Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 43 (2007) 1580–1586
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Short communication

Combined antioxidant effects of rutin and Vitamin C in Triton X-100 micelles

Rong Guo *, Ping Wei, Weiya Liu

School of Chemistry and Chemical Engineering, Yangzhou University, Yangzhou 225002, PR China

Received 18 August 2006; received in revised form 22 November 2006; accepted 22 November 2006
Available online 27 December 2006

Abstract

UV–vis spectra, fluorescence emission spectra and cyclic voltammetric measurements were used to study the influence of Vitamin C on the antioxidant of rutin in Triton X-100 micelles. Rutin can be located in Triton X-100 micelles spontaneously through hydrophobic force, and the binding constant *K* between rutin and Triton X-100 increases with the rutin concentration. The embedment of two hydroxyl groups on rutin into the more hydrophobic micellar microenvironment makes the oxidation of rutin harder and the radical scavenging activity decrease. With low concentration of Vitamin C, the antioxidant capacity of rutin against hydroxyl radical is enhanced, while that capacity is partly inhibited when the concentration of Vitamin C become higher.

Keywords: Rutin; Vitamin C; Triton X-100 micelles; Binding constant; Hydroxyl radical

1. Introduction

There has been an increasing concern for free-radical clearance. Human body contains various biomacromolecules such as proteins, lipids, vitamins, and carbohydrates, which are vulnerable to be attacked by reactive oxygen species (ROS). ROS include superoxide radical anion (O_2^{-}), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$) and singlet oxygen ($^1O_2$), which are normally generated during physiological metabolic activities [1,2]. ROS have been linked with aging and many degenerative diseases such as cancer, inflammation, immune system decline, cardiovascular diseases, neurological diseases, and atherosclerosis [3,4]. While synthetic antioxidants have potential health hazards, the search for natural radical scavengers (antioxidants) is of great interest among scientists [5,6].

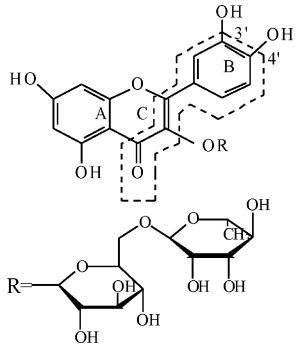
Flavonoids, a large class of phenolic compounds widely distributed in plants and vegetables, have been reported to be strong antioxidants and radical scavengers [7–9]. Rutin (Scheme 1) is a kind of flavonoid glycoside known as Vitamin P and has capacity in antiplatelet, antiviral, antihypertensive, and strengthing the capillaries of blood vessels [10,11], which are the results of its high radical scavenging activity and antioxidant capacity [12–14]. These properties are potentially beneficial in preventing diseases and protecting the stability of the genome.

Vitamin C (ascorbic acid, Scheme 2) is also a well-known natural antioxidant [15–17]. For example, Vitamin C can recycle the lipid-soluble Vitamin E by reducing α-tocopheroxyl radicals in membranes [18]. Besides its ability to scavenge various kinds of free radicals, synergistic antioxidant effects are also present in the combinations of Vitamin C with other phenolic antioxidants [19]. Thus, the co-application of Vitamin C and rutin may provide different protective effects against free-radical oxidation, which will be helpful for oxidation-related diseases prevention.

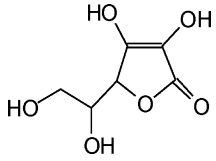
In the present research, the antioxidant capacity of rutin with Vitamin C were investigated in Triton X-100 micelles by UV–vis spectra, fluorescence emission spectra and cyclic voltammetric measurements. Triton X-100 is a kind of nonionic surfactant, which is widely used in pharmic field for its lower toxicity, better biologic compatibility as well as rich diversity in microstructure and the properties [20]. Further, Triton X-100 micelles can be used to simulate the cells and proteins because there are no electrostatic interactions between Triton X-100 and other substances.

* Corresponding author. Tel.: +86 514 7975219; fax: +86 514 7311374.
E-mail address: guorong@yzu.edu.cn (R. Guo).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.11.029



Scheme 1. The structure of rutin.



Scheme 2. The structure of Vitamin C.

2. Materials and methods

2.1. Materials

Triton X-100 (>99%) was obtained from Aldrich. Rutin and Vitamin C (>99%) were purchased from the Merck and Nanjing Chemical Reagent Company, respectively. Hydroxyl radical was generated by Fenton reagents [21] and the final Fe^{2+} and H_2O_2 concentration in the system were 4×10^{-4} mol/l and 1×10^{-3} mol/l, respectively. The water used was double-distilled and the other chemicals were of analytical reagent grade.

