Short Communications

A Simple A.C. Polarography Attachment to a Conventional D.C. Polarograph

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In spite of its several advantages over the conventional d.c. method, the a.c. polarographic method is still much less used in chemical laboratories nowadays. This is partly due to the fact that the method requires, apart from a conventional d.c. polarograph, an additional expense for an a.c. polarographic attachment. Moreover, not all manufacturers of d.c. polarographs supply a.c. polarographic attachments, and products of different manufacturers are not always mutually interchangeable.

There have been several attempts in the past to convert existing commercial d.c. polarographs to a.c. polarographs by changing the internal circuit connection.[1-3] But this procedure is neither generally applicable nor advisable.

In the present paper, an inexpensive and straightforward method of effectuating a.c. polarographic analysis with a conventional d.c. polarograph is described. The "Radiometer PO4 Polariter" was used since this instrument is common in the Scandinavian
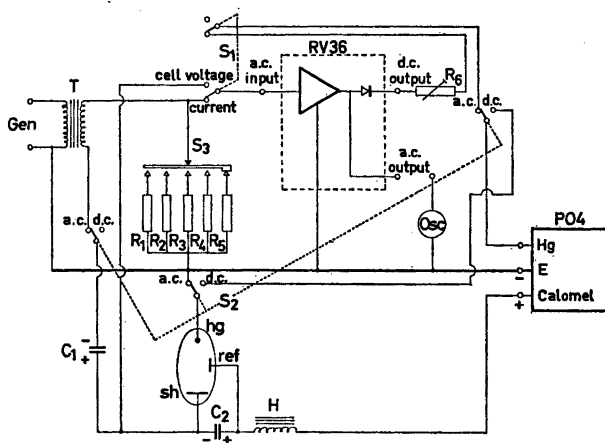

Fig. 1. The circuit diagram of the a.c. polarographic attachment.

Gen: audio-frequency generator. T: audio-frequency output transformer. S_1: interchanging switch between a.c. cell voltage and a.c. polarographic current measurements. S_2: interchanging switch between d.c. and a.c. polarography. S_3: selecting switch. R_1, R_2, R_3, R_4 & R_5: voltage dropping resistors, 10, 20, 50, 100 & 200 Ω, resp. R_6: series resistor 3 MΩ. C_1: d.c. blocking capacitor 2000 μF; d.c. working voltage 6 V. C_2: shunt capacitor 2000 μF; d.c. working voltage 6 V. H: a.c. blocking choke coil. hg: dropping mercury electrode. ref: reference electrode. sh: shunt electrode. RV36: a.c. millivoltmeter. PO4: conventional d.c. polarograph (recorder). Osc: cathode-ray oscilloscope.

countries and elsewhere. A compact-sized transistorized commercial a.c. millivoltmeter with an external d.c. amplifier output terminal was used together with it. If one makes use of a fixed frequency of 50 or 60 Hz from the main supply, as is the case with nearly all commercial a.c. polarographs then the total expenses can be kept low. No circuit modification of the existing d.c. polarograph needs to be undertaken nor any cumbersome calibration of the whole set-up.

The circuit diagram is shown in Fig. 1. As the source of alternating potential "Gen", almost any commercial sine-wave generator of the audio-frequency range can be used. It is usually equipped with an output voltage attenuator.

For a.c. voltage attenuation, an ordinary audio-frequency output transformer "T" can be used. In the present case, a loudspeaker transformer "Lübcke L1−116" (manufacturer: H. & K. Lübcke, Copenhagen; power 2.5 W; primary winding impedance 5 $k\Omega$; secondary winding impedance 15 Ω) was applied. An appropriate step-down ratio of the transformer should be chosen according to the magnitude of the a.c. output voltage from the generator, to give finally a superimposing a.c. voltage of 5−15 mV r.m.s. on the secondary winding.

For routine analysis, however, one could replace the generator "Gen" with the ordinary main supply of 50 or 60 Hz without suffering many disadvantages from it. For more detailed information about this change, reference can be made to an excellent monograph.[4]

For the measurement of the superimposed a.c. cell voltage (switch "S_1" on "cell voltage") and of the actual a.c. polarographic current (switch "S_1" on "current") an a.c. millivoltmeter "Radiometer RV36" was used. The switch "S_2" positioned on "d.c." makes conventional d.c. polarographic measurements possible, preserving all the functional features of the recorder "PO4". Careful shielding and one point earthing must be followed in the part which leads to the input terminal of the millivoltmeter "RV36". By this measure, the hum and noise level at this point can be kept below 20 μV if no excessive external stray fields are present.

The polarity of the electrolytic capacitors "C_1" and "C_2" indicated in Fig. 1 is valid only for the case in which the dropping mercury electrode is polarized in cathodic direction with respect to the reference electrode. In the opposite case, the polarity must be changed.

