Talanta 82 (2010) 560–566

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

A microflow chemiluminescence system for determination of chloramphenicol in honey with preconcentration using a molecularly imprinted polymer

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ARTICLE INFO

Article history:
Received 17 November 2009
Received in revised form 16 April 2010
Accepted 10 May 2010
Available online 16 May 2010

Keywords:
Chloramphenicol
Molecularly imprinted polymer (MIP)
Chemiluminscence
Microfluidics
MicroFIA

ABSTRACT

A novel chemiluminescence (CL) microfluidic system incorporating a molecularly imprinted polymer (MIP) preconcentration step was used for the determination of chloramphenicol in honey samples. The MIP was prepared by using chloramphenicol as the template, diethylaminoethyl methacrylate (DAM) as the function monomer, ethylene glycol dimethacrylate (EGDMA) as the cross-linking monomer, 2, 2′-dimethoxy-2-phenylacetophenone (DMPA) as the free radical initiator and toluene and dodecanol as the solvent. The MIP was pre-loaded into a 10 mm long, 2 mm wide and 150 μm deep channel in a planar glass microfluidic device. When the sample containing chloramphenicol was introduced into the microfluidic device it was first preconcentrated on the MIP then detected by an enhancement effect on the chemiluminescence reaction of tris(2, 2′-bipyridyl) ruthenium(II) with cerium(IV) sulphate in sulphuric acid. A micro-syringe pump was used to pump the reagents. The CL intensity was linear in relationship to the chloramphenicol concentrations from 1.55×10^{-4} to 3.09×10^{-3} μmol L^{-1} ($r^2 = 0.9915$) and the detection limit (3σ) and the quantitation limit (10σ) were found to be 7.46×10^{-6} and 2.48×10^{-5} μmol L^{-1}, respectively. This method offered a high selectivity and sensitivity for quantitative analysis of chloramphenicol in the honey samples.

1. Introduction

Antibiotics are widely used in animals for the treatment of diseases and also as animal growth promoters. The use of antibiotics may lead to drug residues present in animal-derived foods; the side effects of which would threaten public health. To minimize possible exposure to antibiotics, an allowable level of antibiotics in food has been established by the U.S. Food and Drug Administration and European Union.

Chloramphenicol (CAP) is a broad-spectrum antibiotic that was first isolated in 1974 from culture of *Streptomyces venezuelae* [1,2] (Fig. 1). It exhibits activities against both aerobic and anaerobic microorganisms. However, chloramphenicol has been shown to possess several harmful side effects in human, such as grey syndrome, bone marrow suppression and fatal aplastic anaemia [3]. Chloramphenicol is still illegally used in animal farming because of its easy access and low cost. Further, in the EU, application of chloramphenicol to food production has been prohibited since 1994 [4]. Moreover, the EU has defined a minimum required performance limit (MRPL) of 0.3 μg kg^{-1} for chloramphenicol in food of animal origin [5], because a safe level of chloramphenicol dosage has yet to be identified. With growing concerns over food safety and the need to increase sample-throughput in analytical testing laboratories, there is a constant requirement for accurate, simpler, faster and improved analytical methods. The complexity of food matrices and the presence of much potential interference, require specific and selective methods for analysis.

Various methods have been developed for determining chloramphenicol residues, including immunoassay [6], microbiological methods [7], sensors [8] and chromatographic methods using GC–MS [9] and LC–MS/MS [10]. The levels to be determined are very low and the use of molecularly imprinted polymers for analyte enrichment in the determination of chloramphenicol by LC–MS or HPLC with UV detection has been previously described in various sample matrices such as milk, serum, honey and shrimp [11–14]. The molecular imprinting technique combines the advantages of tailor-made sorbents and physical durability for enrichment.

Molecularly imprinted polymers are extensively cross-linked polymers containing specific recognition sites with a predetermined selectivity for analyses of interest [15,16]. The printing process is performed by co-polymerizing functional and cross-linking monomers in the presence of a template molecule. The

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doi:10.1016/j.talanta.2010.05.007