2.2. UV–vis spectra measurements

Rutin and Vitamin C were dissolved in Triton X-100 micelles. After 0.5 h mixing and equilibrium, the spectra were recorded in the range of wavelengths 200–500 nm using an UV-2501 spectrophotometer (SHIMADZU). Distilled water and corresponding solvent were used as the blank.

2.3. Fluorescence spectra measurements

The fluorescence emission spectra of rutin were recorded with a RF-5301 PC spectrofluorophotometer (SHIMADZU) in the range of 480–600 nm when excited at 444 nm. The emission intensity I of rutin in Triton X-100 micelles with Vitamin C or not and the fluorescence intensity I_0 of the system in the absence of Triton X-100 micelles were combined to give the binding constant K [22]:

$$\left[\frac{I}{I_0} - 1\right]^{-1} = \left[\frac{I_M}{I_0} - 1\right]^{-1} \times \left[1 + \frac{1}{\gamma K(C_S - \text{cmc2})}\right] \quad (1)$$

Here, I_M is the maximum fluorescence intensity obtained, C_S the concentration of the surfactant (which is larger than the second critical micelle concentration cmc2), and γ is the extinction coefficient ($\gamma = 1$). The plot of $[(I/I_0) - 1]^{-1}$ versus $(C_S - \text{cmc2})^{-1}$ gives a straight line and the value of the binding constant K can be obtained from the quotient of the intercept and slope of this line.

2.4. Measurements of microenvironment polarity

Pyrene, used as probe, was dissolved in Triton X-100/rutin/Vitamin C mixed solution. Its fluorescence spectra show five emission peaks when exited at 338 nm. The intensity ratio of the first (at about 370 nm) to the third peak (at about 380 nm) can show the polarity of the microenvironment where the probe exists. Furthermore, the location of rutin in the Triton X-100 micelles can be determined from the variety of I_1/I_3 values.

2.5. Determination of the voltammetric properties of rutin

Cyclic voltammetric experiments were carried out with a CHI 660a electrochemical workstation (Zhenghua, Shanghai). The electrode system consists of glass carbon as working electrode (diameter 3.0 mm), platinum as auxiliary electrode, and saturated calomel as reference electrode. NaH_2PO_4 solution (0.59 mol/l) was used as supporting electrolyte.

3. Results and discussion

3.1. The effects of Vitamin C on the interaction between rutin and Triton X-100 micelles

Rutin, a kind of flavonoids, exhibits two main absorption bands, referring to the π–π^* transitions of A–C ring (at about 260 nm) and B–C ring (at about 350 nm) [23]. We choose the B–C ring band of rutin as our object for investigation because the 3′,4′-dihydroxy on the B ring is the active part for antioxidant. It was reported that the ratio of fluorescence intensity of excimer I_e (at about 475 nm) to that of monomer I_m (at about 394 nm) of pyrene, i.e. I_e/I_m has relationship with the structure of surfactant aggregations [24,25]. Therefore, the curve of I_e/I_m versus the concentration of surfactant exhibits three ranges separated by two turning points marked in Fig. 1, which correspond to the concentration for structural changes of surfactant from pre-micelle to spherical micelle (cmc1) and spherical to rod-like micelle (cmc2). Based on this fact, we obtained cmc1 and cmc2 in Triton X-100 aqueous solution (Fig. 1). The values are respectively close to the 3.2×10^{-4} mol/l and 1.3×10^{-3} mol/l obtained by the diffusion coefficient method [26], indicating that the values of cmc1 and cmc2 obtained through this method are credible. Table 1 shows the cmc1 and cmc2 in the systems of Triton X-100 alone, Triton X-100 with rutin, and Triton X-100 with rutin and Vitamin C. There is no evident change between these values, which means that the addition of rutin and Vitamin C has no effect on the cmc of Triton X-100. Then three concentrations of Triton X-100 marked with a, b and c in Fig. 1, which represent pre-micelle, spherical micelle and rod-like micelle, respectively are selected for the following study.

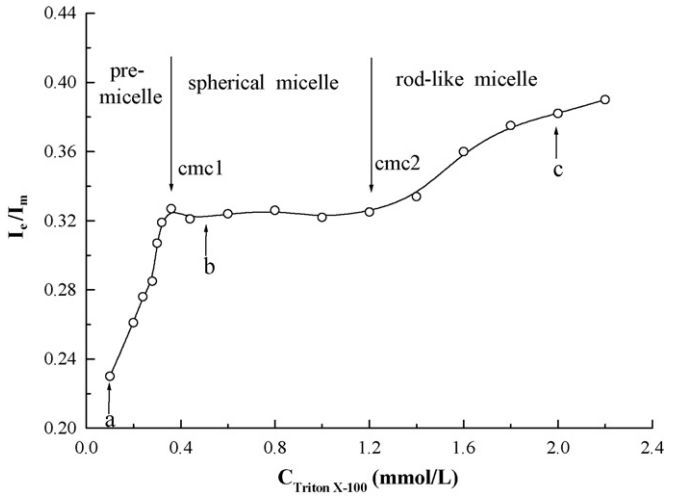


Fig. 1. The I_e/I_m value of pyrene as a function of Triton X-100 concentration.

