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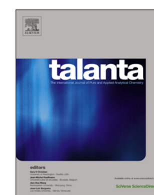

Combination of capillary micellar liquid chromatography with on-chip microfluidic chemiluminescence detection for direct analysis of buspirone in human plasma

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ABSTRACT

Microfluidic based chemiluminescence (CL) detector having novel channel design for enhanced mixing has been developed and investigated in terms of its applicability with micellar mode of liquid chromatography (MLC). The newly developed detector was found to be highly sensitive and an alternative detection technique to combine with capillary MLC. This combination was successfully employed for direct detection of a model analyte using Ru(III)-peroxydisulphate CL system. The selected analyte, buspirone hydrochloride (BUS), was detected selectively at therapeutic concentration levels in human plasma without any sample pretreatment. By incorporating eight flow split units within the spiral channel of microfluidic chip, an enhancement of 140% in CL emission was observed. We also evaluated the effect of non- ionic surfactant, Brij-35, which used as mobile phase modifier in MLC, on CL emission. The CL signal was improved by 52% compared to aqueous-organic mobile phase combinations. Various parameters influencing the micellar chromatographic performance and the CL emission were optimized. This allowed highly sensitive analysis of BUS with limit of detection (LOD) of 0.27 ng mL^{-1} ($3\sigma/s$) and limit of quantification (LOQ) of 0.89 ng mL^{-1} ($10\sigma/s$). The analyte recovery from human plasma at three different concentration level ranges from 88% to 96% (RSD 1.9–5.3%). The direct analysis of BUS in human plasma was achieved within 6 min. Therefore, combining microfluidic CL detection with micellar mode of separation is an efficient, cost-effective and highly sensitive technique that can utilize MLC in its full capacity for various bioanalytical procedures.

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1. Introduction

Developing *green* separation methods with highly sensitive and selective detection techniques is a challenging task. Micellar liquid chromatography (MLC) is among the most promising separation approaches that can provide high selectivity and throughput. In this approach, a mobile phase containing a surfactant at concentrations above its critical micellar concentration (CMC) is used. MLC has received a lot of attention as an attractive alternative to conventional reverse phase chromatography due to its environmental friendly nature, applicability in physicochemical studies, enhanced retention, selectivity and cost effectiveness [1–3]. Another key advantage of MLC is the ability of direct on-column injection of biological fluids and the elimination of laborious and extensive sample pre-treatment steps [4,5]. However, most of the reported MLC methods utilize uv–visible spectrophotometric detection methods. Although, uv–visible spectroscopy is simple and almost universal, the detection limits are usually high, and in many cases, the method is not suitable for the determination of an analyte in biological samples. The major limitation of MLC is its incompatibility with mass spectrometry, a highly sensitive detector commonly used for the determination of analytes in biological fluids. On the other hand, MLC is completely compatible with chemiluminescence (CL) detection techniques. Moreover, the combination of CL and MLC offers an added advantage by enhancing the CL emission of several CL reactions [6–8].

The combination of CL detection with HPLC has been extensively studied. By coupling the CL detection with the selectivity of a powerful separation technique, provides highly sensitive detection that can be easily manipulated for developing many robust applications for biological, pharmaceutical, food and environmental samples [9–11]. CL detection is usually carried out using a post column reaction of column eluent with CL reagents. Moreover, the reactions adopted in HPLC-CL systems are very rapid, and the emission intensity depends on many environmental factors, such as the pH, reagent concentration, temperature, solvent and ionic strength. Therefore, several factors must be considered while interfacing HPLC with CL detection, including compatibility of

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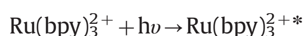
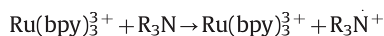
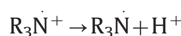
mobile phase conditions and the CL reaction, rate of CL reaction, the mixing efficiency and the volume of flow cell. In most of the cases, and especially in the tris(2,2′-bipyridyl)ruthenium CL system, the use of organic solvents affect the CL emission negatively. This has been observed in the case of methanol, which remarkably increases the background CL signal of the system [8]. It is worth mentioning here that the use of these organic solvents in HPLC-CL is essential for better chromatographic performance, but at the expense of the CL sensitivity. In this work, we propose a micellar mode of separation as an alternative to conventional reverse phase chromatography to utilize the added benefit of enhanced CL intensity due to surfactants in mobile phase.

