Journal of Chromatography B, 879 (2011) 2542–2548

Contents lists available at ScienceDirect

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

A new way of solid-phase microextraction fibers preparation for selected antibiotic drug determination by HPLC–MS

Pawel Olszowy[a], Malgorzata Szultka[a], Jacek Nowaczyk[b], Boguslaw Buszewski[a,*]

[a] Department of Environmental Chemistry and Bioanalytics, Faculty of Chemistry, Nicolaus Copernicus University, Gagarin 7, 87-100 Torun, Poland
[b] Department of Physical Chemistry and Physicochemistry of Polymers, Faculty of Chemistry, Nicolaus Copernicus University, Gagarin 7, 87-100 Torun, Poland

ARTICLE INFO

Article history:
Received 3 March 2011
Accepted 7 July 2011
Available online 18 July 2011

Keywords:
Electropolymerisation
Ozonization
Solid phase microextraction
Linezolid
High performance liquid chromatography
Mass spectrometry

ABSTRACT

The polypyrrole (PPy) and polythiophene (PTh) solid phase microextraction (SPME) coatings were obtained with the use of the electropolymerisation and linear sweep voltammetry. Such fibers were modified by an ozone treatment in a gaseous phase in the concentration of $2.1 \pm 0.2 \times 10^{-5}$ mol dm^{-3}. Both kinds of fibers were applied in the microextraction of linezolid from standard solutions to compare the extraction efficiencies displayed by these sorption phases. In these investigations a better adsorption capacity was obtained for polypyrrole fibers and hence only these kinds of fibers were utilized in the measurements from human plasma. In all measurements the concentrations of the drugs were in the range from 1 to 20 μg ml^{-1} (standard solutions) and 1 to 15 μg ml^{-1} (human plasma). Before the measurements, an optimization of the desorption solution experiments was performed. The correlation coefficients (R) obtained in the standard solution and human plasma were in the range from 0.8399 to 0.9970. The relative standard deviations (RSDs) were in the range of 0.1–7.6%.

1. Introduction

The solid phase microextraction (SPME) is one of the most progressive sample preparation methods. The method was developed in the 1990s by Arthur and Pawliszyn. It combined sampling, extraction, preconcentration and sample introduction in a single step [1]. The transfer of the analytes from the matrices to the fiber coating is running as long as the equilibrium until sample and coating will be reached [2]. That means, the kinetic curves which should be appointed before the measurements with use of the extraction technique, such as SPME, after some extraction time exhibit no difference between the amounts of the extracted analytes. In the extraction method a convection process is also significant. This process allows to obtain an equilibrium state in a shorter time and enables the possibility of getting greater repeatability of extraction [3]. The solid phase microextraction method was developed at the beginning of the environmental analysis. First papers concerning SPME were devoted to the analysis of the analytes from the environmental samples [4–7]. Beside these applications, SPME has also found wide applicability to [8] and biomedical analysis. SPME will provide a possibility for extraction and analysis of different kinds of antibiotics from biological matrices such as urine, plasma and blood [3,9–11]. Up to this time, with the use of this technique adrenolytic drugs [12,13] and antibiotics applied in the bacterial infections treatment, transplantology and psychotropic drugs were analyzed. Using in vitro and in vivo methods analyses with standard deviations on a very low level were performed [14,15]. Beside commercially available SPME coatings, porous adsorbents prepared on the electrochemical polymerisation way have been recently applied as well. Polypyrrole and polythiophene as some of the major conducting polymers were used in the sampling of various kinds of drugs from the standard solutions and biological matrices mentioned above. The results obtained using these fibers allow to get very promising results [13,14]. Hence, in this paper we tried to modify the electropolymerised polypyrrole and polythiophene surfaces in the ozone atmosphere. Ozone was chosen as a modification agent due to its strong oxidation abilities, especially towards conducting polymers. These kinds of materials exposed to the ozone treatment undergo oxidation with the formation of carbonyl group and after a longer time degradation [16]. The intermediate individuals such as solitons, polarons and bipolarons occur to a high concentration especially at the beginning of modification [16–18]. Modified fibers were applied in the analysis of linezolid (Fig. 1). Linezolid was chosen as an analyte because of its strong antibacterial activities. This drug is used especially in the methicillin-resistant *Staphylococcus aureus* treatment. The

* Corresponding author. Tel.: +48 566114308; fax: +48 566114837.
E-mail address: bbusz@chem.uni.torun.pl (B. Buszewski).

1570-0232/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2011.07.007

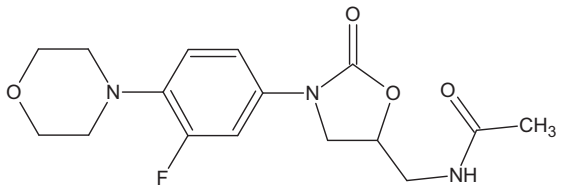

Fig. 1. Linezolid chemical structure.

analysis was performed in the standard solutions and human plasma samples which allowed to estimate a potential application of the prepared fibers in a biomedical analysis.

2. Materials and methods

2.1. Materials

All chemicals and reagents used in our investigations were of an analytical grade. Monomers, pyrrole (98%) and thiophene (99%) purchased from Sigma–Aldrich (Schnelldorf, Germany), were freshly distilled before the use. Linezolid ((S)-N-[[3-[3-fluoro-4-(4-morpholinyl) phenyl]-2-oxo-5-oxazolidinyl] methyl]-acetamide) was provided by Pharmacia & Upjohn GmbH (Erlangen, Germany). Human plasma was obtained from Collegium Medicum, Nicolaus Copernicus University (Torun, Poland). Acetonitrile, methanol and water were supplied from J.T. Baker (Deventer, The Netherlands) and Milli-Q RG system (Millipore Intertech, Bedford, USA), respectively.

