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

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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.

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

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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

NaCl (for a nanoparticles aggregation, final concentration was 0.01 mol L^{-1}) and 10 μL of sample solution (obtained by adding 100 μL of deionized water to a corresponding lyophilized pITP fraction) or standard solution of buserelin at specified concentration level were added to 890 μL of deionized water containing 60 ppm of silver nanocomposite.

2.4. Preparation of samples for ITP

Urine samples used for the pITP fractionation of buserelin were obtained from five healthy volunteers, and they were diluted either 10 or 100 times with deionized water and hydrochloric acid was added to 10 mmol L^{-1} final concentration immediately after obtaining. The samples prepared in this way served as blank urine samples. In the same way prepared urine samples were also spiked with buserelin standard.

3. Results and discussion

3.1. Preparation and characterization of silver nanoparticles

The surface enhanced Raman scattering presents an interesting tool for a trace analysis that can complement other established techniques. Experiments are in a significant number of cases based on the Raman signal enhancement using the surface of nanocomposites. The development of SERS method can be however problematic and some aspects have to be taken into account. Method development described in this paper was based on principles described by Le Ru et al. [44] or by Bell and McCourt [45].

Silver nanocomposites were prepared by a reduction of $AgNO_3$ by a corresponding reduction agent in the basic solution described by Panáček et al. [43]. The influence of reduction agent will be more described later. The method is based on the use of silver nanoparticles prepared using several procedures based on the Evanoff's work [46]. The nanoparticles were prepared using silver nitrate at 3×10^{-3} mol L^{-1} total concentration. The pH value of reaction mixture was set using an addition of ammonium hydroxide to pH 10.0. Synthesized nanoparticles were stabilized by an addition of triethylamine to the total concentration 1×10^{-3} mol L^{-1}. The nanoparticles prepared by a reduction of silver nitrate with glucose have a wide distribution in sizes. On the other hand, the size distribution of nanoparticles prepared by a reduction of silver nitrate with sodium citrate is relatively small (measured using dynamic light scattering microscopy).

The influence of the size of nanoparticles on the single molecule enhanced factor (SMEF) has been previously reported e.g. by Bell and McCourt [45]. SMEF is the SERS enhancement felt by a given molecule at a specific point. SMEF values are calculated using peak area (peak at 1625 cm^{-1} in this case) for analysis with SERS effect and without SERS effect. Ratio of these two areas and respective concentrations gives the SMEF value. The SMEF value is calculated according to Eq. (1):

$$\mathrm{SMEF} = \frac{I_{\mathrm{SERS}}}{I_{\mathrm{RS}}} \quad (1)$$

where I_{SERS} is the SERS intensity of the target molecule under consideration, whereas I_{RS} is the average Raman intensity per molecule for the same probe. The SMEF values obtained with the nanoparticles of different sizes prepared in this work are compared in Table 1. The highest obtained value of SMEF corresponds to nanoparticles prepared by a reduction of silver nitrate with sodium citrate. This nanocomposite was used in all other experiments if not stated otherwise.

Table 1
The summary of parameters of synthesized nanoparticles. Concentration of silver nitrate was 3×10^{-3} mol L^{-1}. The pH value was set to 10.0 by an addition of ammonium hydroxide. Nanoparticles were stabilized using triethylamine that was added to the total concentration 1×10^{-3} mol L^{-1}. SMEF stands for single molecule enhanced factor.

	Reduction agent	Concentration of reduction agent (mg L^{-1})	Particle average size (nm)	SMEF × 10^8
NP1	Sodium citrate	100	58	21.3
NP2	Glucose	100	44	13.8
NP3	Maltose	100	28	6.0

3.2. Preparative ITP experiments

Several alternatives of procedures used in pITP are summarized in the work of Hirokawa and Kiso [47]. Of these, hydrodynamically closed separation system was used in our work. This approach requires using some anticonvective and antiosmotic additive to avoid the separation deterioration. Therefore, both columns were filled with 1% (w/w) solution of hydroxyethylcellulose (high molecular weight anticonvective agent) before pITP runs to minimize the problems with the electroosmotic flow and the convection of solution. Obtained fractions were lyophilized after trapping and after proper reconstitution they can be used in additional analytical techniques. The main advantages of pITP are the well-defined clean-up effect especially when performed in the column-coupling configuration and the increasing concentration of analyte because of the Kohlrausch regulating function.

