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Fibers with polypyrrole and polythiophene phases for isolation and determination of adrenolytic drugs from human plasma by SPME-HPLC

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ABSTRACT

In this study, polypyrrole (PPy) and polythiophene (PTh) SPME coatings and their ability to extract selected adrenolytic drugs with different physico-chemical properties from standard solutions and human plasma samples were evaluated. In measurements metoprolol, oxprenolol, mexiletine, propranolol, and propaphenon were investigated. The main parameters such as extraction time, desorption conditions and pH influence were examined. Inter-day precisions were in range 0.1–2.0%, 1.1–2.9%, 1.3–2.6%, 0.1–2.6% and 0.3–2.1% for metoprolol, oxprenolol, mexiletine, propranolol and propaphenon, respectively. Accuracies were less than 15%, which was evaluated by analyzing preparation samples of five replicates. The method was successfully applied to human plasma samples spiked with selected adrenolytic drugs. The method was linear in the concentration range from 1 to 10 μg/ml for all of studied adrenolytic drugs using human plasma samples. The PTh-SPME coating displayed higher extraction efficiency towards the target analytes in comparison to PPy-SPME. The reproducibility of the extraction using polypyrrole and polythiophene fibers was confirmed by variation coefficients lower than 8% and 3%, respectively.

1. Introduction

In present time pharmaceutical analysis need to meet minimum requirements in terms of reliability and safety. The measurements of drug levels in biological fluids are the corner stone for drug discovery and development as well as for pharmacodynamic, pharmacokinetic studies and drug monitoring. The most relevant matrices to be analyzed for this purpose are plasma or blood, due to providing a good correlation between their concentration and pharmacological effects. Several analytical methods have been described for the quantification of adrenolytic drugs in biological fluids. To determine them different techniques have been applied, e.g. high-performance liquid chromatography (HPLC) which can be connected with very sensitive and selective detectors such as fluorescence or mass spectrum detectors [1–3].

In cases when analytes are present in a complex matrix, e.g. plasma, near quantification method the sample preparation is especially important for quality of the analysis. In addition, miniaturization is a growing trend in area of bioanalysis and analytical chemistry [4,5]. Since few years commonly used sample preparation methods applying to this time for these purposes are liquid–liquid extraction (LLE) and solid-phase extraction (SPE) [6,7]. A significant step in the development towards miniaturization was the introduction at the end of last century solid-phase microextraction (SPME) [8–10]. SPME is a sampling and preconcentration technique which provides a simple means of analyzing drugs and other chemicals in variety matrices [9]. It is also fast as there is typically no or little further sample manipulation required between sampling and instrumental analysis. In spite of SPME was developed mainly for analysis of compound from environmental matrices [11], this method since several years has been shown as an analytical tool for drug monitoring in biological matrices (plasma, whole blood, and tissue) [12–15]. In this technique, a sorbent-coated fiber is exposed to relevant matrices, for a predetermined time. The sorbent possesses high extraction affinity for the selected biologically active compound and low extraction affinity for sample matrix components. After extraction the drugs are desorbed into a small volume of solvent, which is introduced to the chromatographic system. The process takes advantage of an equilibration of drug concentrations between the sorbent phase and the matrices during extraction so that the amount of drug extracted is always proportional to the concentration of free drug in the biological samples. The coatings for SPME may be prepared on different ways. Current commercially available SPME coatings are often prepared on deep or spin coating way [8]. Porosity of these materials has never been investigated.

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Table 1
Polypyrrole and polythiophene fibers thickness, length and content of organic carbon.

Physical parameters	Polypyrrole	Polythiophene
Length [mm]	10.0	10.0
Thickness [μm]	90–95	145–155
Organic carbon [%]	73.6	45.95

In our investigations we are trying to apply a new electrochemical polymerization method to synthesis of various porous SPME coatings. Paper published to this time was focused basically on the creation of the polypyrrole coatings, robustness and short characterizations of the fibers [16]. However, investigations concerned possible interactions between polypyrrole surface and appropriate molecules and consideration about potential application of the fibers prepared on the electrochemical polymerization way as the porous adsorbent in solid-phase microextraction were also performed and published [17,18]. Current work mainly is focused on extraction of five adrenolytic drugs with different physicochemical properties and biomedical applications. Additionally, because applied PPy coatings with some modification in current method preparation were used (with comparison to this polymeric sorption SPME fibers applied in earlier papers) we decided to include in this paper also results from aqueous solutions with use of polypyrrole fibers. This study was designed to compare sorption abilities of polypyrrole (PPy) and polythiophene (PTh) SPME coatings.

