POLAROGRAPHIC DETERMINATION OF 6-β-D-GLUCOPYRANOSYLOXY-7-HYDROXYCOUMARIN

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The polarographic behaviour was studied for 6-β-D-glucopyranosyloxy-7-hydroxycoumarin, a natural compound serving as an optical whitening agent. The substance can be quantitated by tast polarography, differential pulse polarography using a conventional dropping mercury electrode, and differential pulse polarography using a static mercury drop electrode over the regions of 20–1 000, 2–1 000, and 0.2–1 000 μmol l^{-1}, respectively. The methods developed for the quantitation of the compound were applied to its direct determination in a raw product.
Key words: Polarography; 6-β-D-Glucopyranosyloxy-7-hydroxycoumarin.

6-β-D-Glucopyranosyloxy-7-hydroxycoumarin [CAS Name: 6-(β-D-glucopyranosyloxy)-7-hydroxy-2*H*-1-benzopyran-2-one, CAS Registry Number: 531-75-9] was the first compound to be found applicable in practice as an optical whitening agent. The substance can be quantitated by UV spectrophotometry, spectrofluorimetry, or high performance liquid chromatography with photometric or fluorimetric detection[1].

Coumarin[2–4] and its hydroxy and alkoxy derivatives[5–8] are polarographically reducible at rather negative potentials (about –1.5 V vs SCE) and the irreversible wave height and position are usually pH-dependent. A mention of the polarographic behaviour of 6-β-D-glucopyranosyloxy-7-hydroxycoumarin has been made in ref.[9], examining the correlation between the structure and polarographic behaviour of coumarin derivatives in 50% methanol. No details concerning the polarographic behaviour of the substance in question or the application of conventional or advanced polarographic techniques, however, were found in the literature. Therefore, the polarographic behaviour of 6-β-D-glucopyranosyloxy-7-hydroxycoumarin was investigated within the present work, with emphasis on the determination of the substance at low concentrations by tast polarography and by differential pulse polarography (DPP) using the conventional dropping mercury electrode (DME) and using the static mercury drop

electrode (SMDE), techniques that have proved to suit well to the quantitation of some coumarin-based optical whitening agents[10,11].

EXPERIMENTAL

Chemicals

Raw 6-β-D-glucopyranosyloxy-7-hydroxycoumarin obtained from the Chemical Institute, Slovak Academy of Sciences, Bratislava, Slovak Republic, was purified by recrystallization from the water–methanol 1 : 1 mixed solvent. Its purity was checked by melting temperature determination (205 °C, in agreement with the literature[12]), UV absorption spectra measurement in ethanolic solutions (the band positions and intensities agreed with published data[13,14]), and by thin layer chromatography, which gave a single spot when using toluene–chloroform–ethyl acetate 2 : 3 : 1 and toluene–chloroform–ethyl acetate–methanol 2 : 3 : 3 : 3 mobile phases (R_F 0.29 and 0.55, respectively). A stock solution in methanol (c = 50 mmol l^{-1}) was prepared by dissolving 18.3655 g of the substance in this solvent and diluting to 1 l. More dilute solutions were obtained by diluting the stock solution with methanol.

Britton–Robinson buffers were prepared conventionally[15]. The other chemicals used were of reagent grade purity (Lachema, Brno). Water was redistilled in a quartz still.

Thin layer chromatography was performed using a commercial kit with Silufol UV 254 plates (Kavalier, Votice).

Apparatus

A PA 4 polarographic analyzer interfaced to an XY 4105 recorder (both Laboratorni pristroje, Prague, Czech Republic) was used. The working electrode was either an SMDE 1 static mercury drop electrode (Laboratorni pristroje, Prague, Czech Republic) having a capillary diameter of 0.138 mm, with the maximum drop size determined by the valve opening period of 160 ms, or a conventional dropping mercury electrode (DME) whose drop time was 7.04 s and mercury flow rate 0.61 mg s^{-1} at a reservoir height of 36 cm, measured in 0.1 M NaCl at 0 V vs SCE. A saturated calomel reference electrode (SCE), relative to which all potentials are given, and an auxiliary platinum sheet electrode were also used. The potential sweep rate was 5 mV s^{-1}, electronically controlled drop time 1 s, DPP pulse height –50 mV, and DME reservoir height 36 cm, unless stated otherwise. Oxygen was removed from the solutions by purging with nitrogen, which had been purified by passing it through a chromium(II) ion solution in dilute hydrochloric acid (1 : 1) over zinc amalgam. Furthermore, before entering the electrochemical vessel, the nitrogen was passed through a solution with the same methanol content as the solution polarographed.