Preparative isotachophoresis experiments were carried out using sodium cation at 10 mmol L^{-1} concentration as leading ion. Final pH of leading electrolyte was adjusted with acetic acid to 5.00. Beta-alanine at 20 mmol L^{-1} concentration was used as terminating ion and acetic acid was added to pH 4.00. Both electrolyte solutions were prepared in water purified by two-stage system (for details, see Section 2.2). The using of discrete spacers is very useful in preparative isotachophoresis for the isolation of analyte from the potential interfering constituents originating from the complex ionic matrix [27]. We have found the proper mixture of discrete spacers for the isolation of buserelin from the urine matrix consisting of five low molecular weight constituents, i.e., TRIS – trishydroxymethylaminomethane; HIS – histidine; CREAT – creatinine; GABA – γ-amino butyric acid; EACA – ε-amino n-caproic acid. Stock solutions of discrete spacers were prepared at 10 mmol L^{-1} concentration. Discrete spacers divided the mobility span interval between leading and terminating ions into six parts. Isotachopherogram from the analysis of the final mixture of discrete spacers is shown in Fig. 1. The fractions were taken out in such a way that the driving current was switched off when inside the micropreparative trapping valve was present the end of zone having higher effective mobility and the beginning of the zone having lower effective mobility (see Fig. 2A). The valve was turned to the proper position and the fraction was displaced into Eppendorf microtube by air having final volume about 7 μL (see Fig. 2B). Then the channel of the sample from the loop to the microtube was washed with 25 μL volume of water following with 25 μL of methanol. Trapping valve was turned to the next working position and it was filled with leading electrolyte (see Fig. 2C). Finally, the valve was turned back, the driving current was switched on and the isolation of another fraction was possible (see Fig. 2D). The schema of fractionation procedure is shown in Fig. 2. All fractions obtained in pITP experiments were lyophilized and after reconstitution (100 μL deionized water) they were analyzed by SERS.

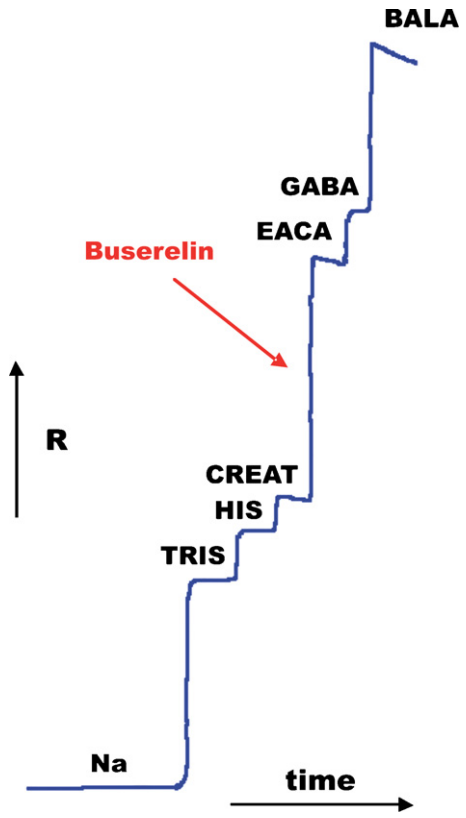

Fig. 1. Isotachopherogram obtained from the ITP analysis of model mixture of discrete spacers. The arrow indicates the migration position of buserelin. R – resistance; Na – sodium; TRIS – trishydroxymethylaminomethane; HIS – histidine; CREAT – creatinine; GABA – γ-amino butyric acid; EACA – ε-amino n-caproic acid; BALA – β-alanine.