2.2. Instrumentations

In the electropolymerisation process, a home-made set-up system coupled with a high performance potentiostat/galvanostat PGSTAT128N series Autolab model (Utrecht, The Netherlands) was applied. The ozone/oxygen mixture was generated by applying Corona Discharge Ozone Generator (ELTO®, Torun, Poland). The concentration of ozone in the gas mixture was determined by applying standard iodometric titration. The ozone concentration was $2.1 \pm 0.2 \times 10^{-5}$ mol dm^{-3} and did not change significantly during the exposition time. The scanning electron microscopy (SEM) was accomplished with LEO 1430VP (Carl Zeiss SMT, Oberkochen, Germany). Furthermore, Fourier transform infrared spectroscopy (FT-IR) was recorded with the use of Spectrometer Spectrum 2000 (Perkin Elmer, Waltham, USA) and polymers in KBr pellets. Additionally, the HPLC 1100 system (Agilent Technologies, Waldbronn, Germany) with a quaternary pump, automatic sample injector, and UV detector (Agilent Technologies, Waldbronn, Germany) were used. The chromatographic separations were performed using the analytical HPLC column Zorbax XDB C8 (150 mm × 4.6 mm, d_p = 5 μm). Agilent Triple Quad mass spectrometer with ESI interface was used. Agilent Technologies ChemStation software was applied for the data acquisition. Moreover, for the sample evaporation a Labconco CentriVap DNA concentrator (Kansas City, USA) was used.

2.3. SPME fibers preparation

The polypyrrole and polythiophene fibers prepared on the electrochemical polymerisation way were used as an adsorbent for the solid phase microextraction. The procedure of fibers preparation was based on a home-made set-up system connected with a new generation potentiostat–galvanostat. In the polymerisation 0.25 M pyrrole and thiophene solutions in 0.1 M tetrabutylammonium tetrafluoroborate in acetonitrile were applied. To perform the polymerisation process, a dynamic voltamperometry (Linear Sweep Voltammetry – LSV) with thresholds potentials at −0.2 to 2.5 V for both monomers was used. Accordingly, a silver metallic electrode as a reference electrode, platinum net as a counter electrode and simultaneously three medical stainless steels (SS, Ni–Cr) as working electrodes were used. The stainless steel support onto which polypyrrole and polythiophene fibers were coated had length and diameter equal to 100 mm and 750 μm, respectively. The polypyrrole and polythiophene fibers were utilized in the sample preparation method. The polythiophene fibers were applied only in the sampling from the aqueous solutions. The polypyrrole fibers were used both for measurements from the aqueous solutions and human plasma samples. The prepared fibers have undergone chemical modification with the use of ozone treatment during six different times: 1.0, 1.5, 2.0, 3.0, 5.0 and 7.0 h. The exposition of the polymer samples to ozone was conducted in the 1 l flasks filled with the O_2/O_3 mixture. Each sample was placed in a separate flask and kept there for an appointed time (mentioned above). After the exposition, all of the samples were characterized and concentration of ozone in the flask was determined. The surface characterization of each polymeric coating was investigated by Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM).

2.4. Surface characterization

The surface evaluation of the PPy and PTh SPME fibers before and after ozonization was performed based on scanning electron micrographs. The infrared absorption spectrum of coating between $\tilde{\nu} = 4000$ and $\tilde{\nu} = 400$ cm^{-1} was obtained with an FTIR spectrometer.

2.5. Chromatographic methodology

The mobile phase applied in the separation of linezolid included methanol (MeOH) and water in composition 50/50 (v/v). The wavelength was adjusted to λ = 251 nm. The flow rate and sample volume injection were 450 μl min^{-1} and 15 μl, respectively. In the measurements, a column Zorbax XDB-C8 150 mm × 4.6 mm and particle size diameter 5 μm was used.

Positive ion selected ion monitoring (SIM) mode was used for the detection and verification of the chemical and molecular structure of linezolid among other chemical individuals in human plasma samples, at m/z = 338.5 corresponding to $[M+H]^+$. The MS conditions were as fallows: capillary temperature: 320 °C, dwell time: 200 ms, drying flow 8.5 l min^{-1}, nebulizer pressure 30 psi.

2.6. Preparation of stock and standard solutions, the validation method

A stock solution of linezolid (125 μg ml^{-1}) was prepared in Milli-Q ultra pure water. Logically, appropriate concentrations of the drug mixtures were prepared by dissolving an adequate volume of stock solution in water and plasma. The stock solution was stored at −20 °C. In addition, the calibration level was set from 1 to 20 μg ml^{-1} (six concentrations). All the measurements were repeated three times.

The validation parameters such as relative standard deviation (RSD), limit of detection (LOD = 25.3 ng ml^{-1}) and limit of quantification (LOQ = 79.0 ng ml^{-1}) were calculated for a calibrators. 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 acceptable precision and accuracy. The calibration curves parameters have shown a good correlation coefficients R = 0.9986 with relative standard deviation RSD = 0.63%.

