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Extraction of stevioside using aqueous two-phase systems formed by choline chloride and K_3PO_4

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ABSTRACT

Limitations and disadvantages of artificial sweeteners in the food and beverage products act as an incentive for paying attention to the extraction and purification of natural sweeteners. Aqueous two-phase systems (ATPSs) are believed to be a desirable method for the extraction and separation of biomolecules. In recent years, researchers have focused on the use of innocuous, benign components having potential to form ATPSs. In this research, choline chloride, as a biocompatible and nutritious constituent, has been utilized to establish an ATPS performing the extraction of stevioside. To assess the efficiency of the ATPS composed of choline chloride and potassium phosphate, the partitioning of stevioside was explored. The effects of such parameters as the weight percents of choline chloride and potassium phosphate on the partitioning of stevioside were studied. All experiments were conducted at four temperatures of 298 K, 303 K, 308 K, and 313 K. Also, the effect of pH on the partitioning of stevioside was investigated. Different regression models were adopted to correlate the empirical results of the stevioside partition coefficient, and through statistical analyses the most reliable regression model was chosen.

1. Introduction

Advanced science of biotechnology has presented a new generation of natural sweeteners which can be widely utilized in industrial as well as domestic applications. One of these natural sweeteners is stevioside which is extracted from a plant called stevia, and is currently available for commercial use. Drawing considerable attention recently, stevioside is regarded as one of the natural and well-known sweeteners in the food and pharmaceutical industries. Stevioside is said to be 300 times sweeter than beet-derived sucrose. This sweetener is comprised of complex molecules besides the building blocks of glucose (Geuns, 2003; Kroyer, 1999).

Known as a no-calorie sweetener, stevoside is not absorbed in the digestive system. Compared to other artificial sweeteners, it has several other benefits such as lack of carcinogenic effects, non-toxicity, potential impact on reducing the obesity and the high blood pressure, and marginal effect on blood glucose. Since the artificial sweeteners are suspected to be carcinogenic, the natural stevioside takes on economic importance in the production of beverages, sweet breads, and dairy (Kroyer, 1999; Stoyanova et al., 2011).

In food industries, the first, and the most important deterrent to the use of natural sweeteners is their rather steep price. Separation and purification of biomolecules, which are considered as downstream processes, are costly and demanding. The downstream costs include 80% of the total costs for the purification of biomolecules (Freire et al., 2012; Lebovka et al., 2011; Waites et al., 2009).

Since stevioside has numerous applications in food industry, pharmaceutical and medical fields, its purity must be very high so as to meet their strict standards. Therefore, the choice of a suitable method for the separation and purification in downstream processing is of great significance. The adopted method must be fast, selective, and must possess the ability to function economically on a large scale.

Food and pharmaceutical industries are frequently faced with major problems related to the extraction and separation of biomolecules. For this reason, researchers and industrialists constantly seek for methods which are suitable and sustainable.

In 1950, Albertsson (1986) suggested the use of ATPSs as an alternative for the biphasic systems containing conventional organic solvents.

Using the aqueous two phase systems (ATPSs), proposed in recent decades, are believed to be the most effective technique for the extraction and separation of biomolecules in one stage. This method seems to be selective and appropriate for the continuous processes on an industrial scale as well. ATPSs have no harmful impacts on the structure of biomolecules, and as reflected by the literature, have many advantages

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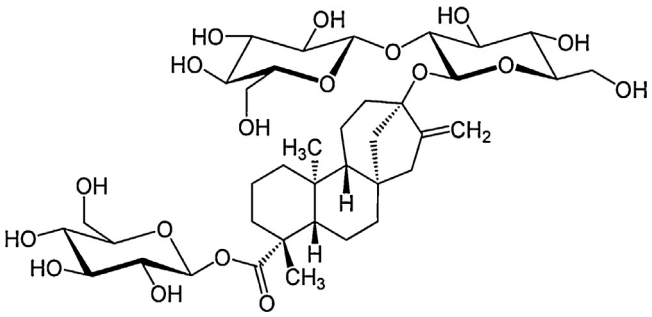

Fig. 1 – Chemical structure of stevioside.

over the other conventional extraction approaches (Freire et al., 2010; In et al., 2005; Wu et al., 2014).

Researchers have shown that separation of a whole range of biomolecules such as pigments (Mageste et al., 2009; Wu et al., 2011), viruses (Liu et al., 1998), enzymes (Sarangi et al., 2011; Shahriari et al., 2010), proteins (Li et al., 2012) and antibiotics (Shahriari et al., 2012; Shahriari et al., 2013) through ATPSs is quite practicable.

ATPSs are formed by mixing two immiscible phases such as two hydrophilic polymers or a hydrophilic polymer and a strong salt (Johansson et al., 1998; Li et al., 2005; Walter, 2012).

Polymer-based ATPSs suffer from such drawbacks as high viscosity and slow phase separation. Gutowski et al. (2003) for the first time, reported the possibility of the ATPSs formation using mineral salts and ionic liquids. Ionic liquids are categorized as salts that are liquid in lower temperatures and can be a good substitute for organic solvents in separation processes. Finding agents that are safer and cheaper than ionic liquids, with similar characteristics, is still a bottleneck.

