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PAPER

A novel approach to the rapid determination of amoxicillin in human plasma by solid phase microextraction and liquid chromatography

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A new approach to the rapid determination of amoxicillin (AMO) in human plasma followed by solid phase microextraction (SPME) fiber coatings based on conducting polymers (polypyrrole and polythiophene) and high performance liquid chromatography (HPLC) has been described. The porous structures of the electrochemically deposited polymer coatings have been characterized by scanning electron microscopy (SEM). The experimental parameters relating to the extraction efficiency of the SPME fibers such as pH, extraction time and desorption conditions (solvents, time) were studied and selected. The SPME/HPLC-UV method was linear over a working range of 1–50 μg ml^{-1}. The inter-day accuracy (expressed as coefficients of variations, CVs) was less than 15% and precision (expressed as the relative standard deviations, RSDs) with percentage values was less than 5.9%. Amoxicillin was found to be stable in the human plasma at room temperature (20 °C) within 8 hours. The developed method was successfully applied to the analysis of real human plasma samples. The limit of detection and limit of quantification for amoxicillin in plasma were 1.21 μg ml^{-1} and 3.48 μg ml^{-1}, respectively.

1. Introduction

Bacterial infections are a wide problem especially in the case of hospitalization and different medical treatments. The multi-resistant bacterial strains, developed during a long-term treatment with the use of the same drug, are now resistant to a vast number of various kind of antibiotics *e.g.* vancomycin and penicillin. The drug which is contemporarily widely used in the medical treatment is amoxicillin. This drug belongs to β-lactam antibiotic and penicillin group. The amoxicillin acts against both Gram-positive and Gram-negative bacteria by inhibiting the synthesis of the bacterial cell wall. Due to the fact that amoxicillin can be susceptible to the degradation by β-lactamase which is formed by bacteria cells, this drug may be given with a small addition of clavulanic acid. This compound can decrease the negative acting of β-lactamase for the amoxicillin and increase their susceptibility to degradation.[1,2]

A fast and precise quantitative and qualitative analysis of amoxicillin in human plasma or directly in the human tissue is exceedingly essential in the case of medical analysis. Pharmacokinetics and pharmacodynamics are useful in a proper dosage of antibiotics for a patient who has to be served an appropriate amount. The problem with amoxicillin is caused by different diffusion of this molecule in the human body.[3] This process depends on the patient's mass, age and numerous other factors. As no investigation of drugs in the target body part or scar is performed in hospital in this kind of treatment, the physicians are not sure that the drug, which was dosed, has reached the target place in the human body. A developing method which will allow to perform a fast and direct (from human tissue or blood) analysis of amoxicillin will help to better adjust the amount of a drug which should be dosed. That method should answer for the physician whether an appropriate drug reached a target scar or other places.[4]

One of the most suitable methods which can allow in the future for a direct analysis of amoxicillin from human tissue (hard heal wounds) or plasma and blood is the solid phase microextraction (SPME).[5,6] This technique is connected with the high performance liquid chromatography (HPLC) coupled with sensitive and selective detectors such as ultraviolet (UV), fluorescence (FLD) or mass spectrometry (MS) and can enable a possibility of quantification of the target compounds at the concentration of analytes lower than ng ml^{-1} (pg ml^{-1} or fg ml^{-1}).[7–13]

The most popular technique for the amoxicillin determination is the liquid chromatography in reversed phase conditions. However, it is difficult to obtain high retention of this compound on the commercial high coverage C_{18} phases because of its low hydrophobicity. From this point of view, the retention on the low coverage density phases may lead to interesting results.[10–12]

The solid phase microextraction technique was developed at the end of the last century and was used for sampling of aromatic compounds[14] especially from the environmental matrices by Pawliszyn and Arthur. It has recently found a wide application in the biomedical analysis.[15] This technique was created on the basis of the solid phase extraction (SPE), which is a precleaning and

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preconcentration technique, and is mainly applied in sampling. The linear dependency with the application of the appropriate adsorbent materials allows quantifying the analytes from extremely complex matrices. SPME has been utilized, in different biomedical applications, up to this time. The analysis of adrenolytic[16] and psychotropic drugs[17] can be found in the literature.

Table 1 shows analytical methods for the quantification of amoxicillin in human plasma.[10,13,18–21] Liquid chromatography coupled to MS, UV or FLD is the method of choice depending on the required sensitivity, the biological matrix, and the applied sample preparation procedures, *e.g.* solid phase extraction or protein precipitation.

In this paper, we focused mainly on the analysis of amoxicillin (applied in bacterial infection treatment) from aqueous and plasma samples. Two different kinds of SPME coatings were used in the experiments: polypyrrole and polythiophene. A deep investigation and development of the HPLC method with UV detection which further allowed for the analysis of amoxicillin in all measurements were both performed before the measurements with the use of SPME. This method provided an analytical tool for the quantification of amoxicillin for pharmacokinetics as well as drug monitoring for biomedical purposes. Sampling of amoxicillin using solid phase microextraction was applied for the first time and has not been under consideration of other research groups yet.