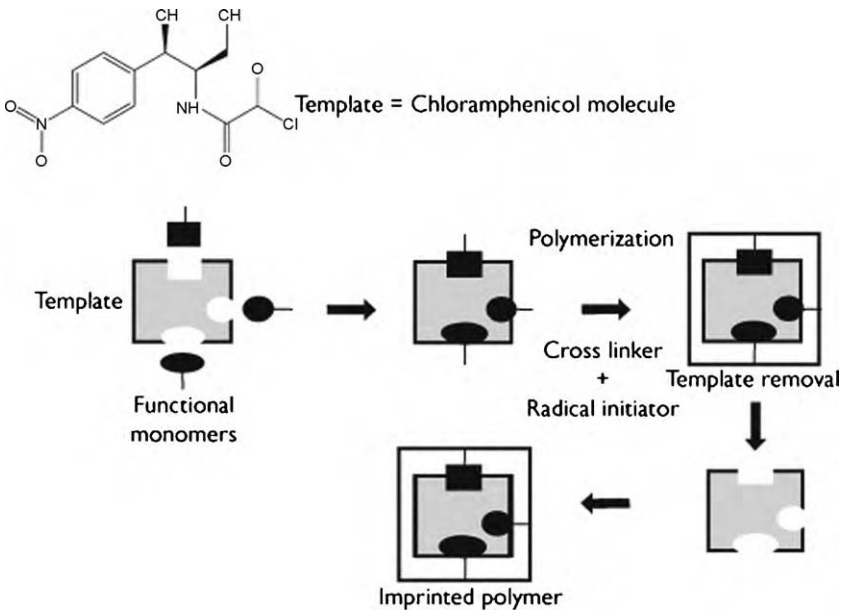

Fig. 1. Molecularly imprinted polymer procedure with chloramphenicol template.

subsequent removal of the imprint molecule reveals binding sites in the polymer network, which are complementary to the template in size and shape. That allows the highly specific rebinding of the template (Fig. 1). Furthermore, MIPs are usually reusable, are inexpensive to prepare, have high mechanical and chemical stability and are applicable to varying parameters of operating conditions. Several reviews have been written summarizing the development of MIP's [17–20] and their applications.

Mena et al. [11] described a molecularly imprinted polymer as a selective solid-phase extraction sorbent for the clean-up and preconcentration of chloramphenicol. The MIP used diethylaminoethyl methacrylate (DAM) as the functional monomer. Detection of chloramphenicol was carried out by square-wave voltammetry. The applicability of the MIP for both clean-up and preconcentration was demonstrated by determining chloramphenicol in ophthalmic solutions. Schirmer and Meisel [14] prepared the MIP by using methacrylic acid (MAA) as the functional monomer. The MIP was applied as a sorbent in solid-phase extraction to selectively extract CAP from honey. This method was shown that recoveries of nearly 100% of a CAP standard solution and up to 94% from spiked honey samples could be obtained after solid-phase extraction.

Chemiluminescence (CL) has been shown to be a powerful analytical tool due to its high sensitivity and low detection limit that can be obtained and chemiluminescence reactions have been used widely for sensitive and selective detection in flow injection and chromatographic analysis [21]. One of the most widely used set of chemiluminescence reactions are those involving the oxidation of tris(2, 2′-bipyridyl) ruthenium(II), $Ru(bipy)_3^{2+}$ to (2, 2′-bipyridyl) ruthenium(III), $Ru(bipy)_3^{3+}$, which is then followed by reduction with an analyte species with subsequence emission of light. The CL reaction between tertiary amine and $Ru(bipy)_3^{2+}$ is very sensitive and has been widely applied. Several methods have been employed to obtain the active oxidised reagent $Ru(bipy)_3^{3+}$, including chemical, photochemical, electrochemical oxidation and *in situ* electrogenerated chemiluminescence. Chemical generation of $Ru(bipy)_3^{3+}$ has been achieved by a range of reagents such as cerium(IV) sulphate [22] as shown below, lead dioxide [23] and potassium permanganate [24,25]:

$$Ru(bipy)_3^{2+} + Ce(IV) \rightarrow Ru(bipy)_3^{3+} + Ce(III) \quad (1)$$

$$Ru(bipy)_3^{3+} + [\text{the radical intermediates}]* \rightarrow [Ru(bipy)_3^{2+}]* + \text{products} \quad (2)$$

$$[Ru(bipy)_3^{2+}]* \rightarrow Ru(bipy)_3^{2+} + hv \quad (3)$$

An alternative approach to generating $Ru(bipy)_3^{3+}$ is to use electrogenerated chemiluminescence [26]. The chemical reaction of electrogenerated chemiluminescence activity of chloramphenicol with $Ru(bipy)_3^{2+}$ has been reported by Lindino in which a decrease in CL emission was monitored [27]. More recently, photochemical reactions and chemiluminescence detection has been investigated for the determination of aromatic amines [28]. Chloramphenicol in aqueous solution at room temperature (29–30 °C) degrades gradually after exposure to sunlight, UV light (365 nm) and red light. The photochemical reaction-chemiluminescence detection for determination of chloramphenicol with a luminol-Co(II) system and potassium permanganate in sulphuric acid medium have been reported by David et al. [29] and Catala Icardo et al. [30], respectively.

In recent years, the miniaturisation of analytical systems has been shown to be advantageous due to the inherent advantages of portability, low reagents consumption and the reduction of analysis time. The microfluidic devices used consist of a network of channels (in the range 10–300 μm cross-section) etched into a solid substrate such as glass. The channel networks are connected to a series of reservoirs containing samples and reagents which forms a complete device or "chip" with overall dimensions of a few cm. To perform a chemical measurement or reaction, reagents are brought together (using a variety of pumping techniques) in a laminar or slug flow diffusive mixing regime in a specific sequence and are allowed to mix and react for a specified time in a controlled region of the device [31,32]. Such devices have been used to detect pesticides usually with immobilised enzymes to achieve selectivity [33]. The incorporation of MIP's within microfluidic devices has been described previously especially for biochip applications [34] with examples such as the detection of viruses (Birnbaumer et al. [35]).