Table 1
cmc1 and cmc2 values in different systems

Systems	Triton X-100	Triton X-100/rutin	Triton X-100/rutin/Vitamin C
cmc1	3.1×10^{-4}	3.5×10^{-4}	3.4×10^{-4}
cmc2	1.3×10^{-2}	1.2×10^{-2}	1.5×10^{-2}

Note: rutin concentration = 4×10^{-5} mol/l; Vitamin C concentration = 1×10^{-4} mol/l.

Fig. 2(A) shows the absorption spectra of rutin in the Triton X-100 micelles with different structures. The peak intensity of rutin decreases without any peak shift in Triton X-100 pre-micelles and spherical micelles, and the peak intensity of rutin further decreases in Triton X-100 rod-like micelles with a bathochromic shift from 353 nm to 363 nm. The fluorescence emission spectra of rutin in three kinds of Triton X-100 micelles are also different from each other: the fluorescence intensity of rutin shows almost no change in Triton X-100 pre-micelle, only a slight increase in spherical micelle, but a distinct increase in rod-like micelle Fig. 2(B). When the concentration of Triton X-100 is low, i.e. there are no micelles, rutin molecules are covered with one or more Triton X-100 molecules through hydrogen bonding between the hydroxyls of rutin and the hydrophilic groups of Triton X-100 molecules. While the concentration of Triton X-100 increases over cmc1, rutin molecules may partially insert into Triton X-100 micelles (both spherical and rod-like micelles) through hydrogen bonding and hydrophobic interaction because micelles provide favorable hydrophobic environment. However, our results show the absorption peak of rutin bathochromic shifts only in Triton X-100 rod-like micelles, not in spherical micelles, which may be due to the differences between spherical and rod-like micelles from the point of view of microstructure: the rod-like micelle is longer than spherical micelle and also the solubilization space is larger. From the structure of rutin (Scheme 1), we can see that rutin tends to insert into Triton X-100 rod-like micelles with the B ring part because of the hydrophilicity of glycoside part and the steric effect of A–C ring. The compact structure of Triton X-100 rod-like micelles makes the rigidity of chromophore at 353 nm of rutin reinforced and the π–π-conjugation effect increased, subsequently causing a bathochromic shift in the absorption peak. The significant increase of rutin fluorescence intensity is also due to the enrichment of rutin in Triton X-100 rod-like micelles and the reduced chance of collisional quenching.

Fig. 3 shows the influence of Vitamin C on the absorption of rutin in Triton X-100 micelles with different structures. The absorption intensity of rutin at the very start increases markedly with the Vitamin C concentration, and then achieves a flat, this phenomenon is just reverse to relationship between pH values and the concentrations of Vitamin C (Fig. 3 curve a). The pH value of the system decreases rapidly with increasing Vitamin C concentration at first, and then this downtrend gets gradually slow when Vitamin C concentration is continually increased, which corresponds to the equilibrium of ionization process of Vitamin C. So it can be inferred that the ionization process of Vitamin C influences the pH value of system, and then affects the absorption intensity of rutin and this deduction is subsequently validated by the absorption of rutin in NaH_2PO_4 buffer solution (figure not shown).

Rutin can be solubilized in Triton X-100 micelles, but where rutin molecules locate in the micelles is of great importance to understand the interaction between rutin molecules and Tri-