2. Experimental

2.1. Materials

All chemicals and reagents used in our investigations were of analytical grade. Monomers, pyrrole (98%) and thiophene (99%) purchased from Sigma–Aldrich (Schnelldorf, Germany), were freshly distilled before use. Adrenolytic drugs: metoprolol, propranolol, oxprenolol, mexiletine and propaphenon (salt forms) and ammonium acetate (99.5%) were purchased from Sigma–Aldrich (Schnelldorf, Germany). Their structures are presented in Table 1. 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, MA, USA), respectively.

2.2. SPME fibers preparation

As an adsorbent for solid-phase microextraction the polypyrrole and polythiophene fibers prepared on the electrochemical polymerization way were used. The procedure of fibers preparation was based on home-made set up system connected with potentiostat–galvanostat and has been described earlier [14,15]. In polymerization 0.25 M pyrrole and thiophene solutions in 0.1 M tetrabutylammonium tetrafluoroborate in acetonitrile were applied. To perform polymerization process dynamic voltamperometry (Linear Sweep Voltammetry-LSV) with thresholds potentials at −0.2 to +2.5 V for pyrrole and −0.2 to +2.7 V for thiophene were used, respectively. Before each polymerization, electrochemical activation of reaction solutions was applied. Silver metallic electrode as a reference electrode, platinum net as a counter electrode and simultaneously three medical stainless steels as working electrodes were used. Example picture of prepared fibers is presented in Fig. 1A and B. Stainless steel support onto which polypyrrole and polythiophene fibers were coated on had length and diameter equal to 100 mm and 750 μm, respectively. Polypyrrole and polythiophene fibers thickness, length and content of organic carbon are shown in Table 1.

Polypyrrole and polythiophene fibers prepared on electrochemical polymerization way were utilized in sample preparation method. Polythiophene fibers were applied in sampling from aqueous solutions. Polypyrrole and polythiophene fibers were used for measurements from aqueous solutions and human plasma. The characteristics of the surface of each polymeric coating were investigated by SEM (Fig. 1). PTh coating has a more porous structure, which significantly increases the extraction capacity in comparison to PPy less porous film.

2.3. Instrumentations

The HPLC 1100 system (Agilent, Waldbronn, Germany) with quaternary pump, automatic sample injector, and UV detector (Agilent, Waldbronn, Germany) was used. Chromatographic separations were performed using the analytical HPLC C18 column (150 mm × 4.6 mm, 5 μm) from Supelco (Bellefonte, USA). Agilent Technologies ChemStation software was used for data acquisition.

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

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

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

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

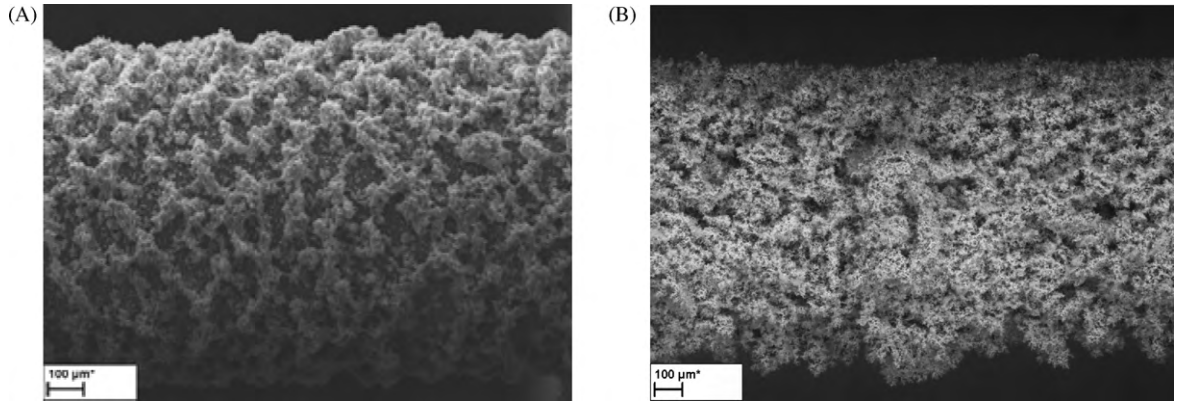

Fig. 1. SEM images of polypyrrole (A) and polythiophene (B) fibers.