2. Experimental

2.1. Chemicals

All chemicals and reagents were *HPLC* grade or analytical grade. Monomers, pyrrole (98%) and thiophene (99%) were purchased from Sigma-Aldrich (Schnelldorf, Germany). Amoxicillin and drug-free human plasma were kindly provided by Collegium Medicum (Nicolaus Copernicus University, Torun, Poland). The methanol for *HPLC* was obtained from J. T. Baker (Deventer, The Netherlands). Water was obtained by means of Milli-Q RG apparatus by Millipore (Millipore Intertech, Bedford, MA, USA) in our laboratory.

2.2. Instrumentation

The HPLC 1100 (Agilent, Waldbronn, Germany) was used as the chromatographic system. It consisted of quaternary pump, degasser, an automatic sample injection and a variable wavelength UV-Vis (λ = 230 nm) detector. Additionally, the chromatographic system was coupled to a mass spectrometer (MS^n) equipped with an electrospray ionization (ESI) interface and operated with Mass Hunter software. Negative-ion selected ion monitoring (SIM) mode was used to detect and verify the chemical and molecular structure of amoxicillin among other chemical individuals in human plasma samples, m/z = 364.1 to corresponding $[M - H]^-$.

For sample evaporation a Labconco CentriVap DNA concentrator (Kansas City, USA) was used.

In the electropolymerization process a home-made set up system coupled with a high performance potentiostat/galvanostat PGSTAT128N series Autolab model (Utrecht, The Netherlands) was applied.

The chemical and mechanical stability experiments were set up with Optical Stereomicroscope model SZX16 (Olympus, Tokyo, Japan) equipped with a CCD camera and CELL software.

The scanning electron microscopy (SEM) was accomplished with LEO 1430VP (Carl Zeiss SMT, Oberkochen, Germany).

2.3. Preparation of SPME polypyrrole and polythiophene coatings

The electropolymerization method published in ref. 22 was used to prepare the polypyrrole (PPy/SPME) and polythiophene (PTh/SPME) fibers. Electrodeposition of polymers was performed with use of three-electrode cell with platinum net (counter electrode), silver metallic electrode (reference electrode) and three medical stainless steel (SS) (working electrode). An electrolyte solution was 0.1 M tetrabutylammonium tetrafluoroborate dissolved in acetonitrile. Pyrrole and thiophene concentrations were 0.25 M. Polymerizations were performed with the use of a dynamic technique with threshold potentials of $-0.2 \div +2.5$ V for both monomers. The SPME fibers have 1.5 cm length of coating and 750 μm diameters with thicknesses in range about 200 μm and 285 μm for polypyrrole and polythiophene, respectively. All the physical parameters of the used fibers are listed in Table 2.

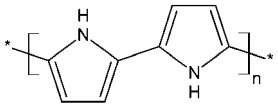
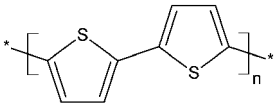
2.4. Preparation of stock and standard solutions

The working standard drug solutions, based on the therapeutic concentrations, were prepared by diluting the stock solutions (0.125 mg ml^{-1} in deionized water) to a proper volume. The stocks solutions were diluted to make working standard solutions of 1–50 μg ml^{-1}. These solutions were stable for 30 days at −20 °C, and at room temperature for 8 hours.

Table 1 Analytical methods for the determination of amoxicillin

Method	Linearity range/μg ml^{-1}	Approx. run time/min	Sample volume/μl	Sample preparation	Intra-day CV (%)	Correlation coefficient
Present method HPLC-UV/MS	4–50	6.64	15	Solid phase microextraction	2.3–5.6	0.9887
Numan *et al.*[13] HPLC-UV	1–80	3.5	20	Protein precipitation	102.00 ± 0.98	0.9998
Fernandez-Torres *et al.*[19] LC-MS	Not reported	16.1	50	Enzymatic-microwave extraction	1.8–9.6	0.999
Fernandez-Torres *et al.*[20] HPLC-DAD-FLD	0.59–20.0	6.76 (7.53)	Not reported	Solid phase extraction	Not reported	0.9993
de Abreu *et al.*[10] HPLC-UV	1–50	4.26	Not reported	Protein precipitation	80–125	0.999
Mascher *et al.*[18] HPLC-FLD	0.1–15	2.77	Not reported	Protein precipitation	4.54–13.18	0.9988
Matar[21] HPLC-UV	0.5–12	6.86	20	Ultrafiltration	1.13–8.16	0.99