The choke coil "H" prevents the superimposed a.c. voltage from interfering with the electrical circuit of the recorder "PO4". Its reactance must be high compared with that of the cell. Its ohmic resistance must, however, be held as low as possible, not to cause a serious ohmic voltage drop which may result in an erroneous evaluation of the summit potential. In the present work, a "Lübcke D115" choke coil was used which has 1 $k\Omega$ internal ohmic resistance and 100 H inductance under the relatively weak d.c. current flow from zero to 100 μA. It was made of the "Radiometal" core with high initial magnetic permeability. Ordinary choke coils of silicon steel core for use in rectifier circuit are not satisfactory.

A cathode-ray oscilloscope may be connected to the a.c. output terminal of the millivoltmeter "RV36" as a monitoring device for the control of a.c. wave form.

The d.c. output from the millivoltmeter "RV36" is then fed over a series resistor "R_6" to the microammeter terminal "Hg" of the recorder "PO4". The magnitude of the "R_6" must be high compared with that of the output impedance of the millivoltmeter "RV36". In the present case, a variable 3 $M\Omega$ resistor was used, which is satisfactory compared with the d.c. output impedance 100 $k\Omega$ and d.c. output voltage 500 mV ($\pm$ 4 %) at full deflection on the millivoltmeter scale) of the "RV36". The final value of the "R_6" was adjusted such that a full scale deflection of the recorder "PO4" on its 0.2 μA range was obtained, when the millivoltmeter "RV36" was fully-deflected. Consequently, a full deflection on the recorder scale reads 1 μA r.m.s. when the voltage dropping resistor "R_4" of 100 Ω, the maximum sensitivity range 1 mV of the millivoltmeter "RV36", and the maximum sensitivity range 0.02 μA of the recorder "PO4" are chosen. This is a recorder sensitivity more than sufficient for usual a.c. polarographic analysis.

Fig. 2 is an example of the a.c. polarograms obtained with the set-up described. The corresponding d.c. polarogram is shown in Fig. 2a.
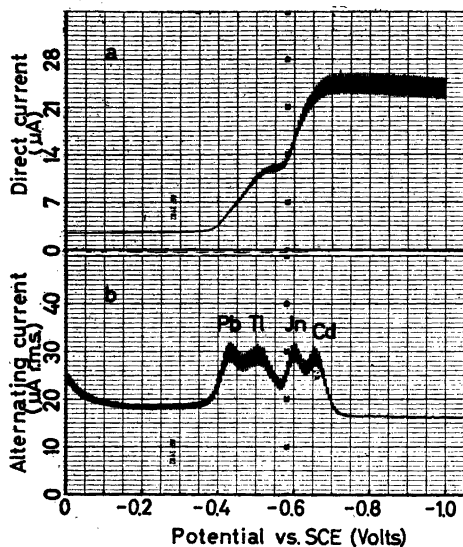

Fig. 2. a) D.c. polarogram of a mixture of 0.3 mM Pb^{2+}, 1.0 mM Tl^+, 0.5 mM In^{3+}, 0.5 mM Cd^{2+}, and 0.1 M HCl in the absence of air oxygen; no extra maximum suppressor added; *b)* a.c. polarogram of the same electrolyte solution but measured in the presence of air oxygen; a.c. frequency 160 Hz; a.c. amplitude 5 mV r.m.s. Damping grade "4" on the recorder "PO4" in both cases.

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On the Role of Δ4-3α-Hydroxysterols in the Biosynthesis of Bile Acids

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In the biosynthesis of bile acids the $Δ^5$-3β-hydroxy configuration of cholesterol is transformed into the 3α-hydroxy-5β configuration of chenodeoxycholic acid and cholic acid by means of the intermediary formation of $Δ^4$-3-ketosteroids.[2] Evidence has been presented that the $Δ^4$-3-ketosteroids are 7α-hydroxycholest-4-en-3-one and 7α,12α-dihydroxycholest-4-en-3-one.[3,4] Studies on the metabolism of these compounds *in vitro* indicate that the major pathway for their further metabolism involves the conversion to the corresponding 3-keto-5β-steroids followed by reduction of the 3-keto group to yield 5β-cholestane-3α,7α-diol and 5β-cholestane-3α,7α,12α-triol.[3-6] However, it has been suggested that in the biosynthesis of 5β-cholestane-3α,7α,12α-triol, 7α-hydroxycholest-4-en-3-one is converted to 5β-cholestane-3α,7α-diol also by means of the intermediate formation of cholest-4-ene-3α,7α-diol.[3] Hutton and Boyd[7] have reported the formation of cholest-4-ene-3α,7α-diol from 7α-hydroxycholest-4-en-3-one in the presence of a partially purified supernatant fraction from rat liver homogenate. Cholest-4-ene-3α,7α-diol was, however, a minor metabolite and the major pathway for the metabolism of 7α-hydroxycholest-4-en-3-one under these conditions involved the conversion into 7α-hydroxy-5β-cholestan-3-one and subsequently 5β-cholestane-3α,7α-diol. Similar findings were made by Björkhem and Danielsson[8] in a study of the formation and metabolism of various $Δ^4$-cholestenols including cholest-4-ene-3α,7α-diol. The results indicated that the major pathway for the metabolism *in vitro* of cholest-4-ene-