

The spectroabsorptiometric and voltammetric behavior of malvin in buffered solutions and its antioxidant properties

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In the present work the structural transformations of malvin in aqueous acetate buffer solutions were investigated over a wide pH range under *in vitro* conditions using electronic absorption spectroscopy. In addition to the spectroabsorptiometric investigation, the voltammetric behavior and the mechanism of the redox process of this molecule were studied, in order to define its antioxidant properties. The electronic absorption spectra reveal that the structural changes of malvin, caused by changes in the pH of the medium, lead to changes in the voltammetric behaviour of this molecule. The dependence of the oxidation potential of malvin on pH, which can be explained by the presence of different electroactive molecular structures of malvin at different pH values, indicates different mechanisms of the electrode process. The voltammetric activity of malvin is compared semiquantitatively with the voltammetric activity of a standard synthetic antioxidant, BHA, and a natural antioxidant, quercetin.

Keywords: malvin, UV-VIS spectra, cyclic voltammetry, antioxidant properties.

INTRODUCTION

The anthocyanin molecules, among which malvin is very important, belong to the class of flavonoid compounds, or, in a more general sense, to the class of polyphenols. As plant pigments these compounds play a prominent role in the phytochemical processes *in vivo*.¹

Structurally, they are heterocyclic π -electron systems in which oxygen is the heteroatom. It has been established that in aqueous medium under *in vitro* conditions changes in the pH value cause changes in the structure of these molecules, which produce different colouring.² As the transformations of these molecules have so far been mostly studied by the relaxation, T jump and pH jump, methods,^{3–5} in the present work spectroabsorptiometry was used to observe the transformations of the malvin molecule over a wide pH range.

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It is known that flavonoid compounds show a relatively high activity in oxidative processes, especially with air oxygen, which makes them good antioxidants.

The aim of the present work was to correlate the spectroabsorptometrically defined structural forms of malvin at different pH values with the voltammetric measurements, and to define their antioxidant properties.

EXPERIMENTAL

The materials used in the present work were malvidin-3,5-diglucoside (malvin 97 %, Fluka Biochemika), quercetin (3,3',4',5,7 pentahydroxyflavone, Fluka Biochemika) and BHA (butylated hydroxyanisole, Merck). The spectrophotometric measurements were performed on a UV-VIS Pye Unicam SP8-100 spectrophotometer with 10 mm optical path quartz cuvettes. Acetate buffer solutions were used. The pH value was adjusted by adding the appropriate amounts of 0.06 M phosphoric acid (85 %, Poole, England) or 0.2 M NaOH (Zorka - Šabac) into a 0.02 M acetate buffer solution (p.a., Merck). The ionic strength of the solution (0.02 M) was adjusted by adding the appropriate amounts of NaCl (p.a., Merck). The pH was measured by an Iskra MA 5730 pH-meter with a combined electrode. The measurements were performed at 298 K. A potassium diphthalate solution (p.a., Merck) was used as a calibration standard buffer solution.

The cyclovoltammetric measurements were carried out on an EG&PAR 173 potentiostat with a PAR 175 programming unit. The cyclic voltammograms were recorded on a PAR RE 0074 X-Y plotter. A three-electrode system was used in the common triangular configuration, which consisted of a working electrode of glassy carbon ($A = 0.85 \text{ cm}^2$), a reference electrode Ag/AgCl (3.0 M KCl), and a platinum wire as auxiliary electrode. Prior to each measurement, the glassy carbon electrode was activated by polishing with alumina (2 μm), and carefully rinsed in bidistilled water.

The cyclovoltammetric measurements were performed over a range of potentials from -0.200 V to $+1.300 \text{ V}$, with a potential sweep rate of 20 mV/s. The cyclic voltammogram of the supporting buffer electrolyte was recorded first, and then an aliquot of the to be investigated solution was added, and a new voltammogram was recorded.

The concentration of malvin was $1 \times 10^{-4} \text{ mol dm}^{-3}$ in all the voltammetric measurements.

RESULTS AND DISCUSSION

a) Spectroabsorptometric measurements

The investigation of the structural transformations of malvin was performed in the pH range of pH 2.00 to pH 11.00. The electronic absorption spectra of malvin in the acidic and neutral media are presented in Fig. 1, whereas the spectra obtained in the alkaline media are presented in Fig. 2.