Table 2 Physical parameters of SPME fibers

Parameter	Polypyrrole (PPy/SPME fibres)	Polythiophene (PTh/SPME fibres)
Length of coating/mm	15.0	15.0
Thickness/μm	200–205	285–295
Organic carbon content (%)	73.6	45.9
Monomer (substrate)	Pyrrole (0.25 M)	Thiophene (0.25 M)
Polymer (product)		
Wire material	Ni–Cr Medical Stainless Steel	Ni–Cr Medical Stainless Steel

The plasma samples were stored at −20 °C. Before use, the plasma was thawed at room temperature and centrifuged at 2500 rpm for 5 min. The spiked plasma samples were prepared by the target analyte (32–400 μl to 1.5 ml plasma) to centrifuged plasma to reach final concentrations of 4–50 μg ml^{-1}.

2.5. Conventional drug analysis and sample preparation for precipitation of protein (PP)

A quantitative determination of the target compound is described briefly as follows. The samples of aqueous standard solutions were subjected to the HPLC system without any pre-treatment. Chromatographic separations were performed using a home-made C18 column (150 × 4.6 mm). The optimal stationary phase with the coverage density of octadecyl bonded ligand 1.11 μmol m^{-2} after end-capping was chosen. The detailed characteristic of the applied column is described in ref. 23. During the separation, the chromatographic column was guarded with a C18 cartridge (10 × 4.6 mm, $d_p = 5$ μm). Additionally, all the chromatographic measurements were carried out in constant room temperature (20 °C). The mobile phase consisted of water and methanol (90 : 10, v/v) and was pumped at a flow rate of 600 μl min^{-1}. The pH of the MeOH/water mixture was 6.32 ± 0.05. UV absorbance detection was performed at $\lambda = 230$ nm. The chromatographic run time was 10 min.

In this study, the plasma samples were treated by adding perchloric acid for the proteins precipitation. To optimize the protein precipitation of the target compound from human plasma samples, different amounts of $HClO_4$ were used. Based on the results, a protocol with the use of 15% of $HClO_4$ for protein extraction was developed (*Section 3.4*). For precipitation of protein, 50 μl of 15% perchloric acid was added to 200 μl plasma and mixed for 3 min vortex. The sample was centrifuged at 3500 rpm for 10 min at room temperature. The upper solution was transferred into a new centrifuge tube, and the precipitation of protein was repeated twice. The calibrators and quality controls were prepared in the same way (4, 7, 10, 15, 30 and 50 mg l^{-1}). Then 15 μl of the supernatant was injected into the HPLC and was analyzed.

2.6. Simultaneous SPME of amoxicillin from human plasma

SPME fiber was preconditioned in MeOH/water (90 : 10, v/v) for 15 min. The adsorption process of amoxicillin was performed manually (without agitation) in 400 μl of human plasma during 10 minutes. Then fibers were washed gently with distilled water and put into 400 μl of methanol/water (50 : 50, v/v) for 5 minutes. Then, samples after desorption were subjected to HPLC without pre-treatment. Meanwhile, samples after adsorption were extracted with utilized protein precipitation (as is described in *Section 2.5*).

The amount of extracted amoxicillin by SPME coatings was calculated using a calibration curve to acquire the relevant concentration. Total amount of the extracted drug (Am_{total}) corresponded to the given equation: $Am_{total} = c_{des} \times V_{des}$, where c_{des} [μg ml^{-1}] is the concentration of drug in the desorption solution, whereas V_{des} [ml] is its volume.

2.7. HPLC-UV method validation

The method was validated under optimized conditions. Each calibration curve was compiled of points covering from 1 to 50 μg ml^{-1}. The accuracy was presented as the ratio of the determined and nominal values of concentrations of the relevant drug and multiplied by 100%. In addition, the precision was defined as the percentage of standard deviation of the relevant values divided by the average of mean values. The limit of detection (LOD = 3 × SD_{xy}/b, where SD_{xy} is the standard deviation and b is the slope) and the limit of quantification (LOQ = 10 × SD_{xy}/b) were calculated with the acceptable precision and accuracy.[20] The recoveries were determined by comparing the peak areas after HPLC-UV measurements of the spiked samples with the direct injection of standard solutions of equal concentrations. Logically, the linearity was obtained by analyzing blank plasma samples ($n = 3$) containing standard solutions of drugs at concentrations of 1–50 μg ml^{-1}. The concentration range was obtained due to the regression curve ($y = ax + b$) and correlation coefficient (R^2). The selectivity of the proposed method was evaluated by analyzing amoxicillin in the presence of linezolid, normally combined with this drug. Convincingly, the stability studies performed on the standard solutions and human plasma samples indicated the thermal stability for at least 30 days stored at −20 °C. The analytical procedure was fully validated according to the appropriate guidelines of the Food and Drug Administration (USA).[24]

3. Results and discussion

3.1. Interactions between stationary phase and target analyte

Chromatographic separations were performed using a home-made C18 column. A detailed discussion was presented in the

previous work.[23] Fig. 1 shows the possible interactions of the target compound with the chromatographic octadecyl stationary phase (SG-C18), which occur through a mixed mechanism involving hydrogen bonds (Fig. 1A) and hydrophobic interactions (Fig. 1B). The first ones are formed between the polar groups of amoxicillin molecule and free silanols on the silica surface. On the other hand, the hydrophobic interactions take place also between the target compound characterized by low hydrophobicity (log $P = 0.87$[25]) and alkyl chains of the chromatographic column stationary phase.