3.3. Off-line combination of pITP–SERS

An off-line SERS analysis of each discrete fraction was based on the measurement of corresponding Raman spectrum and its comparison with a spectrum obtained by the analysis of standard solution containing buserelin ($c = 1 \times 10^{-8}$ mol L^{-1}). Four samples were measured in this way, i.e., buserelin standard, blank urine sample (10 times diluted urine), 10 times diluted urine and 100 times diluted urine spiked with buserelin standard, each sample consisted of six discrete fractions. Human urine was selected as a model matrix of clinical samples because of the high complexity of this body fluid and its noninvasive obtainment. Two dilutions of urine samples (10 times and 100 times) to study the influence of matrix effects were selected. Buserelin was identified in the isotachophoretic fraction 4 of each sample containing target analyte. The resulting spectra of all fractions obtained from the pITP fractionation procedure of the buserelin standard can be seen in Fig. 3b. For a better transparency, Fig. 3a represents a spectrum of buserelin standard without pITP fractionation. Each isotachophoretic fraction contains relatively high concentration levels of spacers (milli-molar concentrations of each spacer) that were used in the process of pITP separation for the isolation of buserelin from the matrix constituents. It can be seen from Fig. 3a and b that the presence of these compounds does not influence the resulting Raman spectra and, therefore, the spacers do not interfere with the Raman spectrum of buserelin. This statement can be proved by a factum that respective spacers were present in the analysis of blank sample (corresponding Raman trace is shown in Fig. 3b), whose fraction 4 contained only spacers and it can be seen that they do not render any Raman signal at this concentration levels and under these experimental

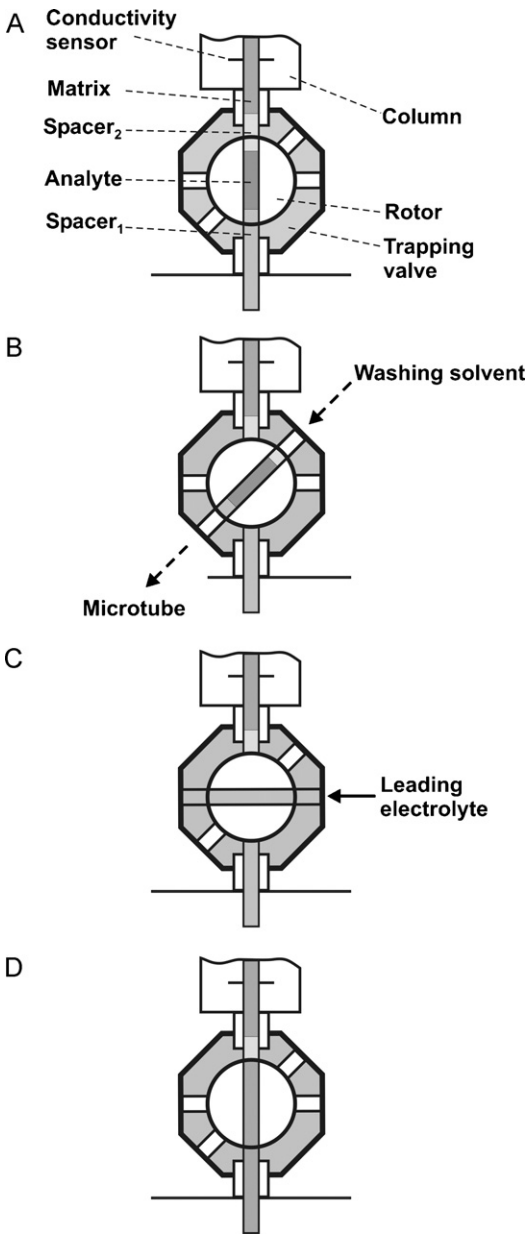

Fig. 2. Schema of fractionation procedure. For details, see Section 3.2.

conditions. Buserelin was identified in the fraction 4 of the corresponding standard sample. The Raman spectrum can be interpreted using existing databases. The peak at 1625 cm^{-1} can be interpreted as a signal of peptide amide groups (Amide I). Peak at 1240 cm^{-1} can be interpreted as Amide III group and other intensive peaks in the spectrum can be interpreted as specific vibrations of aliphatic chains of respective amino acids. The spectrum of the fraction 4 in Fig. 3b was also compared with the spectrum of buserelin standard in Fig. 3a, and an excellent agreement was found.

