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# Isolation of C-phycocyanin from *Spirulina platensis* microalga using Ionic liquid based aqueous two-phase system

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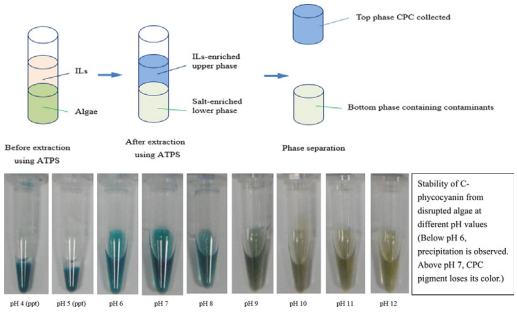
## GRAPHICAL ABSTRACT

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## ABSTRACT

An aqueous two-phase system (ATPS) with ionic liquids (ILs) was used for the isolate of C-phycocyanin (CPC) from *Spirulina platensis* microalga. Various imidazolium ILs and potassium salts were studied. The effect of ILs-ATPS on the extraction efficiency of CPC was also studied. The experimental parameters like pH, loading volume, algae concentration, temperature, and alkyl chain length of IL were well-covered in this report. The experimental results showed that the extraction efficiency, the partition coefficient, and the separation factor for CPC were 99%, 36.6, and 5.8, respectively, for an optimal pH value of 7 and a temperature of 308 K. The order of extraction efficiency for CPC using IL-ATPS was: 1-octyl-3-methylimidazolium bromide (C8MIM-Br) > 1-hexyl-3-methylimidazolium bromide (C6MIM-Br) > 1-butyl-3-methylimidazolium bromide (C4MIM-Br). The isolation process followed the pseudo second-order kinetic model and the thermodynamic results were obviously spontaneous.

## 1. Introduction

Aqueous two-phase systems (ATPSs) are effective extraction processes for downstream processing and have been widely used in the recovery and purification of bio-products (Li et al., 2010; Sivapragasam et al., 2016; Yau et al., 2015). ATPSs have many advantages over conventional solvent extraction processes, such as energy-efficient operating protocol, easy to operate and scale-up, and reducing the processing time and cost (Li et al., 2005; Phong et al., 2018). In recent years, ATPS using ionic liquids (ILs) have been the subject of extensive

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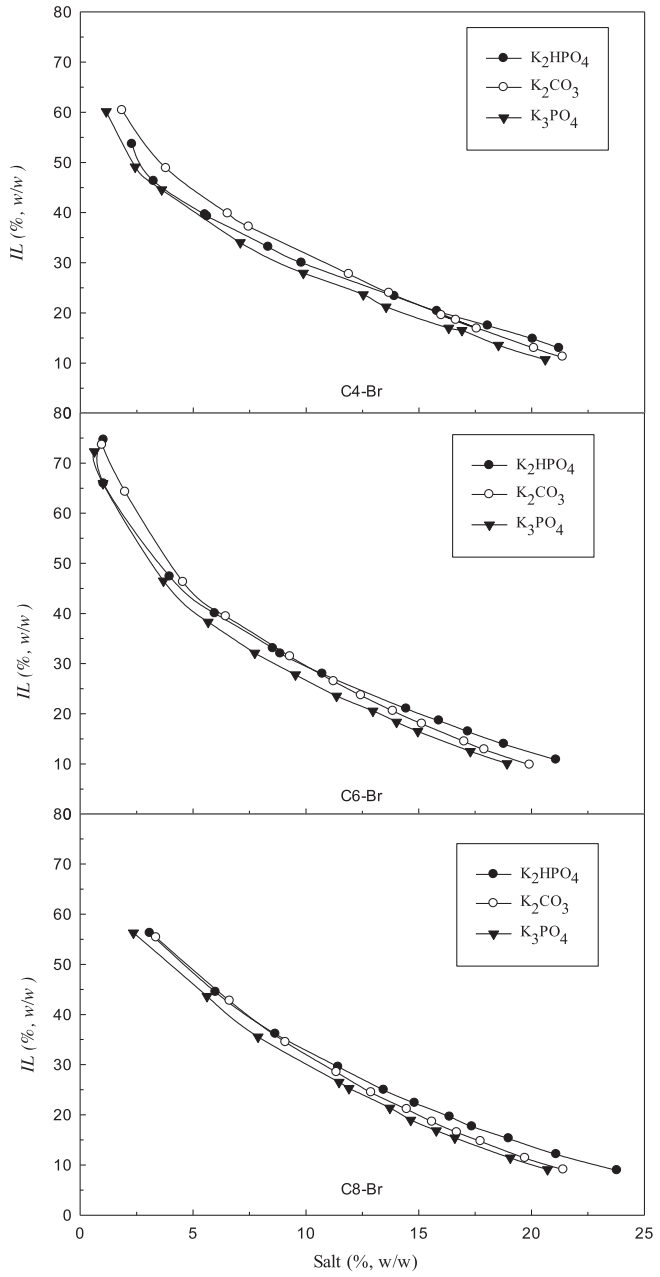


