ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. LVII, 14 SECTIO AA 2002

Antioxidants activity in oil-in-water microemulsion stabilized by anionic surfactant

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Investigations of oxidation kinetics of some antioxidants like: vitamin C, propyl gallate, vitamin E and β-carotene solubilized in the O/W microemulsion formed by SDS, pentanol and water were carried out.
The present results as well as the literature data allow to compare the resistance to oxidation of antioxidants acting separately and together with vitamin C. Vitamin C (water-soluble species) turned out to be the best of them, contrary to the C (totally non-polar, fat-soluble species), which appeared to be the poorest one.
When observing vitamin C, occurring in O/W microemulsion together with vitamin E and β-carotene, we discovered stimulating (not protective) action of vitamin C towards vitamin E oxidation. Vitamin C does not influence the β-carotene behaviour; in the absence and presence of vitamin C its action is similar.

1. INTRODUCTION

The design of microscopic molecular assemblies which mimic the microenvironment present in the biological system can contribute a great deal to the understanding of naturally occurring processes [1-3]. The living cell is a remarkable example of an organized assembly. Biological membranes provide compartments of different sizes, shapes and microenvironments. Cellular functions such as recognition, fusion, endocytosis, exocytosis and transport are all membrane phenomena [4]. Different structures like micelles, black lipid membranes, vesicles, liposomes *etc*. are examples of organized assemblies. The behaviour of a group of molecules in a molecular assembly is quite different from that of individual ones [5-7]. Micellar aggregates not only create organized

structures, but also provide an interface of the hydrophilic and hydrophobic regions. This interface is the simplest chemical model for studying the processes occurring at biomembranes.

It is well known that the antioxidation activity of some species in homogenous solutions may not be the same as that in heterogeneous media [8-16]. In the present paper we consider antioxidant action in microemulsions.

Microemulsions are liquid disperse systems containing a hydrophobic solvent, usually an aliphatic hydrocarbon, water, a surfactant and a medium–chain alcohol acting as cosurfactant. Microemulsions are a special kind of stabilized emulsions in which the dispersed droplets are extremely small (<100nm) and thermodynamically stable. These emulsions are transparent liquids of relatively low viscosity and may form spontaneously. Many substances of hydrophilic and hydrophobic character are solved/ solubilized in microemulsions [17-18].

We investigated the atmospheric oxidation of antioxidants of different solubility in water i.e. vitamin C (H_2A, AA) [8,9], propyl gallate [19,20] (both species can be easily dissolved in water), vitamin E (α-tocopherol, α-T) [9,10,21] (fat-soluble species) and β-carotene [22] (which is totally non-polar and completely insoluble in water) in the microemulsions.

In our investigations we chose the O /W microemulsion formed by water/ pentanol/ sodium dodecyl sulfate [8, 23, 13].

From our own experiment and literature information it follows that all considered antioxidants i.e. vitamin C, propyl gallate, vitamin E and β-carotene are solubilized in SDS surfactant systems.

We found that Vitamin C can be solubilized up to 60% in the microemulsion region formed in the SDS/ pentanol/ water system [8].

Taking into account the fact that the water solubility of propyl gallate and vitamin C is similar, one can expect that, like vitamin C, propyl gallate is easily dissolved in SDS micellar solutions and in the O/W microemulsions studied. This suggestion was confirmed by investigations of Stöckmann and Schwarz [24]. They stated that propyl gallate could be easily solubilized in SDS solutions, in the palisade layer formed by alkyl chains of surfactant molecules in their association structures.

Considering the next antioxidant i.e. vitamin E, Chiu, Yang and Jiang [11,12] found that microemulsions with small size particles and high resistance to oxidation in air, can be obtained by solubilization of vitamin E in non-ionic surfactant solutions, whereas solubilization in sodium dodecyl sulfate solutions results in forming emulsions having small size particles but lacking stability against oxidation.

A number of studies have indicated that carotenoids act as antioxidants in solutions, micelles and liposomes. It was proved that hydrocarbon carotenoids, such as β-carotene are randomly distributed within the hydrophobic part of

micelles and membranes. This fact seems to be a decisive factor influencing the β-carotene antioxidant properties [21, 25-28].

