A METHOD OF MEASURING THE DIELECTRIC PERMEABILITY OF BIOLOGICAL ACTIVE MACROMOLECULES IN AQUEOUS SOLUTIONS

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The problem of measuring the capacity of solutions which in the low-frequency range display principally resistive characteristics, *i. e.* solutions with a high coefficient of dielectric losses tan δ, has been solved by means of Nernst's bridge. This bridge has been modified, both in its circuit and use. Such a measuring system makes it possible to determine dielectric quantities for aqueous solutions of biologically active macromolecules as a function of field frequency. The bridge is designed in such a way that it is now possible to balance independently both impedance components of the solution, *i. e.* C and R.

1. Introduction

Biological macromolecules-including the especially important nucleic acids-assume in aqueous solutions some specific electrical, hydrodynamic and conformational properties, as a result of which solutions of such biopolymers as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), both being carriers of genetic information, become polyelectrolytes. The conductivity of the tested sample with 0.05 mg/ml of DNA amounted to 12 μS/cm, *i. e.* it was about 14 times as high as that of the water used. It is very difficult in such a case to measure the capacity of an object that is characterized by typically resistive properties [2]. This is due to the fact that the sensitivity of the system to resistance changes is often several orders of magnitudes higher than its sensitivity to capacity changes.

The measurements of dielectric characteristics were performed in the range of low frequencies, from 40 Hz to 20 kHz. In this region of field changes there occur such effects as the polarization of electrodes and field divergence, which make measurements even more complicated [5, 6].

Convential bridge systems which enable the capacity and conductivity of an object to be determined may also be used for taking measurements in the case of a strictly deter-

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mined and not too large reactance-resistance ratio. If this ratio is too large (tan $\delta > 1$), anothr suitable method of measurement must be used [1, 6].

This paper discusses an alternative solution of a system which permits the capacity and resistance to be measured as a function of frequency and thus to determine the real component of the dielectric permeability in aqueous solutions of biologically active macromolecules.

The measuring circuit is based, on the whole, on the Nernst bridge with a number of necessary modifications.

2. The measuring system

Of the many bridges which serve to determine the capacity and dielectric losses, the Schering system is the one most often applied [3, 7]. This bridge is unsuitable however, in the case of objects having high losses [3]. The drawing in Fig. 1 presents a modified measuring system of the Nernst bridge.

The measuring circuit is fed by the *RC* generator which is connected to a symmetrical differential transformer. The voltage induced by this transformer is shifted in phase by

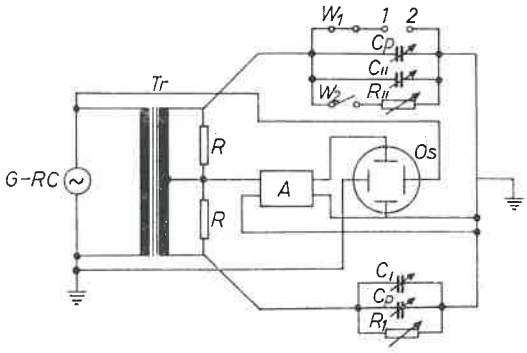

Fig. 1. Basic diagram of the modified Nernst bridge. C_p — standard condenser, C_I, C_{II} — condenser decades, R_I, R_{II} — resistance decades, Os — synchroscope, A — amplifier, *1–2* — dielectric cell

180° and then applied to the object being measured and the standard circuit. In the diagonal of the bridge there is an amplifier and an equilibrium indicator, *i.e.* a synchroscope. In this system a substitution technique is applied which is realised by counterbalancing the bridge twice-once with an attached measuring chamber and the second time after it has been detached. Such a procedure is necessary because it leads to exact and reliable results which are not affected by the mutual capacities of the elements of the system relative to earth. After obtaining the first equilibrium, the chamber is disconnected and the resistance of the mechanically coupled resistance decades corrected. Next, the capacity of the standard capacitor is increased until equilibrium is achieved again. Thus, the capacity change of this capacitor corresponds exactly to the capacity of the object.

