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# Effects of ionic liquid and nanogold particles on high-performance liquid chromatography-electrochemical detection and their application in highly efficient separation and sensitive analysis of five phenolic acids in Xuebijing injection

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ABSTRACT

A novel high performance liquid chromatography-electrochemical detector (HPLC-ECD) analytical system was developed in this study by integratedly utilizing ionic liquid (IL) of 1-butyl-3-methylimidazolium bromide and an additive of gold nanoparticles. The resulted pilot study was first performed to assess the effects of 1-butyl-3-methylimidazolium bromide and gold nanoparticles on the chromatographic characteristics of five phenolic acids in Xuebijing injection, including danshensu (DSS), protocatechuic acid (PA), protocatechuic aldehyde (PAH), hydroxy safflower yellow A (HSYA) and ferulic acid (FA). It was notable to observe that retainability of the phenolic acids were markly lowered by IL addition. Compared with the cases without IL addition, the retention times of DSS, PA, PAH, HSYA and FA have decreased 2.851, 1.532, 1.53, 0.818 and 0.552 min, respectively when 0.6% IL in the mobile phase. In addition, the corresponding theoretical plate numbers and peak areas for these compounds were significantly increased. Area response for DSS, PA, PAH, HSYA and FA were enhanced by 772%, 628%, 584%, 703% and 600%, respectively. It was observed that nano-gold catalysis power enabled peak areas of DSS, PAH, FA and PA to enhance 5.7, 6.2, 8.5 and 66.5 times relative to the case with addition of IL. Altogether, the optimized HPLC-ECD system was successfully applied to the pharmacokinetics study of Xuebijing injection with underlying applicability to in vivo and in vitro analysis of a variety of natural product from Chinese medicine plants, TCM formulae and associated patent TCM preparation.



## 1. Introduction

Many studies indicate that retaining ability of analytical compounds onto the chromatographic column is affected markly by cross-linking reaction resulting from the free silanol groups at the surface of the chromatographic stationary phase silica and its related materials [1,2]. The cross-linking reaction can be intervened by mixing ion-pair reagent into the mobile phase or adjusting its pH value. However, addition of ion-pair reagent to mobile phase often leads to higher pressure, lower tolerance and irreversible damage to stationary phase of the analytical column. Therefore, it can be predictable that the reaction of the free silanol groups poses a great challenge for obtaining ideal analytical performance by using the conventional column.

Because of their distinctive properties, ILs have been broadly applied to many fields, such as heterogeneous catalysis, synthesis, electrochemistry and separation [3–6]. Recently, ILs have also been given increasing attention by scientists of analytical chemistry, who selectively use ILs to improve chromatographic performance [7–12]. ILs are primarily used as modification agent for stationary phase or mobile phase in gas chromatography [13,14] and high performance liquid chromatography [15], for chromatographic analysis of chiral compounds [16] or others. The primary

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reaction of ILs with stationary phase is characterized by cationic precursors of ILs being firstly coated on the surface of stationary phase, where they compete with analytical basic compounds for blinding to stationary phase. After coating, ILs suppress deleterious effects of free silanols and improve chromatographic peak shapes. Then the alkyl chains of the stationary phase proceed cross-linking reaction with the imidazole cation in ILs. Finally, because of the repulsion between imidazolium cation and ionized amines, part of them move with the mobile phase, and the anionic cores of ILs form ion-pair reagent with other cationic ion of the mobile phase [17]. ILs also present a preferred electrochemical activity [18, 19]. However, there are a few cases of approach application the applicability of ILs to electrochemical detector.

In addition, nano-metal particles have received increasing attention in the fields of physics, chemistry, biomedicine, material science and related disciplines [20–25], owing to their special electronic, quantum tunnel, optical and catalytic properties. An increasing number of researchers in electrochemical field also have turned their attention to nanometer materials in attempt to make new style electrode for selective ultra-sensitive detection of target compounds [26–30]. For example, nano-gold particles have been adopted by electro-analytical chemists because of their better biocompatibility, larger specific surface area, good conduction effect and catalytic activity.

Xuebijing injection is a confidential type of species in China, and it is an intravenous preparation in Traditional Chinese Medicine (TCM). Professor Jinda Wang developed it based on the Xuefuzhuyu decoction of Qinren Wang according to the theory of 'bacteria and bacterial toxin treated simultaneously' and dialectical principle of 'Sanzhengsanfa' [31]. The efficacy includes activating blood circulation and removing blood stasis, supporting the healthy energy, strengthening the body resistance, and clearing away and relieving toxin. Its main active component is extracted from *Flos carthami*, *Radix salviae miltiorrhizae*, *Rhizoma chuanxiong*, *Radix angelicae sinensis* and *Radix paeoniae rubra*. Clinically, Xuebijing injection is used in combination with antibiotics to treat pyemia and multiple organ dysfunction syndrome. And it is the unquiet injection of TCM treatment of this disease in China, which makes the TCM become one part of the main stream of emergency medicine [32]. Xuebijing injection may regulate inflammatory reaction and anti-oxidative stress, regulate immune function, ameliorate blood coagulation function, protect endothelial cells and improve microcirculation [33–38].