The retention mechanism on the selected home-made octadecyl columns is similar to both reversed phase and aqueous normal phase systems. However, despite the polar interaction, the efficiency of the separation is in the range 40 000–50 000 theoretical plates per metre. Also some band-broadening is observed but the asymmetry factor is not lower than $f_{AS} = 0.8$ in all measurements. The detailed investigation of the amoxicillin retention on the series of octadecyl packings, including efficiency and peak asymmetry, was presented in ref. 23.

Because of strong amoxicillin interaction with silanol groups this molecule may be adsorbed also on the walls of the vessels during extraction. This effect may be minimized through application of polymer vials in extraction. However there is a limitation of the work in the case of glass vials.

3.2. Stability of analyte

Analyte stability in the samples was evaluated by determining short-term stability. A simple procedure with centrifugation and evaporation was used. The stability of the analyte was studied in triplicates using spiked human plasma samples. 25 μg ml^{-1} of amoxicillin was transferred into tubes and evaporated to dryness at different temperatures (20 °C, 30 °C and 50 °C). Then, they were reconstituted in 100 μl of the HPLC mobile phase. The short-term temperature stability was investigated by keeping the samples for 4 h at the relevant temperature before sample preparation. Fig. 2 shows an HPLC-UV chromatogram of amoxicillin ($c = 25$ μg ml^{-1}) in human plasma samples treated by different temperatures (20 °C, 30 °C and 50 °C). As can be noticed, the peak corresponding to the target compound is well resolved, and additionally there are no interferences from plasma matrix, which confirmed the method specificity. Chromatograms of samples prepared in higher than 20 °C contain two peaks. That allowed to claim that amoxicillin in higher temperature undergoes chemical structures changes. There was no significant degradation of amoxicillin freshly prepared in plasma samples utilized within 24 hours in comparison with the samples stored at −20 °C and kept at room temperature for 8 hours after thawing. Nevertheless, the post-preparation samples were found to be unstable during a treatment with temperature higher than 20 °C. From this reason this value of temperature was chosen in all further experiments.

3.3. Selection of the SPME fiber variables

Polypyrrole and polythiophene SPME coatings were selected from a wide group of conducting polymers for investigation of amoxicillin extraction.

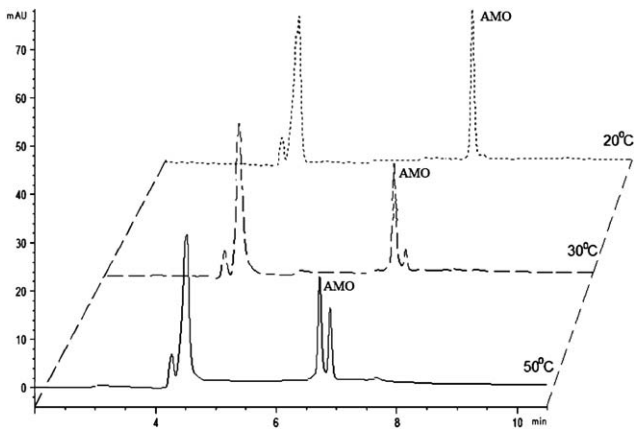


Fig. 2 Thermal instability of amoxicillin (AMO) in different temperatures applied.

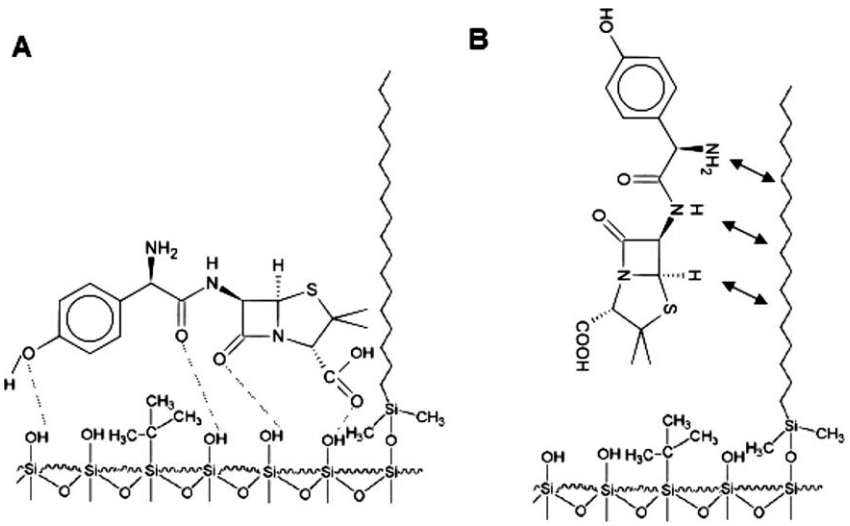


Fig. 1 Possible interactions between polar groups (A) and nonpolar alkyl chain—drug molecule (B).

