



The determination of dissociation constants for active ingredients from herbal extracts using a liquid–liquid equilibrium method



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ABSTRACT

A liquid–liquid equilibrium method to determine dissociation constants was presented in this work using phenolic acids in the water extract of *Salvia miltiorrhiza* as a sample. The models to quantitatively describe the relationship between aqueous phase pH value and apparent distribution coefficient value were built. Apparent distribution coefficients are determined after liquid–liquid equilibrium experiments. The pK_a values of phenolic acids in *S. miltiorrhiza* extracts were fitted successfully. The dissociation constants of active ingredients that are in a mixture in which most components are unknown can be determined using this method. The distribution coefficients of active ingredient molecules and ions can also be obtained. 1-Octanol is a recommended solvent with low solubility, high boiling point, and extra information of K_{ow} .

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1. Introduction

Ionization is one of the key physicochemical characteristics of a drug [1]. It often influences pharmacokinetic property and pharmacodynamics property [2]. Ionization is also a significant concern in the purification process of active ingredients because it always leads to the changes in physical or chemical properties [3]. An important characterization parameter for drug ionization is dissociation constant. Therefore dissociation constant is a key parameter for drug discovery, development, and manufacturing.

Herbal medicines are more and more popular in many countries to deal with chronic diseases. However, the dissociation constants of herbal active ingredients are difficult to determine for several reasons. First, it is difficult or expensive to prepare large amount of pure substances of herbal active ingredients. Sometimes a herbal active ingredient is not stable in aqueous solution when it is the only solute. It means that traditional methods, such as titration method [4], conductivity method [5], and spectrophotometry method [6], cannot be used to determine dissociation constants of these herbal active ingredients. Second, many components in a herbal extract are unknown. Liquid chromatography method or

capillary electrophoresis method can measure dissociation constants of active ingredients in a mixture [7–9]. However, when using these methods to treat herbal extracts, it will be difficult to identify the peaks after changing separation conditions. Therefore new method to determine dissociation constants of herbal active ingredients is still highly required.

In this work, a liquid–liquid equilibrium method is proposed using a sample system of water extract of *Salvia Miltiorrhiza*. There are many phenolic compounds in the water extract of *Salvia Miltiorrhiza*, such as Salvianolic acid B (SaB), Salvianolic acid A (SaA), Danshensu (DSS), lithospermic acid (LA), protocatechuic aldehyde (PA), caffeic acid (CA), rosmarinic acid (RA), and so on [10]. They are considered as main active ingredients of *Salvia Miltiorrhiza* for the treatment of cerebrovascular diseases, coronary artery diseases, myocardial infarction, hepatitis, and menstrual abnormalities [11]. Some of their molecular structures are shown in Fig. 1.

The dissociation constants in water of most phenolic acids in a *Salvia Miltiorrhiza* extract are not reported because some of them are easy to degrade in an aqueous solution. For example, lithospermic acid tends to hydrolyze and form Danshensu and lithospermic acid in aqueous solution [13,14]. Salvianolic acid A is easy to form Salvianolic C and its isomers by oxidation [15,16].

In this work, different solvents were used to extract phenolic acids from water extracts of *Salvia Miltiorrhiza* at different aqueous phase pH values. Models were developed to quantitatively describe the relationship between aqueous phase pH values and apparent

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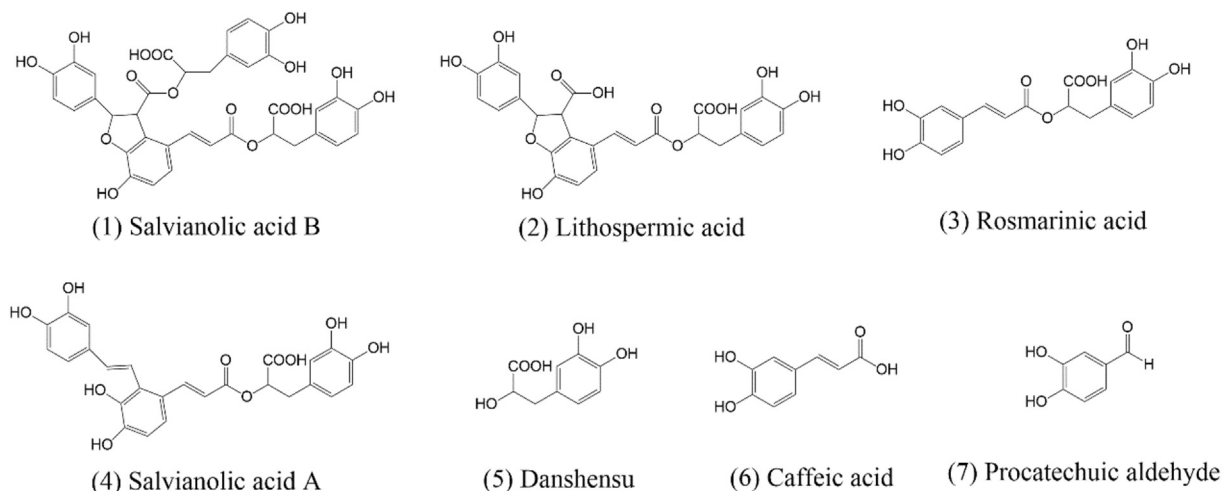


