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A micro-immuno supported liquid membrane assay (μ-ISLMA)

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Abstract

A chemiluminescent (CL) based micro-immuno supported liquid membrane assay (μ-ISLMA) has been developed that enables clean up, enrichment and detection of simazine in a single miniaturised cartridge system. The μ-ISLM cartridge contains a supported liquid membrane (SLM) sandwiched between a donor and an acceptor plate (channel volumes 1.65 μL), the latter being covered by a thin layer of gold on to which anti-simazine antibodies were covalently immobilised via a self assembled monolayer (SAM) of either dithiobis(11-aminoundecane, hydrochloride) (DTAU) or β-mercaptoethylamine (β-MEA). The μ-ISLMA based on DTAU was characterised by both a high apparent extraction efficiency (E^{app} = 136%) and high apparent enrichment factor (E_e^{app} = 544), which resulted in a very high sensitivity for simazine (LOD = 0.1 ng L^{-1}). The paper discusses the influence of the different SAMs and three different anti-simazine-antibody preparations (polyclonal, affinity purified polyclonal and monoclonal) on the extraction parameters and assay sensitivity. The influence of the sample matrix (e.g. mineral water, orange juice and milk) on the simazine μ-ISLMA was also investigated.

Keywords: Micro-immuno supported liquid membrane assay (μ-ISLMA); Micro-immunoassay (μ-IA); Simazine; Chemiluminescence; Self-assembled monolayer; Gold

1. Introduction

In the last 25 years, a new field of chemistry related to microtechnology (Verpoorte, 2000), known as micro total analysis system (μ-TAS), lab-on-a-chip or integrated chemistry lab has grown exponentially. In these systems various chemical operations of conventional analytical processes (e.g. separation, mixing and reaction) are integrated into a miniaturised format (Sato et al., 2003). A small system size presents advantages, such as smaller consumption of reagent, mobile phase, etc. but more importantly it leads to faster analysis times (Reyes et al., 2002) and improved analytical performance due to reduced diffusion distances, conserved laminar flow, improved separation efficiency and increased surface-to-volume ratio (Madou, 1997; Sato et al., 2003; Weigl et al., 2003). All together this has allowed successful applications in many strategic fields (e.g. genomics and proteomics (Sanders and Manz, 2000), drug and explosive residues discovery (Verpoorte, 2002) and pesticides analysis (Yakovleva et al., 2002, 2003)).

Consistent efforts have been undertaken to downsize immunoassays due to their sensitivity and selectivity, and wide range of applications (Sato et al., 2003). In this paper, we report a new miniaturised chemiluminescence (CL) based heterogeneous sequential injection immuno extraction method, referred to as micro-immuno supported liquid membrane assay (μ-ISLMA). The basic principles of immuno supported liquid membrane (ISLM) extraction were recently reported (Thordarson et al., 2000; Tudorache et al., 2004; Tudorache and Emnéus, 2005). The μ-ISLMA is based on a cartridge that contains a supported liquid membrane (SLM, a porous polymeric support impregnated with an organic solvent) sandwiched between a donor and an acceptor polymer plate with identical 1.65 μL volume capacity. The acceptor differs from the donor in that its surface is plated with gold on to which an antibody is immobilised via a self-assembled monolayer (SAM) of a sulfur-containing compound. In this paper, oriented immobilisation of the antibody, via its carbohydrate residues, to two different SAM spacers,

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was evaluated and the influence of the SAM chain length on the unspecific binding and assay sensitivity is discussed. Also, three different antibodies were compared in terms of the achieved extraction parameters and assay sensitivity. Furthermore, the optimum extraction conditions with the herbicides simazine (2-chloro-4,6-di(*N*-ethylamino)-1,3,5-triazine) as the model analyte was determined and the resulting μ-ISLMA applied to simazine spiked mineral water, orange juice and milk; the influence of the sample matrix components is discussed.

2. Experimental

2.1. Reagents and samples

Affinity purified polyclonal anti-simazine immunoglobulin G (IgG) from sheep (AbI) was kindly provided by Dr. Ram Abuknesha (King's College University of London, UK). Monoclonal anti-simazine IgG from mouse (AbII) and the corresponding enzyme tracer Ag^* (Et/S/$(CH_2)_5$CO-HRP) were from Dr. Milan Franek (Veterinary Research Institute, Brno, Czech Republic). Poly-IgG-anti-simazine from rabbit (AbIII) and unspecific tracer butachlor-HRP (Et/Et/*N*($CH_2O(CH_2)_3CH_3$)ClCO-HRP) were generously provided by Prof. Sergei Eremin (Lomonosov Moscow State University, Moscow, Russia). Stock solutions of Ag^* and Ab were kept in the freezer. The working Ag^* and Ab solutions were prepared daily, diluting the stock solution with 10 mM phosphate buffer saline (PBS) at pH 7.4. 0.01% bovine serum albumin (BSA from Sigma–Aldrich, Steinheim, Germany) was added to the Ag^* solution for enzyme label conservation and also for preventing unspecific binding of Ag^* on the acceptor surface.