This work describes the development of a novel microfluidic device incorporating both a selective online enrichment channel with an MIP and a new CL method for the deter-

mination of chloramphenicol. The MIP was synthesized by adapting the approach developed by Schirmer and Meisel [14] with diethylaminoethyl methacrylate (DAM) as the functional monomer, glycol dimethacrylate (EGDMA) as cross-linker and 2, 2′-dimethoxy-2-phenylacetophenone (DMPA) as initiator in the presence of chloramphenicol (Fig. 1). The method has been successfully applied to the determination of chloramphenicol in spiked honey samples.

2. Experimental

2.1. Microfluidic device design

The microchips were fabricated in house [32] using photolithography and wet etching techniques. The channel network was etched into a Superwhite Crown borosilicate glass Plate 50 mm × 40 mm (Alignrite, Bridgend, UK). The glass was obtained precoated with chromium and photoresist layers, which were first, patterned using photolithography. The channels were then etched using aqueous 1% hydrofluoric acid solution buffered with 5% ammonium fluoride for 1 h at 70 °C. This produced the channels network consisting of four channels, intersecting at 90°, 360 μm wide and 100 μm deep in base plate (see Fig. 2a). Three devices were used ((a) 10 mm long, 50 μm deep, (b) 10 mm long, 100 μm deep and (c) 10 mm long, 150 μm deep). The chip was then completed by thermally bonding a 3 mm top plate and base plate of Superwhite Crown borosilicate glass plate. Holes were drilled into the top plate to connect tubing.

2.2. Instrumentation

A custom built, light tight box incorporated the miniaturised photomultiplier tube (H5784, Hamamatsu Photonics, Enfield, UK) and power supply (ITE power supply, ARTESYN, China) 12 V. The PMT was protected by a shutter which opened when the box was shut. The microchip was placed over the shutter and the reagents were pumped through the flow system using syringe pumps (Fusion 100, CHEMYX Inc., US) as shown in Fig. 2b.

A UV light (Handheld UV lamp, UVL-56, Ultra-violet Products Ltd., Cambridge, UK) was utilized for photoinitiation of the polymerization process used to prepare the MIP.

2.3. Reagents

All chemicals and reagents used were of analytical grade. The deionised water used was high purity deionised (18 MΩ cm resistivity, Elgastat UHQ PS, Elga, High Wycombe, UK). The majority of chemicals were supplied by Sigma–Aldrich (Sigma–Aldrich, UK) these included tris(2, 2′-bipyridyl) ruthenium(II) chloride hexahydrate (Sigma–Aldrich, UK) the oxidising agent cerium(IV) sulphate 4-hydrate, the functional monomers diethylaminoethyl methacrylate (DAM) and methacrylic acid (MAA) and the cross-linking monomer ethylene glycol dimethacrylate (EGDMA), the free radical initiator 2, 2′-dimethoxy-2-phenylacetophenone (DMPA) and CAP (99.0% pure). Acetonitrile and methanol (HPLC grade), sulphuric acid, toluene and dodecanol were analytical grade and were supplied by Merck.

2.3.1. Standard and reagent preparations

The stock standard solution of CAP was freshly prepared as a 3.09 μmol L^{-1} (1.00 mg L^{-1}) in aqueous solution by dissolving an accurate weight of 10 mg of CAP (accurately weighed) and diluting to 10 mL with 20 mmol L^{-1} phosphate buffer, pH 8.0 (stored at 4 °C in the dark). This standard solution was further diluted with 20 mmol L^{-1} phosphate buffer, pH 8.0 to give the appropriate concentration for the working standard solutions.