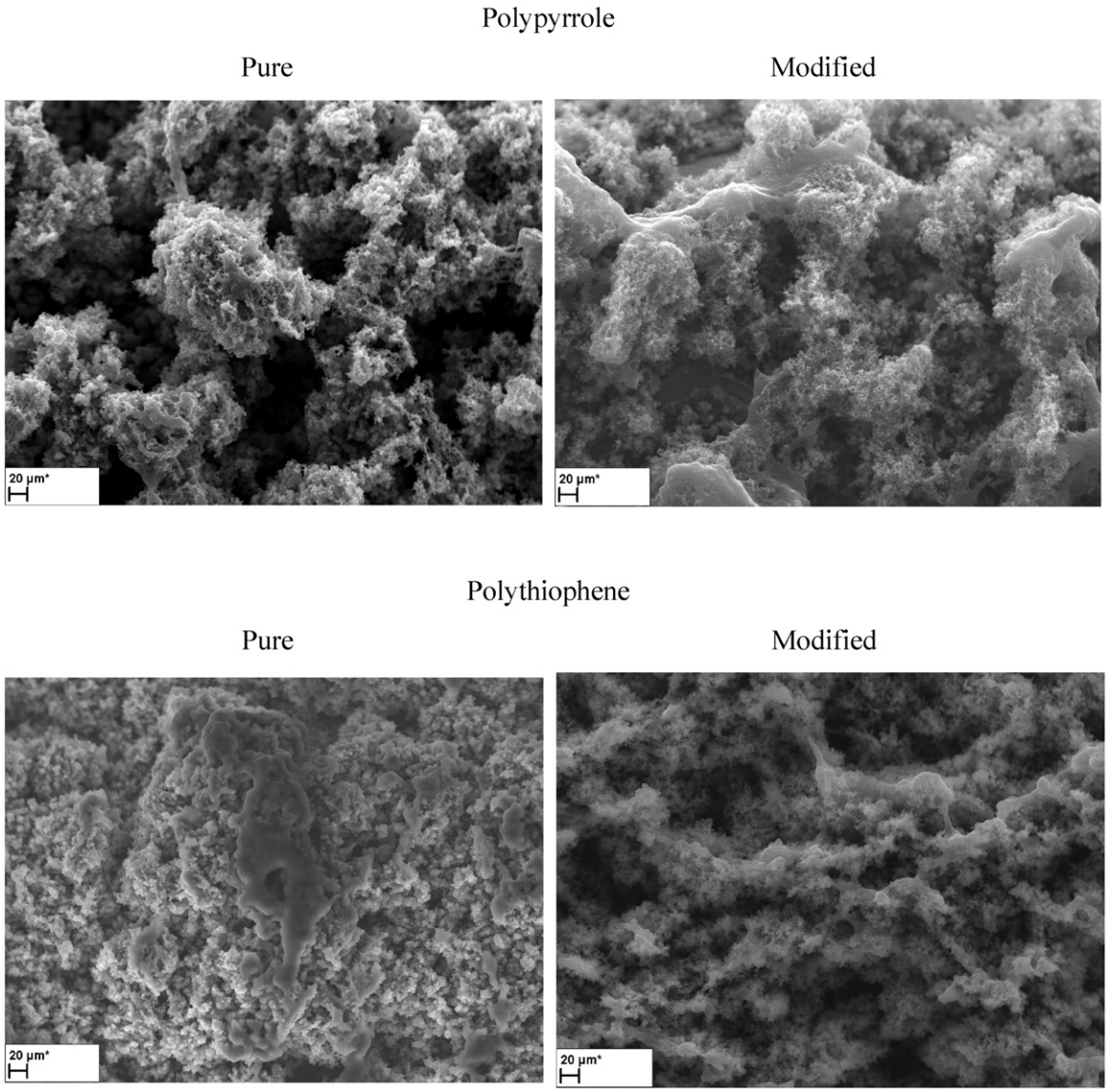

Fig. 2. SEM pictures of polypyrrole and polythiophene fibers before and after modification with ozone treatment.

The freshly fridge plasma was stored at −20 °C. Before use, the plasma was thawed at a room temperature and centrifuged at 2500 rpm for 5 min to complete stratification. Using the stock solutions, six concentrations of linezolid in the range of 1–15 $\mu g\,ml^{-1}$ were prepared in 1.5 ml centrifuge tubes. All the measurements were repeated three times and RSDs values were calculated.

2.7. Extraction and optimization experiments

The extraction time profiles using of polypyrrole and polythiophene fibers were made earlier and arranged at 10 min for adsorption and 5 min for desorption experiments [14,15,22].

Optimization of desorption solutions in the case of polypyrrole fibers was performed with use of five different solutions: methanol, methanol/ammonia, methanol/acetic acid, methanol/water (50/50, v/v), and acetonitrile. Before and after modifications, the fibers were taken into consideration in an attempt to choose the coatings which would exhibit the highest extraction abilities towards linezolid. In these experiments, the extraction was performed from a concentration 15 $\mu g\,ml^{-1}$ of linezolid dissolved in water. Additionally, the adsorption was made in 1.5 ml of linezolid solution and desorption was made in 1.5 ml of mixture methanol/water 50/50 (v/v), components which were chosen in the previous experiments.

Concluding, the polypyrrole fibers exhibit better extraction abilities towards linezolid, and hence these kinds of fibers were used in further investigations. First of all, an experiment in standard solution (water) in six different concentrations (1, 3, 7, 10, 15 and 20 $\mu g\,ml^{-1}$) was performed. The conditions applied here were similar to those described earlier. The experiments on human plasma were performed after having been spiked with a relevant amount of linezolid. The investigated concentrations were similar to those used in the standard solution. All samples after adsorption and desorption in aqueous and human plasma evaporated and then resolubilized in 100 µl of MeOH/H_2O (50:50, v/v). The solution was then moved to µ-insert vials and analyzed with the use of HPLC/UV system and method described in Section 2.5.