Dithiobis(11-aminoundecane, hydrochloride) (DTAU) and β-mercaptoethylamine (β-MEA) were obtained from Sigma–Aldrich (Steinheim, Germany), while sodium periodate (NaI_4O) was obtained from Merck (Darmstadt, Germany).

Simazine was purchased from the Institute of Industrial Chemistry (Warsaw, Poland). The stock solution of simazine (5 mg L^{-1}) was prepared in purified water (Milli-Q/RO4 unit, Millipore, Milford, MA, USA), and diluted in 10 mM PBS solution, to obtain standards for the analyte calibration experiments. Ten millimolar PBS was prepared by diluting a stock solution of 100 mM PBS (80 g NaCl, 2.00 g KCl, 14.30 g $Na_2HPO4{\cdot}2H_2O$ and 3.43 g KH_2PO_4 dissolved in 1 L purified water), adjusting the pH by adding 1 M NaOH solution until a pH of 7.4. All phosphate buffer reagents were purchased from Merck (Darmstadt, Germany).

The HRP substrate (luminol, *p*-iodophenol and hydrogen peroxide) and di-*n*-hexyl ether (97% purity, used to prepare the SLM) were obtained from Sigma–Aldrich (Steinheim, Germany). 0.5 M H_2SO_4 (95–97% H_2SO_4, Merck, Darmstadt, Germany), used to clean the μ-ISLMA system via the donor side at the end of each day, was prepared by dilution in purified water. Twenty five percent of methanol (Merck, Darmstadt, Germany), adjusted to pH 11.5 with 1 M NaOH, was used for regeneration of the immobilised antibody surface.

Mineral water (Ramlösa, Sweden), orange juice with fruit parts (ICA Handlarnas AB, Sweden) and milk (solid milk, Russia) samples were spiked with simazine and analysed by the μ-ISLMA method. Before spiking, mineral water was filtrated (0.45 μm filter, Millipore, Ireland) and the pH adjusted to 7.4 with 1 M NaOH. The orange juice and milk samples were first centrifuged (5 min at 13,000 rpm) to remove the fruit parts or fat and the resulting supernatants were then adjusted to pH 7.4 with 1 M NaOH. Just before extraction, the different samples were diluted 1:1 with 10 mM PBS solution and spiked with simazine (5×10^{-5} to 500 μg L^{-1}).

2.2. Instrumentation

The analytical set-up was based on a simple automatic sequential injection (SI) system containing the following components (Fig. 1A): (1) a μ-ISLM cartridge (Fig. 1B) placed in a metallic holder (Fig. 1C), both units provided by Personal Chemistry, Uppsala, Sweden (Barri et al., 2004), (2) a peristaltic pump (Gilson, Villiers-le-Bel, France) to introduce sample through the donor channel, (3) a syringe pump with a 250 μL syringe capacity (P/N 50300 with six-port valve, Kloehn, Las Vegas, NV, USA) used to aspirate and dispense reagents into the acceptor channel, (4) a multi-position valve (10-position, Valco Instruments Co. Inc., Houston, TX, USA, Model CS-1340EMT) used to select and introduce different reagents into the acceptor channel (i.e. tracer (Ag^*), tracer substrate, standard (Ag) and sample) and (5) a photo multiplier tube (PMT, Hamamatsu Photonics K.K., Japan, Model No. HC135-01 UV–vis). The Winpump Version 3.3 software (P/N 50511, Kloehn, Las Vegas, NV, USA) was used to handle the system components and record the analytical signal. An in-house made software was used for data acquisition.

The Biacore 300 (BIACORE, Uppsala, Sweden) and accompanying software (*Heterogeneous analyte competing reaction*) were used to estimate the antibody affinity constants for the analyte (K) and tracer (K^*). The antibody was in this case immobilised on the gold surface via protein G.

2.2.1. μ-SLM cartridge

The μ-SLM cartridge (Fig. 1B) contained two different plates (donor and acceptor) with identical channel volumes (width 0.75 mm, length 22 mm and volume 1.65 μL). Both plates were from polypropylene material, but distinguishable since the acceptor plate surface was covered with a thin gold layer (used for Ab immobilisation), while the donor was unmodified. The μ-SLM cartridge was assembled by placing a SLM, consisting of a microporous polypropylene membrane support (Celgard 2500 Microporous Membrane, 55% porosity, 0.209 × 0.054 μm pore size, 25 μm thickness),