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Temperature controlled ionic liquid aqueous two phase system combined with affinity capillary electrophoresis for rapid and precise pharmaceutical-protein binding measurements

Deia Abd El-Hady [a,*], Hassan M. Albishri [b]

[a] Chemistry Department, Faculty of Science, University of Jeddah, P.O. 80327, 21589 Jeddah, Saudi Arabia
[b] Chemistry Department, Faculty of Science, King Abdulaziz University, 80203 Jeddah, Saudi Arabia

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ABSTRACT

Temperature controlled ionic liquid aqueous two phase system (ILATPS) was used to improve the precision of pharmaceutical-AGP (human alpha (α1)-acid glycoprotein) binding measurements by affinity capillary electrophoresis (ACE). The effect of different types of short-chain alkyl imidazolium ILs within the concentration range of 10.0–1000.0 μmol L^{-1} on a propranolol (PRO)-AGP model was firstly investigated by ILATPS/ACE system. Use of 100.0 μmol L^{-1} 1-butyl-3-methylimidazolium chloride (BMImCl) in 67.0 mmol L^{-1} potassium phosphate buffer (pH 7.4) containing low concentrations of AGP (5.0–100.0 μmol L^{-1}) gave the highest precision value ($2.98 \pm 0.14 \times 10^5$ L/mol) which is in concord with the reported one (3.00×10^5) under similar experimental conditions. The proposed BMImCl/phosphate solution was a unique temperature controlled system to preserve AGP activity during the pre-analysis and within ACE measurements under lab conditions for about 30 days. This period could be prolonged by converting the one-phase solution into biphasic solution at 4 °C storage temperature and again it could get rapidly back into one-phase by raising the temperature with gentle shaking. This behavior could be attributed to the electrostatic interaction and π–π interaction between BMIm cations and negatively charged AGP ions (pI = 2.7). Moreover, the compatibility of ILATPS with ACE has been the critical factor to avoid precipitation of salts formed by anion exchange in the running buffer. The current ILATPS/ACE system was further used to rapidly and precisely estimate the binding constants of anticancer drugs methotrexate (MTX) and vinblastine (VBL) with human AGP. The obtained binding values have been in good agreements with their findings by high performance affinity chromatography (HPAC). This ILATP/ACE system could similarly be applied to improve the precision of other proteins binding measurements with consuming a small amount of protein and with shortening ACE analysis time.

1. Introduction

The precise determination of protein-pharmaceutical binding is of a particular interest to understand the behavior of a drug during its life cycle in the body. Affinity capillary electrophoresis (ACE) is considered an adequate technique for rapidly and easily accessible protein-pharmaceutical binding measurements due to the presence of several advantages such as low sample and ligand consumption, relatively short analysis times, high efficiency and suitability for probing high and weak affinity interactions [1]. In ACE, a drug is commonly located in the sample solution and a protein usually exists in the background electrolyte (BGE) containing daily prepared aqueous phosphate buffer under physiological conditions [2–5]. Upon applied voltage, the change of the migration time of the analyte (the pharmaceutical) due to its interaction with the protein is monitored and is used for the calculation of binding values [6–9]. Probably, this common behavior could influence the precision of measurements due to the existence of several stress factors on protein during the pre-analysis and within ACE analysis such as sonication, filtration, joule heating, adsorption on the inner

Abbreviations: AC, acetanilide; ACE, affinity capillary electrophoresis; AGP, human alpha (α1)-acid glycoprotein; BMImBr, 1-Butyl-3-methylimidazolium bromide; BMImCl, 1-butyl-3-methylimidazolium chloride; $BMImBF_4$, 1-butyl-3-methylimidazolium tetrafluoroborate; DMImCl, 1-decyl-3-methylimidazolium chloride; EMImCl, 1-ethyl-3-methylimidazolium chloride; HPAC, high performance affinity chromatography; HMImCl, 1-hexyl-3-methylimidazolium chloride; IL, ionic liquid; ILATPS, ionic liquid aqueous two phase system; MTX, L-methotrexate; PRO, propranolol; VBL, vinblastine.

* Corresponding author.
E-mail address: damoustafa@uj.edu.sa (D. Abd El-Hady).

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surface of fused capillary surface, etc [10–14]. Therefore, it is still a challenge to preserve the activity of protein during ACE analysis.

Recently, temperature controlled ionic liquid aqueous two phase system (ILATPS) [15–16] was used to improve the precision of pharmaceutical-human serum albumin (HSA) binding measurements by ACE [17]. This was a unique temperature controlled system in which, hydrophilic imidazolium IL combined with kosmotropic inorganic salts formed a monophasic solution at ambient temperature and easily converted into biphasic solution by cooling it around 4 °C. This could be due to the driving of water from the IL-rich phase to the salt-rich phase by decreasing the temperature, so that the concentration of the IL increases, whereas the salt-rich phase becomes more diluted [18–22]. In another words, salting-out inducing ions acted mainly through entropic effects whereas salting-in inducing ions directly interact with the hydrophobic moieties of the IL. The improvement of HSA stability by hydrophilic ILATPS, specifically short chain imidazolium ILs with low concentrations ($\leq$100.0 μmol L^{-1}), was attributed to the binding of negatively charged HSA molecules under physiological pH with IL through several forces such as electrostatic interactions, π–π interactions and/or hydrogen bonding [17]. These forces did not cause any denaturation of protein or any disturbance in its binding sites with drugs. By combination of ILATPS with ACE, specific features such as enriched aqueous media, relatively low viscosity, and low denaturing properties of IL were also observed. Furthermore, compatibility of water-soluble ILs with phosphate running buffer is a critical factor to avoid precipitation of salts formed by anion exchange. Then, this novel ILATPS/ACE system could be applied successfully to preserve protein activity during pre-analysis and within-ACE analysis leading to improve the precision of ACE binding measurements.