A drawback of bridges which are fed with a sinusoidal voltage is that the two components influence each other during the equilibration; in this way the measuring procedure is protracted, as it is difficult to make out whether the given combination of capacity and

resistance is optimal for the equilibrium state or not. It would be of great advantage, therefore, to find a solution in which the equilibration procedures would take place independently, while the non-equilibrium state parameters are calculated from the obtained data. This problem might be solved by feeding the system with an rectangular wave form, making use of the phenomenon of differentiating and integrating the waves through the components R and C of the investigated object.

In a system which is fed with a sinusoidal voltage this principle can be realized if the horizontal deviation plates X are connected to a synchronizing voltage of a generator, whereas the vertical deviation plates Y are fed with a voltage flowing diagonally through

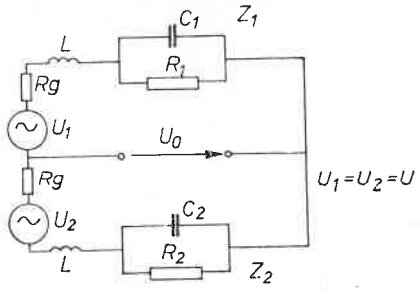

Fig. 2. Simplified bridge circuit used for calculation

the bridge (see Fig. 1). It may be proved that for each frequency either an ellipse or circle appears on the screen of the synchroscope, depending on the ratio of the differences in capacity and resistance of the measured object with respect to the standard capacitor.

The diagram of this system is shown in Fig. 2. The imbalance voltge U_0 in the diagonal of the bridge is

$$U_0 = U[R_1 - R_2 + jR_1R_2\omega(C_1 - C_2)]/[R_1 + R_2 + jR_1R_2\omega(C_1 + C_2)] \tag{1}$$

if the impedance of the source is

$$Z_g = R_g + j\omega L \ll Z_1 = R_1/(1 + j\omega R_1C_1). \tag{2}$$

In the case of $U_0 = 0$, the bridge will have reached equilibrium, and according to Eq. (1)

$$R_1 = R_2 \quad \text{and} \quad C_1 = C_2.$$

When changes of the voltage $U_0 = U(t)$ and the synchronizing voltage $U_x = U_{x0}(t)$ coincide in time, an ellipse is obtained, the major axis of which slopes towards the horizontal axis at an angle α (see Fig. 3).

This analysis shows that $\tan\alpha$ is proportional to $\Delta R = R_1 - R_2$ according to the formula

$$\tan\alpha = \Delta R/(R_1 + R_2). \tag{3}$$

The real component $R_1 = R_2 = R$ having been equilibrated, the major axis of the ellipse coincides with the axis of abscissae. Next, assuming $R\omega C < 1$, Eq. (1) can be approximately given accordingly with the obtained empirical data by Eq. (4), which is of course the equation of an ellipse:

$$U_0^2/\tfrac{1}{2}[UR\omega(C_1 - C_2)]^2 + U_x^2/U_{x0}^2 = 1. \tag{4}$$

It may be deduced therefrom that the value of the half-axis $b = 1/2\ UR\omega(C_1 - C_2)$ depends on the difference between the capacities of the object measured and the standard capacitor. If $C_1 - C_2 = \Delta C \to 0$, then b tends to 0, and the bridges reaches a state of complete equilib-

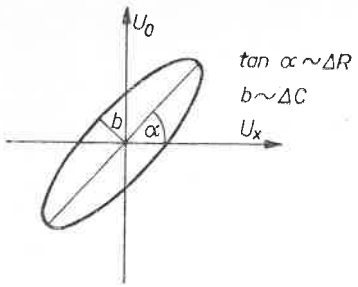

Fig. 3. Figure observed on screen of the synchroscope at non-equilibrium state of the bridge

rium in which the capacity of the measured object is equal to the change in the standard capacity.

It follows from these considerations that the presented system affords possibilities for an independent equilibration of both components, thereby providing means for evaluating the non-equilibrium state of the bridge.

