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In vitro evaluation of new biocompatible coatings for solid-phase microextraction: Implications for drug analysis and *in vivo* sampling applications

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ABSTRACT

A new line of solid-phase microextraction (SPME) coatings suitable for use with liquid chromatography applications was recently developed to address the limitations of the currently available coatings. The proposed coatings were immobilized on the metal fiber core and consisted of a mixture of proprietary biocompatible binder and various types of coated silica (octadecyl, polar embedded and cyano) particles. The aim of this research was to perform *in vitro* assessment of these new SPME fibers in order to evaluate their suitability for drug analysis and *in vivo* SPME applications. The main parameters examined were extraction efficiency, solvent resistance, preconditioning, dependence of extraction kinetics on coating thickness, carryover, linear range and inter-fiber reproducibility. The performance of the proposed coatings was compared against commercial Carbowax-TPR (CW-TPR) coating, when applicable. The fibers were evaluated for the extraction of drugs of different classes (carbamazepine, propranolol, pseudoephedrine, ranitidine and diazepam) from plasma and urine. The analyses were performed using liquid chromatography–tandem mass spectrometry. The results show that the fibers perform very well for the extraction of biological fluids with no sample pre-treatment required and can also be used for *in vivo* sampling applications of flowing blood. A coating thickness of 45 μm was found to be a good compromise between extraction capacity and extraction kinetics. Due to the high extraction efficiency of these coatings, pre-equilibrium SPME with very short extraction times (2 min) can be employed to increase sample throughput. Inter-fiber reproducibility was ≤11% R.S.D. (n = 10) for model drugs examined in plasma, which is a significant improvement over polypyrrole coatings reported in literature, and permits single fiber use for *in vivo* applications.

1. Introduction

Solid-phase microextraction (SPME) is a simple, fast, sensitive and convenient sample preparation technique, which minimizes solvent usage, while integrating sampling and sample preparation steps [1]. SPME was originally developed for use with gas chromatography (GC), but was subsequently successfully coupled to liquid chromatography (SPME–LC) for the analysis of semi- and non-volatile compounds such as drugs [2]. Applications of SPME–LC reported to date in literature include analysis of drugs in biofluids for clinical and forensic applications [3–5], investigation of binding parameters such as drug–protein binding [6], analysis of pesticides and contaminants in food and environmental samples [7–12], and *in vivo* investigations of living systems [6,13]. *In vivo* SPME is a new and a particularly interesting research area as it provides a new tool to directly study a living system without having to withdraw a representative sample. To date, this technique has been applied to study pharmacokinetics of drugs and their metabolites in circulating blood of animals such as beagles and rats by direct intravenous sampling [14–16], to study distribution and fate of pesticides in plants by direct insertion of SPME fiber in plant leaves [17,18], and to study exposure of fish to pharmaceuticals by directly sampling muscle of wild fish in contaminated surface waters [19]. These studies clearly show the potential of *in vivo* SPME as a new tool in life sciences, but further success of this technique is critically dependent on the availability of commercial SPME coatings suitable for use in these types of studies.

Presently, one of the primary limitations of SPME–LC is the availability of a limited number of extraction phases suitable for use with LC applications [3,6]. In fact, only four coatings (polydimethylsiloxane, polydimethylsiloxane/divinylbenzene, polyacrylate and Carbowax-templated resin (CW-TPR)) are commercially available

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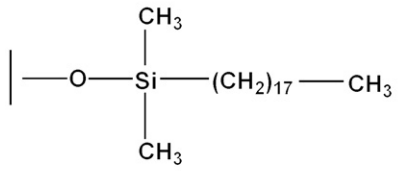
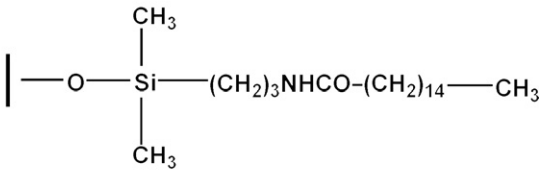
(a) Octadecyl (C18)



(b) RP-Amide C16 (RPA)



(c) Cyano

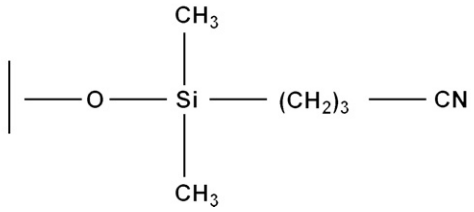


Fig. 1. Chemical structures of coatings (a) Octadecyl (C18), (b) RP-Amide C16 (RPA) and (c) Cyano.

and do not possess important characteristics such as (i) compatibility with commonly used LC-solvents, (ii) good inter-fiber reproducibility and low cost that would permit single-fiber use for *in vivo* applications, (iii) good extraction efficiency for a wide range of analytes and (iv) biocompatibility. The lack of SPME coatings with desired characteristics for *in vivo* and bioanalytical applications limits the development of new applications of SPME–LC and has necessitated further research in this area.

