CHEMIJA. 2011. Vol. 22. No. 3. P. 162–166

Studies of quinone cytotoxicity mechanisms: determination of quinone / semiquinone redox couple potential according to quinone-mediated ascorbate oxidation kinetics

Audronė Marozienė[1],

Aušra Nemeikaitė-Čėnienė[2],

Narimantas Čėnas[1]*

[1] *Institute of Biochemistry of Vilnius University, Mokslininkų 12, LT-08662 Vilnius, Lithuania*

[2] *Center of Innovative Medicine, Molėtų pl. 29, LT-08409 Vilnius, Lithuania*

Quinone / semiquinone redox couple potentials in aqueous media (potentials of single-electron reduction, E_7^1) are usually determined by the pulse-radiolysis technique, but also alternative methods of redox potential determination are of some interest. We have found that the rate of oxygen uptake during ascorbate oxidation by a series of quinones ($n = 11$) with available E_7^1 values is characterized by a well-expressed log (rate constant) *vs.* E_7^1 relationship. It enabled us to obtain previously unknown E_7^1 values for several ($n = 7$) quinones with an error less than ±0.03 V. Our data enabled to demonstrate the role of acyloxy and hydroxyl substituents in the energetics of single-electron reduction of quinones and to clarify the mechanism of cytotoxicity of several quinones in bovine leukemia virus-transformed lamb kidney fibroblasts.

Key words: quinone, reduction potential, ascorbate, cytotoxicity

Abbreviations: E_7^1, potential of quinone / semiquinone redox couple at pH 7.0; Q, quinone; $AscH^-$, ascorbate; k_{app}, the apparent bimolecular reaction rate constant; cL_{50}, the compound concentration for 50% survival of mammalian cells.

INTRODUCTION

Quinones comprise an important group of physiologically active compounds. The cytotoxicity and, in some cases, therapeutical activity of quinones (Q) frequently stems from their single-electron reduction by flavoenzymes dehydrogenases-electrontransferases, e. g., NADPH: cytochrome P-450 reductase (EC 1.6.2.4) or ferredoxin : $NADP^+$ reductase (EC 1.18.1.2), which initiate the redox cycling of their anion-radicals ($Q^{\cdot-}$) with the subsequent formation of superoxide ($O_2^{\cdot-}$) and other activated oxygen species [1, 2]. The single-electron reduction of quinones by above enzymes follows an "outer sphere" electron transfer model [3] and is characterized by linear or parabolic dependence of log (rate constant) on the potential of quinone / semiquinone redox couple (single-electron reduction potential, E^1, or E_7^1 at pH 7.0) [2, 4]. On the other hand, if quinones act mainly through the oxidative stress, their cytotoxicity in mammalian cells increases with an increase in their E_7^1 with a relationship $\Delta\log cL_{50} / \Delta E_7^1 \sim -10\ V^{-1}$, where cL_{50} is compound concentration for 50% cell survival [4]. Thus, the potential of $Q / Q^{\cdot-}$ redox couple is an important parameter for the design of new quinoidal drugs and the analysis of their enzymatic activation. However, due to instability of quinone radicals, the values of E_7^1 are obtained mainly using a sophisticated pulse-radiolysis technique [5]. Therefore, it is of some interest to examine the possibilities of alternative simpler methods of E_7^1 determination, e. g., the linear log (rate constant) *vs.* E_7^1 relationships in enzymatic or chemical reduction reactions, which were previously used to obtain the unavailable E_7^1 values of nitroaromatic compounds [6, 7].

In this paper, we examine the kinetics of O_2 uptake during the quinone-mediated oxidation of ascorbic acid, which proved to be a useful tool for an accurate prediction of un-

* Corresponding author. E-mail: narimantas.cenas@bchi.vu.lt

available E_7^1 values of quinones. Apart from the elucidation of the role of acyloxy and hydroxy substituents in the energetics of single-electron reduction of quinones, these data enabled us to clarify some aspects of quinone mammalian cell cytotoxicity.