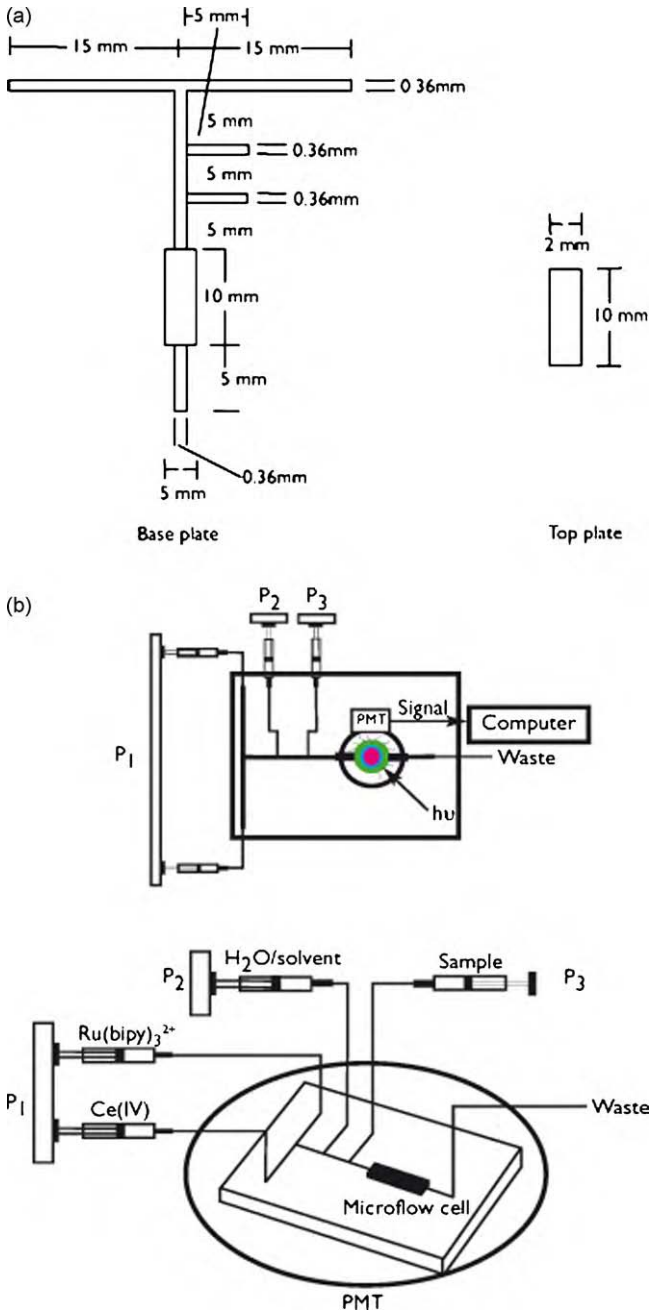

Fig. 2. (a) Schematic diagram of dimension on the micro-channel on chip. (b) Schematic diagram of microflow sensor on chip for determination of chloramphenicol (P_1, P_2, P_3: Fusion 100 micro-syringe pump).

An aqueous of 5.0×10^{-3} mol L^{-1} $Ru(bipy)_3^{2+}$ was prepared by dissolving 0.2500 g of tris(2, 2′-bipyridyl) ruthenium(II) chloride hexahydrate in appropriate amount of 1.0×10^{-1} mol L^{-1} H_2SO_4 solution, then diluting to 100 mL in a volumetric flask. The solution was protected from light by using amber glass bottles. An oxidant solution of 1.5×10^{-2} mol L^{-1} Ce(IV) was prepared by dissolving 3.9424 g of cerium(IV) sulphate 4-hydrate and making up to 100 mL with 1.0×10^{-1} mol L^{-1} H_2SO_4 solution.

2.4. Sample preparation

The honey samples were purchased from local retail markets in United Kingdom. These samples were stored at 4 °C before used; 1.00 g of honey sample was accurately weighed. The honey sample

was then spiked with known variable amounts of chloramphenicol ranging from 3.09×10^{-3} to 3.09×10^{-2} μmol L^{-1}. These samples were diluted with 10 mL of 20 mmol L^{-1} phosphate solution at pH 8.0. Each sample solution was mixed with a vortex mixer and centrifuged (Gilson, ANACHEM) for 10 min. Then the supernatant was filtered through a 0.45 μm filter membrane. The sample blank was prepared in the same way but without the compound-spiking step.

2.5. Procedures

2.5.1. Polymer preparation

The internal wall surface of the glass microchip was first silanized to enable covalent attachment of the monolith to the walls [36]. The microchip was rinsed with acetone and water, activated with a sodium hydroxide (0.2 mol L^{-1}) for 30 min, and finally rinsed with ethanol. A 20% solution of 3-(trimethoxysilyl)propyl methacrylate in 95% ethanol adjusted to pH 5 using acetic acid was then pumped into the channel, and left for 5 h. The channel was then washed with ethanol and dried in a stream of nitrogen.

A pre-polymerization solution consisting of 0.75 mmol L^{-1} (0.25 g) CAP, 5 mmol L^{-1} functional monomer (1.00 mL DAM, or 0.42 mL MAA), 24.5 mmol L^{-1} (4.62 mL) EGDMA, mmol L^{-1} (45 mg) DMPA and 1.10 mL toluene and 8.60 mL dodecanol were prepared in a screw-capped glass vial. The template to monomer molar ratio for the prepared MIPs was 1:2. The solution was sonicated for 20 min, and then purged with a stream of nitrogen for 10 min. The solution was then introduced into the channels A in the microfluidic device (see Fig. 2a). The polymer was then formed by photoinitiation by irradiating the channeled for 30 min using the UV light which emits at 365 nm. The device was then flushed with acetonitrile, followed by 50% methanol in water at flow rate of 2 μL min^{-1} to remove the template and residues of nonreactive species. For comparison non-imprinted polymers (NIP) were prepared simultaneously under the same conditions without the addition of the template.

