Journal of Molecular Liquids xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Molecular Liquids

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

# Kinetic solvent effects on the reaction between flavonoid naringenin and 2,2-diphenyl-1-picrylhydrazyl radical in different aqueous solutions of ethanol: An experimental and theoretical study

Morteza Jabbari [a,*], Hakimeh Mir [a], Ayoob Kanaani [a], Davood Ajloo [b]

[a] School of Chemistry, Damghan University, 36716-41167 Damghan, Iran
[b] School of Chemistry, College of Science, University of Tehran, Tehran, Iran

ARTICLE INFO

*Article history:*
Received 4 December 2013
Received in revised form 12 April 2014
Accepted 18 April 2014
Available online xxxx

*Keywords:*
Kinetic solvent effect
Naringenin
DPPH radical
Ethanol–water mixtures
Reichardt and KAT parameters
DFT calculations

ABSTRACT

Kinetic study of the reaction of flavonoid naringenin with the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was performed in different percentage compositions of aqueous ethanol (50–90% v/v) using spectrophotometric method. The reaction, which follows the mixed second-order rate law, was investigated under pseudo first-order conditions with respect to the DPPH radical, at (25.0 ± 0.1) °C and an ionic strength of 0.1 mol $dm^{-3}$. The rate of reaction was found to decrease with increasing organic solvent content in binary mixture. The reaction mechanism was inferred from the stoichiometry, kinetics, and product identification. Furthermore, the effects of solvent composition on the reaction rate in the mixed solvents were analyzed in terms of Reichardt parameter ($E_T^N$), and Kamlet, Abboud and Taft (KAT) solvatochromic parameters ($\alpha$, $\beta$, and $\pi^*$). To further investigate the solvent effects we theoretically studied the three antioxidant action mechanisms of naringenin using density functional theory (DFT) method. Reaction enthalpies related to these mechanisms were calculated in gas-phase, water, ethanol and 50–90% (by v/v) ethanol–water. It was found that theoretical findings are in good agreement with experimental results.



## 1. Introduction

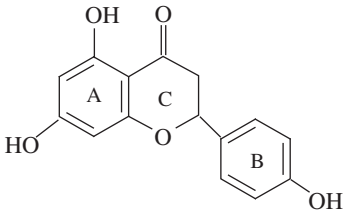
Flavonoids are natural polyphenolic phytochemicals that are found ubiquitously in plants and have been described as health-promoting, disease-preventing dietary supplements and cancer-preventive agents [1]. Moreover, they are extremely safe and low toxicated, which makes them excellent chemopreventive agents. More than 4000 types of biologically active flavonoids have been identified, which can be further divided into flavonols, flavones, flavanols, flavanones, anthocyanidins, and isoflavonoid subclasses [2]. The common structure of flavonoids is the flavan nucleus, which consists of 15 carbon atoms arranged in three rings (phenylchromanone structure, $C_6$–$C_3$–$C_6$). Rings A and B are benzene rings and ring C is a heterocyclic pyran or pyrone. The recent explosion of interest in the bioactivity of the flavonoids of higher plants is due, at least in part, to the potential health benefits of these polyphenolic components as major dietary constituents. Many of pharmacological effects of flavonoids are related to their antioxidant activity, which is a biological function, important in keeping the oxidative stress levels below a critical point in the body. This property of flavonoids may be due to their ability to scavenge free radicals and to synergistic effects with other antioxidants [3].

Naringenin (4′,5,7-trihydroxyflavanone) is one of the polyphenolic compounds that is mostly found in grapefruit and in lower concentrations in tomatoes and tomato-based products [4]. This flavonoid has been shown to inhibit in vitro the growth of cancer cells in human and can exhibit estrogenic, anticarcinogenic, and antioxidative properties [5]. Naringenin has antioxidant and antitumor activity and may play a role in cancer, heart disease, hypertension, circulation, Alzheimer's disease, etc. [6]. Naringenin has also been shown to reduce hepatitis C virus production by infected hepatocytes (liver cells) in cell culture. This seems to be secondary to naringenin ability to inhibit the secretion of very low density lipoprotein by the cells [7].

As has been frequently reported in the literature, phenolic antioxidants are known to act as free radical scavengers via at least three different mechanisms including hydrogen atom transfer (HAT), single-electron transfer–proton transfer (SET–PT) and sequential proton loss electron transfer (SPLET). These mechanisms may co-exist, and depend on solvent properties and radical characters [8]. Some studies have correlated the free radical scavenging activity to the bond dissociation enthalpy (BDE), the ionization potential (IP), the proton dissociation enthalpy (PDE), the proton affinity (PA) and the electron transfer enthalpy (ETE) values [9]. The low BDE, PA and IP values are beneficial to enhance the direct radical scavenging activity in non-polar or polar solvents. However, extremely low IP will enhance the prooxidant danger through direct transfer of an electron to surrounding oxygen [10].

* Corresponding author.
*E-mail address:* m_jabari@du.ac.ir (M. Jabbari).

http://dx.doi.org/10.1016/j.molliq.2014.04.015
0167-7322/




**Scheme 1.** Chemical structure of naringenin.

In this work, we have performed a detailed kinetic study of the reaction between naringenin and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical in different aqueous solutions of ethanol (50 to 90% ethanol by v/v) due to the insolubility of naringenin in water. The mechanistic aspects of the reaction are discussed, and the effects of addition of the organic cosolvent to water in the reaction media are also examined, because the information obtained from the results at mixed aqueous solvents can play a crucial role in understanding antioxidant activity. Apart from the experimental studies, a few theoretical investigations mainly based on DFT calculations have also been performed for understanding the relationship between the structure and the antioxidant mechanism of naringenin in the mentioned solvent mixtures. So that, the reaction enthalpies related to the individual steps of three antioxidant action mechanisms (HAT, SET–PT and SPLET) are computed by using DFT/B3LYP method. These calculations are important for providing insight into molecular parameters and also show which mechanism is thermodynamically preferred.