Analyzes of model samples (spiked human urine) were based on the same approaches. Raman spectra were obtained for each of six discreet fractions, and they were compared with the spectrum of buserelin standard. It can be seen in Fig. 4 that the spectrum obtained from the fraction 4 for both urine samples contains peaks that can be interpreted as the peaks of buserelin. There are no or only insignificant contaminants presented. For the better transparency, also Raman spectrum for fraction 4 of blank solution (human urine without an addition of target analyte) is included.

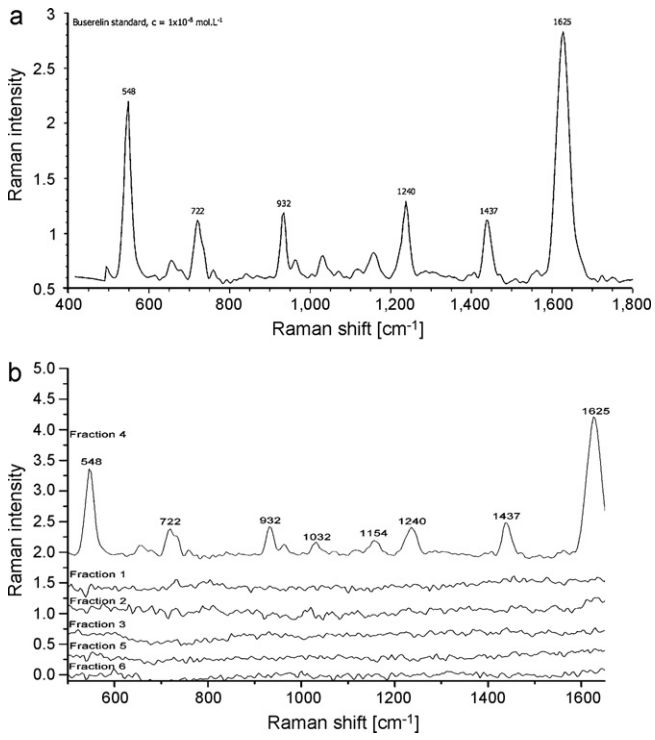

Fig. 3. (a) FT-SERS spectrum of buserelin standard. (b) FT-SERS spectra obtained by an analysis of all fractions (fractions 1–6) obtained from pITP fractionation procedure of buserelin standard. The concentration of buserelin was 1×10^{-9} mol L^{-1}. Spectra are shifted 0.5 units additionally for every spectrum along *Y* axis.

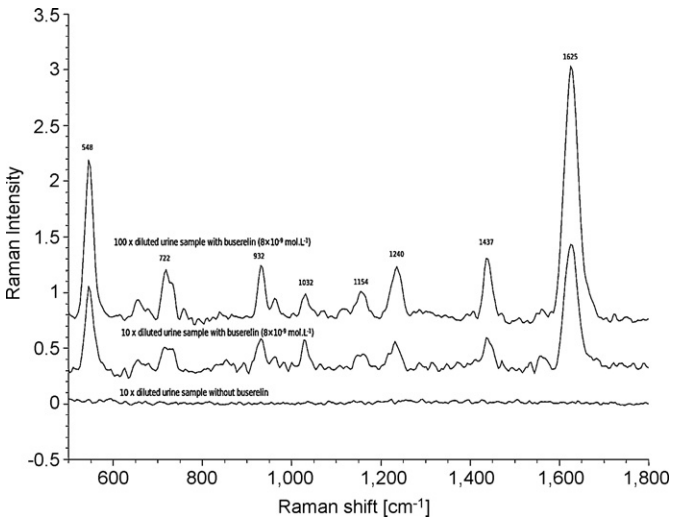

Fig. 4. The comparison of FT-SERS spectra obtained by the analysis of fraction 4 for samples of 10 and 100 times diluted urine and blank (human urine). Spectrum obtained by an analysis of urine without an addition of buserelin (blank) is also included for transparency. Spectra of samples are shifted 0.25 and 0.75 units along *Y* axis.