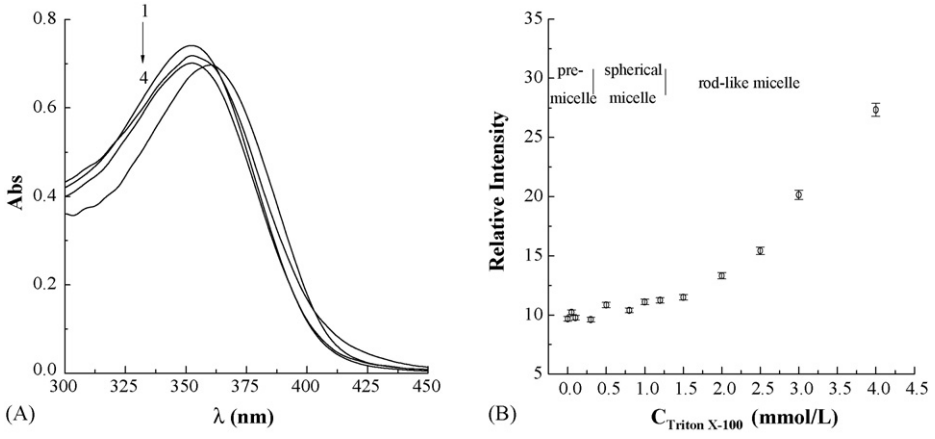


Fig. 2. The effect of Triton X-100 micelles on the absorption (A) and fluorescent (B) spectra of rutin (4.0×10^{-5} mol/l). (A) Concentration of Triton X-100 (mol/l): (1) 0.0, (2) 1.0×10^{-4}, (3) 5.0×10^{-4}, (4) 2.0×10^{-3}; (B) Ex = 444 nm.

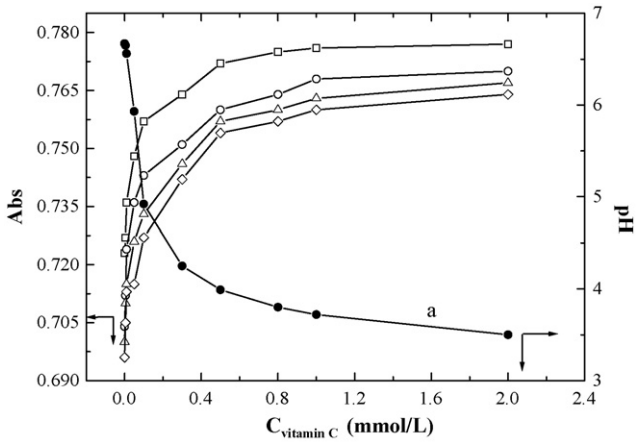


Fig. 3. The influences of the Vitamin C on the absorbance of rutin (4.0×10^{-5} mol/l) in Triton X-100 micelles and the pH value of the system without Triton X-100. Concentration of Triton X-100 (mol/l): (□) 0, (◯) 5×10^{-5}, (△) 5×10^{-4}, (◇) 2×10^{-3}.

ton X-100 micelles. It is known that the I_1/I_3 value of pyrene can be used to measure the polar environment of micelles [20]. The relation between I_1/I_3 value and Triton X-100 concentration with rutin is shown in Table 2. It is clear that the values of I_1/I_3 decrease from 1.610 to 1.354 and to 1.351 with increasing Triton X-100 concentration, indicating that the microenvironment polarity of pyrene decreases with the micelle structure changing from pre-micelle to spherical and to rod-like. The addition of rutin also reduces the I_1/I_3 values of pyrene both in Triton X-100 spherical and rod-like micelles, which means the hydrophobic and hydrogen bond interactions between rutin and Triton X-100 micelles enable rutin to be located in the palisade of Triton X-100 micelles, and thus change the location of the pyrene in the Triton X-100 micelles.

The influence of Vitamin C on the micropolarity of pyrene in Triton X-100 rod-like micelles is also studied, as shown in Table 3. The I_1/I_3 ratio of pyrene in the system of Triton X-100 rod-like micelles with Vitamin C takes an average value of 1.350 for all Vitamin C concentration, implying that Vitamin C has no distinct influence on the microenvironment where pyrene exists because Vitamin C is water-soluble molecule and is located mainly outside Triton X-100 micelles. When Vitamin C is added into the system of Triton X-100 rod-like micelles with rutin, the I_1/I_3 ratio also takes an average value of 1.075, just decreases slight when Vitamin C is over 1×10^{-4} mol/l. The influences of Vitamin C both on the absorption intensity and I_1/I_3 value of rutin/Triton X-100 system indicate that Vitamin C has no influence on the interaction between rutin and Triton X-100 micelles.

Table 2
The micropolarity (I_1/I_3) of pyrene in Triton X-100 micelles with rutin

Triton X-100 (mol/l)	Micelle structure	Rutin ($\times 10^{-5}$ mol/l)			
		0	*s* (‰)	4.0	*s* (‰)
1.0×10^{-4}	Pre-micelle	1.610	4.83	1.344	8.20
5.0×10^{-4}	Spherical	1.354	8.46	1.086	3.83
2.0×10^{-3}	Rod-like	1.351	4.92	1.075	5.98

Table 3
The micropolarity (I_1/I_3) of pyrene in Triton X-100 rod-like micelles with different concentration of Vitamin C

	V_c (mmol/l)						
	0	0.005	0.01	0.05	0.1	0.5	1.0
I_1/I_3 without rutin	1.351	1.349	1.351	1.353	1.347	1.350	1.349
s (‰)	2.78	2.92	3.27	3.12	3.90	3.39	3.86
I_1/I_3 with rutin	1.075	1.076	1.073	1.075	1.069	1.053	1.048
s (‰)	5.29	4.76	4.42	4.57	4.92	5.08	4.20

Note: rutin concentration = 4×10^{-5} mol/l; Triton X-100 concentration = 2×10^{-3} mol/l.

