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Preparative isotachophoresis with surface enhanced Raman scattering as a promising tool for clinical samples analysis

Václav Ranc[a], Andrea Staňová[b], Jozef Marák[b,*], Vítězslav Maier[a], Juraj Ševčík[a], Dušan Kaniansky[b]

[a] Department of Analytical Chemistry, Faculty of Natural Sciences, Palacký University, 17. Listopadu 12, CZ-77146 Olomouc, Czech Republic
[b] Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University, Mlynská Dolina CH2, SK-842 15 Bratislava, Slovak Republic

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This paper is dedicated in memoriam to Prof. Dušan Kaniansky, professor at the Department of Analytical Chemistry, Comenius University in Bratislava, Slovakia.

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ABSTRACT

A surface enhanced Raman scattering (SERS) spectrometry is an interesting alternative for a rapid molecular recognition of analytes at very low concentration levels. The hyphenation of this technique with advanced separation methods enhances its potential as a detection technique. Until now, it has been hyphenated mainly with common chromatographic and electrophoretic techniques. This work demonstrates for a first time a power of preparative isotachophoresis–surface enhanced Raman scattering spectrometry (pITP–SERS) combination on the analysis of model analyte (buserelin) in a complex biological sample (urine). An off-line identification of target analyte was performed using a comparison of Raman spectra of buserelin standard with spectra obtained by the analyses of the fractions from preparative isotachophoretic runs. SERS determination of buserelin was based on the method of standard addition to minimize the matrix effects. The linearity of developed method was obtained in the concentration range from 0.2 to 1.5 nmol L^{-1} with coefficient of determination 0.991. The calculated limit of detection is in tens of pico mols per liter.

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1. Introduction

The development of the sensitive methods for analysis of clinical samples represents one of the frequent analytical tasks. Concerning this type of analytical problem, the concentration levels of target compounds are usually very low and matrices are relatively complex [1,2]. According to the reasons mentioned above, selective and sensitive analytical method is usually necessitated. Separation of target analytes from matrix constituents by a separation technique, e.g., liquid chromatography, electrophoresis or gas chromatography, is usually used in clinical practice. These techniques usually offer high accuracy and short run times, but a requirement of an extensive sample preparation and the limits of quantification can be the limiting factors [3]. In many cases, very complicated procedures of sample preparation can even lead to the unwanted errors in the data evaluation [4].

A Raman spectrometry also plays an important role in the analysis of target compounds at nano-femto molar concentration levels what is mainly given by the application of a surface enhanced Raman scattering (SERS) [5,6]. However, the identification and/or quantification of various target compounds using SERS has high requirements put on the sample composition and thus some corresponding sample preparation procedure has to be carried out. Mainly for this reason, SERS might be used in a combination with suitable separation technique. It has been successfully used in an on-line combination with HPLC [7–9], off-line with TLC [10–13], GC [14], post-column with CZE [15].

Isotachophoresis (ITP) represents another electrophoresis separation technique using two electrolytes system, i.e. leading electrolyte (containing ion with the highest effective mobility) and terminating electrolyte (containing ion with the lowest effective mobility) [16]. Analytes are injected between the zone of leading electrolyte and terminating electrolyte, respectively. Separation of ions in ITP is based on their different effective mobilities under given separation conditions. The concentration of ion in its own zone is adapted to the concentration of leading ion according to the Kohlrausch regulation function and does not depend on the concentration of ion in the sample [17]. Because of its very promising features, ITP was used in on-line coupling with Raman spectroscopy

* Corresponding author at: Department of Analytical Chemistry, Faculty of Natural Sciences, Comenius University, Mlynská Dolina CH-2, SK-84215 Bratislava, Slovak Republic. Tel.: +421 2 60296379; fax: +421 2 60296706.
E-mail address: marak@fns.uniba.sk (J. Marák).

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[18–22] and its SERS arrangement [23]. Unfortunately, the detection limits obtainable by the combination of isotachophoresis with Raman spectroscopy were not good, as they were at the concentration of 5×10^{-6} mol L^{-1} in the analysis of ribonucleotides [18] and at 2×10^{-7} mol L^{-1} concentration in the analysis of pesticides [22]. SERS detection usually provides better limits of detection, but it requires using nanoparticles what is not compatible with the electrophoretic separation techniques performed in narrow capillaries as there are several problems, e.g., a risk of capillary clogging, problems with reproducible capillary filling and last but not the least problem can be an aggregation of nanoparticles. Isotachophoretic free flow electrophoretic focusing with SERS was used for the detection of myoglobin in chip [23] but the authors used very high concentration of myoglobin (410 μmol L^{-1}) what is definitely too high to be applicable in the clinical laboratories. Another aspect of the problems with the on-line combination of ITP and SERS is the time of data acquisition. When low concentrations of analytes are analyzed by ITP, due to the Kohlrausch regulation function the analytes are migrating as very narrow zones (in the boundary layer) [16] and their detection time is about 1 s. This time interval is too short to obtain sufficient amount of data and resulting signal-to-noise (S/N) ratio is very low. When the off-line combination of ITP with SERS is used, one can expect increasing S/N ratio as there is enough time to measure many Raman spectra and the problems with the mixing of nanoparticles with the sample constituents are also eliminated in this way.