EXPERIMENTAL

2,3-Dihydroxy-1,4-naphthoquinone, 5-acetyloxy-1,4-naphthoquinone, 2-chrysanthemoyloxy-1,4-naphthoquinone (7), 2-chrysanthemoyloxy-3-hydroxy-1,4-naphthoquinone (15) and 1-hydroxy-2-chrysanthemoyloxy-9,10-anthraquinone (17) (trans-isomers), 5-chrysanthemoyloxy-1,4-naphthoquinone (8) (the ratio of cis- and trans-isomers, 1:2) (Fig. 1) were a generous gift of Prof. Avtandil Dolidze (P. Melikishvili Institute of Physical and Organic Chemistry, Tbilisi, Georgia), and were obtained as described [8, 9]. 2,5-Diaziridinyl-3-(hydroxyethyl)-6-methyl-1,4-benzoquinone (RH1) (Fig. 1) was a generous gift of Dr. Jonas Šarlauskas (Institute of Biochemistry, Vilnius). All other compounds were obtained from "Sigma" and used without further purification. The rate of oxygen consumption during the reduction of quinones by ascorbic acid was monitored in 0.1 M K-phosphate buffer (pH 7.0) containing 1 mM EDTA at 25 °C, using a Digital Model 10 Clark electrode (Rank Brothers Ltd.). During the experiments, the initial rates (0–5 min) of ascorbate autooxidation were recorded, and then quinones from the stock solution in DMSO (factor of dilution 1 : 200) were introduced into the cell. In order to obtain the bimolecular reaction rate constants, the reaction rates were corrected for the rate of ascorbate autooxidation. The rate of oxygen consumption was calculated from the slope of $[O_2]$ *vs* time. The initial O_2 concentration was assumed to be 250 μM [5].

The culture of bovine leukemia virus-transformed lamb kidney fibroblasts (FLK line) was grown and maintained in Eagle's medium supplemented with 10% fetal bovine serum and antibiotics at 37 °C as described previously [4]. In the cytotoxicity experiments, cells (2.5×10^4/ml) were seeded on glass slides in 5 ml flasks in the presence or absence of compounds, and were grown for 24 h. Further, the slides were rinsed 3–4 times with phosphate buffer saline and stained with trypan blue. Cells on the slides were counted under a light microscope.

The statistical and multiparameter regression analysis was performed using Statistica (version 4.3) (Statsoft Inc., 1993). The pK_a values of hydroxynaphthoquinones were calculated using an ACD / ChemSketch (version 4.02, Advanced Chemistry Development, Toronto, Ontario, Canada).

RESULTS AND DISCUSSION

It has been previously shown that the uptake of O_2 occurs during the single-electron reduction of quinones (Q) by ascorbate ($AscH^-$) [10,11]:

$$Q + AscH^- \rightarrow Q^{\cdot-} + Asc^{\cdot-} + H^+, \quad (1)$$

$$Q^{\cdot-} + O_2 \rightarrow Q + O_2^{\cdot-}, \quad (2)$$

$$Asc^{\cdot-} + Asc^{\cdot-} + H^+ \rightarrow AscH^- + \text{dehydroascorbate}, \quad (3)$$

$$O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow O_2 + H_2O_2. \quad (4)$$

In this case, a linear relationship exists between the E_7^1 of low-potential quinones ($E_7^1 \leq -0.08$ V) and the log of the apparent rate constant of quinone reduction, k_{app}, which is expressed as

