Journal of Molecular Liquids 216 (2016) 216–223

Contents lists available at ScienceDirect

Journal of Molecular Liquids

journal homepage: www.elsevier.com/locate/molliq

The dependence on ionic strength of the protonation of water-insoluble flavonoids chrysin and daidzein in NaClO$_4$ non-aqueous solutions

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ARTICLE INFO

Article history:
Received 26 November 2015
Accepted 31 December 2015
Available online xxxx

Keywords:
Proton dissociation equilibria
Chrysin
Daidzein
Ionic strength effect
SIT approach
NaClO$_4$ non-aqueous ethanol media

ABSTRACT

The acid–base equilibria of two water-insoluble flavonoids, chrysin and daidzein, were studied using combined spectrophotometric/potentiometric measurements at constant temperature (25.0 ± 0.1) °C. The investigation was performed over a wide range of ionic strengths of 0.101–3.503 mol kg^{-1} NaClO$_4$ medium in ethanol-water mixture (1:1 v/v). The Gran's method was used for glass electrode calibration to obtain pH readings based on the concentration scale (p$_c$H). Data process for calculation of the protonation constants was done using the STAR computer program. The dependence on ionic strength of the constants was successfully modeled by means of the Brönsted–Guggenheim–Scatchard Specific Ion Interaction Theory (SIT). Other than the specific interaction parameters of the ionic species, the protonation constants at infinite dilution (zero ionic strength) were obtained. The thermodynamic functions ΔG^0 (standard Gibbs free energy change) for the protonation processes were also calculated at different ionic strengths.

1. Introduction

An accurate knowledge about the acid–base properties of bioactive and pharmaceutical compounds is very important in many fields, since provide basic data to predict the extent of ionization of them as a function of pH and thus help in understanding the action mechanism of these substances in both chemical and biological processes. Moreover, these information are essential for the measurements of pH-dependent the pharmacologic properties such as lipophilicity, permeability, protein binding and solubility which in turn directly affects pharmacokinetic characteristics like absorption, distribution, metabolism, and excretion [1,2].

One of the largest classes of polyphenolic phytochemicals is flavonoids or bioflavonoids which are found in most plants particularly fruits and vegetables and thus is considered important constituents of our daily diet. Based on structural characterizations, the flavonoids are categorized into flavonols, flavones, flavanones, isoflavones, flavonols, and anthocyanidins. The common structure of flavonoids is the flavan nucleus, which consists of 15 carbon atoms arranged in three rings including two benzene rings A and B and a heterocyclic pyran or pyrone ring C. Different compounds of the flavonoid class have been distinguished by the number and the position of functional groups, mainly hydroxyls, introduced on the rings A and B. During the past decades, flavonoids have generated growing interest due to their numerous biological and pharmacological activities such as antioxidant, vasoprotective, anti-inflammatory, anti-viral and anti-fungal actions [3–5]. Many of these beneficial effects are related to their molecular structure, for instance their antioxidant activity is because of their ability for hydrogen atom donating to free radicals and also the presence and number of hydroxyl groups in the molecule. The hydroxyl moiety deprotonation has influence in the intrinsic antioxidant potential of the flavonoid, because deprotonation generally enhances the antioxidant action of the flavonoid [6–8]. Therefore, the knowledge of their physicochemical parameters, such as protonation constants, is important to predict their antioxidant capacity.

The bioflavonoid chrysin, with the formula C$_{15}$H$_{10}$O$_4$ (5, 7-dihydroxy-2-phenyl-4 H-chromen-4-one, Fig. 1a) is present at high levels in honey and propolis and in low concentrations in fruits, vegetables, and certain beverages. In addition to its reported anticarcinogenic, antiviral, antioxidant, and anti-inflammatory activities [9], chrysin has shown to be a potent inhibitor of aromatase, decreasing the levels of estrogen in the body and of human immunodeficiency virus activation in models of latent infection. It has also shown cancer chemopreventive activity via induction of apoptosis in diverse range of human and rat cell types. However, the molecular mechanisms underlying chrysin anticancer effects are not well understood [10].