It can be seen that the concentration levels of contaminants are below their limits of detection and that preparative isotachophoresis is suitable method for the separation and preparation of target analytes for SERS measurements from complex matrices.

SERS quantification of buserelin (target analyte) was based on the method of standard addition to avoid the possible matrix effects. It has been shown, that the development of quantification methods based on the SERS approaches can be difficult and some overcoming has to be performed. Quantification of buserelin in two model samples containing different concentration levels of human urine (10 times and 100 times diluted human urine) was based on the addition of buserelin standard to the measured sample and its quantity was calculated using standard approaches for selected method. Firstly, five points calibration curve was measured using buserelin standard solutions. According to the theory of SERS effect, the linear range for selected approach is very limited [48] and thus the calibration range was selected to reflect the expected low concentration range of the analyte in model samples. Calibration range was from 1×10^{-10} to 1×10^{-8} mol L^{-1}. The linearity of signal was obtained in the range from 0.2 to 1.5 nmol L^{-1} of buserelin. Statistical evaluation of obtained data discovered a significant curvature of calibration line behind these limits. The curvature behind the limit of 1×10^{-8} mol L^{-1} was probably caused by a limited effective surface of given nanoparticles and the curvature on the other side of the line may be caused by an instrumental limitations. Limit of detection and limit of quantification, respectively, were calculated using signal to noise ratio (3 and 10, respectively) of the highest peak in the spectrum (wavenumber 1625 cm^{-1}). Limit of detection was 60 pmol L^{-1} and limit of quantification was 200 pmol L^{-1}. Obtained coefficient of determination was 0.991. The results obtained by a calibration are summarized in Table 2.

Quantification of buserelin in both samples was based on the addition of known amount of standard solution of buserelin into the measured sample. Results obtained by the analyses are shown in Table 2. The sample 1 (containing 10 times diluted urine) contained 8×10^{-9} mol L^{-1} of target analyte and 2.7×10^{-9} mol L^{-1} was found. The sample with 100× diluted urine contained 8×10^{-9} mol L^{-1} of target analyte and 3.7×10^{-9} mol L^{-1} was found. The average recovery of this method is 40%. Recovery was measured as a percentage difference between the actual concentration level of target analyte (buserelin) in the given sample and an experimentally obtained value. This phenomenon of relatively lower recoveries can be caused by the losses of target analyte in the separation process (for example an adsorption on a capillary wall) or, moreover, because of the concurrent adsorption of the target analyte to the nanoparticles during the SERS experiments, caused by a limited effective surface of given nanoparticles. How-

Table 2
The results obtained by a quantification of buserelin in model samples (spiked human urine). SD stands for standard deviation, RSD for relative standard deviation, respectively. Limits of detection and quantification were calculated according to the procedure based on the signal to noise ratio of the most intensive peak in the corresponding Raman spectrum.

Calibration parameters						
Intercept (nmol L^{-1})	SD	Slope (nmol L^{-1})	SD	LOD (pmol L^{-1})	LOQ (pmol L^{-1})	R^2
−0.028	0.003	1.033	0.031	62	198	0.991

	Concentration (nmol L^{-1})			
Samples ID	Added	Found	RSD (%)	Average recovery (%)
100 times diluted urine	8	3.74	5.1	46.8
10 times diluted urine	8	2.71	4.5	33.9

ever, obtained recovery is reproducible in time calculated using the measurements of five independent samples measured across five days interval. According to calculated limits of detection and/or quantification, respectively, this does not represent any further complication.