The main interest of the present paper is the interdependence of the antioxidant solubility character (sometimes identified with hydrophobicity) and its behaviour in O/W microemulsions.

2. EXPERIMENTAL

Materials. The materials used were as follows: L-ascorbic acid, propyl gallate, α-tocopherol, β-carotene, sodium dodecyl sulphate (SDS), and 1-pentanol, all Fluka Chemie Ag and RdH Laborchemicalien GmbH & Co. KG production and double distilled water.

Methods. The method of making microemulsions depends on the antioxidants solubility. For water-soluble agents, such as ascorbic acid and propyl gallate, the microemulsion was prepared by titration of pentanol and SDS mixtures with antioxidant water solution. For fat-soluble species, such as α-tocopherol and β-carotene, the microemulsion was prepared by titration of pentanol (with a proper amount of α-tocopherol or β-carotene dissolved) and SDS mixtures by water. These processes were accompanied by vigorous mixing of sample during the titration.

The kinetics of antioxidants decomposition was studied in the system whose composition corresponds to one line of SDS concentration (6% *wt.*) in microemulsion region of the phase diagram. This line passes through the inverse micellar solution (the W/O microemulsions, >72% water), a bicontinous part (6% SDS, 72% water, 22% pentanol), and the aqueous micellar solution (the O/W microemulsion <72%) [8,21]. In the present paper we investigated antioxidant behaviour in O/W microemulsion (6% SDS and >72% water i.e.: 94%, 93%, 91%, 89%, 87% up to 72%).

The kinetics of antioxidants decomposition was determined by ultraviolet spectroscopy using double-beam spectrophotometer, Specord M-42 by Carl Zeiss Jena. The UV adsorption measurements were taken at wavelength range 220-600nm. The UV spectrum of ascorbic acid reveals a λ_{max} of 265nm [9]. The UV spectrum of propyl gallate has two characteristic peaks: λ_{max1} 217nm and λ_{max2} 274nm (the present results). The UV spectrum of α-tocopherol in ethanol has λ_{max} at 292 nm [29,30]. The UV spectrum of β-carotene has two characteristic peaks: λ_{max1} at 460nm and λ_{max2} at 490nm (the present results). The citied values of λ_{max} depend on a medium in which investigated antioxidant is dissolved [13].

The values of the initial antioxidant concentration were taken from a region of linearity of concentration/absorbance relationships. The initial concentrations of antioxidant in micellar solutions were respectively: of vitamin C - 0.002%

wt. (value of absorbance about 1.2), of propyl gallate - 0.002% *wt.* (value of absorbance was about 1.2), of α-tocopherol - 0.015% *wt*. (value of absorbance was about 1.2). The initial concentration of β-carotene was - 0.0005% *wt*. – absorbance was about 0.8. The value β-carotene concentration used corresponds to the maximum amount of β-carotene, which can be solubilized in 6% SDS aqueous solution.

As reference samples the microemulsion of water, pentanol and SDS of the same composition as the investigated ones were prepared. The spectra were recorded at regular time intervals to monitor the atmospheric oxidation kinetics of the antioxidant.

3. RESULTS AND DISCUSSION

The investigations are based on the well-known system: water, sodium dodecyl sulphate, and pentanol (Figure 1 [8,21]). It contains an inverse micellar solution (I), the basis for W/O microemulsions, a bicontinous part (II) and aqueous micellar solution (III) that forms the basis for the O/W microemulsions.

The results of the kinetics determination of ascorbic acid decomposition/ oxidation in the O/W microemulsion are presented in Figure 2. It is easy to observe the ascorbic acid oxidation enhancement with increased pentanol concentration in the system. With increasing amounts of pentanol the micelles become larger and the surface becomes less charged: a feature interpreted as more favorable to oxidation because the H_2A molecules have more tendency to locate at the less charged surfaces [8,31].

As expected, for propyl gallate we can observe a similar relationship between its oxidation and pentanol amounts in the O/W, Figure 3, like for vitamin C. However, in this case the dependence of the oxidation rate on alcohol concentration is more difficult to observe because the oxidation process is much slower than for vitamin C. For both the antioxidants the process is faster in O/W microemulsion than in SDS micellar solutions.