The optimization parameters including the extraction time, kind of desorption solution and pH of organic extraction solvent were selected. The influence of the solvent pH on the amoxicillin extraction was evaluated by investigating different pH values from 4.9 (15% acetic acid) to 10.7 (25% ammonia). In the centrifuge tubes, the acetic acid or ammonia was added to 200 μl of plasma sample, spiked with the standard solution. To determine the appropriate desorption conditions: solvent (methanol, acetonitrile and methanol/water mixture), desorption time (2, 5, 7 and 10 min) and the control of the memory effect were thoroughly studied.

The parameters which have influence on the extraction efficiency were optimized using a standard solution containing 25 μg ml^{-1} of amoxicillin. The selected parameters included solvent type for desorption, pH value of desorption solution and extraction time.

Fig. 3 shows the representative time extraction profiles (2–10 min). This experiment was performed using PPy and PTh fibers. At the beginning, a small amount of amoxicillin was extracted. Then, after 5 minutes the highest extraction efficiency occurred. At the last two points, the desorption-time profile seemed to be closer to reach the expected equilibrium. This also confirmed the practicable factor due to unnecessary long exposure time of the fiber to the medium. The relative standard deviations (RSDs) obtained in these experiments were calculated on a very low level in the range from 0.84 to 2.26% for polypyrrole and from 0.64 to 1.89% for polythiophene SPME fibers.

According to the high solubility of amoxicillin in water, some relevant organic extraction solvents were chosen. These contained: pure methanol, pure acetonitrile as well as some mixture of methanol with acetic acid, ammonia and water, respectively. According to Fig. 4, MeOH/water (50 : 50, v/v) effected the higher extraction efficiency, thus it was chosen for further investigations. As a matter of fact, the highest gap is observed for the PPy-coated SPME fibers in the case of MeOH/water (50 : 50, v/v). However, good results were obtained with the use of this kind of coating in acidic conditions. Unfortunately, in the same case the usage of the PTh coating was realized with no success at the extraction efficiency. This result can be explained by the high polarity of the target compound.

Additionally, amoxicillin is an amphoteric drug ($pK_{a1} = 2.8$ and $pK_{a2} = 7.2$[26]), hence the pH value of the plasma sample will play an essential role in the amount of the extracted analyte. The results also proved that the acidic conditions will provide higher extraction efficiency for the polypyrrole SPME coating. On the other hand, the polythiophene-coated SPME fibers preferred neutral or slightly basic medium, probably because of the lack of hydrogen atom in its polymeric chain (Table 2).

The fibers reproducibility was investigated with the use of six PPy and PTh fibers prepared in the same conditions. The results from this short experiment gave the relative standard deviations (RSDs) ranging from 5 to 11%. For these experiments three consecutive experiments were performed using six fibers of each coating type. The SPME coatings were compared in terms of interfiber reproducibility and sorption of the coating upon repeated use. The PPy SPME coating was found to have the highest extraction efficiency for compound tested. Moreover, the SPME coating based on polythiophene showed the best extraction reproducibility for the three extractions.

Additionally, both the chemical and mechanical stabilities were investigated. Neither during the extractions from standard solutions nor plasma samples, was there any significant loss in the extraction abilities or even destruction of the sorption surface of SPME fibers. The carryover was found to be well below 3%. The chemical and mechanical stability and robustness of the PPy and PTh coatings were confirmed after treatment in different solvents (water, methanol, THF, acetonitrile, MeOH/H_2O (50/50, v/v), 2-propanol, MeOH/CH_3COOH and MeOH/NH_3) and during various time (5–15 min), and there were no significant differences in the porous surfaces of PPy-SPME and PTh-SPME fibers. These experiments were performed with the use of an optical stereomicroscope.