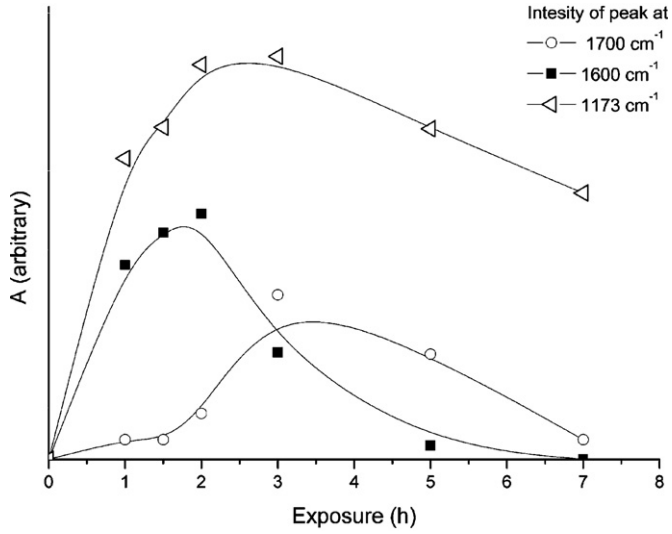

Fig. 3. The change of intensity of carbonyl related peaks as a function of polypyrrole ozonization time.

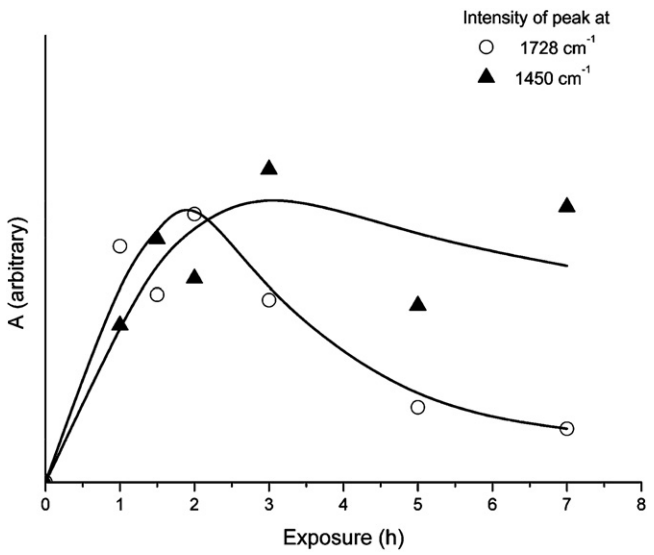

Fig. 4. Intensity changes of important peaks on the IR spectra of ozonized polythiophene.

3. Results and discussion

3.1. Surface characterization

3.1.1. SEM measurements and visualization of PPy and PTh coated SS surfaces before and after modification

Scanning electron microscope (SEM) is a very useful tool for the visualization and short characterization of the solid state materials. In our case, besides the abilities to recognize the dimension of porosity exhibited by our materials, this method also allows for the measurement of fibers' thickness and ground (basic) layer on the SS surface. The scanning electron micrographs of polypyrrole and polythiophene before and after modification, with the use of ozone as a strong oxidizing agent, allow to conclude that from the morphological point of view this kind of modification caused a wide scale swelling of polymeric structure. A similar approach and conclusion were achieved in an investigation performed for polypenthylthiophene thin films by Nowaczyk et al. [17]. In case of thin films, "a huge jostle of primary exceptionally thin cracking after ozone treatment" was reported [17]. In our investigations, the calculation of these parameters was not possible; however, as mentioned above, the swelling of the pores was evidently observed. SEM micrographs showing polypyrrole and polythiophene before and after ozone treatment are shown in Fig. 2. Fibers before ozonization have a thickness in range 275–285 μm for polypyrrole and 205–215 μm for polythiophene. In case of fibers after modification fibers thickness was changed (150–175 μm for polypyrrole and 310–325 μm for polythiophene). In polythiophene fibers more swelling effect may be visible and hence thickness increasing were measured. In case of polypyrrole decrease of fibers thickness were observed.

3.1.2. FT-IR spectroscopy

The infrared spectra were performed for the polymer samples in the form of KBr pellets (1 wt% of polymer). The interpretation spectra were made based on IR spectra tables and general information available in literature as well [19].

The spectra of polypyrrole show specific bands of the wavenumber $\tilde{\nu} = 2965\,cm^{-1}$ which is typically assigned to C–H stretching mode of hydrogen atoms attached directly to the pyrrole ring. The spectra of ozone treated polymer did not reveal any significant changes in this region. The broad band above $\tilde{\nu} = 3000\,cm^{-1}$ refers to O–H stretching vibrations. This band presence in the spectra of pristine polymer suggests the presence of adsorbed moisture, despite the fact that polymer was kept in a desiccator. The changes in this area during the ozone exposure were not interpretable. The peak at about $\tilde{\nu} = 1623\,cm^{-1}$ distinctive for C=C stretching was quite weak and its intensity fluctuated rather chaotically in the exposition time. For samples treated with ozone it was difficult to derive an exact intensity of the peak at $\tilde{\nu} = 1623\,cm^{-1}$ since there was an intensive broad band at about $\tilde{\nu} = 1600\,cm^{-1}$ that overlapped it. The latter can be assigned to carbonyl C=O bond stretching and its intensity increased over time for the first 2 h. For the samples exposed to ozone longer than 2 h, the intensity of band at $\tilde{\nu} = 1600\,cm^{-1}$ decreased with the exposure length. There is also another carbonyl-related band appearing in the ozonized sample spectra at about $\tilde{\nu} = 1700$ and $\tilde{\nu} = 1173\,cm^{-1}$. The last band is related to C–C(O)–C skeletal vibrations. The change of intensity of prominent carbonyl bands is depicted in Fig. 3 The band also contained as well the bands at $\tilde{\nu} = 1544\,cm^{-1}$ for ring skeletal vibrations [20] $\tilde{\nu} = 1533\,cm^{-1}$ for C–N stretching, $\tilde{\nu} = 1150$ and $\tilde{\nu} = 890\,cm^{-1}$ for C–H in plane and out-of-plane vibrations, respectively.