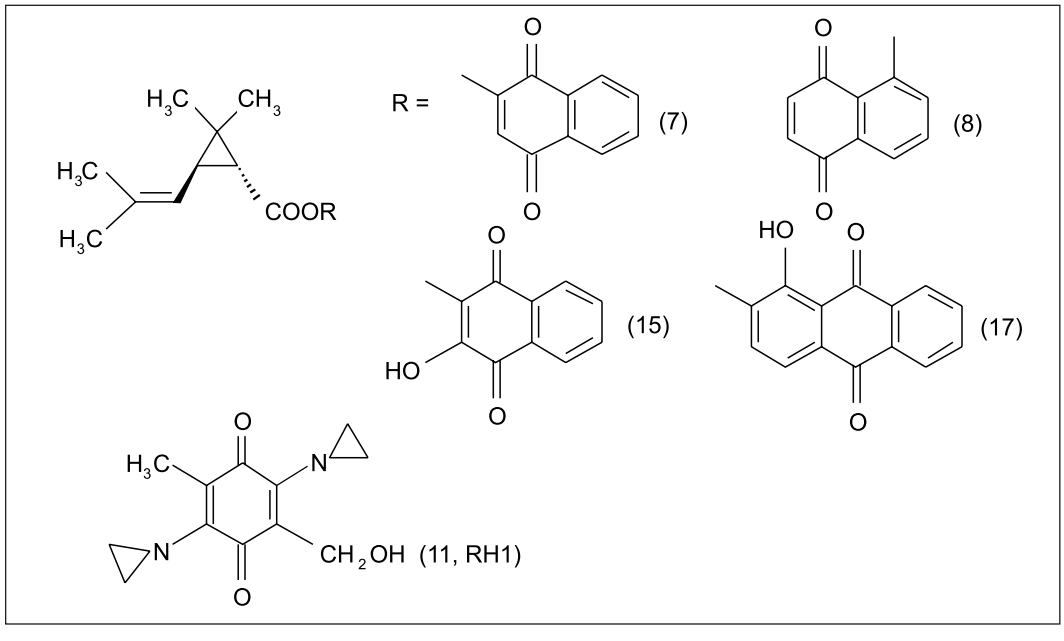

Fig. 1. Formulae of nontrivial quinone compounds used in the study. Their numbers correspond to those in Table

$$k_{app} = v / [Q][AscH^-], \quad (5)$$

where v is the initial rate of O_2 uptake [11]. Because at a high conversion degree the reaction deviates from pseudo-first order kinetics, the initial reaction rates were used to calculate the rate constants instead of data linearization in semilogarithmic coordinates [11]. We found that at fixed ascorbate concentration, 1.0 mM, the initial rate of O_2 uptake was directly proportional to the quinone concentration (1.0–20 μM for compounds 1–9 and 20–150 μM for slowly reacting compounds 10–18 (Table)). Alternatively, at a fixed concentration (2.0 μm) of quinones 1, 3, 6 (Table), the rate of O_2 uptake was proportional to ascorbate concentration 0.2–1.0 mM. On the other hand, in accordance with previous observations [10], the O_2 uptake starts to attain a constant rate at ascorbate concentrations above 1.5 mM (data not shown). This may be attributed to the increased rate of reaction (6) at a high ascorbate concentration, which prevents the oxidation of $Q^{\cdot-}$ by O_2 [12]:

$$Q^{\cdot-} + AscH^- + H^+ \rightarrow QH_2 + Asc^{\cdot-}. \quad (6)$$

Therefore, the values of k_{app} for quinones (Table) were determined in the presence of 1.0 mM ascorbate. In this case, the ascorbate autooxidation rate was equal to 0.1 μM/min, and the rates of quinone-mediated ascorbate oxidation varied from 70 μM/min to 0.2 μM/min. We observed a well pronounced linear correlation between log k_{app} and E^1_7 of quinones (Fig. 2):

$$\log k_{app} = (2.923 \pm 0.182) + (11.875 \pm 0.770)\, E^1_7, (r^2 = 0.964), \quad (7)$$

which enabled us to calculate the unavailable E^1_7 values for compounds 7–10, 12, 14, and 16 (Table). Because the differences between the experimentally determined E^1_7 values and $E^1_{7(calc.)}$ of quinones did not exceed ±0.03 V (Table), the values of $E^1_{7(calc.)}$ are considered to be realistic. For example, the $E^1_{7(calc.)}$ for alizarin (compound 14) is similar to that of other dihydroxyanthraquinones (compounds 13, 16, Table).