Vitamin E is a strong lipophilic radical-scavenging antioxidant, but its activity is much smaller in biomembranes than in solution, because of the reduced mobility of phytyl side chains which is essential for its biopotency [9].

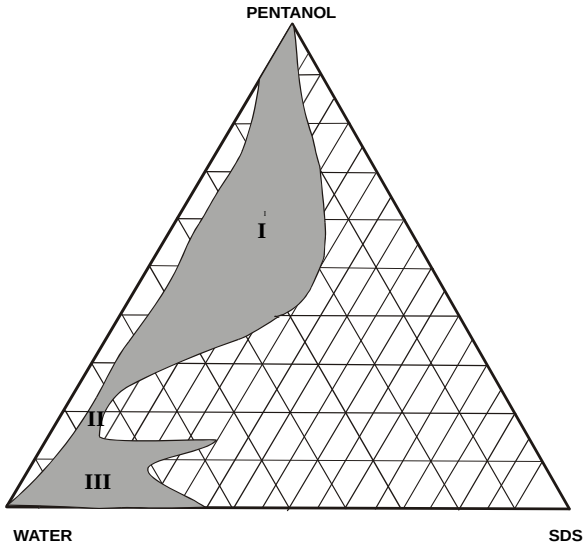

Fig. 1. The system of water, sodium dodecyl sulfate (SDS) and pentanol showing an isotropic solubility region with three kinds of amphiphilic association structures: (I) inverse micelar region; (II) bicontinous micellar region; (III) aqueous micellar region.

In our earlier studies [8] we stated that with increasing amount of alcohol in the mixture of SDS, pentanol and water, the vitamin E oxidation process becomes slower. It means that vitamin E is more resistant to oxidation in W/O than in O/W and in bicontinous microemulsions. Pentanol solutions of vitamin E and solution, which consist of water 10%*wt.* and pentanol 90%*wt.* (no SDS) and dissolved vitamin E are very stable in air. Vitamin E behavior described above is opposite to that found for vitamin E solubilized in anionic surfactant system, in the presence of Vitamin C (vitamin C to vitamin E weight ratio 0.13 [8]). Addition of vitamin C to the system changes the region of vitamin E antioxidation activity. In the presence of H_2A vitamin E is a better antioxidant in W/O microemulsion than in O/W. As long as vitamin C is in its non-oxidized form in the system, it stimulates vitamin E decomposition. Thus, when both vitamins are not completely decomposed, their action is similar – Figure 4. This effect seems to be in contradiction to a general opinion about vitamin C and E action when they exist together in a system. Numerous publications have addressed [9,26,32,33] the determination of vitamin C role as a protection against vitamin E oxidation.

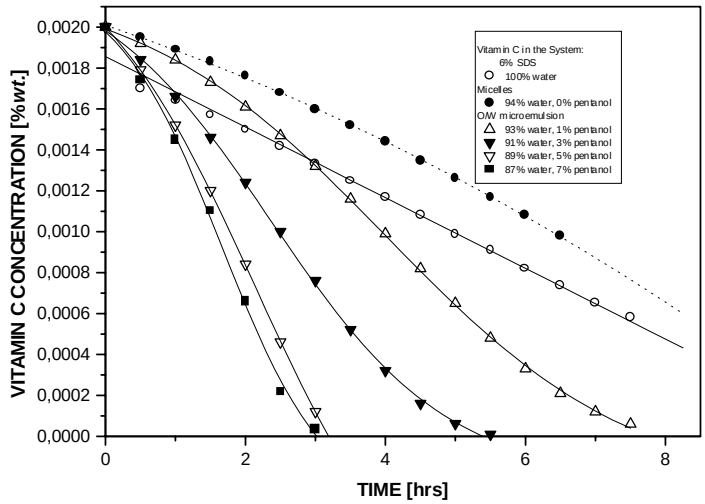

Fig. 2. The kinetics of 0.002% vitamin C atmospheric oxidation in O/W microemulsion formed by SDS, pentanol, water. Remainder of vitamin C in the system.