2.5.2. Online preconcentration and detection of chloramphenicol

A schematic diagram of the instrument set up is shown in Fig. 2b. The procedure for analyte enrichment and determination could be summarized in six steps:

Step 1, Pump 1 was used to deliver the $Ru(bipy)_3^{2+}$ and Ce(IV) solutions into the microfluidic device at a flow rate 10 μL min^{-1} for 1 min. The CL signal obtained was used as the blank signal.
Step 2, Pump 1 was stopped pump 2 was then used to remove any remaining $Ru(bipy)_3^{2+}$ and Ce(IV) solutions for 2 min before introducing pH 8.0 buffer solution at the same flow rate for preconditioning the MIP.
Step 3, Pumps 1 and 2 were stopped and pump 3 was used to introduce the sample into MIP channel at a flow rate 5 μL min^{-1} for 6 min, during which time the chloramphenicol was trapped on to the MIPs.
Step 4, Pump 2 was used to pump water into the MIP channel at 5 μL min^{-1} for 2 min, to wash the surface of the MIP.
Step 5, $Ru(bipy)_3^{2+}$ and Ce(IV) solutions were then introduced by pump 1 at flow rate 10 μL min^{-1} for 1.5 min. These reagents interacted with the trapped chloramphenicol and the CL intensity was measured. The concentration of chloramphenicol was quantified by the peak height corresponding to CL intensity.
Step 6, Pump 2 was then started and the system was cleaned by methanol then water before the process was restarted.

The final conditions used are shown in Table 1.

Table 1
The optimum conditions for determination of CAP.

Parameter studied	Range	Optimum
H_2SO_4 concentration (mol L^{-1})	5.0×10^{-2} to 1.0×10^{-2}	1.0×10^{-1}
$Ru(bipy)_3^{2+}$ concentration (mol L^{-1})	1.0×10^{-3} to 5.0×10^{-3}	2.0×10^{-3}
Ce(IV) concentration (mol L^{-1})	5.0×10^{-3} to 3.0×10^{-2}	2.0×10^{-2}
pH of phosphate buffer solution	3.0–10.0	8.0
Sample injection volume (μL)	3–50	30
$Ru(bipy)_3^{2+}$ + Ce(IV) volume (μL)	5–25	15
Flow rate of $Ru(bipy)_3^{2+}$ (μL min^{-1})	2–10	8
Flow rate of Ce(IV) (μL min^{-1})	2–10	8
Cleaning time (min)	1–5	2

3. Results and discussion

The microfluidic device was designed to have simple operation but allow a preconcentration step prior to CL detection. The chloramphenicol is an amide not a tertiary amine, and therefore the Ce(IV) has two roles in this particular CL reaction, one being in its usual role to oxidise the $Ru(bipy)_3^{2+}$. The other role is to oxidise the amide to form a transient intermediate which then reacted with the $Ru(bipy)_3^{3+}$ to give the CL emission signal observed. Careful evaluation of the variables relating to the CL reaction was required to achieve maximum sensitivity.

3.1. Evaluation of the MIP

Of the microfluidic devices investigated the design with the deepest channel (150 μm deep) gave the highest sensitivity and was selected for further work. The method for the preparation of the MIP was adapted from that described by Schirmer and Meisel [14]. The ability of the MIP to trap the chloramphenicol was initially evaluated in bulk using CL detection where the DAM and MAA were compared as the functional monomers. Fig. 3 shows the results obtained in comparison to the polymer obtained without the incorporation of the template (blank). It can be seen from Fig. 3 that much better enrichment is seen with DAM. Fig. 4 shows the scanning electron micrographs for the two different monoliths, these show a porous surface with pore diameter distributions from 2 to 5 mm.

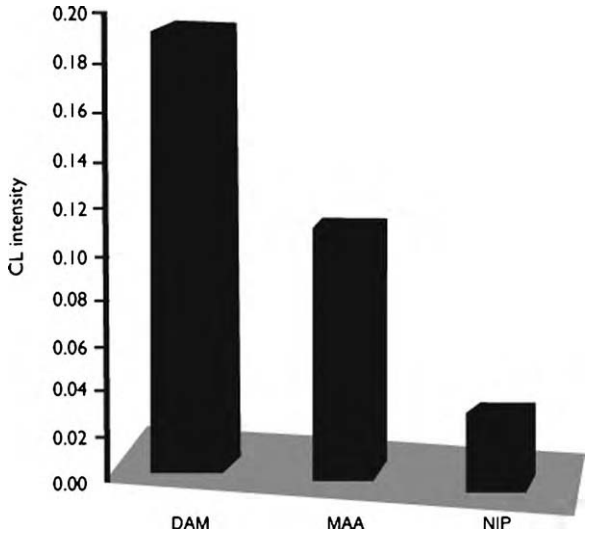

Fig. 3. The CL intensity of sample with effect of adsorption comparison between DAM and MAA monomers synthesis for microflow sensor on chip for determination of chloramphenicol (3.09×10^{-3} μmol L^{-1} of chloramphenicol).

