an adsorption on the mercury electrode, a certain length of the chain is required. This assumption has been supported by our finding that upon hydrolysis of the polyphosphate the indentations disappear. The action of the supporting electrolyte is also of importance. Whilst in alkaline media polyphosphates do not produce any indentations, the indentations are well defined in acid media. Obviously the effect of suppression of the dissociation of the polyphosphate molecule and thus also the attending decreasing solubility, which is known to be in direct relationship to the formation of capacity indentations [12], are playing their part.

With the other substances, i. e. dextransulphate, heparin, hyaluronate and chondroitinsulphate, both the polymeric state of these substances and the presence of groups that can dissociate so that the molecules of these substances are the bearers of the electric charge, are most probably the main factor responsible for the origin of a capacity indentation. It is of interest that with dextransulphates having a varying number of sulphogroups their oscillopolarographic differentiation can also be effected according to the position of the indentation (Table 2).

Our experiments dealing with the following of the oscillopolarographic activity of polymeric substances have not yet been brought to a conclusion and certain preliminary results obtained with polynucleotides and polypeptides show that also here it will be possible to obtain interesting results by this method.

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OSCILOGRAFICKÁ POLAROGRAFIA NIEKTORÝCH ANORGANICKÝCH POLYFOSFÁTOV A POLYANIÓNOV IZOLOVANÝCH Z BIOLOGICKÉHO MATERIÁLU

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Polyfosfáty, začínajúc P_6, v kyslom základnom elektrolyte poskytujú na krivkách $dE/dt = f(E)$ charakteristické zárezy. Zisťovala sa závislosť hĺbky získaných zárezov od molekulovej váhy skúmaných polyfosfátov.

ОСЦИЛЛОГРАФИЧЕСКАЯ ПОЛЯРОГРАФИЯ НЕОРГАНИЧЕСКИХ ПОЛИФОСФАТОВ И НЕКОТОРЫХ ПОЛИМЕРНЫХ ВЕЩЕСТВ ПОЛИАНИОНОВОГО ХАРАКТЕРА, ИЗОЛИРОВАННЫХ ИЗ БИОЛОГИЧЕСКОГО МАТЕРИАЛА

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Полифосфаты начиная P_6 дают в кислых средах зубцы на осциллополярографических кривых. Изучалась зависимость глубины этих емкостных зубцов от молекулярного веса полифосфатов.

Preložil I. Smoleň

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