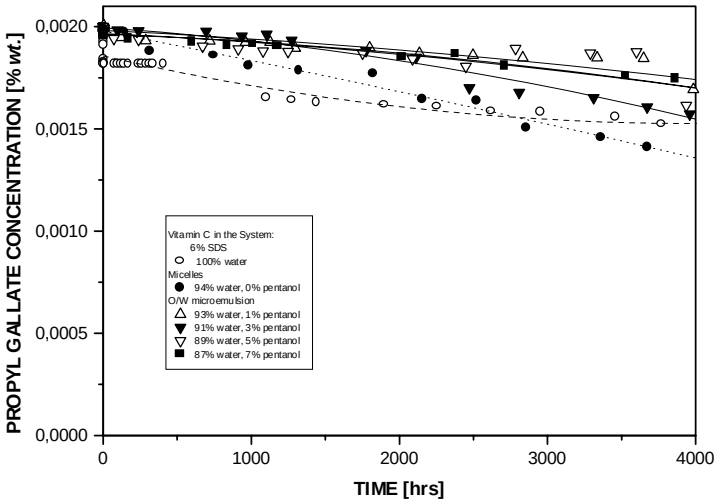

Fig. 3. The kinetics of 0.002% propyl gallate atmospheric oxidation in the presence of 0.002% vitamin C in O/W microemulsion formed by SDS, pentanol, water. Remainder of propyl gallate in the system.

This oxidation goes through a hydrogen-transfer mechanism that involves the oxidation of ascorbic acid to convert α-tocopherylquinone (vitamin E oxidation product) back to α-tocopherol. It was found that there exists an optimum value of the vitamin C to vitamin E ratio for which vitamin C protects vitamin E against oxidation (1:1 by weight). The results presented in our earlier

work [8] were obtained for the systems with vitamin C to vitamin E ratio equal to 0.13 i.e., much smaller than the optimum value. This may be the reason why we found the stimulating (not protective) action of vitamin C towards vitamin E oxidation.

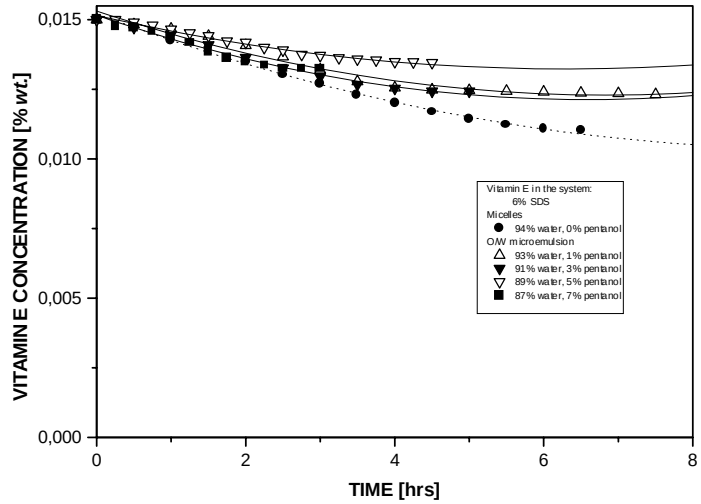

Fig. 4. The kinetics of 0.15% vitamin E atmospheric oxidation in the 0.002% vitamin C presence in O/W microemulsion formed by SDS, pentanol, water. Remainder of vitamin E in the system.

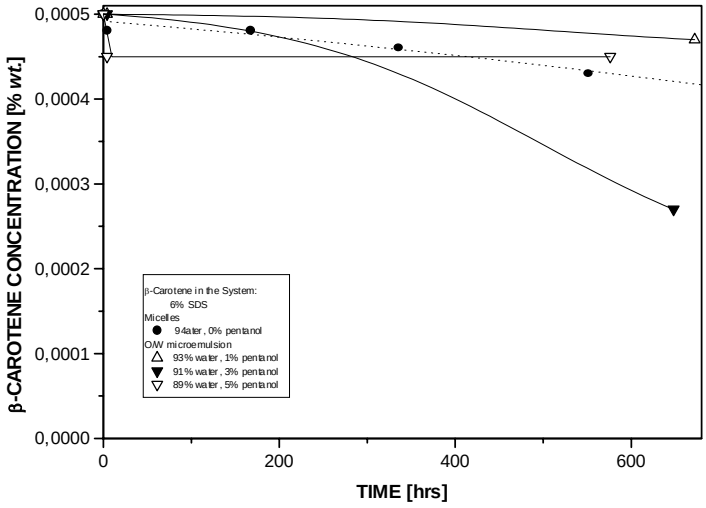

Fig. 5. The kinetics of 0.0005% β-carotene atmospheric oxidation in O/W microemulsion formed by SDS, pentanol, water. Remainder of β-carotene in the system. (The solid lines are drawn to help the eye).