In the case of polythiophene the IR spectra contained peaks at $\tilde{\nu} = 3430\,cm^{-1}$ for O–H stretching. $\tilde{\nu} = 2963\,cm^{-1}$ for C–H stretching, $\tilde{\nu} = 1631\,cm^{-1}$ for conjugated C=C bonds vibrations, $\tilde{\nu} = 1482\,cm^{-1}$ for thiophene ring =C–C= stretching, $\tilde{\nu} = 1084\,cm^{-1}$ for in plane anti-symmetrical C–H rocking vibrations, $\tilde{\nu} = 918$ and $\tilde{\nu} = 784\,cm^{-1}$ for out-of-plane anti-symmetrical and symmetrical C–H vibrations, respectively, and $\tilde{\nu} = 885\,cm^{-1}$ for ring deformations. Some peaks related to electrolyte remnants in the spectra were also present. These bands are similar in the spectra of PPy and PTh and appear at $\tilde{\nu} = 1384$, 1319 and $1034\,cm^{-1}$.

The vibrational spectroscopy of polythiophene and its ozone treated samples show that the formation of carbonyl groups is reflected by the band at about $\tilde{\nu} = 1700\,cm^{-1}$. Another significant changes in the appearance of the shoulder at about $\tilde{\nu} = 1450\,cm^{-1}$ can be assigned to the CH_2 scissor deformation. The last significant change in the spectra is the initial decrease and further slight increase of the peak at $\tilde{\nu} = 784\,cm^{-1}$ which could be assigned to the skeletal deformation of the thiophene rings. It was discovered that the skeletal vibrations of main chain decrease when there are ozone molecules adsorbed to the chain [21]. The interpretation of changes apparent in the spectra is proposed as follow – the ozone molecules first adsorb to the polymer surface. Then the next

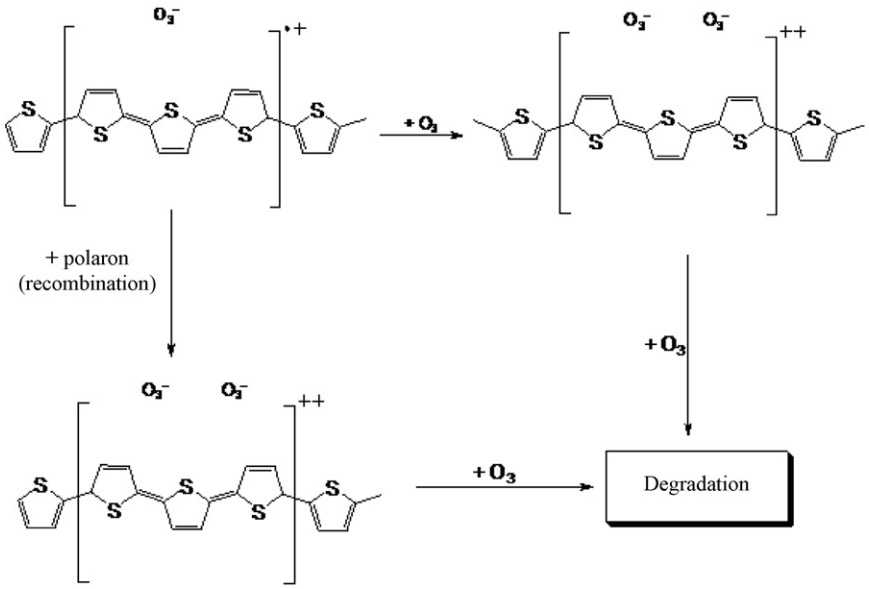

Fig. 5. Ozonization mechanism of heterocyclic conducting polymers (polythiophene).

process is followed by the oxidative modification of polymeric material that results in the formation of a wide variety of carbonyl bearing moieties. According to the ring cleavage in polythiophene alkyl, CH_2 groups appear to rise to a signal at about 1450 cm^{-1} and lifting background line in region $\tilde{\nu} = 2900–2850\ cm^{-1}$. A chart exhibiting the changes in this polymer is presented in Fig. 4. The analysis of FTIR spectra revealed significant differences in the mechanism of ozone reaction between PPy and PTh. In case of the former, the distinct formation of carbonyl, especially ketonic carbonyl, moieties is a leading process in the later formation of stable O_3 adducts with thiophene ring aside to be the predominant effect. The evolution of spectra in time, i.e. subsequent decrease of carbonyl bands intensity, can be explained by the fact that the intensive oxidation of polymers like PPy and PTh leads to the evolution of CO_2.

3.2. Ozonization mechanism of heterocyclic conducting polymers

According to the obtained FT-IR spectra and literature reports [16,21], one can predict a potential mechanism of ozonization. At the beginning of the oxidation, the formation of polarons is visible. The further oxidation leads to the formation of bipolarons, creation of carbonyl groups and, at the end, degradation. An illustration describing this reaction is presented in Fig. 5.