The interaction between rutin and Triton X-100 micelles is further studied by the determination of the binding constant K between rutin and micelles and then the thermodynamic functions of the binding process can be obtained from the following equations:

$$\Delta G = -RT \ln K \tag{2}$$

$$\ln K = -\frac{\Delta H}{RT} + C \tag{3}$$

$$\Delta G = \Delta H - T\Delta S \tag{4}$$

Here, ΔG is the Gibbs energy, ΔH the enthalpy, ΔS the entropy, C a constant and T is the absolute temperature. The binding constant K and the thermodynamic functions of the binding process are listed in Table 4. From Table 4, we can see that the binding of rutin to the Triton X-100 rod-like micelles is a spontaneous behavior ($\Delta G < 0$), and the value of the ΔG becomes further negative with the increase of rutin content. $\Delta H < 0$ indicates that the binding process of rutin with the Triton X-100 rod-like micelles is an exothermic reaction, so the rise of the temperature will decrease the binding of rutin with the Triton X-100 rod-like micelles. In addition, the value of ΔH is smaller than that of the interaction energy of the chemical bond (>100 kJ/mol) [27], indicating that the interaction force between rutin and Triton X-100 is weak intermolecular force, mainly hydrophobic force here. Also the binding constant K between rutin and Triton X-100 micelles with 1×10^{-4} mol/l Vitamin C is listed in Table 4. Comparing these values, it validates the conclusion above that

Table 4
The binding constant of rutin with Triton X-100 micelles and the thermodynamic functions of the binding process

T (°C)	Rutin ($\times 10^{-5}$ mol/l)	*K*	ΔG (kJ/mol)	ΔS (J/(mol K))	ΔH (kJ/mol)
25.0	2.0	96.45 (94.39)	−11.32	11.38	
	3.0	104.5 (107.2)	−12.52	12.04	
	4.0	121.6 (120.8)	−12.90	14.30	
	5.0	134.7 (134.3)	−13.15	15.15	
	6.0	183.1 (182.4)	−13.91	16.70	−7.93
35.0	4.0	117.6	−12.51	13.88	
45.0	4.0	104.4	−12.29	13.71	
55.0	4.0	72.99	−11.70	11.49	
65.0	4.0	45.72	−10.74	8.30	

Note: the concentration of Vitamin C is 1×10^{-4} mol/l in the parentheses.

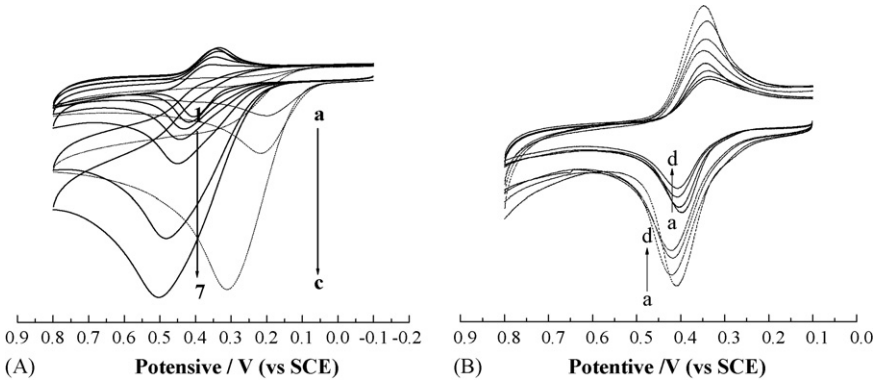


Fig. 4. (A) Cyclic voltammograms of Vitamin C with (solid line) and without (dashed line) rutin (4.0×10^{-5} mol/l). Concentration of Vitamin C (mol/l): (a) 1.0×10^{-5}, (b) 1.0×10^{-4}, (c) 1.0×10^{-3}; (1) 0.0, (2) 5.0×10^{-6}, (3) 1.0×10^{-5}, (4) 5.0×10^{-5}, (5) 1.0×10^{-4}, (6) 5.0×10^{-4}, (7) 1.0×10^{-3}. (B) Cyclic voltammograms of rutin (4.0×10^{-5} mol/l) in Triton X-100 micelles with (dashed line) and without (solid line) Vitamin C (1.0×10^{-4} mol/l). Concentration of Triton X-100 (mol/l): (a) 0.0, (b) 1.0×10^{-4}, (c) 5.0×10^{-4}, (d) 2.0×10^{-3}. Scan rate: 100 mV s^{-1}.