Several different types of coatings have been proposed in literature to address some of the above limitations of existing commercial coatings. For example, biocompatible coatings proposed in research include polypyrrole coatings [14,20–22], coatings based on restricted access materials [23–26], and coatings based on mixtures of SPE sorbents (coated silica particles) with biocompatible polymers [27,28]. Monolithic silica coatings [29,30] and polymer monolithic coatings [31,32] were introduced to improve extraction kinetics as well as to improve extraction of polar compounds by incorporation of appropriate functional groups. Various tailor-made sol–gel coatings have also been reported in order to improve chemical and mechanical stability as well as extraction efficiency, but these have predominantly been used in GC applications [33].

The main objective of this work was to evaluate *in vitro* the performance of new biocompatible coatings produced by Supelco for the extraction of drugs from biological fluids. The performance of these coatings with other classes of compounds commonly analyzed in biological fluids by LC such as riboflavin (water-soluble vitamin), adenosine (nucleoside) and hydrocortisone and progesterone (hormones) is also briefly reported. The proposed coatings were immobilized on the metal fiber core and consisted of a mixture of proprietary biocompatible binder and various types of coated silica (octadecyl, polar embedded RP-Amide and cyano) particles. The chemical structures of these coatings are shown in Fig. 1. These coatings are available in two formats (1) regular SPME assembly for *in vitro* testing and (2) new commercial assembly housed inside of a hypodermic needle for *in vivo* sampling. Their performance was compared against the commercially available Carbowax-templated resin coating, which is the most commonly used SPME coating for LC applications.

2. Experimental

2.1. Chemicals and materials

All drug standards and chemicals were purchased from Sigma–Aldrich (Oakville, ON, Canada), unless otherwise indicated. Diazepam was purchased from Cerilliant (Round Rock, TX, USA) as a 1 mg mL^{-1} methanolic solution. Carbamazepine-d10 (CBZ-d10) was used as an internal standard for the analysis and was purchased from Alltech (Deerfield, IL, USA) as a 100 μg mL^{-1} methanolic solution. Acetonitrile (HPLC grade), methanol (HPLC grade), and glacial acetic acid were purchased from Fisher Scientific (Ottawa, ON, Canada). Human plasma with EDTA was purchased from Cedarlane Laboratories Limited (Burlington, ON, Canada). Drug-free human urine was collected from a healthy volunteer and stored frozen until use. Commercial CW-TPR coating (50 μm thickness) and all prototype SPME coatings evaluated in this study were obtained as research samples from Supelco (Bellefonte, PA, USA). The coatings were immobilized on an inert, flexible metal alloy and consisted of biocompatible binder and 5 μm coated silica particles. The following coating types were evaluated (i) Discovery^{TM} C18 15 μm thickness, (ii) Discovery^{TM} C18 30 μm thickness, (iii) Discovery^{TM} C18 45 μm thickness, (iv) Discovery^{TM} C18 60 μm thickness, (v) Ascentis^{TM} RP-Amide (RPA) 45 μm thickness and (vi) Discovery^{TM} Cyano 45 μm thickness. All coatings were 15 mm long and prepared with silica particles of 5 μm size. Phosphate-buffered saline (PBS) solution pH 7.4 was prepared by dissolving 8.0 g of sodium chloride, 0.2 g of potassium chloride, 0.2 g of potassium phosphate and 1.44 g of sodium phosphate in 1 L of purified water and adjusting the pH to 7.4, if necessary.

2.2. SPME procedure

Organic content of all spiked samples and standards used for extraction was kept constant at 1% v/v methanol. PBS buffer was included in the current study for two main reasons. PBS buffer does not contain any binding matrix, so in bioanalytical SPME methods, it is often used to perform matrix-free calibration in order to determine free (unbound) analyte concentration [15]. PBS buffer is also a good and simple alternative to use for *in vitro* SPME optimization experiments in order to avoid unnecessary exposure to human biological fluids and to avoid lot-to-lot differences in the composition and pH of urine or plasma during SPME method development experiments. Spiked analytes in biological fluids (urine and plasma) were pre-incubated for 60 min prior to the extraction to allow for the binding to proteins to occur. For carbamazepine, the experiments for the extraction from plasma were repeated after increasing pre-incubation time to 12 h as discussed in Section 3.

Fibers were preconditioned for minimum of 30 min in methanol/water (1/1, v/v) prior to use. Fibers were housed either inside a regular SPME assembly that fits into a manual SPME holder or inside an *in vivo* SPME assembly. The design of the *in vivo* SPME device as described in detail by Musteata et al. [16], was modified to accomodate an 0.008 in. flexible metal wire with 1.5 cm coating immobilized on the bottom portion (Fig. 2a). SPME was performed using a 1.5 mL sample solution placed in a 2 mL amber HPLC vial unless otherwise indicated in the text. The extraction was performed at room temperature using pre-equilibrium SPME conditions with the extraction times of 2 min (PBS buffer and urine) and 5 min (plasma) unless otherwise indicated in the text. Extraction time in plasma was increased to improve method sen-