Acidity of the solutions was measured with an OP-208/1 precision digital pH-meter (Radelkis, Hungary) using a combined glass/silver chloride electrode.

Absorption spectra were measured on a Pye Unicam 8800 UV/VIS spectrophotometer (Philips) using quartz cells 1 cm or 2 cm optical pathlength.

Small volumes of solutions were added by using Varipipette 3000 micropipettes type A-20, A-200, or A-1000 (Plastomed, Poland).

An M 415 centrifuge (Chirana, Czech Republic) and a type 350 rotary vacuum evaporator (Unipan, Poland) were employed in the purification and separation processes.

Procedures

For the polarographic measurements, the required amount of the 6-β-D-glucopyranosyloxy-7-hydroxycoumarin solution in methanol at the required concentration was added to a 10 ml volumetric flask and diluted to the mark with the corresponding Britton–Robinson buffer. The buffers and methanol were stored in glass vessels because if polyethylene vessels were used, substances affecting unfavourably the determination of the lowest concentrations of analyte were leached out from the walls.

The solutions were nitrogen purged for 10 min prior to measurement. The calibration dependences were measured in triplicate and subjected to statistical processing.

The limit of determination L_Q was established as tenfold the standard deviation of 7 analyte determinations at the concentration corresponding to the lowest point of the calibration straight line[16].

The following procedure was applied to determine 6-β-D-glucopyranosyloxy-7-hydroxycoumarin in the raw product by DPP using a DME: About 0.1 g of the product was weighed in, dissolved in a small volume of methanol, and diluted with methanol to 100 ml in a volumetric flask. An 0.1 ml aliquot was added to a 10 ml volumetric flask and diluted with the Britton–Robinson buffer at pH 7.9, and the differential pulse polarogram was recorded. The analyte content was read from the calibration plot obtained by using the pure substance.

The procedure for quantitating the analyte in the raw product by UV spectrophotometry was as follows. About 0.1 g of the product was weighed in, dissolved in a small volume of methanol, and diluted with this solvent to 100 ml in a volumetric flask, and the spectrum was recorded. The analyte content was read from a calibration plot obtained with the pure substance.

RESULTS AND DISCUSSION

Stability of Stock Solutions of 6-β-D-Glucopyranosyloxy-7-hydroxycoumarin

The absorption spectrum of 6-β-D-glucopyranosyloxy-7-hydroxycoumarin in methanol exhibits maxima at 227 and 334 nm.

Molar absorptivity of the analyte solution at 334 nm is $\varepsilon_{334} = 1.32 \cdot 10^4$ l mol^{-1} cm^{-1}, as obtained over the region of 10–100 μmol l^{-1} where Beer's law is obeyed. Stored in darkness, the stock solutions of the substance in methanol at c = 10–100 μmol l^{-1} exhibited absorbances that remained constant within the limits of experimental error during a week, and decreased less than 2% and 4% in 2 and 4 weeks, respectively. Therefore, fresh solutions (at the above concentrations) were prepared every week.

Tast Polarography and Differential Pulse Polarography of 6-β-D-Glucopyranosyloxy-7-hydroxycoumarin Using a Dropping Mercury Electrode

The effect of pH on the polarographic behaviour of the substance at c = 100 μmol l^{-1} in Britton–Robinson buffers containing 1% (v/v) methanol over the region of pH 2–12 is demonstrated in Table I. The wave and peak shapes are shown in Figs 1 and 2, respectively, demonstrating that waves or peaks are only observed at pH > 6, whereas at lower pH values they are overlapped by the supporting electrolyte decomposition current.

At pH < 10, the half-wave potential $E_{1/2}$ and peak potential E_p shift to more negative values with increasing pH; the slope of this shift is 38 mV per pH unit. At pH > 10, the potentials remain virtually constant. The $E_{1/2}$ values of 6-β-D-glucopyranosyloxy-7-hydroxycoumarin are more negative than those of unsubstituted coumarin, apparently due to the substituent effect.

The I_{lim} and I_p values remain virtually constant across the region of pH 6–12; changes observed were rather due to the accuracy of their reading from the records. The decrease at pH > 12 is apparently associated with basic hydrolysis of the compound as indicated in Eq. (*A*).