Vitamin C nearly has no influence on the interaction between rutin and Triton X-100 micelles.

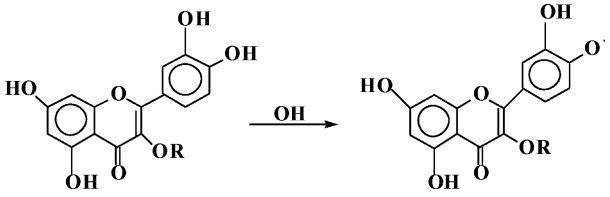
3.2. The influence of Vitamin C on the antioxidancy of rutin in Triton X-100 micelles

The response of 3′,4′-adjacent hydroxyl groups of rutin at a glass carbon electrode is a two-electron and two-proton electrode reaction [28,29]. The similar result is obtained in the present research.

Fig. 4(A) shows the influence of Vitamin C on the redox process of rutin. The addition of Vitamin C to rutin solution makes the oxidant peak of rutin move to higher potential and the oxidation peak current increases rapidly but the reduced peak disappears gradually, which is due to the overlapping of oxidation peaks of rutin and Vitamin C, because the oxidation peak current and potential of Vitamin C both increase with its concentration and the electrode reaction of Vitamin C is irreversible (see the dashed line a–c in (A)). These facts are valuable that Vitamin C is more easily oxidated than rutin and Vitamin C may reduce the rutin *ortho*-quinone back to rutin because of its higher reducing property. Thus, Vitamin C can serve as a protector to prevent the oxidation of rutin, and thus improve the antioxidancy of rutin. The influence of Triton X-100 on the redox property of rutin is shown in part (B) of Fig. 4. It can be seen that the oxidant peak of rutin moves to a higher potential with decreased peak current in the Triton X-100 micelles, which means the presence of Triton X-100 micelles makes the oxidation of rutin harder. That is because the interaction of rutin with Triton X-100 micelles makes the electroactive site of rutin (3′,4′-hydroxyl) shielded to some extent so the oxidation of rutin became more difficult and the peak current decreases.

In order to clarify whether the Vitamin C has a synergistic antioxidant effect on rutin or not, we investigated the radical-scavenging ability of rutin with and without Vitamin C in Triton X-100 micelles. The hydroxyl radicals originated from Fenton reaction are shown below [30]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + OH^- \quad (5)$$



(6)

It can be clearly seen from Eq. (6) that 3′,4′-hydroxyls of rutin are oxidated to *ortho*-quinone by the attack of hydroxyl radicals [31], thus the hydroxyl radicals are cleared and the absorption intensity of rutin will decrease (inset, Fig. 5). The influence of Triton X-100 micelles with different structures on the rutin decay is also shown in Fig. 5. The absorption of rutin drops rapidly within the first 10 min and after that this downtrend gets slow. The reason is that there are a great amount of hydroxyl radicals at the beginning which makes rutin easily oxidated to rutin radicals without absorption at 353 nm. But the amount of

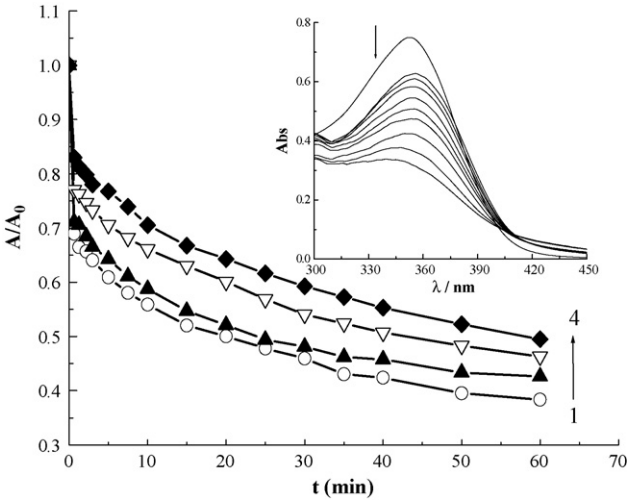


Fig. 5. The ratio of absorption intensity of rutin (4.0×10^{-5} mol/l) with time in Triton X-100 micelles. Concentration of Triton X-100 (mol/l): (1) 0.0, (2) 1.0×10^{-4}, (3) 5.0×10^{-4}, (4) 2.0×10^{-3}. Inset: absorption spectra of rutin (4.0×10^{-5} mol/l) at different time (t) reacted with hydroxyl radical. Time (min) on the arrow is ordinal 0, 0.5, 3, 5, 10, 20, 30, 45, 60.