Daidzein with the molecular formula C$_{15}$H$_{10}$O$_4$ [7-hydroxy-3-(4-hydroxyphenyl) chromen-4-one, Fig. 1b] belongs to the isoflavone subclass of flavonoids, which is the most commonly ingested and most intensely studied type of phytoestrogen, often found in fruits, nuts, soybeans, and soy-based products. It has been reported that daidzein exhibits a variety of beneficial effects on human health [11], including the chemoprevention of cardiovascular diseases and cancer, and it can serve as an alternative for estrogen replacement therapy to prevent and treat osteoporosis in post-menopausal women with bone loss.

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http://dx.doi.org/10.1016/j.molliq.2015.12.115

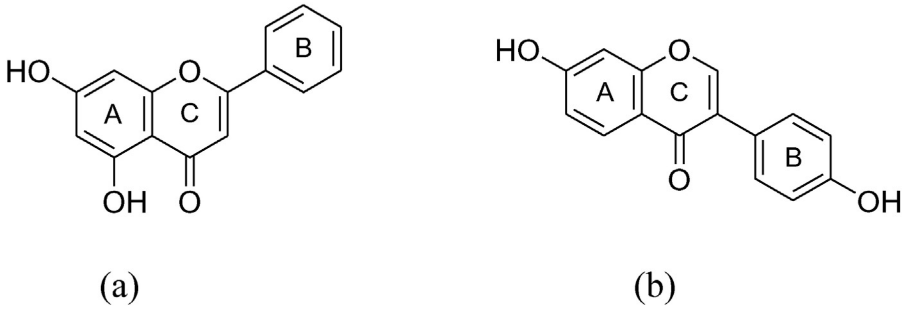

Fig. 1. Chemical structure of (a) chrysin and (b) daidzein.

Daidzein has also antioxidant, antidipsotropic, anticarcinogenic and antiatherogenic activity [12,13].

The selection of a suitable analytical method for the acid dissociation constants (pK_a's) determination of organic ionizable compounds can be done taking into account the properties of the studied compounds. Among a wide range of methodologies, using a combination of pH-metric titration and multi-wavelength spectrophotometric method is an accurate and good-reproducibility technique for the pK_a's determination of each compound that possesses pH-dependent light absorption due to the presence of a chromophore in proximity to ionizable group(s). This method only requires small amounts of analyte and solubility is not a serious concern [14,15]. Flavonoids chrysin and daidzein are practically insoluble in pure water but they are sufficiently soluble in a water-miscible organic solvent, hence it is possible to determine their acid dissociation constants in co-solvent mixtures pH-metrically. Ethanol–water binary mixtures are often used because they have a lower polarity than pure water, but keeping a similar environment, also having low toxicity. Despite the importance of this family of bioactive compounds as already mentioned above, the information related to acid–base equilibria of them is relatively few or sparse in the literature, in particular in media with high ionic strengths. Therefore, in this work we perform a systematic study on the protonation process of two antioxidant flavonoids chrysin and daidzein by a UV/pH titration method in different aqueous ethanol solutions (1:1 v/v) of $NaClO_4$ (0.101–3.503 mol kg^{-1}) as background electrolyte. The dependence of acid–base equilibria on ionic strength of medium was modeled by the specific ion interaction theory (SIT), with the aim of obtaining better understanding with regards to their variation as a function of charges involved in the acid dissociation process.

2. Experimental

2.1. Reagents and chemicals

Flavonoids chrysin and daidzein were purchased from Sigma-Aldrich as analytical reagent grade material and were used without further purification. The NaOH and hydrochloric acid solutions (Titrisol, 1.00 mol dm^{-3}) were prepared from Merck. Sodium perchlorate supplied from Merck and was kept at room temperature in vacuum at least 72 h before use. The organic solvent, ethanol was of analytical grade, obtained from Merck. All dilute solutions were prepared by mixing doubly distilled water, the specific conductivity of which did not exceed 0.05 ± 0.01 μS cm^{-1}.