3. Dielectric measuring chamber

The proper choice of the measuring chamber depends in principle on two factors: the assumed range of working frequencies and applied measuring technique. It has to ensure elimination of the influence of electrode polarization on the admittance value.

In the measuring device use has been made of a dielectric chamber described by Schwan [6], which was chosen for exact measurements of the dielectric permeability of solutions in the frequency range from 10 Hz to 200 kHz.

4. Determination of real values from measured data

One of the main sources of error when determining the impedance of aqueous solutions of biological active macromolecules is polarization of the electrodes. This phenomenon consists in the generation of a constant potential between the metal electrode and the contacting liquid. If a variable field is applied, the polarization potential is modulated by the variable voltage. The electrode polarization becomes particularly obvious when investigating solutions feature high conductivity in the range of low frequencies. This effect

should be taken into consideration in measurements, as the values obtained for the components of the object are subject to fairly large errors. A detailed study of electrode polarization as well as the correction techniques may be found in Schwan's paper [6]. An interesting correction has been suggested by Moser and his co-workers [4], who have applied a reference solution of the same conductivity.

The design of the dielectric chamber, which permits the distance between the electrodes, to be changed was influenced by the acceptance of a technique which renders possible the elimination of the phenomenon of polarization [8]. Also related to this problem is the application of platinum electrodes covered with platinum black, so that the polarization impendance becomes about four orders of magnitudes smaller than in the case of clean surfaces [6].

5. Accuracy of measurements

The proportion of the error arising while determining the capacity and resistance of the object depends on the degreee of accuracy of the elements in the measuring system. The use of a standard air condenser, provided with an additional micrometric scale, makes it possible to read the capacity with an accuracy of 0.125 pF in the range of 50–600 pF and 0.25 pF in the range of 50–1200 pF. The condenser decades were calibrated with an error of 0.5%. The accuracy of the applied resistance decades was 0.2%.

The insensitivity error of the system depends on the frequency and amounts on the average to 0.3% for resistance and 0.5% for capacity. These values have been obtained at a supply voltage of 20 V and a sensititivity of the set (consisting of an amplifier and a synchroscope) amounting to 8 mV/cm. The investigated sensitivity depends on the width of the picture line, which distinctly increases in the case of considerably stronger amplification.

a) The intrinsic error of the measuring method

This error arises because the method is developed on the basis of certain simplifying assumptions. In the case of the discussed method these errors may be caused, among other things by the occurrence of additional capacitative couplings between the structural elements of the bridge, leakage, and the resistance and reactance of the assembling and leading ducts of the measuring system. The determination of the intrinsic error of the method is based on comparing the obtained results with the actual values. It is usually assumed that the actual value of the resistance or capacitative element is equal to the corresponding standard. In the method discussed here the determination of the intrinsic error consisted in comparing the values of the parallelly connected circuit of the standards, *viz.*, the resistance and capacity which were inserted instead of the measuring chamber, with the values which are obtained directly in the measurements. Table I presents the percentage values of the intrinsic errors for the resistance and capacity as a function of frequency. The nominal values of the standards R and C are given at the top of the table for three different combinations of a parallel circuit: $R = 10{,}000\Omega$ and $C = 50$ pF, $R = 20{,}000\Omega$ and $C = 100$ pF, and $R = 20{,}000\Omega$ and $C = 500$ pF.

TABLE I

f	R	C	R	C	R	C
[Hz]	10 kΩ	50 pF	20 kΩ	100 pF	20 kΩ	500 pF
	%	%	%	%	%	%
50	0.25	0.71	0.31	0.52	0.33	0.52
100	0.28	0.71	0.31	0.52	0.33	0.5
500	0.32	0.72	0.32	0.47	0.33	0.46
1000	0.34	0.65	0.33	0.4	0.33	0.35
10000	0.41	0.66	0.35	0.3	0.42	0.33
20000	0.45	0.43	0.35	0.3	0.41	0.32

b) Spurious errors in the determination of the capacity, resistance and electrical permeability of a solution