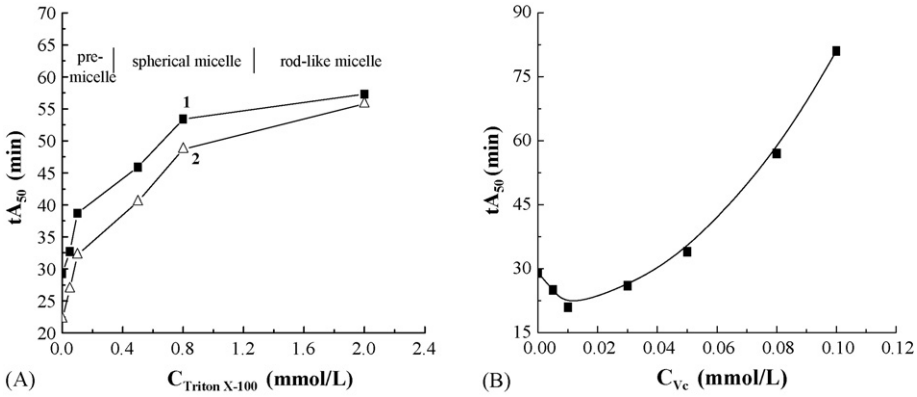


Fig. 6. The effects of Triton X-100 (A) and Vitamin C (B) on tA_{50} of rutin. Concentration of Vitamin C (mol/l): (1) 0, (2) 1.0×10^{-5}.

hydroxyl radical is reduced and the probability of rutin reacting with hydroxyl radical is accordingly reduced with the reaction going on.

Assuring the amount of hydroxyl radicals is excessive, then the tA_{50} is defined as the time necessary to decrease the absorption intensity of rutin aqueous (A_0) to half after clearing hydroxyl radicals. The smaller the tA_{50}, the higher the antioxidant activity of rutin. Fig. 6(A) curve 1 shows tA_{50} in Triton X-100 micelles with different structures. tA_{50} continuously increases with the concentration of Triton X-100, which means the reaction between rutin and hydroxyl radical becomes harder and harder. That is to say, the reaction between rutin and hydroxyl radicals is partly blocked with the solubilization of rutin in Triton X-100 micelles. So, the value of tA_{50} is doubled compared with that in aqueous solution.

However, the radical scavenging ability of rutin is affected by Vitamin C. It can be clearly seen from Fig. 6(B) that there is a lowest point on the curve, which corresponding concentration of Vitamin C is 1×10^{-5} mol/l, and tA_{50} is about 20 min, nearly one-third of that without Vitamin C. It has been mentioned that lower tA_{50} value represents higher antioxidant activity, so Vitamin C can improve the antioxidancity of rutin when the concentration is below 4×10^{-5} mol/l. However, tA_{50} of the system increases distinctly with Vitamin C when the concentration of Vitamin C is over 1×10^{-4} mol/l, which suggests that Vitamin C inhibits the antioxidancy of rutin rather than improves it under this condition. Vitamin C cannot only scavenge many kinds of free radicals but also regenerate several phenolic agents [19]. In our research, Vitamin C on the one hand can react with hydroxyl free radical, so that the reaction rate between rutin and hydroxyl radical is inhibited; on the other hand, it can reduce the rutin radical to rutin and the reduced rutin can scavenge hydroxyl radicals again. These two effects are both exhibited here and the integrated effect is that the process of rutin scavenging hydroxyl radical is improved at lower Vitamin C concentrations, and inhibited at higher concentrations of Vitamin C.

It is necessary to make clear whether the synergistic effect of Vitamin C could be kept in Triton X-100 micelle solutions. It can been seen from Fig. 6(A) curve 2 that the synergistic effect is weakened with the structure of Triton X-100 aggregation changing from pre-micelles to spherical micelles and to rod-like micelles. In Triton X-100 rod-like micelles, the process of synergistic antioxidant gets even harder because the active parts of rutin are solubilized in micelles as discussed before.

4. Conclusion

Rutin can be partially solubilized in Triton X-100 rod-like micelles spontaneously ($\Delta G < 0$) through hydrogen bond and hydrophobic force, and the binding constant K increases with rutin concentration. The embedding of two hydroxyl groups on the phenyl of rutin into Triton X-100 micelles makes the oxidation peak of rutin move to higher potential with decreased peak current. Vitamin C can accelerate the free radical scavenging rate of rutin when its concentration is lower, but the free-radical scavenge activity of rutin is inhibited when the concentration of Vitamin C is higher.