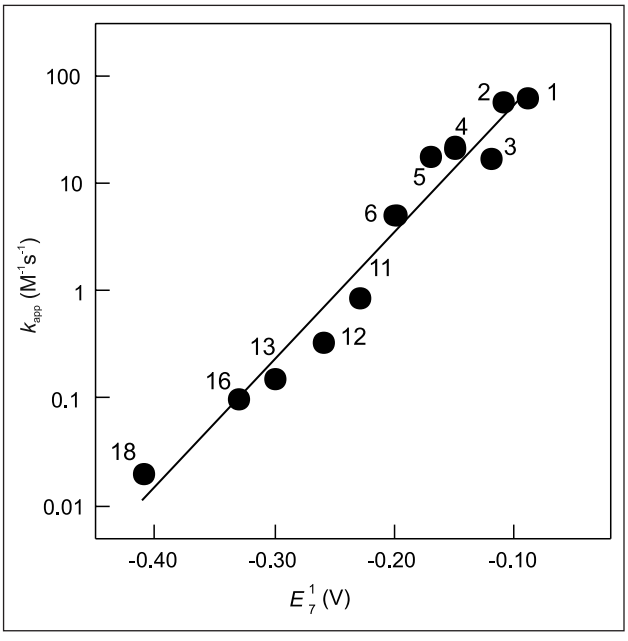

Fig. 2. Correlation between the apparent rate constant of ascorbate oxidation by quinones and quinone E^1_7 values. The numbers of compounds are taken from Table

Table. **Apparent rate constants of quinone reduction by ascorbic acid (k_{app}) at pH 7.0 and 25 °C, calculated according to Eq. (5), the experimentally determined values of single-electron reduction potentials of quinones (E^1_7) [2, 5], their E^1_7 values calculated according to Eq. (7) ($E^1_{7(calc.)}$), and their concentrations for 50% survival of FLK cells (cL_{50})**

No.	Quinone	k_{app} ($M^{-1}s^{-1}$)	E^1_7(V)	$E^1_{7(calc.)}$ (V)	cL_{50} (μM)
1.	5-Hydroxy-1,4-naphthoquinone	62.5 ± 1.5	–0.09	–0.10	0.50 ± 0.10[a]
2.	5,8-Dihydroxy-1,4-naphthoquinone	58.3 ± 2.0	–0.11	–0.10	0.33 ± 0.05[a]
3.	9,10-Phenanthrene quinone	16.7 ± 1.0	–0.12	–0.15	0.70 ± 0.08[a]
4.	1,4-Naphthoquinone	21.6 ± 1.9	–0.15	–0.14	1.60 ± 0.10[a]
5.	Trimethyl-1,4-benzoquinone	18.0 ± 2.0	–0.17	–0.14	5.00 ± 1.20
6.	2-Methyl-1,4-naphthoquinone	5.0 ± 0.6	–0.20	–0.19	3.50 ± 0.30[a]
7.	2-Chrysanthemoyloxy-1,4-naphthoquinone	4.5 ± 0.5	–	–0.19	7.40 ± 0.50
8.	5-Chrysanthemoyloxy-1,4-naphthoquinone	2.0 ± 0.2	–	–0.22	1.90 ± 0.10[b]
9.	5-Acetyloxy-1,4-naphthoquinone	1.9 ± 0.2	–	–0.22	3.30 ± 0.40
10.	2,3-Dihydroxy-1,4-naphthoquinone	0.70 ± 0.09	–	–0.26	63.0 ± 6.00[b]
11.	RH1	0.85 ± 0.10	–0.23	–0.25	0.11 ± 0.01[a]
12.	Tetramethyl-1,4-benzoquinone	0.33 ± 0.05	–0.26	–0.28	16.0 ± 3.00[a]
13.	1,8-Dihydroxy-9,10-anthraquinone	0.15 ± 0.02	–0.30	–0.31	120 ± 15.0[a]
14.	1,2-Dihydroxy-9,10-anthraquinone	0.13 ± 0.01	–	–0.32	83.0 ± 10.0
15.	2-Chrysanthemoyloxy-3-hydroxy-1,4-naphthoquinone	0.11 ± 0.03	–	–0.33	100 ± 14.0[b]
16.	1,4-Dihydroxy-9,10-anthraquinone	0.10 ± 0.01	–0.33	–0.33	240 ± 20.0
17.	1-Hydroxy-2-chrysanthemoyloxy-9,10-anthraquinone	0.10 ± 0.02	–	–0.33	123 ± 20.0
18.	2-Hydroxy-1,4-naphthoquinone	0.02 ± 0.003	–0.41	–0.38	700 ± 100[a]