Finally we studied the atmospheric oxidation of β-carotene in the anionic surfactant system as well as the role the impact of ascorbic acid on β-carotene behaviour.

Looking at Figure 5 one can see that β-carotene oxidation process is very slow. Due to that as well as to the low solubility of β-carotene in O/W microemulsion the obtained results may not be very accurate. However, there is no doubt that β-carotene acts as an antioxidant in the O/W microemulsion, whereas in the W/O microemulsion it remains stable.

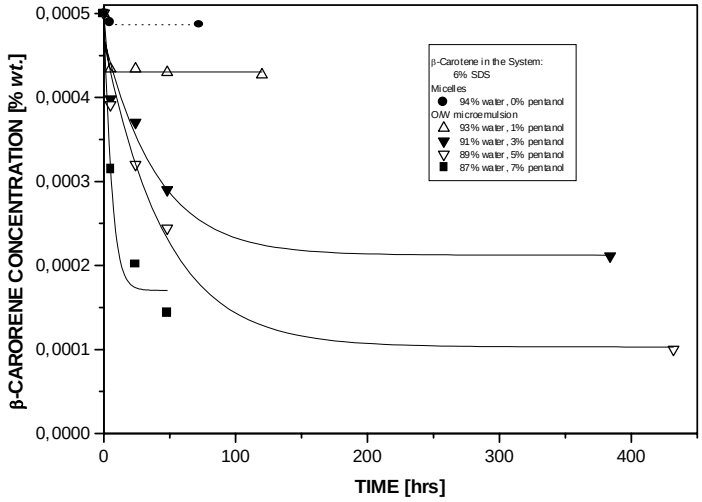

Fig. 6. The kinetics of 0.0005% β-carotene atmospheric oxidation in the presence of 0.002% vitamin C in O/W microemulsion formed by SDS, pentanol, water. Remainder of β-carotene in the system. (The solid lines are drawn to help the eye).

This conclusion is in accordance with the results of electrochemical oxidation of β-carotene in non-ionic Triton X-100 surfactant, which were published by He and Kispert [22] and Burke and co-workers [27,28].
They suggested that the hydrophobic barrier of association structures of surfactants could hinder electron transfer from β-carotene molecules. Such behaviour was also observed for vitamin E molecules which are less hydrophobic than β-carotene [10,16,13,26].

The mutual action of β-carotene and vitamin C in anionic surfactant system is illustrated in Figure 6, where the results of β-carotene decomposition in the presence of vitamin C are shown. One can see that vitamin C addition into the system does not influence the behaviour of β-carotene, which still acts as antioxidant in O/W microemulsion.

The comparison of antioxidation abilities of vitamin C, propyl gallate, vitamin E and β-carotene in the micellar systems studied [8,10, present results] revealed that the most hydrophobic of them is the poorest one in terms of antioxidation abilities. We agree with the statement of Pryor and co-workers [32] that β-carotene seems to be a better antioxidant in liposomal and microsomal systems than in micellar ones.

4. CONCLUSIONS

1. Antioxidants of different solubility in water i.e. vitamin C (H_2A, AA), propyl gallate (both species can be easily dissolved in water), vitamin E (α-tocopherol, α-T) (fat-soluble species) and β-carotene (which is totally non-polar and extremely insoluble in water) solubilize in the O/W micro-emulsion formed by SDS, pentanol and water.
2. Among the antioxidants investigated vitamin C (water-soluble species) turned out to be the best in O/W microemulsion.
3. β-Carotene (totally non-polar, fat-soluble species) solubilized in O/W microemulsion was more resistant to oxidation than other antioxidants in air.
4. We found stimulating (not protecting) action of vitamin C towards vitamin E oxidation.
5. Vitamin C does not influence the β-carotene behaviour; in the absence and presence of vitamin C its action is similar.

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