TABLE I
Effect of pH on tast and differential pulse polarograms of 6-β-D-glucopyranosyloxy-7-hydroxycoumarin (c = 100 μmol l^{-1}) in Britton–Robinson buffers containing 1% (v/v) methanol

pH[a]	$E_{1/2}$, V	I_{lim}, μA	E_p, V	I_p, μA
5.0	–[b]	–[b]	–[b]	–[b]
6.0	–1.59[c]	2.4	–1.57	3.4
6.5	–1.62	3.0	–1.60	3.7
7.9	–1.67	3.8	–1.65	4.4
9.1	–1.72	4.2	–1.70	4.1
10.2	–1.75	3.8	–1.73	4.1
11.4	–1.75	3.6	–1.73	3.3
13.4	–1.75	2.7	–1.74	2.3

[a] In solution containing 1% (v/v) methanol; [b] cannot be evaluated due to coincidence with the supporting electrolyte decomposition current; [c] crude value.

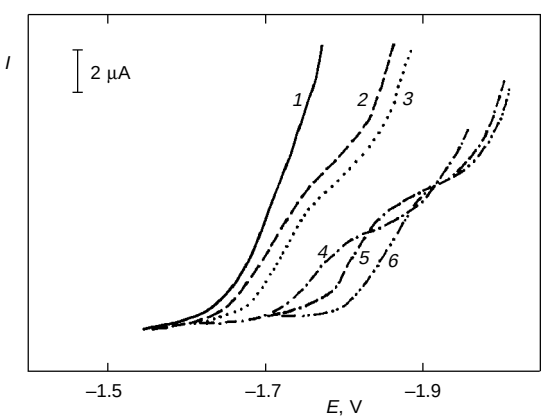
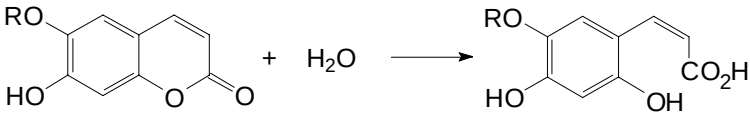


FIG. 1
Tast polarograms of 6-β-D-glucopyranosyloxy-7-hydroxycoumarin (c = 100 μmol l^{-1}) in Britton–Robinson buffers containing 1% (v/v) methanol. pH: *1* 5.0, *2* 6.0, *3* 6.5, *4* 7.9, *5* 9.1, *6* 11.4



(*A*)

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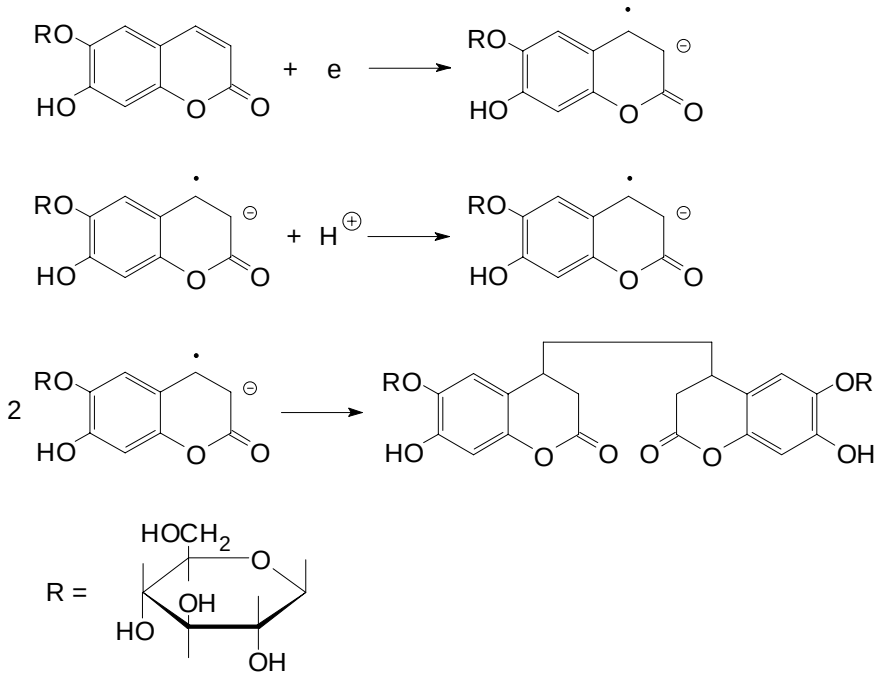
The wave height of 6-β-D-glucopyranosyloxy-7-hydroxycoumarin is comparable to that of unsubstituted coumarin. In analogy with the polarographic behaviour of coumarin[2,4], which has been confirmed recently[17], 6-β-D-glucopyranosyloxy-7-hydroxycoumarin can be assumed to be irreversibly reduced with the exchange of one electron within a diffusion-controlled process as shown in Scheme 1, the dimerization of the radical formed being faster than its further reduction if any.