[a] From Ref. [4]. [b] From Ref. [16].

Our data highlighted the influence of acyloxy substituent on the energetics of single-electron reduction of quinones, which, to our best knowledge, has not been addressed before. According to Hammett constants for inductive (σ_I) and resonance effects (σ_R), acyloxy substituent possesses both weak electron accepting ($\sigma_I = 0.41$) and electron donating ($\sigma_R = -0.19$) properties [13]. For comparison, the methyl group is characterized by $\sigma_I = -0.05$ and $\sigma_R = -0.13$ and the nitro group by $\sigma_I = 0.65$ and $\sigma_R = 0.16$ [13]. A comparison of $E^1_{7(calc.)}$ values for compound (7), 2-methyl-1,4-naphthoquinone (6), and 1,4-naphthoquinone (4) (Table) shows that the electron donating properties of the acyloxy group prevail in this case because, in general, the presence of electron donating groups decrease the reduction potentials of quinones. In contrast, the H-bond of the 5-OH group of juglone (1) with the quinone carbonyl group stabilizes its semiquinone and increases its E^1_7 as compared with 1,4-naphthoquinone (Table), irrespectively of the electron donating properties of the OH group [14]. The esterification of 5-OH group disrupts the H-bond and decreases the reduction potential of compound (8). The interpretation of the difference between the E^1_7 values for 2-hydroxy-1,4-naphthoquinone (18) and compound (15) (Table) is less straightforward. On the other hand, an increasse in the $E^1_{7(calc.)}$ value of compound (10) with respect to that of 2-hydroxy-1,4-naphthoquinone (18) (Table) may be explained by the formation of intramolecular H-bonds. At pH 7.0, the 2-OH group in the quinone ring is deprotonated ($pK_a = 4.2$ [15]) due to strong electron accepting properties of the quinone moiety. Calculations using ACD / ChemSketch software show that the presence of a second –OH group in 2,3-dihydroxy-1,4-naphthoquinone increases its pK_a, giving $pK_{a(calc.)} = 7.3$. This value may be even higher, because for 2-hydroxy-1,4-naphthoquinone $pK_{a(calc.)} = 4.0$, and for 5,8-dihydroxy-1,4-naphthoquinone with $pK_a = 7.85$ [14] $pK_{a(calc.)}$ is equal to 7.3. Thus, an introduction of a second –OH group into the quinone ring results in the formation of two H-bonds that stabilize semiquinone and may increase the E^1_7 of the compound. In accordance with this, 2,3-dihydroxy-1,4-naphthoquinone is reduced by single-electron transferring ferredoxin: $NADP^+$ reductase ten times faster than 2-hydroxy-1,4-naphthoquinone [16].