The preparative modification of isotachophoresis (pITP) has inherently high production rate [16,17,24] offering tools for isolation of required analyte from the sample in a shorter time than any other electrophoresis technique. Using of pITP also offers: (a) high load capacity; (b) minimum contaminations of the zones of interest by the electrolyte system constituents and (c) well-defined concentrations of the isolated constituents [17,24–28]. The amount of isolated analyte is sufficiently high to be used in another analytical technique providing the improvement in the selectivity and/or sensitivity of analytical procedure. The pITP isolation of analyte of interest was used before ITP [25,26], CZE [24,29,30], agarose gel electrophoresis [31], two-dimensional polyacrylamide gradient gel electrophoresis (2D-GGE) [32], HPLC [27,33], MS [34,35].

The surface enhanced Raman scattering represents a tool for a trace analysis of target compounds, but its use can also bring a set of difficulties that have to be overcome. The combination of SERS with an advanced pITP separation procedure enhances a method application potential and allows analyzing the samples with complex matrices containing target analytes at trace concentration levels [36,37]. The analytical aim of this work was to prospect the analytical potential of the combination of powerful separation technique with sensitive detection on the analysis of model therapeutic drug (buserelin) present in a complex biological matrix (human urine). Buserelin is a nonapeptide (D-Ser(Tbu) ^{6}EA^{10}LHRH, Mw = 1299.48) used for a treatment of breast and prostate cancer [38]. It is normally delivered as a nasal spray, but it is also available in the form of injection applied subcutaneously [39]. Therapeutic blood levels are in the hundreds of micro grams per liter. About 60% of applied dose is eliminated unchanged in urine. Analysis of buserelin in model samples has been previously performed using HPLC–MS [40,41] and in biological sample using CE–MS [42], where detection limits are in the hundreds of micro grams per liter and the analysis times are in tens of minutes.

The results of this work led to an establishment of the potent analytical combination of preparative isotachophoresis–surface enhanced Raman scattering spectrometry (pITP–SERS) that has been demonstrated on the analysis of clinical samples (buserelin in human urine). Based on these preliminary results, this analytical combination can be potentially used as a complementary technique in an analysis of ultra trace concentration levels of Raman active compounds in clinical samples in general.

2. Experimental

2.1. Apparatus

All pITP experiments were performed using modified isotachophoretic analyzer ZKI-001 (Villa – Labeco, Spišská Nová Ves, Slovak Republic) with the high voltage power supply capable to deliver the driving currents up to 1 mA and it was used for trapping the fractions. A separation unit provided with a column-coupling system consisted from a pre-separation column of 1.8 mm I.D. (120 mm to a detector) and the analytical column of 0.8 mm I.D. (160 mm to the detector). Both columns were made of fluorinated ethylene–propylene copolymer (FEP). Higher I.D. of the pre-separation column provides the possibility to apply the higher current (600 μA) in comparison with the current used in the analytical capillary (200 μA) to provide sufficient power within a short time period [17]. Injection valve with 44 μL volume of the inner sample loop and/or microsyringe (Hamilton) was used for the sample injection. The on-column conductivity detectors were used for the detection of isotachophoretic zones. Preparative fractionation valve with ca. 7 μL volume of the inner loop was part of the analytical column. Concentrator 5301 (Eppendorf AG, Hamburg, Germany) was used for the lyophilization of the collected fractions.

Concerning FT-SERS experiment, FT-IR Nicolet 6700 spectrometer with NXR Raman extension (Thermo – Finnigan, U.S.A.) was used. The instrument is equipped with an argon laser (wavelength = 1064 nm) and with a germanium detector cooled by a liquid nitrogen. The experimental conditions were tested using the standard solution of buserelin with silver nanoparticles (1 mL of solution contained 60 ppm of silver nanocomposite having 56 nm mean size (measured using dynamic light scattering microscopy), 1 mol L^{-1} NaCl used for aggregation, buserelin at the concentration level 1×10^{-6} mol L^{-1}, all components were dissolved in deionized water). Laser power was set up to 100 mW. Each spectrum is an average from 512 scans. Each sample was measured five times if not stated otherwise.

2.2. Chemicals

All chemicals used for the preparation of the leading and terminating electrolytes and the stock solutions of discrete spacers were obtained from Merck (Merck, Darmstadt, Germany). Water used for preparation of electrolytes and solutions of standards was cleaned in two stages by Pro-PS unit (Labconco, Kansas City, U.S.A.) and Simplicity (Millipore, Molsheim, France). Buserelin standard was obtained as noncommercial sample from Merck.

Silver nitrate (p.a.), sodium chloride (p.a.), water (gradient grade), sodium citrate (p.a.), glucose (p.a.), maltose (p.a.), triethylamine (p.a.), ammonium hydroxide (25% v/v, p.a.) were bought from Sigma–Aldrich (San Jose, MA, U.S.A.).

2.3. Preparation of samples for SERS

Buserelin standard solution (1 g L^{-1}) was prepared by solution of 1 mg of standard in 1 mL of water (HPLC grade). Stock solution (c = 10 mg L^{-1}) was prepared by a dilution of buserelin standard solution with water (HPLC grade). This solution mixture was used for a preparation of all calibration standards and for a standard addition method. Calibration samples were prepared at concentration levels 1×10^{-10}, 5×10^{-9}, 1×10^{-9}, 5×10^{-8} and 1×10^{-8} mol L^{-1} by a standard dilution of work solutions.

The measured sample for UV/vis experiments contained 60 ppm of silver nanocomposite. Regarding SERS experiments, 100 μL of

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