**Fig. 1.** The phase diagram for an IL/salt aqueous two-phase system.

studies (Ng et al., 2013; Phong et al., 2017b; Sankaran et al., 2017; Show et al., 2012). ILs are green solvents that exhibit unique properties, such as high thermal and chemical stability, strong solubility and solvating power and low vapor pressure. Typically, ILs that are based on imidazolium and pyridinium have the highest UV/visible absorption wavelengths at 210 and 264 nm, respectively. This feature is important for the determination of IL concentrations (Li et al., 2005; Sivapragasam et al., 2016). The unique features of IL mean that they are eminently suitable for the extraction of metal ions, organic molecules and proteins (Dreyer and Kragl, 2008; Moniruzzaman et al., 2010; Pei et al., 2009; Tzeng et al., 2008) (see Fig. 1.).

*Spirulina* is a functional food in the prevention and treatment of diseases because of its health promoting effects (Leng et al., 2018). It is a blue-green alga that belongs to the Oscillatoriaccae family. Two of the most common spirulina species are *Spirulina platensis* (*Arthrospira platensis*) and *Spirulina maxima* (*Arthrospira maxima*), which have a high nutritional value and exhibit functional properties. *S. platensis* has been extensively studied in the fields of food science and medicine, because of its broad market potential (Antelo et al., 2008). *S. platensis* has antiviral, anti-free radical, anti-inflammatory, anti-oxidation, anti-parasitic, anti-bacterial and anti-fungal and other functional properties (Kuddus et al., 2013). It also contains many important nutrients, including carbohydrates, proteins, vitamins, minerals and fatty acids. It is also an important source of protein and its dry weight contains about 60–70% protein (Khan et al., 2005; Kulshreshtha et al., 2008).

C-phycocyanin (CPC) is a natural blue pigment that belongs to the phycobiliprotein group. *S. platensis* is the main source of CPC. CPC has been widely used in the food processing, cosmetics and nutrition industries as a replacement for artificial dyes (Cheah et al., 2016; 2015; Chew et al., 2017; Yu et al., 2017). CPC has also been proven to have a role in preventing cancer, modulating lipid and carbohydrate metabolisms and indirectly controlling the onset of chronic diseases, such as diabetes, obesity, cardiovascular disease and heart disease (Arad and Yaron, 1992; Khan et al., 2005).

The extraction and purification of CPC from *Spirulina platensis* has been extensively studied using ammonium sulfate precipitation, ultrafiltration, ATPS and ion exchange, hydrophobic interaction or gel filtration chromatography (Jian-Feng et al., 2007; Moraes et al., 2010; Patil et al., 2006; Patil and Raghavarao, 2007; Ramos et al., 2011). In this study, APTSs that have imidazolium ions were used to extract CPC from S. *platensis*. Different combinations of IL and potassium salts were used to determine the optimal extraction process for ATPS. The optimum phase-forming components for ATPS were determined by varying the operational parameters, such as the pH value, the loading volume, the algae concentration, the working temperature and the alkyl chain length for the ILs. Kinetic and thermodynamic studies of ATPS that use IL were also undertaken.

## 2. Materials and methods

### 2.1. Materials

N-methylimidazole, a series of alkyl bromides, potassium carbonate ($K_2CO_3$), monopotassium phosphate ($KH_2PO_4$) dipotassium phosphate ($K_2HPO_4$) and tripotassium phosphate ($K_3PO_4$) were purchased from Merck (Darmstadt, Germany). Dried *Spirulina platensis* powder was purchased from Charming & Beauty Co. Ltd. (Taipei, Taiwan). All chemicals used were of analytical grade. Algae cells were disrupted using an ultrasonicator (Misonix Model S-4000, Sonics & Materials, Inc. MA, USA). A UV–vis spectrophotometer (Model Ultrospec 3100 pro, GE Healthcare Biosciences, Uppsala, Sweden) was used to measure the absorbance value of the CPC sample.

### 2.2. Preparation of IL

Imidazolium IL (i.e., 1-butyl-3-methylimidazolium bromide (C4MIM-Br), 1-hexyl-3-methylimidazolium bromide (C6MIM-Br) and 1-octyl-3-methylimidazolium bromide (C8MIM-Br) were prepared as described in references (Dreyer and Kragl, 2008; Pei et al., 2009). The $^1H$ NMR spectra for the IL were measured using the method for previous studies and the results were in agreement with the data that has been reported in these studies (Li et al., 2005; Pei et al., 2009; Tzeng et al., 2008).

### 2.3. Construction of phase diagram

The phase diagram for the ATPS using IL was determined using the cloud-point method (Albertsson, 1960). The weight of the IL (i.e., C4MIM-Br, C6MIM-Br and C8MIM-Br) and the potassium salts (i.e., $K_2CO_3$, $K_2HPO_4$ and $K_3PO_4$) were measured using an analytical balance and these masses were given as a percentage of the mass of the entire system (%, w/w). The bimodal curve in the phase diagram was plotted by varying the concentrations of the IL and the potassium salts (Dreyer