The obtained E^1_7 values of quinones enabled us to clarify the mechanism of the cytotoxicity of chrysanthemoyl-substituted quinones (compounds 7, 8, 15, 17, Table) in FLK cells whose several representatives have been studied previously [16]. The esters of chrysanthemic acid are essential building blocks of pyrethroids, a class of natural insecticides [17], and chrysanthemic acid and its methyl ester in FLK cells possess cL_{50} values of 850 ± 120 μM and 720 ± 90 μM, respectively [16]. Thus, it is important to assess whether the presence of the chrysanthemate group affects quinone cytotoxicity. We found that the log cL_{50} values of chysanthemoyl-substituted and unsubstituted quinones, except RH1, follow a well pronounced negative dependence on their $E^1_{7(calc.)}$ (Table, Fig. 3) with the coefficient $\Delta \log cL_{50} / \Delta E^1_{7(calc.)} = -10.845 \pm 0.935\ V^{-1}$

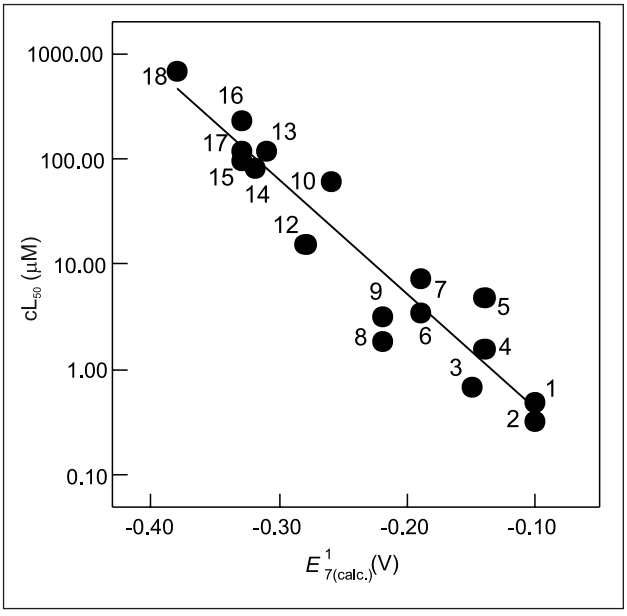

Fig. 3. Correlation between the cytotoxicity of quinones in FLK cells and their $E^1_{7(calc.)}$ values. The numbers of quinones are taken from Table

($r^2 = 0.900$). RH1 was omitted fom the correlation because of its enhanced toxicity due to the presence of aziridinyl groups [4]. Taken together with the protective effects of antioxidants against the cytotoxity of 5-chrysanthemoyl-1,4-naphthoquinone [16], it implies that chrysanthemoyl-substituted quinones act mainly through the oxidative stress and that the role of the chrysanthemate-depending mode is insignificant.

CONCLUSIONS

Data of this study show that the monitoring of the kinetics of O_2 uptake during the quinone-mediated oxidation of ascorbic acid may be a useful tool for an accurate prediction of unavailable E^1_7 values of quinones in the range –0.10 – –0.40 V. Apart from the elucidation of the role of acyloxy and hydroxy substituents in the energetics of a single-electron reduction of quinones, these data enabled us to clarify some aspects of quinone mammalian cell cytotoxicity.

ACKNOWLEDGEMENTS

The support of the Scientific Council of Lithuania (the Project of the Global Grant Measure No. VP1-3.1-ŠMM-07-K01-103) is gratefully acknowledged. We thank Professor Avtandil Dolidze (Institute of Physical and Organic Chemistry, Tbilisi, Georgia) for his generous gift of chrysanthemoyl-substituted and other quinones and Dr. Jonas Šarlauskas (Institute of Biochemistry, Vilnius) for calculations using ACD / ChemSketch software.