Primarily malvin transformations are observed that produce no change in its flavylium structure, *i.e.*, the process of deprotonation (Scheme 1) that occurs as the pH value increases is observed. This is important for the explanation of the redox properties of this molecule, as will be seen in the further text.

In the acidic medium at pH 2.00 (Fig. 1, curve 1) malvin exists primarily in its cationic form (AH^+). At that pH, the spectrum of malvin shows a characteristic absorption band of the cationic structure, with a maximum at λ_{max} 518 nm. After an increase in the pH value to 2.50 (Fig. 1, curve 2), the cation band undergoes a bathochromic shift to λ_{max} 520 nm, accompanied by a significant hypochromic shift. Further decrease of the acidity to pH 3.65 (Fig. 1, curve 3) produces a further bathochromic shift (λ_{max} 525 nm)

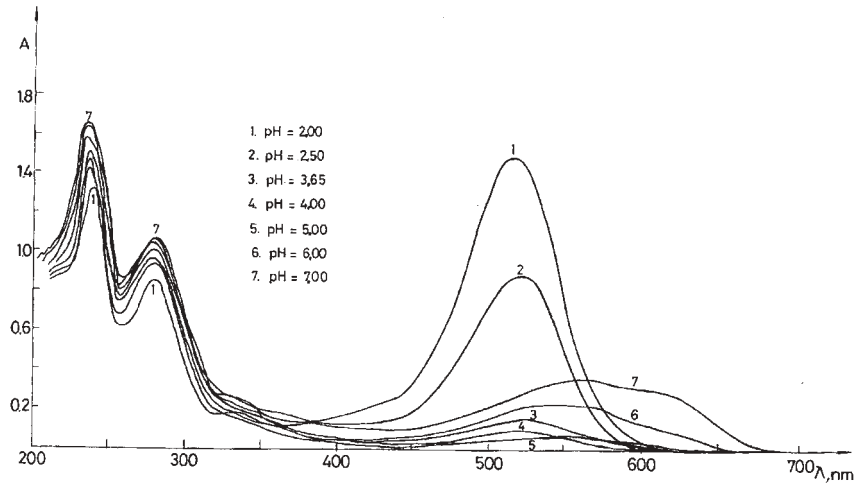


Fig. 1. Absorption spectra of malvin in acidic and neutral buffer solutions.

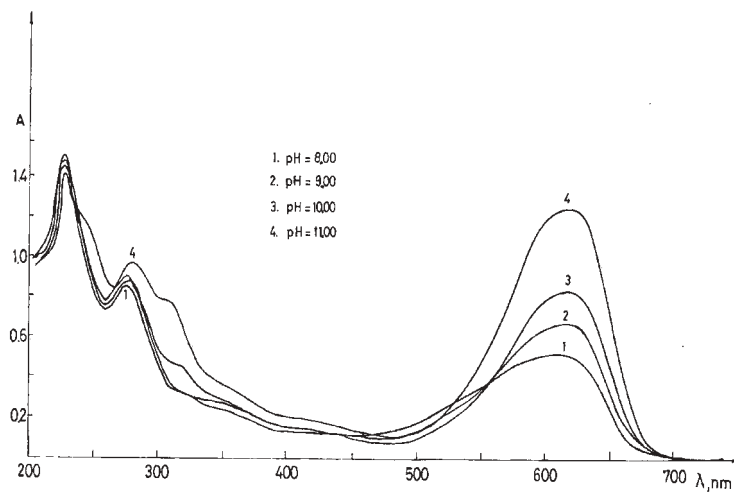
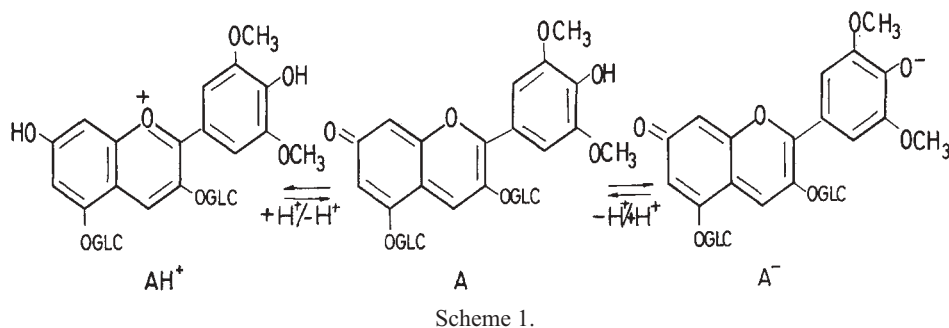