In short, the optimized conditions for the extraction of amoxicillin in water and human plasma samples with prepared SPME fibers were: extraction time, 5 min; desorption solution,

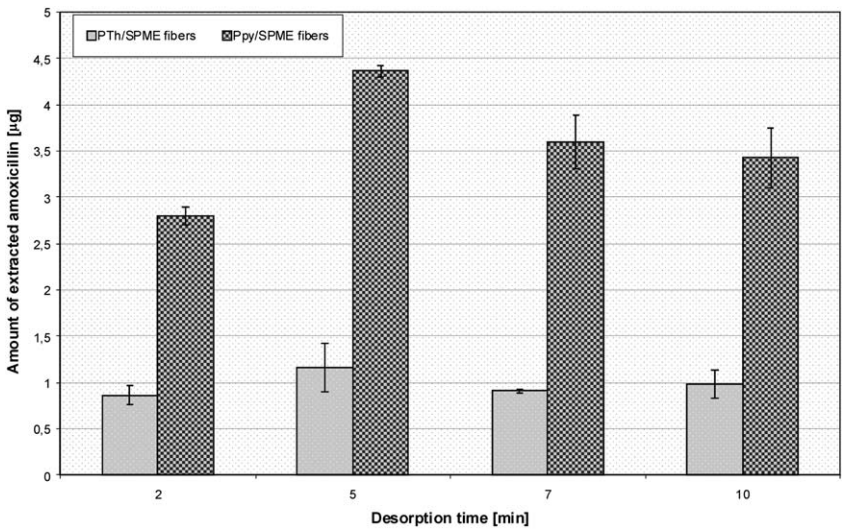


Fig. 3 PPy/SPME and PTh/SPME desorption time profile of amoxicillin in standard solution.

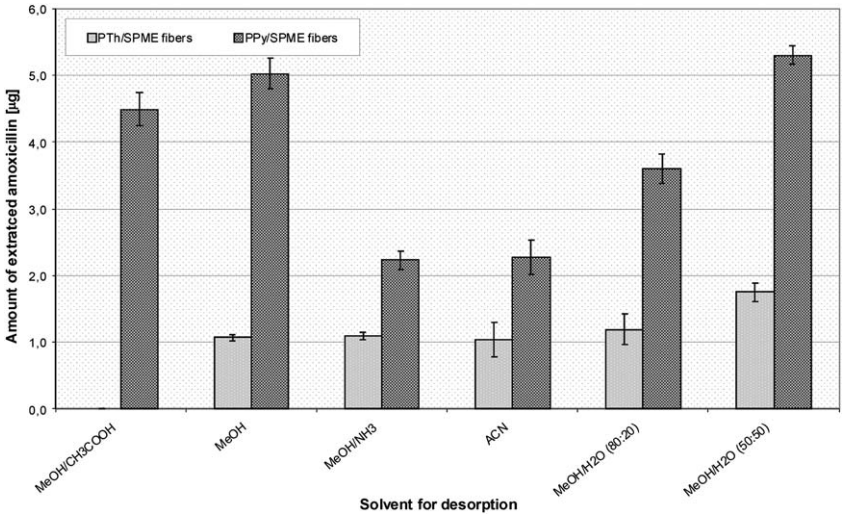


Fig. 4 Influence of solvent for desorption on PPy/SPME and PTh/SPME extraction efficiency. Desorption time: 5 min.

MeOH/water (50 : 50, v/v); desorption time, 5 min; no adjustment of ion strength, no agitation during the extraction.

3.4. Optimization of proteins precipitation protocol

To optimize the protein precipitation of the target compound from the human plasma samples, a selected percentage of the perchloric acid solutions were tested. The protocol with the use of 15% of $HClO_4$ for the protein extraction was developed. Moreover, during the precipitation of proteins, the target analyte did not precipitate together with the endogenous compounds from the plasma samples.

3.5. Interactions between polymer coating and target analyte

The characteristics of the PPy/SPME and PTh/SPME fibers were investigated by the scanning electron microscope (SEM). The images (Fig. 5) evidently show that the morphological properties of the prepared polymer films affect the differences between the sorption capacities among them. Additionally, SEM pictures proved the porous structure of the prepared fibers. However, detailed measurements of the polymeric coatings should be employed to get acquainted with the pore size distribution. The stability and robustness of these three kinds of coatings are comparable. The porous structure (PPy) exhibits higher extraction efficiency in comparison to rather less porous, but with thicker PTh structure. Furthermore, it could mean that thickness is effective on the sorption surface up to a certain value due to the porous structure of the film. Hence, it is not true that the extraction efficiency and sensitivity enlarged with the increase of the thickness of the coating. As can be concluded, there is no relationship between the obtained short equilibrium times with the thicknesses of the presented coatings. It can be also proved by comparison of the amounts of extracted drug (discussed in *Section 3.3*). Consequently, in spite of a thicker polythiophene fiber, the more porous polypyrrole hydrophilic phase showed higher results. Another possible description of this phenomenon could be related to the chemical structure of two prepared polymer coatings. The porous-structured PPy possesses –NH groups in its chain which could counterbalance a higher surface area for the interactions with the analyzed compound than the PTh one.