Received 12 May 2011
Accepted 22 June 2011

References

1. P. J. O'Brien, *Chem.-Biol. Interact.*, **80**, 1 (1991).
2. N. Čėnas, Ž. Anusevičius, H. Nivinskas, L. Misevičienė, J. Šarlauskas, *Methods Enzymol.*, **382B**, 258 (2004).
3. R. A. Marcus, N. Sutin, *Biochim. Biophys. Acta,* **811**, 265 (1985).
4. A. Nemeikaitė-Čėnienė, J. Šarlauskas, Ž. Anusevičius, H. Nivinskas, N. Čėnas, *Arch. Biochem. Biophys.*, **416**, 110 (2003).
5. P. Wardman, *J. Phys. Chem. Ref. Data*, **18**, 1637 (1989).
6. J. Šarlauskas, H. Nivinskas, Ž. Anusevičius, L. Misevičienė, A. Marozienė, N. Čėnas, *Chemija*, **17**, 31 (2006).
7. M. Uchimiya, L. Gorb, O. Isayev, M. M. Qasim, J. Leszczynski, *Environ. Pollut.*, **158**, 3048 (2010).
8. N. Kavtaradze, T. Kurtskhalia, T. Korkia, T. Shatakishvili, R. Chedia, A. Dolidze, *Georgia Chem. J.*, **5**, 561 (2005).
9. I. Jinikashvili, N. Kavtaradze, A. Dolidze, R. Chedia, *Georgian Engineer. News*, **1**, 251 (2006).
10. Ž. Anusevičius, A. Ramanavičius, J. Šarlauskas, *Chem. Papers,* **52**, 643 (1998).
11. V. A. Roginsky, T. K. Barsukova, H. B. Stegmann, *Chem.-Biol. Interact.*, **121**, 177 (1999).
12. V. Roginsky, C. Michel, W. Bors, *Arch. Biochem. Biophys.*, **384**, 74 (2000).
13. C. Hansch, A. Leo, R. W. Taft, *Chem. Rev.*, **91**, 165 (1991).
14. E. J. Land, T. Mukherjee, A. J. Swallow, J. M. Bruce, *J. Chem. Soc. Faraday Trans. I.*, **79**, 391 (1983).
15. S. I. Bailey, I. M. Ritchie, *Electrochim. Acta*, **30**, 3 (1985).
16. A. Dolidze, R. Chedia, N. Kavtaradze, L. Misevičienė, V. Miliukienė, A. Nemeikaitė-Čėnienė, N. Čėnas, *Chemija*, **20**, 116 (2009).
17. Y. Katsuda, *Pestic. Sci.*, **55**, 775 (1999).

Audronė Marozienė, Aušra Nemeikaitė-Čėnienė, Narimantas Čėnas

CHINONŲ CITOTOKSIŠKUMO MECHANIZMŲ TYRIMAI: CHINONO / SEMICHINONO REDOKS POROS POTENCIALO NUSTATYMAS PAGAL CHINONŲ KATALIZUOJAMOS ASKORBATO OKSIDACIJOS KINETIKĄ

S a n t r a u k a

Kadangi chinono / semichinono redoks poros potencialai (vienelektroninės redukcijos potencialai (E_7^1)) vandeninėje terpėje yra įprastai nustatomi taikant sudėtingus impulsinės radiolizės metodus, susidomėjimo verti ir alternatyvūs E_7^1 nustatymo metodai. Tirdami deguonies suvartojimo greitį askorbato oksidacijos chinonais metu, nustatėme, kad daugelio chinonų ($n = 11$) su žinomais E_7^1 reaktingumas yra aprašomas gerai išreikšta tiesine greičio konstantos logaritmo priklausomybe nuo E_7^1. Tai leido mums nustatyti kelių ($n = 7$) chinonų anksčiau nežinomus vienelektroninės redukcijos potencialus su mažesne nei ±0,03 V paklaida. Mūsų duomenys leido atskleisti aciloksi- ir hidroksipakaitalų reikšmę vienelektroninės chinonų redukcijos energetikoje ir patikslinti kai kurių chinonų citotoksiškumo mechanizmus galvijų leukemijos virusu transformuotuose ėriuko inkstų fibroblastuose.