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Flavonoid-surfactant interactions: A detailed physicochemical study

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ABSTRACT

The aim of this article is to study the interactions between flavonoids and surfactants with attention of finding the probable location of flavonoids in micellar media that can be used for controlling their antioxidant behavior. In present study, the micellar and interfacial behavior of twin tailed anionic surfactants *viz.* sodium bis(2-ethylhexyl)sulfosuccinate (AOT) and sodium bis(2-ethylhexyl)phosphate (NaDEHP) in the presence of two flavonoids, namely quercetin (QUE) and kaempferol (KFL) have been studied by surface tension measurements. UV–visible, fluorescence and differential pulse voltammetric (DPV) measurements have been employed to predict the probable location of flavonoids (QUE/KFL) within surfactant (AOT/NaDEHP) aggregates. Dynamic light scattering (DLS) measurements further confirmed the solubilization of QUE/KFL in AOT/NaDEHP aggregates deduced from increased hydrodynamic diameter (D_h) of aggregates in the presence of flavonoids. Both radical scavenging activity (RSA) and degradation rate constant (k) of flavonoids are found to be higher in NaDEHP micelles as compared to AOT micelles.

1. Introduction

Flavonoids, among the natural antioxidants such as vitamin A (retinol), C (ascorbic acid), and E (tocopherol), are particularly interesting being most biologically active and common dietary antioxidants present in significant amounts in foods and beverages [1–5]. Flavonoids play an important role in combating the oxidative stress and to keep its levels below a critical point in the body.

Quercetin (QUE) is a common dietary flavonoid which is one of the most biologically active flavonoid showing potent antioxidant (scavenging ROS) and anti-inflammatory effects in vivo [6,7]. Among its antioxidant activities, it shows high free radical scavenging activity toward hydroxyl radical, peroxyl and superoxide anion compared to other flavonoids [8]. Recent studies on QUE report that it can inhibit proliferation of multiple cancer cell type, including lung cancer cells, colon cancer cells, prostate carcinoma cells, and pancreatic tumor cells. QUE by itself, and paired with ascorbic acid, reduce the incidence of oxidative damage to neurovascular structure in skin, and inhibit damage to neurons caused by experimental glutathione depletion [9]. On the other hand, kaempferol (KFL) is a common flavonoid and largely used for the treatment of diabetic, ulcer, cough, cataract, bronchial asthma, epilepsy and anxiety because of its anti-inflammatory effects [10]. KFL is also known to exhibit cancer chemopreventive (especially ovarian cancer), neuroprotective, antidepressant, antiatherogenic and anxiolytic effects [11,12]. The employment of flavonoids to combat these various deceases is restricted because of their poor water solubility and instability under various conditions such as temperature, light, pH, enzymes, oxygen during food or pharmaceutical products processings, in the gut or during storage. These issues limit their health benefits in functional food or pharmaceutical products [13]. Such limitations can be overcome by use of surfactant nano-cavities, known to improve bioavailability and resist degradation of pharmacologically active molecules [14]. Therefore, surfactant-antioxidant interactions and the consequent influence on the antioxidant activity has been the subject of a number of studies [15–25]. Most of these studies are concentrated on a limited number of single tailed surfactants. Moreover these studies either focused only on solubilisation and location of flavonoids in micelles or relative antioxidant activity of flavonoids. In contrast to these studies, the present work is focused on both probable location as well as antioxidant activity of flavonoids (QUE, KFL) in micellar media of twin tailed anionic surfactants. To the best of our knowledge, there is no in-depth scientific study of twin tailed anionic surfactants and flavonoids reported in literature.

Sodium bis(2-ethylhexyl)sulfosuccinate (AOT), is a biocompatible twin tailed anionic surfactant, used as a common ingredient in consumer products especially as laxatives, an excipient in the production of tablets (as a lubricant) and in suspensions (as emulsifier) [26–28]. AOT is an approved oral, topical and intramuscular excipient due to its low toxicity. On the other hand, sodium bis(2-ethylhexyl)phosphate (NaDEHP) is also a twin tailed anionic surfactant and has been used extensively as an extracting agent for the separation and purification of variety of chemicals, basic and quaternary drugs [29–31].

In this work, the interactions of surfactants (AOT/NaDEHP) with flavonoids (QUE/KFL) in bulk and at air/solution interface have been studied by surface tension measurements. Differential pulse voltammetric (DPV) and spectroscopic (fluorescence and UV–visible) measurements

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have been employed to probe the possible location of flavonoids (QUE/KFL) in surfactant (AOT/NaDEHP) micelles. The radical scavenging activity of flavonoids towards hydroxyl radicals generated through Fenton's reagent was monitored by UV–visible measurements. In short, the objective of this study was to investigate the influence of location of QUE/KFL within micellar media on its radical activity toward •OH radicals. This report is expected to have relevance in understanding the antioxidant mechanism of QUE/KFL in real complex foods and biological systems.

2. Material and method

2.1. Materials

Quercetin (QUE), kaempferol (KFL), sodium bis(2-ethylhexyl) sulfosuccinate (AOT), bis(2-ethylhexyl)phosphate (HDEHP), acetic acid and sodium hydroxide with purities ≥98% were purchased from Sigma Aldrich. Sodium bis(2-ethylhexyl)phosphate (NaDEHP) was synthesized by the procedure reported in literature [32]. The molecular structures of surfactants (AOT and NaDEHP) and both flavonoids (QUE and KFL) are shown in Scheme 1.