Acknowledgement

This research work was supported by the National Nature Science Foundation of China (no. 20233010).

References

[1] D.O. Kim, O.K. Chun, Y.J. Kim, H.Y. Moon, C.Y. Lee, J. Agric. Food Chem. 51 (2003) 6509–6515.
[2] K.D. Croft, Ann. NY Acad. Sci. 854 (1998) 435–442.
[3] B.N. Ames, M.K. Shigenaga, T.M. Hagen, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 7915–7922.
[4] F. Visioli, C. Galli, J. Agric. Food Chem. 46 (1998) 4292–4296.
[5] S. Bastinaetto, R. Quirion, Neurobiol. Aging 23 (2002) 891–897.
[6] M.G.L. Hetog, P.C.H. Hollman, D.P. Venema, J. Agric. Food Chem. 40 (1992) 1591–1598.
[7] A.C. Rice-Evans, J.N. Miller, G. Paganga, Free Radic. Biol. Med. 20 (1996) 933–956.
[8] O.V. Brenna, E. Paliarini, J. Agric. Food Chem. 49 (2001) 4841–4844.
[9] A. Papadopoulou, J.R. Green, A.R. Frazier, J. Agric. Food Chem. 53 (2005) 158–163.
[10] E.E. Deschner, J. Ruperto, G. Wong, H.L. Newmark, Carcinogenesis 12 (1991) 1193–1196.
[11] M.I. Chung, K.H. Gan, C.N. Lin, F.N. Ko, C.M. Teng, J. Nat. Prod. 56 (1993) 929–934.
[12] J.A. Dugas, J. Castaneda-Acosta, C.G. Bonin, L.K. Price, H.N. Fisher, W.G. Winston, J. Nat. Prod. 63 (2000) 327–331.

[13] S. Nagai, K. Ohara, K. Mukai, J. Phys. Chem. B 109 (2005) 4234–4240.
[14] D.O. Kim, K.W. Lee, H.J. Lee, C.Y. Lee, J. Agric. Food Chem. 50 (2002) 3713–3717.
[15] S. Nagaoka, M. Inoue, C. Nishioaka, Y. Nishioku, S. Tsunoda, C. Ohguchi, K. Ohara, K. Mukai, U. Nagashima, J. Phys. Chem. B 104 (2000) 856–862.
[16] M.I. Gil, F.A. Tomás-Barberán, B. Hess-Pierce, A.A. Kader, J. Agric. Food Chem. 50 (2002) 4976–4982.
[17] K.P. Wright, A.A. Kander, Postharvest Biol. Technol. 10 (1997) 39–48.
[18] G. Gramlich, J.Y. Zhang, M.W. Nau, J. Am. Chem. Soc. 124 (2002) 11252–11253.
[19] M.L. Liao, M.C. Yin, J. Agric. Food Chem. 48 (2000) 2266–2270.
[20] W.Y. Liu, R. Guo, X. Guo, J. Dispers. Sci. Technol. 24 (2003) 219–228.
[21] W.G. Deng, X.W. Fang, J.L. Wu, Radiat. Phys. Chem. 50 (1997) 271–276.
[22] R. Guo, W.Y. Liu, G.K. Fan, Acta Phys-Chim. Sin. 17 (2001) 1062–1065.
[23] J.M. Zhang, B. Mary, B.M. Satterfield, S.J. Brodbelt, J.S. Britz, B. Clevidence, A.J. Novotny, Anal. Chem. 75 (2003) 6401–6407.
[24] H. Siu, J. Duhamel, Macromolecules 39 (2006) 1144–1155.
[25] T. Asakawa, T. Okada, T. Hayasaka, K. Kuwamoto, A. Ohta, S. Miyagishi, Langmuir 22 (2006) 6053–6055.
[26] R. Guo, Y.H. Ding, T.Q. Liu, Acta Chim. Sin. 57 (1999) 943–952.
[27] W.Y. Liu, R. Guo, Acta Phys-Chim. Sin. 290 (2005) 564–573.
[28] J.L. Tang, Z.Y. Wu, J.G. Wang, E.K. Wang, Electrochem. Commun. 2 (2000) 796–799.
[29] I.F. Cheng, K. Breen, Biometals 13 (2000) 77–83.
[30] D. Nematollahi, M. Malakzadeh, J. Electroanal. Chem. 547 (2003) 191–195.
[31] W.G. Deng, Y.K. He, X.W. Fang, J.L. Wu, Radiat. Phys. Chem. 53 (1998) 629–633.