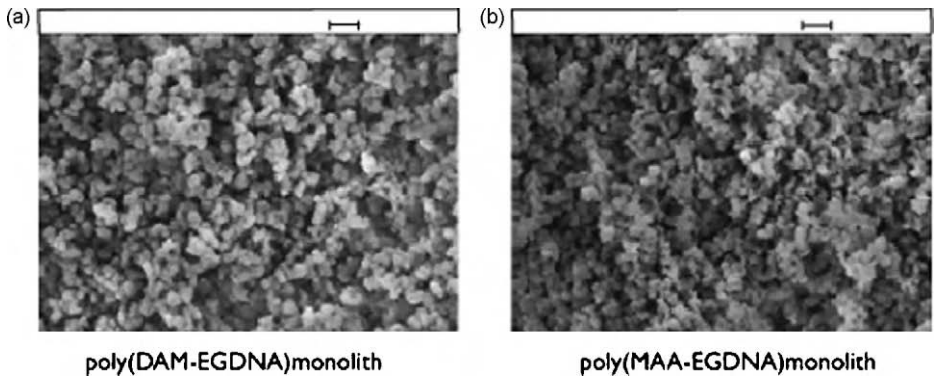

Fig. 4. Scanning electron micrographs of the MIPs by photo-polymerization method (a) DAM and (b) MAA monolith.

3.2. Investigation of parameters for CL detection

As a starting point for this study the initial reagent concentrations were selected to be 5×10^{-3} mol L^{-1} for $Ru(bipy)_3^{2+}$, 1.5×10^{-2} mol L^{-1} for Ce(IV) in H_2SO_4 and 3.09×10^{-3} μmol L^{-1} for the chloramphenicol standard studied. The average of three measurements is reported for all results.

3.2.1. H_2SO_4 concentration

The effect of H_2SO_4 concentration was investigated in the range of 5×10^{-2} to 1 mol L^{-1}. The result showed that the strongest CL intensity was obtained in the concentration 1×10^{-1} mol L^{-1} of H_2SO_4 and was selected as the optimum concentration for the CL system.

3.2.2. The pH effect of phosphate buffer solution for carrier solution

The pH effect of phosphate buffer solution on preconcentrating the chloramphenicol was evaluated in the pH range of 3–10. It was found that the pH 8.0 of phosphate buffer solution gave maximum CL intensity. This pH provides the best pH for trapping the chloramphenicol on the MIP.

3.2.3. $Ru(bipy)_3^{2+}$ concentration

The effect of the concentration of $Ru(bipy)_3^{2+}$ on the CL intensity was investigated in the range of 1×10^{-3} to 5×10^{-3} mol L^{-1}. It was found that the CL intensity increased with the increase of $Ru(bipy)_3^{2+}$ concentration up to 2×10^{-3} mol L^{-1} as shown in Fig. 5(a). The 2×10^{-3} mol L^{-1} $Ru(bipy)_3^{2+}$ was therefore selected as the optimum concentration for the CL system.

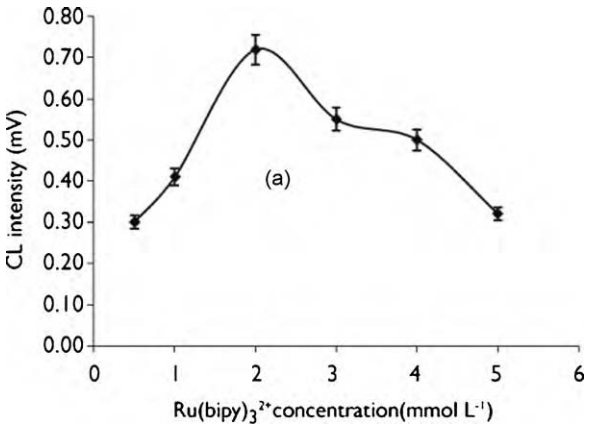

Fig. 5. The effects of reagent concentration (a) $Ru(bipy)_3^{2+}$ and (b) Ce(IV) in 1.0×10^{-1} mol L^{-1} H_2SO_4.

3.2.4. Oxidant concentration

As would be expected, especially in this reaction where the Ce(IV) had a dual role, the CL emission was strongly affected by the Ce(IV) concentration (Fig. 5b). The influence of Ce(IV) concentration on the CL system was examined in the range of 5×10^{-3} to 3×10^{-2} mol L^{-1}. It was found that the 2×10^{-2} mol L^{-1} Ce(IV) concentration gave maximum CL intensity and this was used for further work.