The analysis of the aforesaid errors was based on the results obtained for an aqueous solution of deoxyribonucleic acid of a concentration of 0.025 mg/cm^3. The experiments were carried out using the sodium salt of highly polymerized DNA from a calf's thymus gland, produced by the Sigma Chemical Company. The value of the DNA concentration was tested spectrophotometrically by measuring the optical density (OD) at a wavelength of 260 nm. The measurements were taken at a temperature of 25°C. The capacity C_s and resistance R_s of the investigated solution were determined indirectly, taking into account the phenomenon of polarization of electrodes [8, 9]. Measurements were made for two different positions of a movable electrode. In the first position the distance between the electrodes was 0.35 cm, whereas in the second position it was 0.55 cm. The magnitudes C_s and R_s are a function of the capacity C_1 and resistance R_1 for the first distance between the electrodes, and the values C_2 and R_2 for the second distance between the electrodes [6].

The mean square of the arithmetic mean ΔC_s for the capacity of the investigated solution C_s is expressed by the value obtained by means of the following formula (3):

$$\Delta C_s = \pm \sqrt{\left(\frac{\partial C_s}{\partial C_1} M_{C1}\right)^2 + \left(\frac{\partial C_s}{\partial C_2} M_{C2}\right)^2 + \left(\frac{\partial C_s}{\partial R_1} M_{R1}\right)^2 + \left(\frac{\partial C_s}{\partial R_2} M_{R2}\right)^2} \qquad (5)$$

where

$$M_{C1} = \frac{\sigma_{C1}}{\sqrt{n}}; \quad M_{C2} = \frac{\sigma_{C2}}{\sqrt{n}}; \quad M_{R1} = \frac{\sigma_{R1}}{\sqrt{n}}; \quad M_{R2} = \frac{\sigma_{R2}}{\sqrt{n}}. \qquad (6)$$

The quantities σ_{C_1}, σ_{C_2}, σ_{R_1}, σ_{R_2} denote the mean square errors (standard deviations) of the capacity and resistance in the first and second positions, respectively; n is the number of measurements performed.

The value of the resistance in the solutions is determined directly from measurements, as the polarization effect of electrodes on this quantity is negligible in the range of low frequencies [6].

The random error in the determination of the electrical permeability $\Delta\varepsilon_s'$ of a solution is proportional to the value ΔC_s. Table II gives the absolute values of random error of the capacity, resistance and electrical permeability as a function of field frequency.

Table III, on the other hand, contains the relative values of spurious errors for the capacity C_s and resistance R_s.

The value of the relative error $\Delta C_s/C_s$ increases with the decrease of the measurement frequency, amounting in the case of 40 Hz to $\pm 5.1\,\%$, *i.e.*, it is comparatively high. Measure-

TABLE II

f[Hz]	40	50	70	100	200	500	1000
ΔC_s[pF]	± 23.4	±16.56	±10.1	± 4.87	± 1.67	± 0.625	± 0.16
ΔR_s[Ω]	± 31.6	±82.1	±31.2	±29.9	±30.8	±29.5	±31.2
$\Delta\varepsilon_s'$	±126.7	±89.4	±54.9	±26.3	± 9.03	± 3.37	±00.80

TABLE III

f[Hz]	40	50	70	100	200	500	1000
$\Delta C_s/C_s$[%]	±5.1	±4.0	±3.5	±2.9	±2.9	±2.5	±0.85
$\Delta R_s/R_s$[%]	±0.104	±0.106	±0.103	±0.099	±0.102	±0.098	±0.11

ments in the range of lower frequencies were associated with much greater errors, so that this frequency value is the lowest limiting frequency value of the measuring method.

The results obtained for the dielectric changes of a heterogenic population of DNA molecules undergoing thermal helix-coil phase transition, and the hysteresis of the melting curve, are comparable with those obtained by other physico-chemical methods. The described technique can be of use for some conformational investigation of biological active macromolecules.

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