This assumption is also borne out by the cyclic voltammogram of 6-β-D-glucopyranosyloxy-7-hydroxycoumarin using a hanging mercury drop electrode (Fig. 3) as well as by the linear dependence of the DC polarographic wave height at pH 7.9 on the square root of the mercury reservoir height.

From the analytical point of view, the best-developed waves or peaks were obtained in Britton–Robinson buffer at pH 7.9 in the presence of 1% (v/v) methanol. The dependences of the tast polarographic wave height on the analyte concentration are linear



SCHEME 1

in this system over the concentration region of 20–1 000 μmol l^{-1}; for the DPP peak, the linearity range is 2–1 000 μmol l^{-1}. The parameters of the dependences are given in Table II. Polarograms recorded at the lowest attainable concentrations are shown in Fig. 4.

Lower concentrations could not be quantitated even if the supporting electrolyte concentration was decreased. Increasing the methanol content of the solution polarographed brought about a decrease in the DPP peak height, apparently due to a lowering of the reduction rate as a result of a partial blocking of the working electrode surface by adsorbed molecules of methanol.

Differential Pulse Polarography of 6-β-D-Glucopyranosyloxy-7-hydroxycoumarin Using a Static Mercury Drop Electrode

Due to the highly negative half-wave potential of the substance, no suitable conditions for the quantitation of the analyte at low concentrations by DPP using a hanging mercury drop electrode could be found. However, a considerable reduction in the limit of

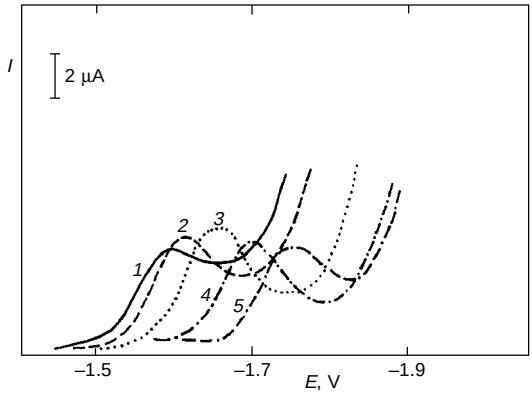


FIG. 2
DP polarograms of 6-β-D-glucopyranosyloxy-7-hydroxycoumarin (c = 100 μmol l^{-1}) in Britton–Robinson buffers containing 1% (v/v) methanol. pH: *1* 6.0, *2* 6.5, *3* 7.9, *4* 9.1, *5* 11.4

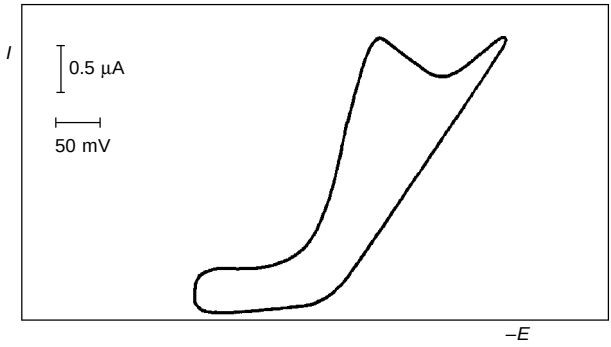


FIG. 3
Cyclic voltammogram of 6-β-D-glucopyranosyloxy-7-hydroxycoumarin (c = 10 μmol l^{-1}) using a hanging mercury drop electrode in Britton–Robinson buffer containing 1% (v/v) methanol at pH 7.9. Initial potential –1.5 V; potential sweep rate 100 mV s^{-1}

determination as compared to DPP using a DME could be achieved by employing the static mercury drop mode. In this case, too, the optimum conditions were found in the Britton–Robinson buffer at pH 7.9 in the presence of 1% methanol; the concentration dependence was linear in this system over the concentration range of 0.2–1 000 μmol l^{-1} (Table III). The differential pulse polarograms for the lowest attainable concentrations are shown in Fig. 4. It is clear that the lowest analyte concentrations that can be deter-