3.2.5. Effect of flow rates and sample volume

The flow rate of the different reagents was an important factor influencing the response of the CL system. The inclusion of the MIP into the device caused backpressures within the system. If the flow rate was too high, the high backpressure made it difficult to transfer the reagents and cause leaks, however, if the flow rate were too low, the analysis time would increase. It was found that excellent reproducibility, stability and good CL response was achieved when the flow rate of the $Ru(bipy)_3^{2+}$ and Ce(IV) solution channel (pump 1, Fig. 2b) was fixed at the range of 2–10 μL min^{-1}. As well as causing back pressure problem if the flow rate of the sample introduction channel (pump 3, Fig. 2b) was too high there would be insufficient time for the chloramphenicol to be trapped on the MIP. The maximum CL intensity was achieved when the sample flow rate was fixed at 8 μL min^{-1}. This flow rate also gave sufficient cleaning time for the enrichment step.

The effect of the volume of sample introduced was then investigated at different volumes from 5 to 30 μL. As would be expected the experimental results showed that the CL intensity increased as volume of sample increased. The 30 μL volume of sample was selected for subsequence analysis because it gave high CL intensity, short analysis time and the MIPs become saturated beyond this point (Fig. 6).

3.3. Binding characteristic of the MIP incorporated in the microfluidic device

The binding characteristic of the DAM MIP for chloramphenicol in the microfluidic device was investigated by comparing the results using the MIP and the polymer without the incorporation of the template (NIP blank). When the solutions containing chloramphenicol in the concentration range of 3.09×10^{-3} to 7.55×10^{-3} μmol L^{-1} flowed through the MIP the chloramphenicol was selectively adsorbed on the MIP and the CL intensity increased. The results can be seen in terms of CL intensity in Table 2. No further selectivity studies, except for a recovery experiment, were made as these have already been reported by Schirmer and Meisel [14] and the properties would not be changed by incorporation within the microfluidic device.

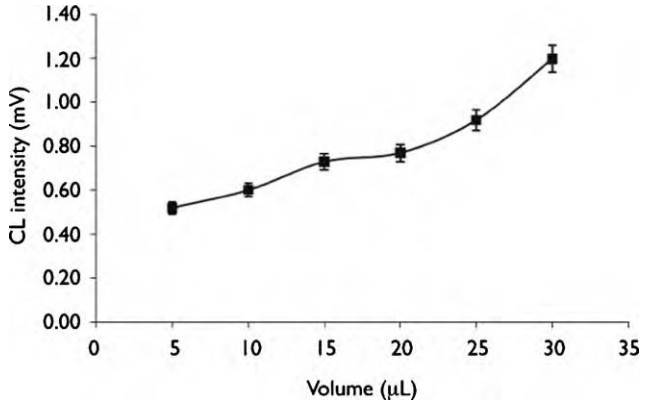

Fig. 6. The effects of volume of sample on CL intensity (flow rate 15 μL min^{-1}; sample 3.09×10^{-3} μmol L^{-1} of chloramphenicol).

3.4. The lifetime of the microflow sensor

The lifetime of microflow sensor was evaluated by comparing the CL intensity of the same chloramphenicol concentration over time. The results showed that the microflow sensor could be used more than 300 times/month (100 samples in triplicate) before the CL intensity began to decrease. This was possibly due to loss of binding sites, however it was easy to replace the MIP in the channel.

3.5. Analytical characteristics of the microfluidic device for chloramphenicol determination

Using the selected conditions as shown in Table 1, the linear calibration range, the detection limit (LOD) and the quantitation limit (LOQ) for the determination of chloramphenicol were investigated.

A linear calibration curve was obtained for chloramphenicol over the concentration range of 1.55×10^{-4} to 3.09×10^{-3} μmol L^{-1} which would covers a range to below the EU MRLP if 1 g of honey was diluted to 10 mL for analysis (MRLP 9.28×10^{-4} μmol kg^{-1}). The equation of the line was $y = 53.753x + 0.0216$ where y is relative CL emission in mV and x is chloramphenicol concentration in μmol L^{-1} with a correlation coefficient of 0.9919.

3.5.1. Sensitivity, detection limit and quantitation limit

The sensitivity value of this method, defined as the slope of calibration curve was found to be 53.75 mV μmol^{-1} L. The detection limit (3σ) and the quantitation limit (10σ) were found to be 7.46×10^{-6} and 2.48×10^{-5} μmol L^{-1}, respectively. These limits are well below the level required to measure the MRLP and are achieved due to the high sensitivity of the CL detection.

3.5.2. Precision

Fig. 7 shows the traces obtained for the method. The relative standard deviation of the proposed method (peak height in mV) calculated from five replication of 1.55×10^{-3} and 1.55×10^{-4} μmol L^{-1} of chloramphenicol were found to be 7.81% and 4.86%, respectively.