Fig. 2. Absorption spectra of malvin in alkaline buffer solutions.

and a decrease in the intensity. These changes follow the same course in the pH 4.00 buffer solution (Fig. 1, curve 4). It is obvious that the cationic form of malvin exists in the acidic buffer solutions used, but its percentage decreases with increasing pH. In the pH 5.00 buffer solution (Fig. 1, curve 5), the main absorption maximum significantly broadens, with a notable hypochromic effect. At pH 6.00 (Fig. 1, curve 6), the absorption spectrum changes significantly. A new absorption band appears, featuring a double, moderately expressed maximum, with peaks at λ_{\max} 530 nm and λ_{\max} 565 nm, as well as an inflection at λ_i 610 nm. The same trend is observable at pH 7.00 (curve 7) as well. These changes show that the process of deprotonation of the flavylium structure had taken place, resulting in the formation of a coloured anhydrous base (A, Scheme 1). It is believed that deprotonation begins first at position 7, and then continues at position



4'.² The anhydrous base formed possesses an additional double bond, which increases the number of π -electrons relative to the flavylum cation, causing an increased delocalization of the π -electrons, *i.e.*, a significant bathochromic shift of the main absorption band (λ_{\max} 570 nm). On further increasing of the pH, the process of deprotonation continues, it is assumed at position 4' of ring B, with the appearance of the anionic form of malvin, *i.e.*, the ionized form of the anhydrous base (A^- , Scheme 1). The beginning of the formation of the anionic form is indicated by the inflection in the main absorption band (λ^i 610 nm), already in the neutral environment. It can be said that the deprotonation of position 4' begins near neutrality, immediately after the deprotonation of position 7. The total deprotonation of position 4' and the appearance of the anionic form ends at pH 8.00 (Fig. 2, curves 1–4).

b) Voltammetric measurements

The antioxidant properties of malvin at different pH values were investigated by cyclic voltammetry. The recorded malvin cyclic voltammograms indicate its electrochemical activity over a wide range of pH values of the supporting electrolyte (pH 2.00 – 11.00). The cyclic voltammograms of malvin at three different pH values of the supporting electrolyte are presented in Fig. 3. In the range of pH values investigated only anodic – oxidation CV waves are present, whereas the absence of the cathodic – reduction CV waves at some pH values indicates the irreversibility of the electrode process.⁶ Although cathodic waves also appear, between pH 2.50 and pH 4.00, the ratios of the potentials *vs.* currents of the peaks confirm the general irreversible electrochemical behavior of malvin, according to the Nicholson-Shain criteria.⁷

Two OH groups are present in the structure of malvin, which indicates a possibility of electrochemical oxidation, following the same mechanism as in the case of phenol. On the other hand, these OH groups are, due to π -delocalization, in resonance, so that the electrochemical behavior of malvin can be compared with that of dihydroxy phenols, *e.g.*, hydroquinone.

The parameters of the CV peaks of malvin obtained for a series of pH values are presented in Table I. It is observable from Fig. 4 that the dependence of $E_p(\text{ox})$ on the pH is linear in the pH range between 2.00 and 5.00, with a slope of -60 mV/pH. The diffusion nature of the voltammetric peaks was established at pH 3.65, from the linear relationship between the current of the CV peak and the square root of the potential sweep

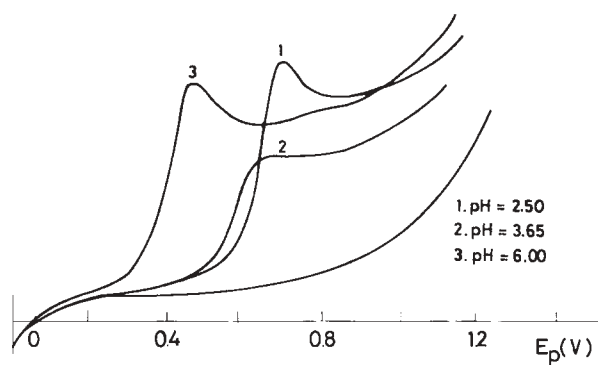


Fig. 3. Cyclic voltammograms of malvin ($c = 5 \times 10^{-4} \text{ mol dm}^{-3}$) for different pH values of the supporting electrolyte: 1) 2.50 2) 3.65 3) 6.00 (sweep rate 20 mV/s).