The two most important plasma proteins with respect to drug binding are HSA and human alpha (α1)-acid glycoprotein (AGP). Although AGP has a much lower concentration than HSA in serum, it can be a major binding protein for a broad spectrum of drugs. To the best of our knowledge, there are no reports about the use of hydrophilic imidazolium ILATPS containing AGP as a running buffer for precise ACE binding measurements. Therefore, in the current work, the study of different short chain imidazolium ILs and their use to improve the precision of AGP binding with several pharmaceuticals ((+)-propranolol (PRO), methotrexate (MTX) and vinblastine (VBL)) were firstly investigated. The influences of key factors such as alkyl side chain length of ILs, type of IL-anions, concentrations of ILs and concentrations of AGP on ACE measurements were also studied. These ACE binding values were further confirmed by alternative high performance affinity chromatography (HPAC) or by comparing with the reported value under similar conditions. Moreover, the stability of the stored AGP solution in ILATPS for a long time under ambient lab conditions was investigated.

2. Materials and methods

2.1. Materials and solutions

AGP ($\geq$99% agarose gel electrophoresis), acetanilide (AC), potassium dihydrogen phosphate, dipotassium hydrogen phosphate dehydrate, sodium hydroxide, sodium nitrate, hydrochloric acid and 2-propanol were purchased from Sigma-Aldrich, Germany. PRO, MTX and VBL were obtained from Fluka (Steinheim, Germany). All short alkyl chain ionic liquids: 1-ethyl-3-methylimidazolium chloride (EMImCl), 1-butyl-3-methylimidazolium chloride (BMImCl), 1-hexyl-3-methylimidazolium chloride (HMImCl), 1-decyl-3-methylimidazolium chloride (DMImCl), 1-butyl-3-methylimidazolium bromide (BMImBr) and 1-butyl-3-methylimidazolium tetrafluoroborate ($BMImBF_4$) were obtained from Aldrich (Steinheim, Germany). Water was purified by Milli-Rx apparatus (Millipore, Milford, MA, USA).

Standard stock solutions of MTX (250.0 μg mL^{-1}), VBL (250.0 μg mL^{-1}) and PRO (100.0 μg mL^{-1}) were prepared by dissolving the appropriate amounts of analytes in 25 mL phosphate buffer. AC (1500.0 μg mL^{-1}) was prepared in 25 mL phosphate buffer. Phosphate buffer (pH 7.4) was prepared by mixing 80.2 mL of 67.0 mmol L^{-1} dipotassium hydrogen phosphate and 19.8 mL of 67.0 mmol L^{-1} potassium dihydrogen phosphate. The working solutions were prepared by diluting the appropriate volume of stock solutions in 5.0 mL phosphate buffer. Before dilution, 1.0 mL of AC (EOF marker) was added into each sample solution in order to give a concentration of 300.0 μg mL^{-1}. AGP solution was prepared by dissolving the appropriate amount of AGP in a mixture solution of 67.0 mmol L^{-1} phosphate buffer (pH 7.4) and 100.0 μmol L^{-1} of BMImCl. All solutions were prepared under ambient lab conditions (temperature 22 ± 2 °C and normal fluorescent light). The injected solutions were filtered through 0.22-μm syringe filters prior to their application into the ACE system.

2.2. Apparatus

The CE experiments were performed using Agilent 7100 System (Waldbronn, Germany) equipped with a diode array detector. Bare fused-silica capillaries (Agilent, Moers, Germany) with 50 μm ID, 8.5 cm outlet and 56 cm effective length were used. Electropherograms were monitored using a CE Chemstation software. The mobility ratio (R) of the drug was calculated by the equivalent relationship $R = t_{eof}/t_{drug}$ where t_{eof} is the migration time for a neutral marker (AC) and t_{drug} is the migration time of the targeted drug [8].

The HPAC experiments were performed on Shimadzu HPLC-20AD (Kyoto, Japan) system consisted of a degasser unit (DGU-20A 3R), a pumper unit (LC-20AD), an autosampler (SIL-20A), an oven (CTO-20AC) and a diode array detector (SPD-M20A). AGP column (2 × 100 mm, particle size 5 μm) was purchased from Sigma-Aldrich (Germany). The spectra were automatically obtained by LC solution analysis software that is controlled by "dell" desktop. The pH was measured using a microprocessor pH meter (pH 211 Hanna instruments, UK).

2.3. Operating conditions

2.3.1. ACE measurements

The new uncoated fused silica capillary was conditioned by flushing it with 1.0 mol L^{-1} NaOH for 40 min followed by H_2O for 10 min. At the beginning of each day, the capillary was rinsed with 0.1 mol L^{-1} HCl for 3.0 min and then H_2O for 6.0 min. Between runs, the capillary was rinsed with 0.1 mol L^{-1} HCl for 1 min followed by H_2O (2 min) and running buffer (4 min). At the end of each day, capillary was washed with 0.1 mol L^{-1} HCl for 2.0 min and then H_2O for 20.0 min. All washing steps were performed by applying 940.0 mbar of pressure. The analytes were hydrodynamically injected at 50.0 mbar for 5.0 s. All injections were performed at the inlet end of the capillary (farthest from the detector). The separations were achieved by maintaining a voltage of 20.0 kV with positive polarity. Temperature of capillary was maintained at 23.0 °C. Wavelengths were 285 nm for PRO and 250 nm for MTX and VBL. All experiments were performed by injecting a constant concentration of a drug onto an AGP/IL/Phosphate running buffer. The binding constants were calculated by the following non-linear regression equation [6]:

$$K \cdot c(L) = \frac{R_f - R_i}{R_i - R_c}$$