3.3. Selection of modification time

The polypyrrole and polythiophene fibers modified in different periods of time were applied in the investigations to choose time in which the extraction efficiencies of linezolid will be the highest. Using the sample concentration and procedure described above, we realized that in the case of the polypyrrole coatings the extraction efficiencies is higher than in polythiophene one. This can be recognized also as a selectivity increasing of linezolid using PPy SPME fibers. The obtained results are presented in Fig. 6 (for polypyrrole) and Fig. 7 (for polythiophene). Parallel with the increasing modification time, the amount of adsorbed drugs is raising up to 0.8158 μg after 3 h of ozonization from 0.2730 μg indicated by raw polypyrrole fibers. After 3 h of modification in case of polypyrrole and 2 h in case of polythiophene the extraction abilities towards the applied drug were the highest. In the case of the polythiophene coatings a significant saturation effect (changes in amounts of adsorbed drug in the range of 0.6108–0.5547 μg after 2 h of modification) can be noticed. In the case of the desorption profiles polypyrrole fibers allow to extract about twice the amount of linezolid than in the case of polythiophene. After 3 (PPy) and 2 (PTh) h of ozonization, the amounts of extracted drugs were equal to 0.2289 and 0.1073 μg using polypyrrole and polythiophene fibers, respectively. With the application of polypyrrole fibers, according to the increasing

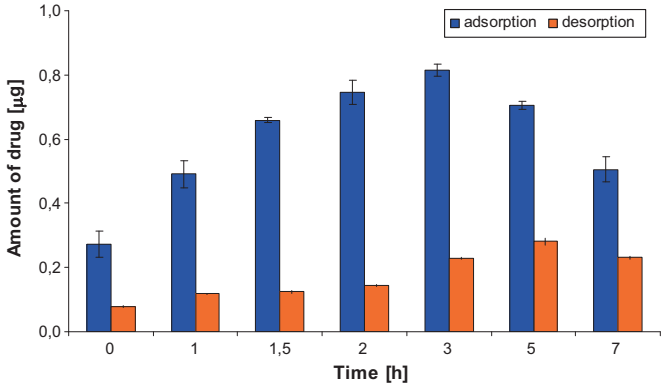

Fig. 6. Amount of extracted drug according to increasing modification time using polypyrrole fibers.

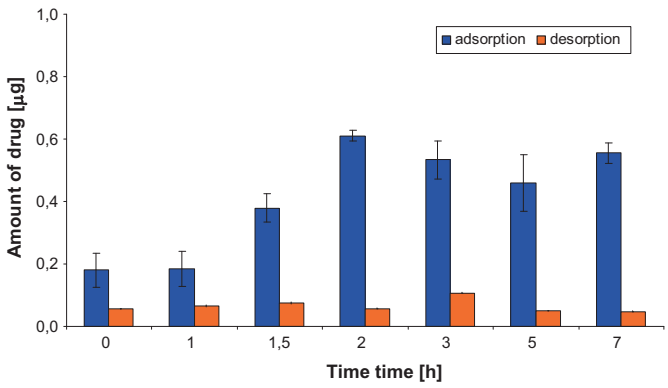

Fig. 7. Amount of extracted drug according to increasing modification time using polythiophene fibers.

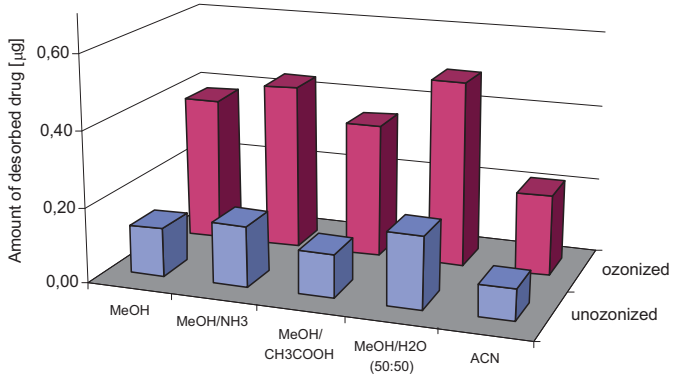

Fig. 8. Optimization of desorption solution using pure and modified polypyrrole coatings.

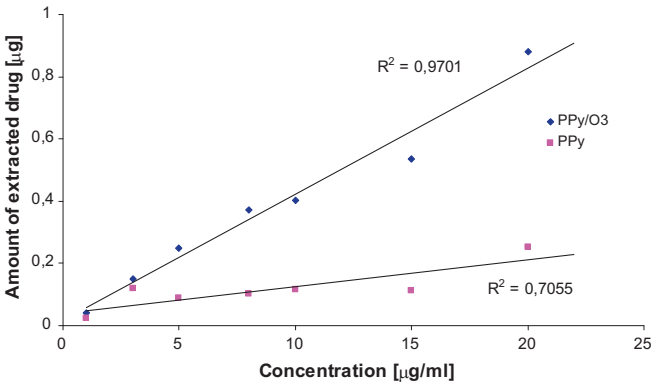

Fig. 9. Extraction profile of linezolid from standard solutions.