4. Conclusions

The analysis of clinical samples represents a difficult task, mainly because of complex character of matrices and related matrix effects. The concentration levels of various target analytes can be even very low. The off-line hyphenation of preparative isotachophoresis with surface enhanced Raman spectrometry allowed a development of analytical method for a confirmation and/or quantification of buserelin in the human urine. The influence of matrix effects was evaluated using 10 times and 100 times diluted human urine. There was found that target analyte (buserelin) can be successfully confirmed in both spiked samples and there were no significant interferences present in Raman spectrum of such complex matrix (urine). There was five points calibration curve constructed to evaluate the possibilities of pITP–SERS technique for quantification purposes. The calculated concentration limit of detection for buserelin is 60 pmol L^{-1}, the limit of quantification is 200 pmol L^{-1}. Obtained coefficient of determination in the measured concentration range (1×10^{-10} to 1×10^{-8} mol L^{-1}) is 0.991. Quantification of the target analyte in two model samples was based on the standard addition method. Ten times and 100 times diluted urine was selected as an example of complex matrix, respectively. Analyte was quantified at the 3.74 ± 0.18 and 2.71 ± 0.12 nmol L^{-1} concentration level, respectively. The recovery of the developed pITP–SERS method is 40%. This phenomenon can be caused by the losses of target analyte during the separation process or, moreover, due to the concurrent adsorption of impurities to nanoparticles during the SERS measurements.