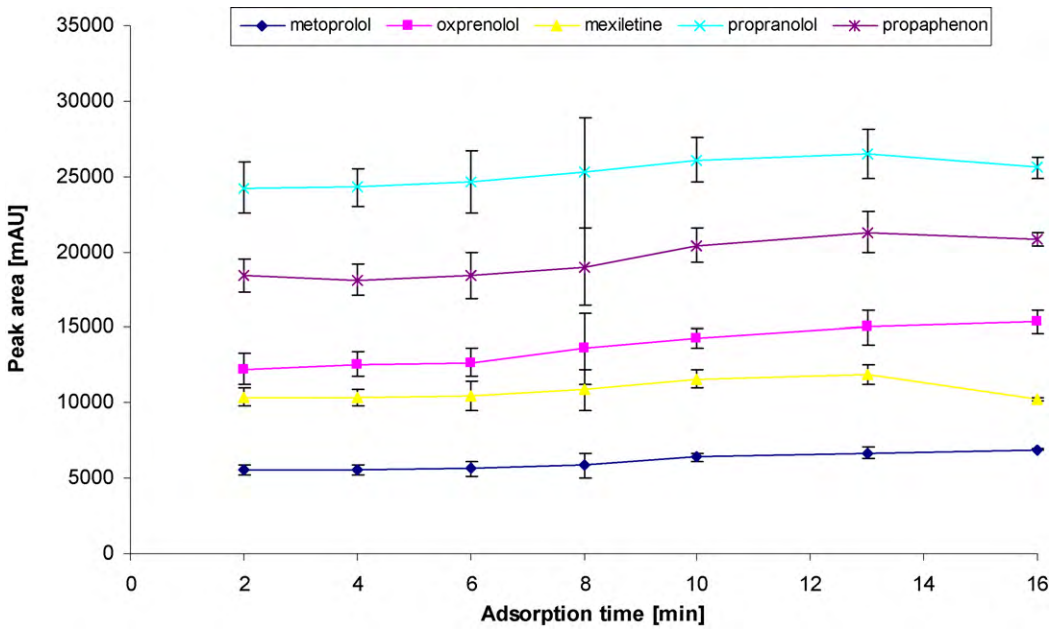

Fig. 2. Effect of adsorption time on the extraction efficiency of selected adrenolytic drugs from aqueous sample by PTh-coated SPME fibers.

2.4. Chromatographic procedure

Mobile phase applied in separation was acetonitrile (MeCN) and water with addition of 5 mM ammonium acetate. Gradient settings were adjusted from 25% MeCN at the start of analysis through 35% in 5 min, 45% in 7.5 min and 80% in 15 min. Wavelength was adjust at $\lambda = 205$ nm. The flow rate and sample volume injection were 0.6 ml/min and 15 μl, respectively.

2.5. Preparation of stock and standard solutions, validation

A stock solution (500 μg/ml) was prepared in water. An appropriate concentration of drugs mixtures was prepared by dissolving an adequate volume of stock solutions in water and plasma. All stock solutions were stored at −20 °C. Calibration level was set from 1 to 150 μg/ml (eleven concentrations). All measurements were repeated five times. Quality parameters such as accuracy, precision, standard deviation (SD), relative standard deviation (RSD), limit of detection (LOD) and limit of quantification (LOQ) were calculated. Accuracy was presented as the ratio of the determined and nominal values of concentrations of relevant drug and multiple by 100%. Precision was defined as the percentage of standard deviation of the relevant values divided by the average of mean values. The limit of detection ($LOD = 3 \times SD_{xy}/b$, where SD_{xy} is the standard deviation and b is the slope) and the limit of quantification ($LOQ = 10 \times SD_{xy}/b$) were calculated with acceptable precision and accuracy according to Konieczka [19].