modification time amounts of desorbed drugs have saturation profiles after 3 h (in the range 0.2289–0.2310 μg). In case of polythiophene, the amount of desorbed linezolid was on a similar level for all modification times in the range between 0.0559–0.0474 μg. The relative standard deviations (RSDs) calculated for adsorption and desorption experiments were in range of 0.31–0.54 and 1.86–1.30%, respectively. The differences in amounts of adsorbed and desorbed amount of linezolid may derive from several factors. The mechanism of ozonization of heterocyclic compounds was going through a formation of radical-cations (polarons) and di-cations (bipolarons) (Section 3.2). Such a kind of individual is a subject of further chemical transformation (reaction) and more stable ozonides are formed. After that transformation of formed compounds, they are going through the creation of carbonyl groups and finally degradation of polymers. As in our study two different kinds of polymers were taken under consideration, we have to deal with two kinds of potential reactions. Followed by Cataldo and Omastova [16] in case of polypyrrole the ozonization reaction is going through an interaction of ozone with the polarons and bipolarons defects created in the previous step of modification. This fact may be responsible for the initially increasing extraction abilities observed in case of polypyrrole. Further reaction induced a degradation of polymer chains including the cleavage of heterocyclic rings, carbonyl formation and also according to [16] also hydroxide groups. This fact may be observed after longer ozonization when the extraction abilities of polypyrrole fibers slowly decreased. The processes which are happening in the polythiophene after ozone treatment are a little bit different than those in polypyrrole. From FT-IR spectra (Section 3.1.2) and SEM (Section 3.1.1) figures, it may be concluded that degradation is occurring much faster than in the case of polypyrrole, which is a main reason for a worse extraction abilities exhibited by the polythiophene fibers.

Moreover, the polypyrrole fibers used in the preliminary investigations exhibited better sorption capacity in comparison to polythiophene ones, and hence we decided to continue our consideration with only the first one (pure PPy and PPy after 3 h of modification). The applied method for the preparation of the SPME polythiophene fibers did not occur with relevant to obtain a selective phase, sorption abilities of which could be compared to those applied in Ref. [22] where linezolid was extracted from human plasma and whole blood samples with satisfactory extraction efficiencies.

3.4. Selection of desorption solution

In the measurements five different solutions were taken under consideration: methanol, methanol/ammonia (25% ammonia), methanol/acetic acid (15% acetic acid), methanol/water (50/50, v/v), and acetonitrile. According to the performed measurements, in Fig. 8, it was shown that the most appropriate desorption solution is a mixture methanol/water (50/50, v/v) which was used in all further experiments. This fact might be due to a different elution force presented by an appropriate applied mixture.

3.5. Application of pure and modified polypyrrole SPME fibers in analysis of linezolid from aqueous solutions and plasma samples

The prepared fibers were applicated after the optimization experiments in the analysis of linezolid from aqueous solution and from human plasma. These experiments were performed in 7 concentrations (1–20 μg ml^{-1}) for standard solutions and 6 concentrations (1–15 μg ml^{-1}) for human plasma samples.

3.5.1. Aqueous solutions

First investigations were performed using standard aqueous solutions prepared on the dilution of stock solution way. Both pure polypyrrole and modified fibers, a linear dependence in whole applicated range (Fig. 9) may be observed. However, the highest concentration, especially 20 μg ml^{-1}, is evidently leading to a decrease of correlation coefficient in both cases. This parameter was equal with R = 0.8399 and 0.9849 for pure polypyrrole coating and for polypyrrole after modification, respectively. Some details about the influence of individuals created on the ozonization way on the extraction abilities of linezolid by polypyrrole fibers may be noticed from this figure. In almost each concentration, the amount of the extracted drugs was about 4 times higher with the use of modified fibers. Only in one case, for the lowest concentration, extraction abilities were increased almost twice. This fact may be additionally confirmed by differences in slope of concentration curve. Slope values for pure and modified fibers were equal 0.0085 and 0.0403, respectively. That means ideas about the creation of polar groups and the appearance of radical center in polymer chains have a significant influence on the adsorption of drugs molecule such as applied linezolid. The relative standard deviations (RSDs) calculated for all fibers and concentration were in range of 0.21–7.56%.

3.5.2. Real human plasma samples

The solid phase microextraction is a technique which, in the future, may allow to the direct sampling of drugs from human vessels, tissue or blood and hence we decided to perform also experiments from human plasma samples in our research. It was stressed that ions existence in this matrice is the reason why in this case a higher affinity towards drugs by SPME coatings should be observed. We performed measurements using pure polypyrrole and fibers after modification. Similar to the results obtained from the measurements in aqueous solution, fibers after modification exhibit also higher extraction abilities towards linezolid. In this

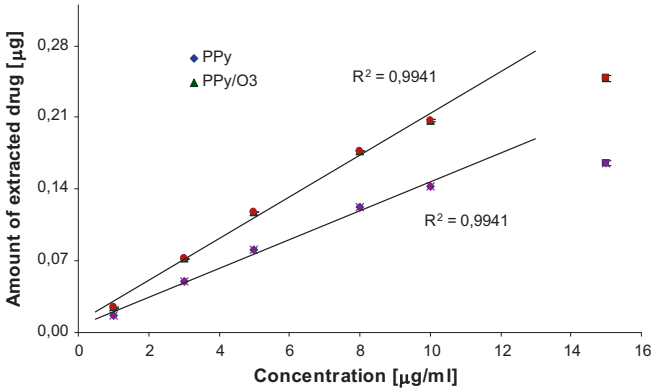

Fig. 10. Extraction profile of linezolid from human plasma samples.

case, the differences in the extracted amount of drug between both fibers were in the range of from 44 to 50%. Differences in selectivity between applied fibers were smaller than these used for standard solution. Slope values were 0.0140 and 0.0204 for pure and modified PPy SPME coatings, respectively. Smaller differences between both fibers used there may come from matrice components. An ion species existing in plasma could be bonded to modified polar polymer surface more than to pure polypyrrole.