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References

[1] T. Uematsu, Asia Pac. J. Pharmacol. 9 (1994) 43.
[2] G. Lippi, G. Banfi, M. Buttarello, F. Ceriotti, M. Daves, A. Dolci, M. Caputo, D. Giavarina, M. Montagnana, V. Miconi, B. Milanesi, A. Mosca, M. Morandini, G.L. Salvagno, Clin. Chem. Lab. Med. 45 (2007) 728.
[3] M.A.Z. Arruda (Ed.), Trends in Sample Preparation, 2007, p. 304.
[4] J.M. Rosenfeld, Sample Preparation for Hyphenated Analytical Techniques, 2004, p. 226.
[5] W.E. Smith, Method Enzymol. 226 (1993) 482.
[6] R. Wilson, S.A. Bowden, J. Parnell, J.M. Cooper, Anal. Chem. 82 (2010) 2119.
[7] R. Sheng, F. Ni, T.M. Cotton, Anal. Chem. 63 (1991) 437.
[8] B. Sägmüller, B. Schwarze, G. Brehm, G. Trachta, S. Schneider, J. Mol. Struct. 661 (2003) 279.
[9] G. Trachta, B. Schwarze, B. Sägmüller, G. Brehm, S. Schneider, J. Mol. Struct. 693 (2004) 175.
[10] Y. Wang, J.Z. Zhang, X.Y. Ma, Spectrosc. Spect. Anal. 24 (2004) 1373.
[11] Y. Wang, S.Y. Wang, Y.X. Zhao, G.F. Ren, F.L. Zi, Spectrosc. Spect. Anal. 22 (2002) 43.
[12] Y. Wang, S.Y. Wang, Y.X. Zhao, F.L. Zi, Y.F. Wang, H.J. Wang, J.B. Zhang, Chem. J. Chin. U 22 (2001) 48.
[13] Y. Wang, F. Zi, Y. Wang, Y. Zhao, X. Zhang, S. Weng, Spectrosc. Spect. Anal. 19 (1999) 824.
[14] E. Roth, W. Kiefer, Appl. Spectrosc. 48 (1994) 1193.
[15] L. He, M.J. Natan, C.D. Keating, Anal. Chem. 72 (2000) 5348.
[16] F.M. Everearts, J.L. Beckers, Th.P.E.M. Verheggen, Isotachophoresis – Theory, Instrumentation and Applications, Elsevier, Amsterdam, 1976.
[17] L. Arlinger in, Z. Deyl (Eds.), Electrophoresis: A Survey of Techniques and Applications, Part A, Elsevier, Amsterdam, 1979, pp. 363–377.
[18] P.A. Walker III, W.K. Kowalchyk, M.D. Morris, Anal. Chem. 67 (1995) 4255.
[19] P.A. Walker III, J.M. Shaver, M.D. Morris, Appl. Spectrosc. 51 (1997) 1394.
[20] P.A. Walker III, M.D. Morris, J. Chromatogr. A 805 (1998) 269.
[21] H. Li, P.A. Walker III, M.D. Morris, J. Microcolumn Sep. 10 (1998) 449.
[22] P.A. Walker III, M.D. Morris, M.A. Burns, B.N. Johnson, Anal. Chem. 70 (1998) 3766.
[23] M. Becker, C. Budich, V. Deckert, D. Janasek, Analyst 134 (2009) 38.
[24] A. Cifuentes, X. Xu, W.T. Kok, H. Poppe, J. Chromatogr. A 716 (1995) 141.
[25] D. Kaniansky, E. Šimuničová, E. Ölvecká, A. Ferancová, Electrophoresis 20 (1999) 2786.
[26] D. Kaniansky, V. Zelenská, I. Zelenský, J. Chromatogr. 256 (1983) 126.
[27] D. Kaniansky, V. Madajová, M. Hutta, I. Žilková, J. Chromatogr. 286 (1984) 395.
[28] M. Hutta, D. Kaniansky, E. Kovalčíková, J. Marák, M. Chalányová, V. Madajová, E. Šimuničová, J. Chromatogr. A 689 (1995) 123.
[29] V. Madajová, E. Šimuničová, D. Kaniansky, J. Marák, V. Zelenská, Electrophoresis 26 (2005) 2664.
[30] D. Kaniansky, E. Krčmová, V. Madajová, A. Ferancová, Electrophoresis 18 (1997) 260.
[31] A. Böttcher, C. Möllers, K.J. Lackner, G. Schmitz, Electrophoresis 19 (1998) 1110.
[32] A. Böttcher, J. Schlosser, F. Kronenberg, H. Dieplinger, G. Knipping, K.J. Lackner, G. Schmitz, J. Lipid Res. 41 (2000) 905.
[33] A.C. Schoots, F.M. Everaerts, J. Chromatogr. 277 (1983) 328.
[34] E. Kenndler, D. Kaniansky, J. Chromatogr. 209 (1981) 306.
[35] H.R. Udseth, J.A. Loo, R.D. Smith, Anal. Chem. 61 (1989) 228.
[36] F.M. Everaerts, T.P.E.M. Verheggen, E.F.P. Mikkers, J. Chromatogr. 169 (1979) 21.
[37] D. Kaniansky, Ph. D. Thesis, Comenius University, Bratislava, 1981.
[38] H.M. Everest, J.N. Hislop, T. Harding, J.B. Uney, A. Flynn, R.P. Millar, C.A. McArdle, Endocrinology 142 (2001) 4663.
[39] A. El-Nemr, M. Bhide, Y. Khalifa, E. Al-Mizyen, C. Gillott, A.M. Lower, T. Al-Shawaf, J.G. Grudzinskas, Eur. J. Obstet. Gynecol. Reprod. Biol. 103 (2002) 140.
[40] V. Sanz-Nebot, I. Toro, J. Barbosa, J. Chromatogr. A 870 (2000) 335.
[41] V. Sanz-Nebot, F. Benavente, I. Toro, J. Barbosa, Chromatographia 53 (2001) 167.
[42] A. Staňová, J. Marák, V. Maier, V. Ranc, J. Znaleziona, J. Ševčík, D. Kaniansky, Electrophoresis 31 (2010) 1234.
[43] A. Panáček, L. Kvítek, R. Prucek, M. Kolár, R. Večeřová, N. Pizúrová, V.K. Sharma, T. Nevěčná, R. Zboil, J. Phys. Chem. B 110 (2006) 16248.
[44] E.C. Le Ru, E. Blackie, M. Meyer, P.G. Etchegoin, J. Phys. Chem. C 111 (2007) 13794.
[45] S.E.J. Bell, M.R. McCourt, Phys. Chem. Chem. Phys. 11 (2009) 7455.
[46] D.D. Evanoff, G. Chumanov, J. Phys. Chem. B 108 (2004) 13948.
[47] T. Hirokawa, Y. Kiso, J. Chromatogr. A 658 (1994) 343.
[48] M. Lin, L. He, J. Awika, L. Yang, D.R. Ledoux, H. Li, A. Mustapha, J. Food Sci. 73 (2008) T129.