The stock solutions of both flavonoids were prepared from its crystals by dissolving them in a small amount of ethanol [33]. Aqueous solutions of flavonoids of desired concentration were freshly prepared just before the experiments by diluting the stock solution of ethanol with double distilled water/buffer [acetic acid-sodium acetate (AcOH-NaAc) of concentration of 5 mM and pH 5.0].

2.2. Methods

2.2.1. Surface tension measurements

The surface tension (γ) measurements of surfactants in the absence and presence of flavonoids were carried out by using Du Nouy ring Tensiometer (Kruss Easy Dyne tensiometer) from Kruss Gmbh (Hamburg, Germany) equipped with thermostat, using platinum ring. The samples were thermostatted at 25 ± 1 °C. The surface tension of doubly distilled water, 71.6 ± 0.4 mNm^{-1} was used for calibration purposes. The series of measurements were repeated at least three times. All measurements were performed in AcOH-NaAc buffer of pH 5.0.

2.2.2. Spectroscopic measurements

The UV–visible absorption spectra were recorded on a UV-1800, Shimadzu UV–visible spectrophotometer with quartz cuvette having path length of 10 mm at 25 ± 1 °C. The absorption spectra were recorded in range of 240 nm to 450 nm. All measurements were repeated at least three times.

The intrinsic fluorescence measurements were carried out by using F-4600 FL fluorescence spectrophotometer from Hitachi, Japan using a 10 mm path length quartz cuvette at 25 ± 1 °C. The emission spectra were recorded between 400 nm to 600 nm at excitation wavelength of 364 nm by keeping the excitation and the emission slits width of 5 and 10 nm respectively. All spectroscopic measurements were carried out at least three times in double distilled water.

2.2.3. Differential pulse voltammetric measurements

Differential pulse voltammetric (DPV) measurements were performed on a PC controlled CHI660D (Austin, USA) electrochemical workstation with conventional electrochemical cell containing three-electrode system. The glassy carbon (GC) electrode, a platinum wire and a Ag/AgCl electrode were served as working, counter and reference electrodes respectively. GC electrode was sequentially polished with slurry of alumina powder and rinsed with doubly distilled water prior to each measurement. All measurements were performed thrice in AcOH-NaAc buffer.

2.2.4. Dynamic light scattering measurements

Dynamic light scattering (DLS) measurements were performed using a light scattering apparatus (Zetasizer, Nano series, Nano-ZS, Malvern Instruments) equipped with a built-in temperature controller having an accuracy of ± 0.1 K. All the scattered photons were collected at 173° scattering angle. The scattering intensity data was processed using the instrumental software to obtain the hydrodynamic diameter (D_h) and the size distribution of the scatterer in each sample. The solutions were filtered through membrane filters (0.45 mm) to remove dust particles. DLS experiments were done in triplicate with 15 runs per measurement and are reported with an uncertainty of less than 5%.

2.2.5. Evaluation of hydroxyl-radical (•OH) scavenging activity

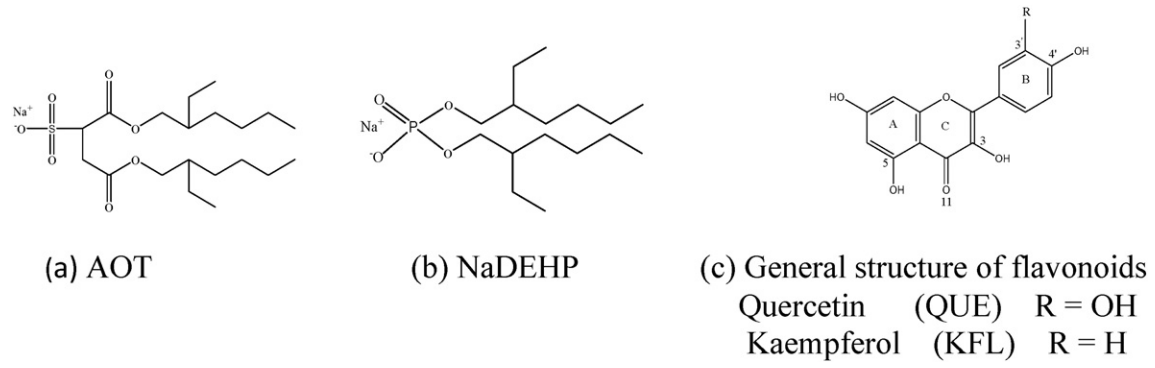
Hydroxyl radical scavenging activity (RSA) of flavonoids (QUE/KFL) in the presence of surfactants (AOT/NaDEHP) was determined by first dissolving the flavonoids in surfactant micellar solution followed by addition of Fenton's reagent to the mixture. The decrease in absorbance at the absorption wavelengths of QUE (366 nm) and KFL (362 nm) after every 60 s interval was measured at 25 ± 1 °C. The decay in absorbance, an index of the oxidation of •OH radicals, was measured for 45 min. Concentrations of QUE/KFL, $FeSO_4$ and H_2O_2 were fixed at 0.03 mM, 0.0125 mM and 0.125 mM respectively. All experiments were repeated at least three times.

3. Results and discussion

3.1. Surface tension measurements

3.1.1. Critical micelle concentration (cmc)

The representative plots of surface tension (γ) *vs.* log[surfactant] for pure surfactants (AOT/NaDEHP) and in the presence of flavonoids (QUE/KFL) are shown in Fig. 1. The cmc_1 values of pure surfactants (AOT/NaDEHP) were measured both in water as well as in buffer solution (AcOH-NaAc) of pH 5.0. The cmc_1 values for pure AOT and NaDEHP are 2.36 mM and 19.95 mM respectively in water indicating pure AOT



Scheme 1. Molecular structure of twin tailed anionic surfactants and flavonoids.