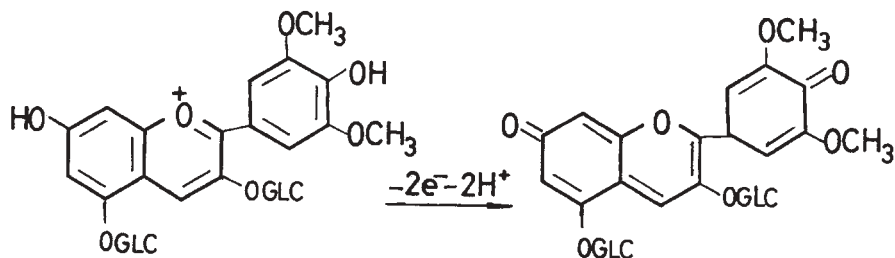
rate. The value of the diffusion coefficient of malvin, equal to $1.15 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$, was calculated from the equation for an irreversible process.⁸

TABLE I. The potentials, E_p , and currents, I_p , of the oxidation peaks of malvin (1) ($c = 1 \times 10^{-4} \text{ mol dm}^{-3}$), BHA (2) ($c = 1 \times 10^{-4} \text{ mol dm}^{-3}$), and quercetin (3) ($c = 1 \times 10^{-4} \text{ mol dm}^{-3}$), in dependence on the pH of the supporting electrolyte

pH	E_p^1 / V	$I_p^1 / \mu\text{A}$	E_p^2 / V	$I_p^2 / \mu\text{A}$	E_p^3 / V	$I_p^3 / \mu\text{A}$
2.00	+ 0.800	3.4				
2.50	+ 0.736	4.2	+ 0.660	6.4	+ 0.460	9.6
3.65	+ 0.676	1.4	+ 0.612	8.2	+ 0.400	8.2
4.00	+ 0.632	0.6				
5.00	+ 0.612	2.2	+ 0.556	5.4	+ 0.325	5.6
6.00	+ 0.468	2.6	+ 0.532	6.8	+ 0.260	9.4
7.00	+ 0.412	2.6	+ 0.476	6.2	+ 0.180	7.8
8.00	+ 0.376	3.6	+ 0.440	6.0	+ 0.120	5.4
9.00	+ 0.384	3.0				
10.00	+ 0.368	3.2				
11.00	+ 0.336	3.8				

The oxidation of malvin is a two-electron process in the pH range studied, following the eHHe type mechanism established for hydroquinones by Driebergen⁹ (Scheme 2).

A change of the dependence of E_p on the pH occurs in the pH range between 5.00 and 6.00 (Fig. 4). The slope of the curve increases, which indicates a change in the mechanism of the electrode oxidation, as a consequence of the change in the structure of the reduced form (malvin). The behavior, and the results of the spectroabsorptiometry in the pH range studied, lead to the conclusion that a quinoidal base is formed, represented by structure A (Scheme 1). In this pH range the other OH group also oxidizes according to the eH mechanism⁶ (Scheme 3). The values of the oxidation potentials in this pH range are significantly lower than those at lower pH values, indicating that the oxidation is facilitated by easier deprotonation.



Scheme 2.

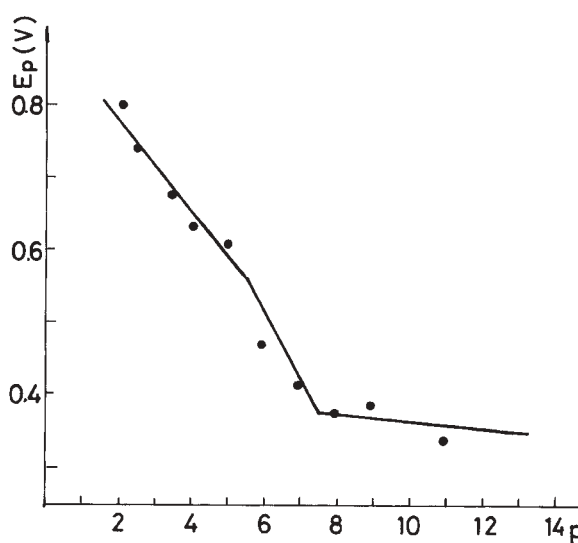
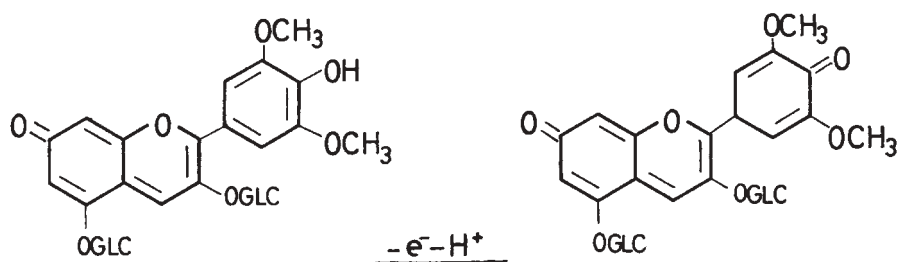