Because of the sensitivity of biomolecules and their applications, particularly in the food and pharmaceutical industries, certain restrictions on the ATPS ingredients should be set. Recently published articles introduced a new class of salts having the ability to overcome these limitations. The cholinium-based salts are promising candidates and seem to be viable alternatives to conventional salts used for the preparation of ATPSs (Pereira et al., 2013b; Shahriari et al., 2013).

Cholinium chloride (choline chloride) was discovered by Adolph Strecker who separated the platinum salts from the pig bile (Strecker, 1862). He showed that choline is an integral part of the egg yolk lecithin. Choline is classified as a vitamin and belongs to the B-group vitamins, which acts similar to amino acids or essential fatty acids. Choline chloride ([Ch]Cl) is a tetravalent ammonium salt [(2-hydroxyethyl) trimethylammonium chloride, or vitamin B_4] which is an essential nutrient for healthy growth of animals, particularly poultry, pigs and pets. Thanks to its role in the human organism, [Ch]Cl is also a pharmaceutical ingredient, as confirmed by the Institute of Medicine in 1998 (Zeisel and Da Costa, 2009). Its proven benefits are: ease of preparation, greater stability in water and air, biocompatibility, biodegradability, and being relatively cheap and more hydrophilic compared to other choline-based salts (Shahriari et al., 2013). Also, Shahriari et al. showed that the viscosity of choline-chloride-rich phase is lower than that of corresponding phases with other choline-based salts. The lower viscosity benefits mass transfer and makes the phase separation procedure easier when it comes to scaling-up and industrial extraction (Shahriari et al., 2013).

A review of the previous works reveals that a few papers on the use of [Ch]Cl-based ATPS have been published, aimed at extracting and separating biomolecule (Liu et al., 2013; Pereira et al., 2014). For instance, Freire et al. (2010) pointed out the safety of these systems for the extraction of the antibiotics. Hydrophilic choline chloride together with tripotassium phosphate are practical options to stablish an ATPS. It is worth noting that this salt is readily miscible in the aqueous phases (Zempleni et al., 2007).

Tripotassium phosphate is a strong, inexpensive salt which is frequently used in food processing. Thanks to its great potential for salting-out, it has drawn a lot of attention in the field of ATPSs (Shahriari et al., 2013).

The aim of this study is to assess the partitioning of stevioside in the ATPSs containing [Ch]Cl and tripotassium phosphate. The partition coefficient and recovery percent of stevioside have been taken into consideration to evaluate the extent of applicability of these favorable two-phase systems. Aiming at determining the most optimal conditions for the separation of the stevioside, the effects of the weight percent of [Ch]Cl, the weight percent of tripotassium phosphate, pH, and four different temperatures of (298 K, 303 K, 308 K, and 313 K) on the partition coefficient of stevioside have been investigated. The optimal recovery of stevioside in the bottom phase was determined in terms of the volume ratio of phases.

2. Materials and methods

2.1. Materials

Choline chloride and tripotassium phosphate, with a purity of 99%, were purchased from Alfa Aesar Company. Stevioside (>98%) was procured from Sigma-Aldrich Company, USA. Distilled water was provided by RO-LAB, DW65 equipment utilizing twice distillation reverse osmosis. The molecular structure of stevioside has been illustrated in Fig. 1.

2.2. Methods

2.2.1. Phase diagrams and tie-lines

In this study, the phase diagram was obtained using the cloud point titration method at a temperature of 298 K (±1 K) and the ambient pressure in accordance with our previous work (Pourebrahimi et al., 2015). The experimental data of the binodal curve were correlated according to the following equation proposed by Merchuk et al. (1998).

$$[\mathrm{ChCl}] = A\ \exp[(B * [\mathrm{K_3PO_4}]^{0.5}) - (C * [\mathrm{K_3PO_4}]^3)] \quad (1)$$

where [ChCl] and $[K_3PO_4]$ are the choline chloride and the tripotassium phosphate weight percentages, and A, B, and C are constants which can be found by the regression of the experimental binodal data (Pourebrahimi et al., 2015).

The tie lines (TLs) were measured using a gravimetric method presented by Merchuk et al. (1998). Also, the TLs were calculated by the application of a mass balance along with the data of phase diagram related to Eq. (1). The mixture concentrations of [Ch]Cl and potassium phosphate are determined by their corresponding values in the top and bottom phases, adopting the lever-arm rule (the mass balance expression). To determine each TL, the following four equations are solved:

$$[\mathrm{ChCl}]_T = A\exp[(B * [\mathrm{K_3PO_4}]_T{}^{0.5}) - (C * [\mathrm{K_3PO_4}]_T{}^3)] \quad (2)$$

$$[\mathrm{ChCl}]_B = A\exp[(B * [\mathrm{K_3PO_4}]_B{}^{0.5}) - (C * [\mathrm{K_3PO_4}]_B{}^3)] \quad (3)$$

$$[\mathrm{ChCl}]_T = \left(\frac{[\mathrm{ChCl}]_M}{\alpha}\right) - \left(\frac{1-\alpha}{\alpha}\right) \times [\mathrm{ChCl}]_B \quad (4)$$

$$[\mathrm{K_3PO_4}]_T = \left(\frac{[\mathrm{K_3PO_4}]_M}{\alpha}\right) - \left(\frac{1-\alpha}{\alpha}\right) \times [\mathrm{K_3PO_4}]_B \quad (5)$$

In the above equations, B, T, and M refer to the bottom phase, the top phase, and the mixture, respectively. α is the ratio between the bottom phase mass and the total mixture mass.