Utilizing a porous SPME coating short equilibrium time with an equivalent extraction efficiency (at extraction time less than 5 min) was achieved. These results could stand closely to the fact that the analyzed drug can diffuse through the fiber pores and during the migration adsorb onto the surface. The amoxicillin NH_2 group undergoes hydrogen bonding with the NH– fragment of pyrrole rings. Additionally, this type of interaction occurs between the –OH group of the target compound and polymer NH– part.

The chemical behavior of amoxicillin can change depending upon the conditions of the medium. If the pH increases, the ionization of amoxicillin decreases and induces a decrease of the extraction efficiency. If the pH becomes too high, the amine group of the analyte is neutralized, accordingly the interactions

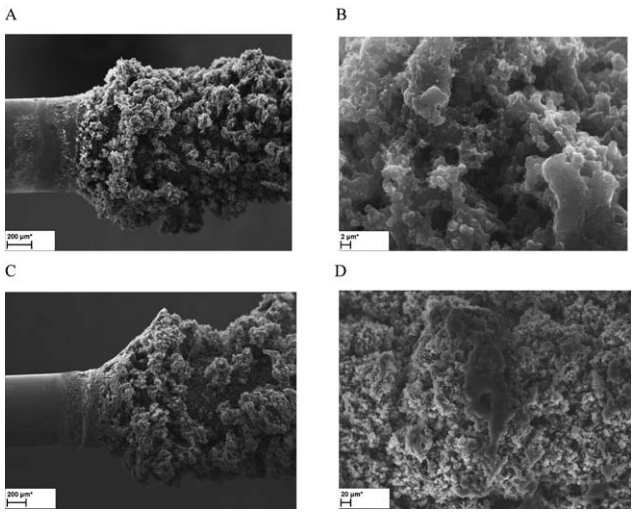


Fig. 5 Scanning electron micrograph of: PPy/SPME (A) and PTh/SPME (C) coatings, respectively; top view of the polypyrrole (B) and polythiophene (D) sorption surfaces, respectively.

between the polymer coating and drug are no longer strong enough. On the other hand, the acidic conditions limit the possibility of any bonding formation in the PTh case. Under pH = 7 the amino group of amoxicillin is completely protonated. Hence, the sorption mechanism occurs and a significant extraction efficiency due to strong intermolecular hydrogen bonds between PPy and the analyzed drug is observed. The contrasting behavior of the weak or strong interactions is rationalized by the difference in the sorption mechanism of the zwitterions structure in which the amoxicillin exists under the relevant conditions. According to the chemical structure of amoxicillin, it can be noticed that more than one active site existed for the applied SPME fiber coatings for a selective sorption. Additionally, the phenol ring, as well as $-NH_2$ group and –COOH, may participate in the sorption mechanism with a polymeric layer. Amoxicillin exists as a zwitterion under the appropriate pH conditions. This fact can affect the different extraction efficiency. Additionally, the presence of the phenol ring, which can be also negatively charged, stabilizes the zwitterions formation and decreases the extraction efficiency. The straightforward procedure allows obtaining a selective extraction by PPy/SPME fibers with extraction efficiency 58% and very low RSD values (3.11%, $n = 3$). Concerning the PTh/SPME fibers, the extraction efficiency of 32% was achieved with RSD values lower than 4.58% ($n = 3$).

3.6. Analytical validation

The obtained validation parameters are on a satisfactory level of sensitivity of amoxicillin that can be found in human plasma. Additionally, the limit of detection found was comparable with this corresponding to administrated therapeutic levels described before.[18,27] The calibration curve parameters, presented in Table 2, show good correlation coefficients (R^2) of 0.9994 and 0.9887 for standard solutions and plasma samples, respectively. The accuracy of the method ranged from 89% to 113%. The linearity of the developed method was determined with the plasma samples spiked with the analytical standard. The quantitative analysis of amoxicillin in biological samples was preceded by fixing the limit of quantification (LOQ = 3.48 μg ml^{-1}) and the limit of detection (LOD = 1.21 μg ml^{-1}). The results obtained show that liquid chromatography with UV detection at a wavelength $\lambda = 230$ nm may be applied for determining this analyte at low concentrations. These values are in agreement with the relevant Food and Drug Administration guidelines which suggested the mean value to be within 15% of the actual value.[24]

The selectivity of the developmed analytical method is proved by the chromatogram of the plasma sample spiked with amoxicillin at the therapeutic concentration level (Fig. 6). As can be noticed, there are no significant interferences from the endogenous plasma components. Table 3 shows the results for the validation of the method in the human plasma samples and standard solutions.

3.7. Application to real samples

To assess the applicability of the proposed PPy/SPME and PTh/SPME coatings in real samples, the experiment to determine amoxicillin in human plasma samples was conducted. The samples were spiked at three concentration levels and were analyzed by two kinds of fibers under optimal conditions. The extraction efficiency for the samples obtained with the polypyrrole sorption phase was higher than polythiophene one. The extraction capacities were 43% and 26% for PPy/SPME and PTh/SPME coatings.