2.2. Apparatus and procedure

To determine the protonation constants of each flavonoids tested, the potential values of the potentiometric cell, E_{cell}, in potentiometric titrations were measured using a WTW inoLab (model 720) research pH meter, equipped with a combined glass-pH electrode (model N 6000 A). The electrode response can be read to the third decimal place in terms of pH units with a precision of ±0.001 and the potential with a precision typically of the order of ±0.1 mV. The electrode was soaked for 10 to 15 min in the ethanol–water mixture (1:1 v/v) before the potentiometric measurements. All titrations were carried out in a 80 mL thermostatted commercial double-walled glass vessel and the test solution was stirred magnetically for homogenizing it. To exclude any the atmospheric $CO_2(g)$ and $O_2(g)$ trace from the system, a stream of purified $N_2(g)$ was passed through a sodium hydroxide solution and then bubbled slowly during the course of titrations. Spectrophotometric measurements were performed on a Perkin Elmer (model LAMBDA 25) diode array UV–Vis spectrophotometer in conjunction with a Peltier temperature controller, using quartz cells of path 10 mm.

All experiments related to measuring the pK_a values of the flavonoids chrysin and daidzein were performed at constant temperature (25.0 ± 0.1)°C and ionic strengths of 0.101, 0.513, 1.051, 2.212 and 3.503 mol kg^{-1} $NaClO_4$. The working solutions were prepared freshly for each experiment, and the container was covered with foil to protect the solution from light. Before the potentiometric titration, the glass electrode system calibration was done in each of ionic strengths examined according to the Gran's method [16]. For this purpose, a measured amount of an acidic solution (total volume of 20 mL) was placed in the double-wall glass vessel. The electrode was immersed in the solution in the titration vessel and the acidic solution was titrated potentiometrically with a strong base (0.10 mol dm^{-3} NaOH) both at the same conditions of temperature and ionic strength to be used in later experiments. Sufficient time (normally 2–3 min) was allowed to reach a reasonably stable E_{cell} reading before the next titrant addition and the recorded E_{cell} values were then used to obtain calibration constants. Usually, 10 or 12 additions of sodium hydroxide solution were enough for these constants to be accurately determined.

In the next step, a suitable volume (1.0–2.0 mL) of stock solution of the flovonoids studied (0.0005 mol dm^{-3}) was added to the pre-titrated background solution. By continuing the titration, in each addition of titrant, after waiting for the potential reading to be stable, a UV–Vis spectrum was recorded with 1 nm resolution, all relevant data were stored in computer, and a new volume of titrant was added to restart the cycle. Each set of experiments (in different ionic strengths studied), including calibration of the glass electrode and the UV–Vis spectrometric titrations was at least twice repeated under carefully controlled experimental conditions. The total time taken for each experiment was around 3 h. Typically, the experimental values of E_{cell} as a function of the added volume of alkaline titrant for the combined glass electrode calibration and potentiometric titration are listed in Tables 1 and 2 in 1.051 mol kg^{-1} $NaClO_4$ ionic strength for the flavonoids chrysin and daidzein, respectively. The typical potentiometric titration curves (a plot of pH or E_{cell} against V) for chrysin and daidzein at an ionic strength of 1.0 mol dm^{-3} $NaClO_4$ medium are shown in Fig. 2. The first step was clearly observed in the potentiometric curves which relates to the A-ring 7-OH group. The second step was not clearly seen as expected and it is related to the hydroxyl group at the positions 5 and 4′ for chrysin and daidzein, respectively [17,18]. In addition, Fig. 3 shows the electronic spectral changes of the flavonoids, in the pH ranges 2.0–12.5 at an ionic strength of 1.051 mol kg^{-1} $NaClO_4$. The clear changes