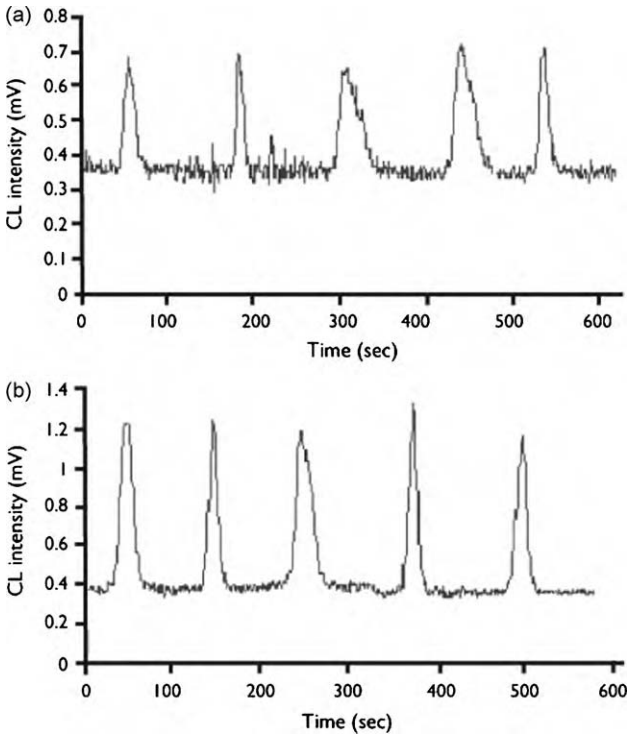

Fig. 7. Precision of the proposed method in the concentration range of (a) 1.55×10^{-4} μmol L^{-1} and (b) 1.55×10^{-3} μmol L^{-1}.

3.6. Analytical applications

The proposed MIP-CL method was successfully applied to the determination of chloramphenicol in honey samples using the standard addition method. Known concentrations of standard chloramphenicol (1.55×10^{-4}, 7.75×10^{-4} and 1.55×10^{-3} μmol L^{-1}) were added to accurately weighed 1.00 g of honey sample, respectively (using the procedure mentioned in Section 2.4) and then analyzed using the proposed method. The standard addition curve was established by plotting the chloramphenicol relative CL emission in mV versus the added concentration of chloramphenicol in μmol L^{-1}. The amounts of chloramphenicol residue could then be calculated from the standard addition curve. It was found that there was no chloramphenicol residue present in the honey samples that had been purchased.

The percentage recoveries were also determined by the standard addition method. A triplicate determination of each concentration was conducted, along with statistical evaluation showing the standard deviations at difference values. The mean percentage recoveries are presented in Table 3. These results indicated that the proposed method provided highly accurate results.

Table 2
The binding characteristics of the MIP in the microfluidics device.

Chloramphenicol concentration (μmol L^{-1})	CL intensity (mV MIP[a])	CL intensity (mV NIP[b])	R.S.D. (%)
Reagent blank	0.030	0.025	5.26
3.09×10^{-3}	0.190	0.030	4.76
1.55×10^{-3}	0.110	0.028	9.09
7.55×10^{-4}	0.060	0.032	4.11

[a] MIP average of three net CL intensity signals.
[b] NIP average of three net CL intensity signals.

Table 3
Analytical recovery of chloramphenicol added to honey sample solution.

Honey sample	Concentration		Recovery[a] (%)
	Added (μmol L^{-1})	Found (μmol L^{-1})	
Sample no. 1	7.75×10^{-4}	7.41×10^{-4}	97.00
	1.55×10^{-3}	1.52×10^{-3}	98.12
	3.09×10^{-3}	2.91×10^{-3}	94.08
Mean ± S.D.			96.40 ± 2.08
Sample no. 2	7.75×10^{-4}	7.44×10^{-4}	78.07
	1.55×10^{-3}	1.33×10^{-3}	86.11
	3.09×10^{-3}	2.41×10^{-3}	96.20
Mean ± S.D.			86.79 ± 9.08
Sample no. 3	7.75×10^{-4}	8.06×10^{-4}	89.08
	1.55×10^{-3}	1.46×10^{-3}	94.12
	3.09×10^{-3}	2.76×10^{-3}	104.20
Mean ± S.D.			95.80 ± 7.69

[a] Average of three determinations.

4. Conclusion

In this work a simple, selective and highly sensitive system has been developed for the analysis of chloramphenicol using a microfluidic device incorporating a chloramphenicol-imprinted polymer and CL detection system. The simple instrumentation, low flow rates and reagent usage means the system could easily be developed into a portable system with computer controlled pumping. The high sensitivity means that the system could be used for screening samples to detect for chloramphenicol at the EU MRPL.

Acknowledgements

The authors gratefully acknowledge the Thailand Research Fund (TRF) that supported a Royal Golden Jubilee Ph.D. research assistantship to Wisanu Thongchai (Project No. PHD/0223/2548 Code 5.G.CM/48/A.1). They also would like to express their sincere thank to the Graduate School together with the Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University for their partial support. Great thanks are offered to the Department of Chemistry, The University of Hull, UK for providing the research facilities.

Special thanks are also expressed to Center for Innovation in Chemistry: Postgraduate Education and Research Program in Chemistry (PERCH-CIC); Chemistry Department, Faculty of Science, Chiang Mai University; TRF and Commission on Higher Education for their very kind partial support.

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