Freshly fridge plasma was stored at −20 °C. Before use, the plasma was thawed at room temperature and centrifuged at 2500 rpm for 5 min to complete homogenization. With use of

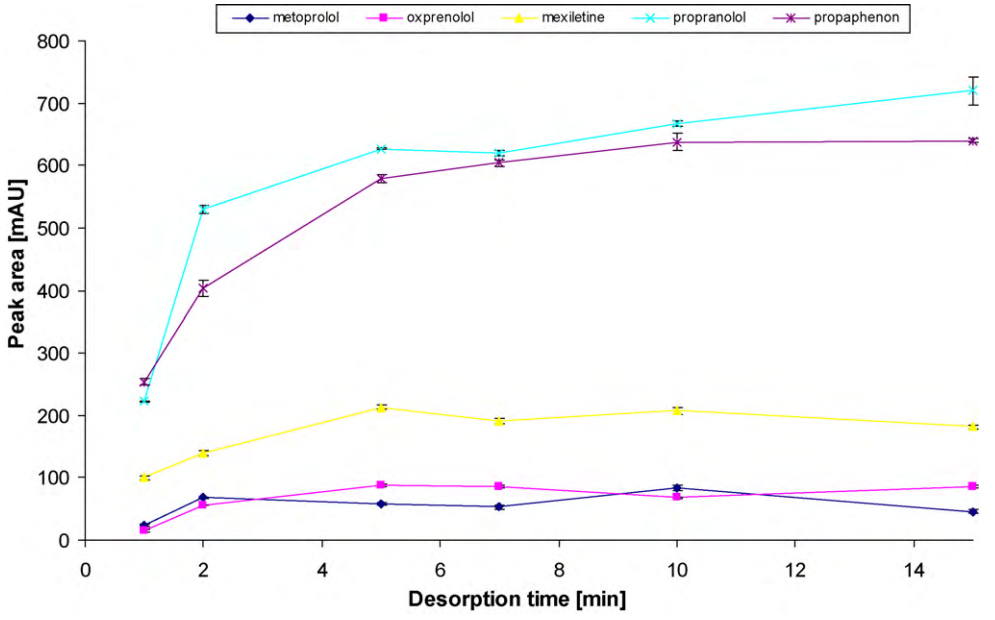

Fig. 3. Effect of desorption time on the extraction efficiency of selected adrenolytic drugs from aqueous sample by PTh-coated SPME fibers.

Table 2
Linear regression, LOQ and LOD for calibration curves ($n = 5$).

Parameters	Metoprolol	Oxprenolol	Mexiletine	Propranolol	Propaphenon
Retention time [min]	6.6	7.8	8.9	10.8	12.6
Linear range [μg/ml]	1–150				
Slope	20.467	65.804	61.603	126.420	47.562
Intercept	−3.412	−41.555	−35.534	−90.179	−90.179
LOD [ng/ml]	31.0	28.0	49.0	12.0	63.0
LOQ [ng/ml]	93.0	85.0	148.0	37.0	189.0
R^2	0.9962	0.9932	0.9994	0.9977	0.9947
RSD	1.0	2.1	2.0	1.0	0.9

stock solutions six concentration of mix drugs in range 5–30 μg/ml was prepared in 1.5 ml centrifuge tubes. All measurements were repeated three times and RSD values were calculated.

2.6. Extraction and optimization experiments

After preparing each fiber was stored through 1 day in MeOH/water (90:10; v/v) solution. After that fibers were preconditioned while three cycle adsorption (10 min)/desorption (5 min) in water and methanol. That procedure allows increasing adsorption abilities to choose drugs. These experiments were performed in 1.5 ml of water and methanol.

Adsorption and desorption kinetics were performed with use of polythiophene fibers. These experiments were performed in concentration of each drugs equal to 15 μg/ml. Experiments were performed in 1.5 ml of water and methanol. Adsorption times applied in measurements were 2, 4, 6, 8, 10, 13 and 16 min. Desorption were made with use of best adsorption time which was 10 min. Desorption time chosen to investigate were 1, 2, 5, 7, 10 and 15 min. Samples after extraction were evaporated, dissolved in 100 μl of methanol and analyzed with HPLC/UV system.

Extraction experiments from aqueous solution with use of polythiophene fibers and eleventh concentration of mix drugs were performed. Applied concentrations were in range of 1–150 μg/ml. Adsorption and desorption were performed in 1.5 ml, then evaporated, resolubilized and analyzed with use of HPLC/UV.