TABLE II
Regression straight line parameters for the concentration dependences of the polarographic wave or peak heights of 6-β-D-glucopyranosyloxy-7-hydroxycoumarin in the Britton–Robinson buffer pH 7.9 in the presence of 1% (v/v) methanol

Method	c, μmol l^{-1}	Slope mA l mol^{-1}	Intercept μA	r^a	$L_Q{}^b$ μmol l^{-1}
Tast	100–1 000	31	0.1	0.9993	–
	20–100	34	–0.14	0.9956	29
DPP	100–1 000	43	0.00	0.9997	–
	10–100	42	–0.03	0.9989	–
	2–10	36	–0.011	0.9962	3.4

[a] Correlation coefficient; [b] limit of determination.

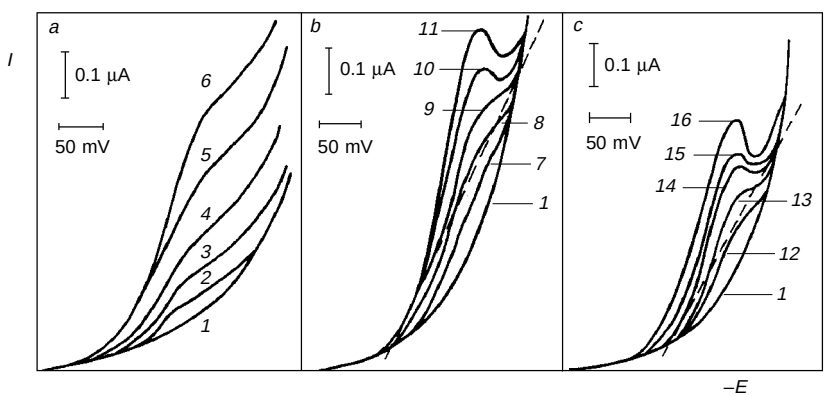


FIG. 4
Polarograms of 6-β-D-glucopyranosyloxy-7-hydroxycoumarin in Britton–Robinson buffer containing 1% (v/v) methanol at pH 7.9. Initial potential –1.5 V. c (μmol l^{-1}): *1* 0, *2* 20, *3* 40, *4* 60, *5* 80, *6* 100; *7* 2, *8* 4, *9* 6, *10* 8, *11* 10; *12* 0.2, *13* 0.4, *14* 0.6, *15* 0.8, *16* 1.0. *a* Tast polarography using DME; *b* DP polarography using DME; *c* DP polarography using SMDE. Broken line is the base line for peak hight evaluation

mined are one order of magnitude lower in the static mercury drop electrode mode than in the conventional dropping mercury electrode mode.

Practical Applications

The 6-β-D-glucopyranosyloxy-7-hydroxycoumarin content in the technical material was determined by DPP in the DME mode. The observed content was 84.1%; the standard deviation estimate of the determination performed in triplicate was 1.2%. The analyte content found spectrophotometrically was 85.0%, with a standard deviation of 1.1% for the determination performed in triplicate. Moore's u-test gave evidence that the results of the polarographic and spectrophotometric determination do not differ at the 95% confidence level.

Insufficient selectivity of the polarographic method that might be perceived in complex matrices such as waste waters could be rectified by inserting a suitable preconcentration step, such as extraction combined with thin layer chromatography using mobile phases indicated in the Experimental.

TABLE III
Regression straight line parameters for the concentration dependences of the differential pulse polarographic peak heights (SMDE mode) of 6-β-D-glucopyranosyloxy-7-hydroxycoumarin in the Britton–Robinson buffer pH 7.9 in the presence of 1% (v/v) methanol

c, μmol l^{-1}	Slope, mA mol^{-1}	Intercept, μA	r^a	$L_Q{}^b$, μmol l^{-1}
100–1 000	165	2.1	0.9989	–
10–100	196	–0.20	0.9997	–
1–10	212	–0.04	0.9982	–
0.2–1	218	–0.008	0.9909	0.3

[a] Correlation coefficient; [b] limit of determination.

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