Up to now, much attention has been focused on the clinical research and chemical composition analysis of Xuebijing injection [39, 40], and there are no reports on the chemical composition analysis by ECD or the pharmacokinetics of main component. Most publications described methods for the analysis of DSS, PA, PAH, HSYA or FA in biological samples by high-performance liquid chromatography with UV detector [41–45], few analyzed with ECD or mass detector [46, 47]. The poor sensitivity of the HPLC–UV method always leads to failure in the complicated sample analysis.

This work aims to characterize the effects of IL and nano-gold as additives in HPLC-ECD analytical system by comparatively assessing retention time, number of theoretical plate and corresponding peak area of five acids in Xuebijing injection. Furthermore, the optimized HPLC-ECD method was applied to profiling pharmacokinetics features Xuebijing injection.

## 2. Materials and methods

### 2.1. Apparatus

The experiments were performed on an Agilent HPLC system (Series 1100, Agilent Technologies, USA) equipped with a G1311A quaternary pump, G1379A vacuum degasser, G1316A column thermostat, G1315B DAD and G1313 auto manual sample injector. In addition, a 790 VA programmer electrochemical detector (Metrohm, Switzerland) and a G1311A quaternary pump (Series 1100, Agilent Technologies, USA) were used. The chromatography data were recorded and processed with HP chemstation software.

### 2.2. Chemicals and reagents

DSS (Lot no.: 110855–200506), PA (Lot no.: 101800–200205), PAH (Lot no.: 110810–200205), HSYA(Lot no.: 111637–200905) and FA (Lot no.: 11173–201012) were purchased from the National Institute for Control of Pharmaceuticals and Biological Products (Beijing, China), and their purity was over 98% by HPLC analysis. The ionic liquid, 1-butyl-3-methylimidazolium bromide, was supplied by Shanghai Chengjie Chemical Reagent Co., Ltd. (Shanghai, China). Xuebijing injection was purchased from Tianjin Chasesun Pharmaceutical Co., Ltd. (Tianjin, China). Methanol was of HPLC grade and obtained from Fisher Scientific Products (Fair Lawn, USA), and triply distilled water was employed. The other chemicals, reagents and solvents used were all of analytical grade.

### 2.3. Chromatographic condition

The analytical column was an Aglient TC-$C_{18}$ (250 mm × 4.6 mm, 5 μm) column coupled with a $C_{18}$ guard column. The column temperature was controlled at 30.0 ± 0.1 °C. The mobile phase was a mixture of methanol (A) -water containing 0.1% (v/v) formic acid (B) and different concentrations (0.0∼0.6%, v/v) of ILs by gradient elution mode.(0–10 min, 15% A; 10–25 min 15–25% A; 25–40 min, 25–60% A; 40–55 min, 60–100% A). The flow rate of the mobile phase was 0.6 mL/min. The equilibration time for each run was 5 min. The sample injection volume was 20 μL.

For electrochemical detection, a glassy carbon electrode was used as the working electrode, an Ag/AgCl was the reference electrode and a Pt wire was used as the counter electrode.

### 2.4. Effects of the IL and nano-gold on the HPLC-ECD analytical system

Preparation of nano-gold sol: take 1 mL $HAuCl_4$ aqueous solution (10 mg/mL) into 100 mL aqueous solution, heat to boil and maintain for 5 min, then drip 0.7 mL 1% $C_6H_5Na_3O_7$ (mass fraction) quickly followed by keeping for boiling for 15 min until the solution turns into wine-red. After cooling the solution at the room temperature, settle to 100 mL, then store in dark.

#### 2.4.1. Investigation of the chromatographic behavior

The applied working potential of the electrochemical detector was set at +900 mV. The mobile phase includes different concentrations of additive IL (0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, v/v). Mobile phases without additives were also employed for comparative purpose. The variation of the retention time and theoretical plate number of DSS, PA, PAH, HSYA and FA were investigated.

#### 2.4.2. Effects of IL and nano-gold particles on sensitivity of ECD

The working potentials, as the part of the entire methods, were set as +600, 700, 800, 900, 1000, and 1100 mV respectively. In order to acquire the optimal condition for HSYA, DSS, FA, PAH and PA detection in Xuebijing injection, the methods were set as follows:

(1) The chromatographic behavior and the electrochemistry detection for the five phenolic acids with non-ILs and nano-gold added took as 2.3.