Polypyrrole and polythiophene extraction abilities from plasma samples were checked with use of 6 different concentrations (in range 5–30 μg/ml) of mix adrenolytic drugs. Desorption was performed in three different pH (4.9, 7.0 and 8.6) which were adjusted by small addition to methanol acetic acid (15%) or ammonia (25%). Adsorption and desorption time were 10 and 5 min, respectively. Experiments were repeated three times which allowed to calculate relative standard deviations (RSD).

3. Results and discussion

3.1. Selection of extraction time and conditions

In extraction methods one from the most important parameters which should be taken under considerations before each experiments are adsorption and desorption kinetics. In our experiments performed for adsorption in seven different times and in six times for desorption (Fig. 3) were assigned the most optimal extraction times. To find the best adsorption time, a time range of 2–16 min was taken into account to be studied. Fig. 2 shows the adsorption time profiles for the studied adrenolytic drugs from aquatic samples. For each drug, the plateau trend occurred at the beginning of time dependence. Although for metoprolol and oxprenolol it looks that they needed more time to reach the equilibrium, an adsorption time of 10 min was selected to shorten the analysis time. Such equilibrium rate might find the reason of meaning in the chemical structure of applied SPME coatings and extraction abilities possessed by them. On the other hand only a small increase in responses and constant dependence till the end of studied time range can be explained by a competitive sorption mechanism between target compounds and SPME coatings. Such trend is observable also in case of other drugs (such as tricycle antidepressants) but a mechanism of this peculiar dependence might be a background of separate investigations.

In case of desorption differences are higher between each times. These curves has saturated character which allow to choose 5 min as the best desorption time.

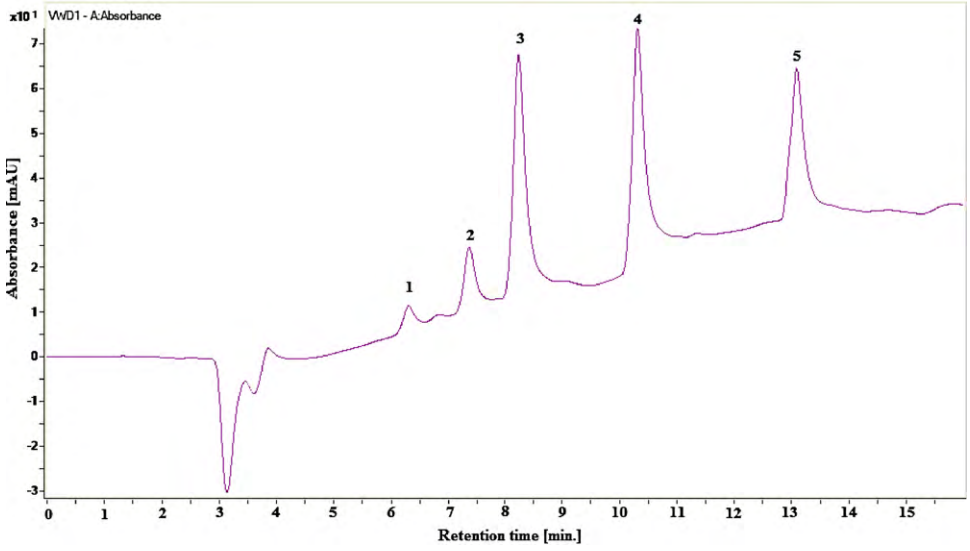

Fig. 4. Separation of investigated adrenolytic drugs from human plasma sample. 1-Metoprolol, 2-oxprenolol, 3-mexiletine, 4-propranolol, and 5-propaphenon.