In plasma matrice, the linear range was observed from 1 to 10 $\mu g\,ml^{-1}$ for both SPME coatings. In these concentrations, the correlation coefficient was constant and equal 0.9970. The relative standard deviations (RSDs) were in the range of 0.1–2.1%. The values obtained for the highest concentration allow claiming that concentration dependence occurred as a saturated behavior. All the performed experiments have a plot description and are presented in Fig. 10.

4. Conclusions

The main aims of our investigations were synthesizing a polypyrrole and polythiophene solid phase microextraction coatings and modification of their structure using of the ozone treatment. According to the earlier investigations, a modification using such strong oxidizing agent caused wide changes in the polymeric structure. In polymer chains, new carbonyl groups were created and cleavages of monomer rings were observed in the IR spectra. SEM picture proves an earlier prediction about swelling of the polymeric structure. The extraction abilities of polypyrrole and polythiophene fibers before and polypyrrole also after modification were measured using linezolid from aqueous solutions and human plasma samples as well. In all cases, it can be noticed that the modified fibers allow for higher extraction abilities of linezolid. Linear range in case of aqueous solutions was observed in the whole range of applied concentrations; however, in human plasma linearity was kept until 10 $\mu g\,ml^{-1}$. The correlation coefficients for all measurements were higher than 0.99 what allows to claim that the optimization was executed well. All the performed measurements proved earlier presumption that modification of conducting polymers using strong oxidizing agent caused an evident increase of the extraction abilities of used fibers towards linezolid. This fact showed that in the contemporary science there is no necessity to synthesize new, high generation, and a very expensive compound. Sometimes small modification of very well known molecules using present knowledge is enough to diffuse their utility for various purposes.

Acknowledgements

This work was supported by the Foundation for Polish Sciences (FNP) Professor's Subsidy "Mistrz", Ministry of Science and Higher Education (Warsaw, Poland) Grant no. N N204 268038, Alexander von Humboldt Foundation (no. 3.4. Fokoop-POL/1003705) and by European Social Found, Polish National Budget, Kujawsko-pomorskie Voivodship Budget (within Sectoral Operational Programme Human Resources) "Krok w przyszlosc".

References

[1] Z. Zhang, M.J. Yang, J. Pawliszyn, Anal. Chem. 66 (1994) 844A.
[2] J. Pawliszyn, Solid Phase Microextraction: Theory and Practice, Wiley-VCH, Inc., New York, 1997.
[3] X. Zhang, A. Es-haghi, J. Cai, J. Pawliszyn, J. Chromatogr. A 1216 (2009) 7664.
[4] D.W. Potter, J. Pawliszyn, J. Chromatogr. 625 (1992) 247.
[5] J. Pawliszyn, Trends Anal. Chem. 14 (1995) 113.
[6] S. Magdic, J.B. Pawliszyn, J. Chromatogr. A 723 (1996) 111.
[7] K. Jinno, T. Muramatsu, Y. Saito, Y. Kiso, S. Magdic, J. Pawliszyn, J. Chromatogr. A 754 (1996) 137.
[8] M. Ligor, B. Buszewski, J. Chromatogr. A 847 (1999) 161.
[9] D. Vuckovic, R. Shirey, Y. Chen, L. Sidisky, C. Aurand, K. Stenerson, J. Pawliszyn, Anal. Chim. Acta 638 (2009) 175.
[10] L. Junting, C. Peng, O. Suzuki, Forensic Sci. Int. 97 (1998) 93.
[11] S.D. Brown, D.J. Rhodes, B.J. Pritchard, Forensic Sci. Int. 171 (2007) 142.
[12] B. Buszewski, J. Nowaczyk, T. Ligor, P. Olszowy, M. Ligor, B. Wasiniak, W. Miekisch, J.K. Schubert, A. Amann, J. Sep. Sci. 32 (2009) 2448.
[13] B. Buszewski, P. Olszowy, T. Ligor, M. Szultka, J. Nowaczyk, M. Jaworski, M. Jackowski, Anal. Bioanal. Chem. 397 (2010) 173.
[14] M. Szultka, R. Kegler, P. Fuchs, P. Olszowy, W. Miekisch, J.K. Schubert, B. Buszewski, R.G. Mundkowski, Anal. Chim. Acta 667 (2010) 77.
[15] J.K. Schubert, W. Miekisch, P. Fuchs, N. Scherzer, H. Lord, J. Pawliszyn, R.G. Mundkowski, Clin. Chim. Acta 386 (2007) 57.
[16] F. Cataldo, M. Omastova, Polym. Degrad. Stab. 82 (2003) 487.
[17] J. Nowaczyk, P. Olszowy, P. Cysewski, A. Nowaczyk, W. Czerwinski, Polym. Degrad. Stab. 93 (2008) 1275.
[18] E.T. Kang, K.G. Neoh, K.L. Tan, F.C. Loh, Synth. Met. 84 (1997) 59.
[19] G. Socrates, Infrared and Raman Characteristic Group Frequencies. Tables and Charts, John Wiley & Sons, Chichester, 2001.
[20] S. Jin, X. Liu, W. Zhang, Y. Lu, G. Xue, Macromolecules 33 (2000) 4805.
[21] J. Nowaczyk, W. Czerwinski, E. Olewnik, Polym. Degrad. Stab. 91 (2006) 2022.
[22] P. Olszowy, M. Szultka, P. Fuchs, R. Kegler, R. Mundkowski, W. Miekisch, J. Schubert, B. Buszewski, J. Pharm. Biomed. 53 (2010) 1022.