In most commercial HPLC-CL systems, coiled-tubing flow cells with T- or Y-shaped connectors are used as CL flow cells [12,13]. Many research groups fabricated flow cells for CL detection using Perspex, Teflon, Polycarbonate or White Acetal disks or thick sheets with spiral or serpentine channels machined or engraved into them and sealed with transparent plates or films [14]. These flow cells had many merits over the coiled-tubing approach in terms of mixing and fabrication capabilities; however, they have not received widespread acceptance. One of the major reasons is due to the usage of relatively high quantities of CL reagents for efficient mixing. The total internal volume of these cells are moderately high (133–275 μL), and therefore, it is necessary to infuse the CL reagents at high flow rates [15]. Conventional HPLC systems can be operated at 0.8–1.2 mL min^{-1}, but the CL reagents need to be infused at a rate of minimum 2–3 mL min^{-1} for efficient mixing. Even flow rate as high as 15 mL min^{-1} was also reported [16]. The high CL reagent flow rates in HPLC-CL systems result in additional dilution of the analytes, post column band broadening and poor resolution. Microbore HPLC columns have been utilized as an alternative to reduce the consumption of CL reagents, resulting in improved resolution and sensitivity; however, the flow rates of CL reagents remain high compared with typical flow rates of capillary systems [17–20]. In capillary HPLC systems, the minimum CL reagent flow rate reported is 400 μL min^{-1} while a typical mobile phase flow rate of 100 μL min^{-1} is used and even a CL-flow rate as high as 2 mL min^{-1} was used with 0.2 μL min^{-1} flow rate of the mobile phase [20]. Generally, high flow rates of CL reagents are used in HPLC-CL to achieve good sensitivity which confines developing applications for routine analysis due to the expensive and hazardous nature of CL reagents. Additionally, long and wide tubes used in conventional CL detector can have an impact on the separation and band broadening. These limitations make HPLC-CL systems relatively unpopular even though it possesses many analytical merits. Microfluidics is an ideal alternative to overcome the limitations associated with high flow rates as it is a proven efficient mixing device at low flow rates. The operating flow rate of capillary HPLC instruments with microbore columns (20–200 μL) is very well suitable and the tolerable flow rates suitable for reagent mixing in microfluidic chips generally falls within these limits. In addition, it can be easily fabricated for enhanced mixing according to the rate of CL reaction.

Here, we introduce a novel approach to overcome the problems associated with high flow rates generally used in CL detectors of microbore HPLC systems by interfacing it with on-chip detection system. Microfluidic chips can be easily fabricated to be used simultaneously as a *microflow cell* and *micromixer* for CL reaction. By incorporating suitable channel designs, it is possible to enhance the reagent mixing with analyte and resultant chemiluminescence efficiency. We have investigated the appropriateness and advantages of such setup in combination with capillary HPLC and their impacts on CL emission. Tris(2,2′-bipyridyl)ruthenium(III)-ammonium peroxydisulphate (Ru-Oxidant) CL system with buspirone hydrochloride (BUS) was selected as a model system. BUS belongs to the azapirone chemical class of drug molecules, which are generally used as anxiolytics and antipsychotics drugs. Generally, these molecules are suitable for CL detection using Ru- Oxidant due to the presence of an aliphatic tertiary nitrogen atom in the molecule. The $Ru(bpy)_3^{3+}$, generated via photoinduced chemical oxidization of Ru(II) using peroxydisulphate in presence of light, can be reduced by tertiary amine to an electronically excited $[Ru(bpy)_3^{2+}]^*$ species from which light emission is observed. A reducing intermediate of amine with sufficient energy to produce an excited species was supposed to be formed by one-electron oxidation followed by deprotonation and the resultant neutral amine radical subsequently reduces the $[Ru(bpy)_3^{3+}]$ to $[Ru(bpy)_3^{2+}]^*$ (Scheme 1) [21–23]. The effect of pH on the CL intensity substantiates the deportation step as slightly basic pH was always observed as the most suitable condition for maximum CL emission.







Scheme 1

In this paper, we demonstrated for the first time the development, performance and validation of an instrumental combination of a capillary HPLC in micellar mode of separation and on-chip chemiluminescence detection with novel microfluidic channel design for enhanced luminescence emission of Ru(III)-Peroxydisulphate CL system.

We also presented, a highly selective and sensitive analytical method for the determination of BUS hydrochloride. The feasibility of direct on column injection of biological fluids using *micellar-capillary HPLC with microfluidic CL* detection has been explored. Several validated methods are available for the determination of BUS and other drugs in azapirone class, including radioimmunoassay [24], GC–MS [25], liquid chromatography [26–30] with different detection techniques, such as electrochemical detection [31], mass spectrometry and tandem mass spectrometry [32]. Detection limits as low as 0.2 ng mL^{-1} have been reported for BUS using MS and MS/MS. However, these methods require extensive sample cleaning, protein separation and pre-concentration of analyte. To the best of our knowledge, no such method has been reported for the analysis of any such drugs from this class, including BUS, using capillary micellar liquid chromatography and on-chip CL detection. Additionally, the developed HPLC method is a cost-effective "*green*" analytical solution.

2. Experimental

2.1. Chemicals and reagents

All reagents were of analytical grade unless otherwise specified and consumed without further purification. The solutions were prepared with ultrapure water obtained from a MilliQ water system (Millipore S.A.S. France). Acetonitrile (HPLC Grade), Brij 35, ammonium peroxydisulphate, sodium hydroxide and potassium dihydrogen phosphate (Fluka Biochemika, Ultra for Molecular biology) were purchased from Sigma Aldrich, Germany. Tris(2,2′-bipyridyl)ruthenium(II) chloride ($Ru(bpy)_3^{2+}$) was purchased from Aldrich (USA). The buspirone hydrochloride standard was a gift from a quality control laboratory, Ministry of Health (Muscat, Sultanate Oman).

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