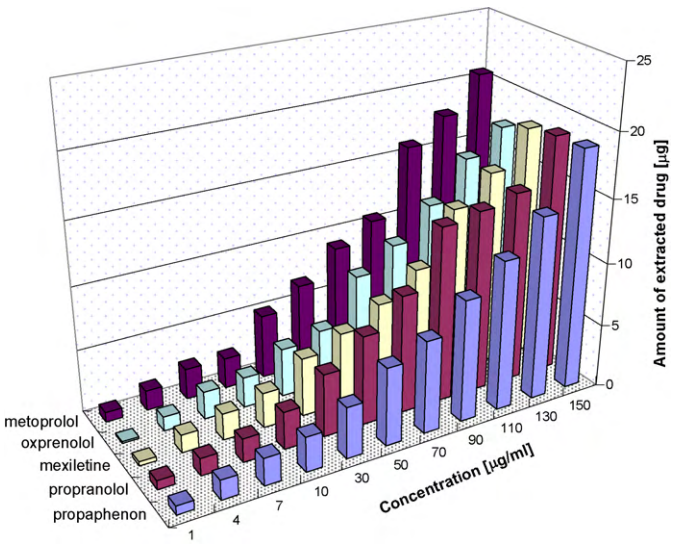

Fig. 5. Amounts of metoprolol, oxprenolol, mexiletine, propranolol and propaphenon extracted by PTh-coated SPME fibers from aqueous solutions.

Fig. 2 shows time extraction profiles (2–16 min) at room temperature (21 °C). Based on this, an extraction time of 10 min was selected for later experiments. Time profile for desorption is presented in Fig. 3. Desorption time at 5 min was chosen because it allowed to obtain the best results for most target drugs. Relative standard deviations (RSDs) obtained in these experiments were calculated on the very low level in range from 1.0 to 2.7% for adsorption and from 0.54 to 1.48% for desorption experiments.

Pure methanol yielded the highest result amongst the desorption solvents studied (MeOH/H_2O, acetonitrile, and acetone). The recovery obtained with use of MeOH/H_2O mixture, acetonitrile and acetone were 55%, 40%, 35% and 20%, respectively relative to the recovery obtained by pure methanol.

The chemical stability and robustness of the PTh coating 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 was no significant difference in the porous surface of PTh-SPME fibers. These experiments were performed with use of optical stereomicroscope.

3.2. Method calibrations

Developed procedure which allowed performing quantitative and qualitative analysis of five drugs in one chromatogram was firstly deeply validated. Calibration measurements were performed by preparing eleventh concentrations of adrenolytic drugs mixture. Each concentration was prepared and analysis was repeated five times which gave at the end fifteen values for one concentrations. Calibrations experiments which were performed in aqueous solutions are described in detail in Section 2.4. Calibration curve parameters, presented in Table 2, showed a good correlation coefficient (R^2) from 0.9932 to 0.9994 and relative standard deviations (RSDs) from 0.9 to 2.1%. The selectivity of applied method is presented on chromatogram of human plasma sample spiked with adrenolytic drugs at concentration 10 µg/ml (Fig. 4).

Table 3
Interassay accuracy and precision for analyzed adrenolytic drugs ($n = 5$).

Compound	Concentration [µg/ml]	Calculated concentration	Accuracy (%)	RSD (%)
Metoprolol	1	0.96	96	0.2
	4	3.76	94	0.1
	7	6.68	95	0.9
	10	10.87	109	0.9
	30	30.77	103	2.0
	50	55.56	111	0.7
	70	69.14	99	1.9
	90	93.97	104	2.0
	110	114.16	104	0.1
	130	129.93	100	0.4
	150	142.96	95	1.4
Oxprenolol	1	0.89	89	2.3
	4	4.09	102	2.9
	7	6.55	94	2.5
	10	11.09	111	1.1
	30	32.46	108	2.7
	50	52.96	106	1.5
	70	73.72	105	1.2
	90	87.14	97	2.3
	110	107.67	98	2.3
	130	127.42	98	2.0
	150	150.51	100	2.1
Mexiletine	1	0.93	93	1.9
	4	3.92	98	2.1
	7	6.73	96	1.2
	10	11.43	114	1.3
	30	30.72	102	2.5
	50	46.07	92	2.6
	70	64.81	93	2.1
	90	81.66	91	2.1
	110	111.69	102	2.5
	130	130.47	100	1.3
	150	155.82	104	2.4
Propranolol	1	0.91	91	2.6
	4	4.11	103	2.1
	7	6.62	95	2.0
	10	9.69	97	0.5
	30	33.85	113	0.4
	50	54.01	108	0.7
	70	77.64	111	1.6
	90	96.06	107	0.8
	110	106.99	97	0.2
	130	132.77	102	0.1
	150	139.31	93	0.3
Propaphenon	1	0.87	87	0.3
	4	3.84	96	2.1
	7	6.28	90	0.6
	10	11.14	111	0.4
	30	34.20	114	0.9
	50	52.89	106	0.3
	70	74.88	107	0.8
	90	95.53	106	1.6
	110	112.52	102	1.8
	130	125.4	96	0.5
	150	144.51	96	0.6

Table 4
Linearity, correlation coefficient and relative standard deviations for extraction by PTh-coated SPME fibers from aqueous solutions ($n = 3$).