The plasma extracts from PPy/SPME were remarkably clean in comparison with the use of the precipitation of protein as a sample preparation technique, indicating that the last process is influenced by the sample matrix effect.

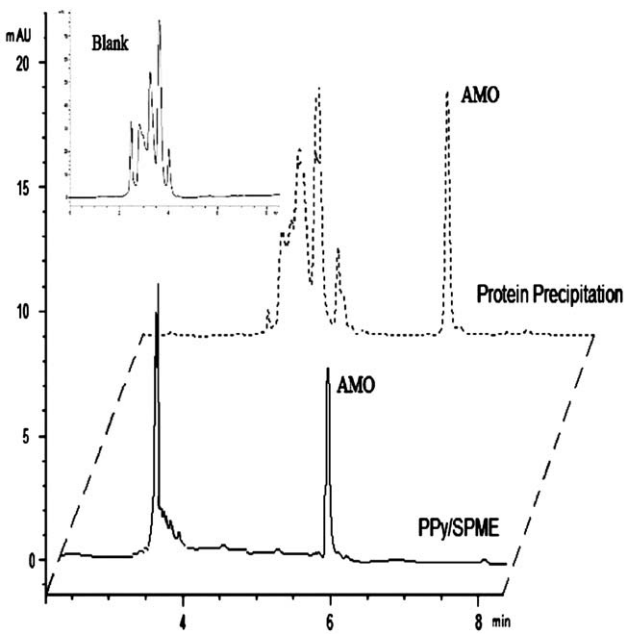


Fig. 6 Chromatograms from a human plasma samples spiked with 10 μg ml^{-1} amoxicillin and extracted by PPy/SPME or precipitation of protein. The inset corresponds to a blank using PPy.

Table 3 Validation parameters of calibration of the amoxicillin in standard solutions and plasma samples determined by HPLC

Medium	Linear range/ μg ml^{-1}	Intraday precision (%RSD) 4 μg ml^{-1}	10 μg ml^{-1}	30 μg ml^{-1}	LOD/ μg ml^{-1}	LOQ/ μg ml^{-1}
Standard solution	1–50	5.6	4.2	2.9	0.13	0.39
Plasma	4–50	5.9	4.4	2.3	1.21	3.48

Table 4 Comparison of amount of extracted target compound by PPy *vs.* PTh fibers from aqueous solutions and human plasma samples ($n = 3$)

Concentration/ μg ml^{-1}	Amount of extracted amoxicillin/μg Aqueous solutions PPy *vs.* PTh	Plasma samples PPy *vs.* PTh
1	0.15/0.13	0.20/0.19
4	0.45/0.17	0.39/0.17
7	0.81/0.45	0.49/0.26
10	1.02/0.67	0.74/0.52
15	1.41/1.11	1.16/0.78
30	2.28/1.29	1.53/0.91

Table 5 Extraction of amoxicillin from aqueous solutions and plasma samples for polypyrrole (PPy) and polythiophene (PTh) fibers ($n = 3$)

Medium	Parameter Fiber	Slope	Intercept	R^2	RSD (%)
Aqueous solutions	PPy	0.0720	0.2164	0.9792	0.11–2.02
	PTh	0.0430	0.1559	0.8641	0.16–2.31
Plasma samples	PPy	0.0469	0.2286	0.9412	0.28–2.44
	PTh	0.0283	0.1551	0.8495	0.31–3.08

Table 4 compares extraction efficiency in aqueous solutions to that obtain in human plasma samples. Polypyrrole SPME coatings provide better sorption efficiencies than polythiophene SPME fibers for both matrices studied.

Table 5 presents the linear regression parameters calculated for PPy and PTh fibers.

4. Conclusions

A rapid and sensitive solid phase microextraction and high performance liquid chromatography-UV detection (SPME/HPLC-UV) analytical method for analysis of amoxicillin in human plasma samples was described. This miniaturized sample preparation technique focused on reducing the sample volume required, analytical time, cost and the solvent elimination.

The main aim of this investigation was to apply a fast and sensitive extraction technique using electrochemically prepared polymeric coatings as sorbents for SPME. The potential of polypyrrole and polythiophene SPME fibers for the selective extraction of the amphoteric drug was demonstrated considering the results obtained for the standard solutions and applied to human plasma samples. The developed polymer sorption fibers can provide a lower memory effect and short equilibrium times. Amoxicillin was analyzed by HPLC-UV for the reason that the sensitivity of the UV was reported to be sufficient for our purpose according to drug monitoring with the use of the human plasma samples.[28] This method can be used for the quantitative analysis of amoxicillin, and provide a potential application to study the metabolism and pharmacokinetics of other drugs from different medical classes from the human plasma.

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