Fig. 1. Structural formulae of some phenolic compounds [12].

distribution coefficients (D_{app}). The pK_a values of SaB, SaA, CA, DSS, LA, and RA were calibrated. Standard errors of fitted results were also obtained. The influences of equilibrium temperature and dry matter concentration were also investigated. The obtained pK_a values were compared with published values. The characteristics of this method and solvent selection were also discussed.

2. Materials and methods

2.1. Materials and chemicals

Standard compounds and reagents, as shown in Table 1, were used in this work. Two concentrated extracts of *Salvia miltiorrhiza* were kindly provided by Chiatai Qingchunbao Pharmaceutical Co., Ltd. (Hangzhou, Zhejiang, China). Ultrahigh-purity water was produced using a Milli-Q academic water purification system (Milford, MA, USA). All materials were used as received without any further purification.

2.2. Procedures

Two concentrated extracts of *Salvia miltiorrhiza*, namely CE A and CE B, were diluted with water to obtain initial aqueous solutions before contacting with organic solvents. MTBE, 1-heptane,

butyl acetate, MIBK, 1-butanol, and 1-octanol were used as the solvents. Liquid–liquid equilibrium experiments were carried out in a conical flask. After adding a diluted concentrated extract and a solvent to a conical flask, a small volume of HCl solution was also added. The flask then was sealed and placed in a temperature-controlled oscillator (DSHZ-300, Taicang City Experimental Equipment Factory). After shaken at 140 rpm for more than 12 h, the mixture then was centrifuged for 20 min at a rotation speed of 3000 rpm using a centrifuge (5810R, Eppendorf) to separate two phases. The concentrations of DSS, PA, CA, RA, LA, SaB, and SaA in two phases were determined. The pH values of aqueous phase were also determined using a pH meter (S40, Mettler-Toledo Instruments Co., Ltd.) after liquid–liquid equilibrium experiments.

To investigate the effects of initial aqueous solution composition, equilibrium temperature, and solvent type on determination results, the experiments were carried out with different conditions, which are listed in Table 2. All the experiments were carried out at atmospheric pressure.

2.3. Analytical methods

The concentrations of DSS, PA, CA, RA, LA, SaB, and SaA were determined by high performance liquid chromatography analysis according to the method published by Cao et al. [17]. The method is

Table 1
Sources and purities of standard compounds and reagents used in this work.

Chemical name	Source	CAS number	Mass fraction purity ^a
Salvianolic acid B (SaB)	Shanghai Winherb Medical Science (China)	121521-90-2	0.9941
Salvianolic acid A (SaA)	Shanghai Winherb Medical Science (China)	96574-01-5	0.9891
Sodium Danshensu	Shanghai Winherb Medical Science (China)	67920-52-9	0.9910
Lithospermic acid (LA)	Shanghai Winherb Medical Science (China)	28831-65-4	0.9923
Procatechuic aldehyde (PA)	Shanghai Winherb Medical Science (China)	139-85-5	0.9961
Caffeic acid (CA)	Shanghai Winherb Medical Science (China)	331-39-5	0.9967
Rosmarinic acid (RA)	Shanghai Winherb Medical Science (China)	20283-92-5	0.9943
Methyl tert-butyl ether (MTBE)	Shanghai Lingfeng Chemical Reagent (China)	1634-04-4	≥0.990
1-Heptane	Shanghai Lingfeng Chemical Reagent (China)	142-82-5	≥0.985
Butyl acetate	Shanghai Lingfeng Chemical Reagent (China)	123-86-4	≥0.990
1-Octanol	Shanghai Lingfeng Chemical Reagent (China)	111-87-5	≥0.990
4-Methyl-2-pentanone (MIBK)	Aladdin (China)	108-10-1	0.9901
1-Butanol	Chinasun Specialty Products (China)	71-36-3	≥0.995
Hydrochloric acid	Jiani Chemical (China)	7647-01-0	0.36–0.38
Phosphoric acid	Sigma–Aldrich (USA)	7664-38-2	0.85–0.90
Acetonitrile	Merck (Germany).	75-05-8	>0.999
Formic acid	Roe Scientific (USA).	64-18-6	>0.99

^a As reported by the supplier.

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