## 2. Experimental section

### 2.1. Chemicals

Naringenin (4′,5,7-trihydroxyflavanone), Scheme 1, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) as free stable radical were purchased from Sigma-Aldrich. The solvent ethanol of HPLC gradient grade and tetra-n-butylammonium chloride (TBAC) were obtained from Merck. All chemicals were of reagent grade and were used without further purification. Stock solutions of naringenin and DPPH were freshly prepared by directly dissolving the required amounts of substances in ethanol–water mixture. Dilute solution of these compounds were prepared by adding a known volume of water and organic solvent before recording any kinetic run. The doubly distilled deionized water (conductivity of $1.2 \pm 0.1\ \mu\Omega^{-1}$) was used throughout.

**Table 1**
The values of $k_{obs}$ at different concentrations of naringenin, constant ionic strength 0.1 mol dm$^{-3}$ (TBAC) and 25 °C.

| $10^3 k_{obs}/s^{-1}$ | | | | | |
|---|---|---|---|---|---|
| $10^4$ [Nar] | Ethanol % | | | | |
| | 50 | 60 | 70 | 80 | 90 |
| 1.80 | 5.1 | 5.5 | 6.2 | 7.1 | 7.4 |
| 2.16 | 5.9 | 6.2 | 6.9 | 7.7 | 7.9 |
| 2.52 | 6.3 | 6.7 | 7.5 | 8.2 | 8.4 |
| 2.88 | 7.2 | 7.4 | 8.3 | 8.9 | 9.1 |
| 3.24 | 7.9 | 8.2 | 8.7 | 9.6 | 9.8 |

Uncertainties in the pseudo rate constants are 0.1 or lower.

### 2.2. Kinetics measurements and stoichiometry

The reaction between flavonoid naringenin and the DPPH radical was followed spectrophotometrically by recording the absorbance changes of DPPH at its absorption maximum of 520 nm as a function of time. Earlier it was verified that there is negligible interference from the other reagents at this wavelength. The kinetic measurements were carried out at different percentages for ethanol–water solvents ranging from 50% to 90% (v/v). The progress of the reaction was followed on a UV–vis Cary-50 diode array spectrophotometer (Varian) in conjunction with a Julabo F12 circulating thermobath, using quartz cells of path 10 mm. The temperature was maintained at (25.0 ± 0.1) °C by circulating a thermostated liquid through hollow, thermospacer plates on either side of the cell compartment.

The kinetic runs were performed under pseudo first-order conditions by keeping a large excess of naringenin over the DPPH ($1.8 \times 10^{-5}$ mol dm$^{-3}$) in all percentages of ethanol–water mixture (with the ratios 18:1, 16:1, 14:1, 12,1, and 10:1 of naringenin to DPPH) and at constant ionic strength (0.1 mol dm$^{-3}$ TBAC). The pseudo first-order rate constants, $k_{obs}$, for different runs were calculated from the slope of the linear least-square fits of $\ln[1/(A_t - A_\infty)]$ versus time plots, according to the equation: $\ln(1/[A_t - A_\infty]) = k_{obs}t - \ln[A_0 - A_\infty]$, where $A_0$, $A_t$ and $A_\infty$ are the values of absorbance at zero time, at any time and at the end of the reaction. The $A_\infty$ for each run was taken as the experimentally determined values. The pseudo first-order plots, in all cases, were linear

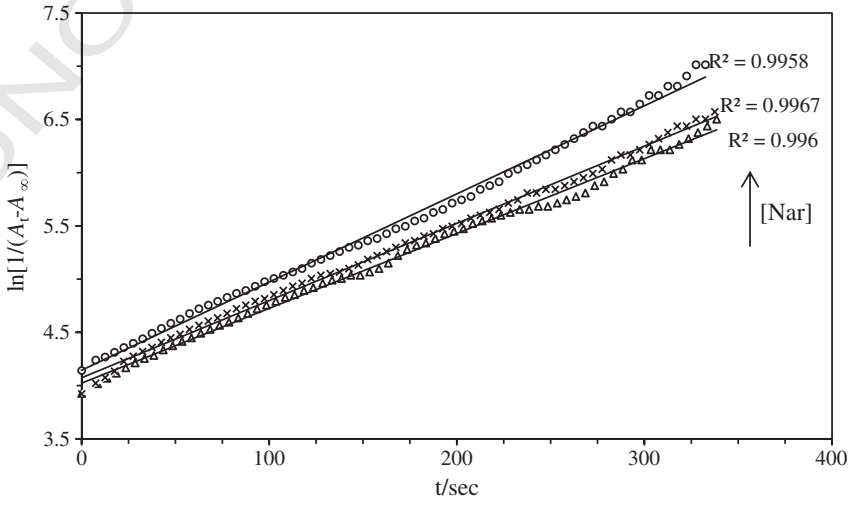



**Fig. 1.** Plots of $\ln[1/(A_t - A_\infty)]$ versus time in different concentrations of naringenin in 70% ethanol.



Please cite this article as: M. Jabbari, et al., J. Mol. Liq. (2014), http://dx.doi.org/10.1016/j.molliq.2014.04.015