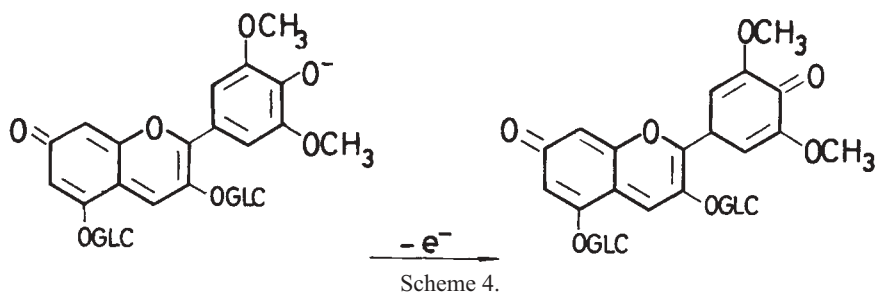
Fig. 4. Effect of the pH of the supporting electrolyte on the CV peak potentials of malvin.



Scheme 3.

At higher pH values, between pH 8.00 and pH 11.00, the dependence of $E_p(\text{ox})$ on the pH changes. The relationship is still linear, but with a very small slope, which leads to a conclusion that protons no longer participate in the electrode oxidation process, *i.e.*, that the reduced form is already chemically deprotonated (Scheme 4).

A comparison of the potentials of the oxidation CV peaks of malvin, BHA, and quercetin (Table I) shows that the antioxidant capacity in the pH range studied in-



creases in the series: malvin < BHA < quercetin. It is also to be expected that the peroxide numbers of these compounds are in the same order.^{10,11}

CONCLUSION

From the results of the cyclic voltammetric measurements of malvin, which are in complete correlation with its spectroabsorptiometric behavior, it can be concluded that this molecule possesses antioxidant properties in the pH range studied.

From the oxidation potentials obtained it can be assumed that the antioxidant capacity of malvin is significantly lower in acidic media than in neutral or basic media. At lower pH values, between pH 6.00 and pH 7.00, the oxidation potentials are much lower, indicating an increased antioxidant capacity. In the pH range between pH 8.00 and pH 11.00 the oxidation potential can be considered constant.

It can be concluded that the antioxidant capacity of malvin is mostly expressed in media with pH values between pH 6.00 and pH 7.00. This is supported by the results obtained from the electronic absorption spectra which reveal the structural transformations of malvin.

ИЗВОД

СПЕКТРОАПСОРПЦИОМЕТРИЈСКО И ВОЛТАМЕТРИЈСКО ПОНАШАЊЕ МАЛВИНА У ПУФЕРСКИМ РАСТВОРИМА И ЊЕГОВЕ АНТИОКСИДАТИВНЕ ОСОБИНЕ

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У овом раду испитиван је, у условима *in vitro*, преко електронских апсорпционих спектра, структурни трансформациони ток малвина у ацетатним воденим пуферским растворима широког опсега рН вредности. Поред спектрохемијског испитивано је и волтаметријско понашање као и механизам редокс процеса овог молекула у циљу дефинисања његових антиоксидативних особина. Преко електронских апсорпционих спектра констатовано је да промене у структури малвина, које су условљене рН вредностима средине, условљавају и различито волтаметријско понашање овог молекула. Различита зависност оксидационог потенцијала малвина од рН која се може објаснити присуством различитих структура молекула малвина, електрохемијски активних на различитим рН вредностима, указује на промену механизма електродног процеса. Волтаметријска активност малвина семиквантитативно је упоређена са вол-

таметријском активношћу стандардног синтетског антиоксиданса, ВНА, и природног антиоксиданса, кверцетина.

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