Parameters	Metoprolol	Oxprenolol	Mexiletine	Propranolol	Propaphenon
Linear range [µg/ml]	1–150				
Slope	319.72	146.9	131.65	966.62	1025.5
Intercept	1054	667.4	465	830.9	4212.2
R^2	0.9856	0.9605	0.9644	0.9773	0.9879
RSD	0.9	1.2	1.1	1.1	1.8

To complete validation method an accuracy and RSD values for all concentration of drugs were also calculated and are shown in Table 3. Concentration calculated from calibration curves and derived from these values had an accuracy (and also RSD's) showed that prepared and validated method can be used in further measurements with the use of SPME fibers. The accuracy of the method ranged from 87% to 114%. These values are in the agreement to the relevant Food and Drug Administration guidelines which suggested the mean value to be within 15% of the actual value [20].

3.3. Application of polypyrrole and polythiophene SPME fibers in analysis of selected adrenolytic drugs from aqueous solutions and plasma samples

3.3.1. Aqueous solution

Experiments performed in aqueous solution allow getting a dependence, which indicates an affinity of each drug to applied adsorbent material. Results presented in Fig. 5 revealed the highest adsorption abilities of metoprolol on the polythiophene surface. Mexiletine, propranolol and propaphenon were adsorbed on the polymeric surface on the similar level. The smallest extraction efficiency was visible for oxprenolol. Amount of extracted drugs at the highest concentration (150 μg/ml) were in range since 17.45 μg for oxprenolol and 20.67 μg for metoprolol. With comparison to results obtained for polypyrrole fibers and presented in [10] changes in selectivity of SPME fiber were observed. In polypyrrole case the highest adsorption abilities were demonstrated towards mexiletine and metoprolol. Oxprenolol and propranolol were adsorbed in smaller scale on the polypyrrole surface. The smallest extraction efficiency was pointed out for propaphenon which was adsorbed on the polythiophene surface much better.

Parameters that described amount of extracted drugs versus concentration in output aqueous solutions were collected and are presented in Table 4. Correlation coefficients (R^2) for all drugs are in range from 0.9605 for oxprenolol to 0.9879 in case of propaphenon. Relative standard deviations (RSD's) calculated from investigations in aqueous solutions which were in range 0.9–1.8% showed that repeatability of adsorption with use of this kind of fiber is very high.

3.3.2. Real human plasma samples

Extraction of adrenolytic drugs exhibited by polypyrrole and polythiophene fibers from aqueous solutions showed differences in selectivity towards these molecules. Because of that we decided to include in measurements from plasma beside polythiophene also polypyrrole fibers. Target analytes are a basic drug and the pH of organic solvent solutions is known to play a crucial importance in the extraction efficiency of them. Desorption after extraction from plasma samples were performed in different pHs 4.9, 7.0 and 8.6. Results obtained from these measurements are shown in Table 5. From this table it is clearly seen that pH has a high influence for an extraction efficiency. In case of each drugs the highest amount of extracted drugs were visible in alkaline solution at pH 8.6. In analysis of adrenolytic drugs the smallest extraction was observed after desorption in acidic solution at pH 4.9. Studied adrenolytic drugs are bonded to plasma proteins at different levels in range 12–97% for metoprolol and propaphenon, respectively. Additionally, in pH value of 7.0 basic compounds are partially in the ionic form. At low pH values PPy and PTh coatings are positively charged, and probably in connection with electrostatic repulsion decreased the extraction abilities each of them towards the basic analytes.

Results presented in Table 5 were good background to calculate a concentration curves parameters. Example plot with results obtained for polythiophene fibers at pH 8.6 is presented in Fig. 6.

Linear regression parameters calculated for a polypyrrole and polythiophene fibers in dependency with a pH are presented

Table 5
Comparison of amount of extracted drug in different pH values by PPy vs. PTh fibers in human plasma samples ($n = 3$).

Concentration [μg/ml]	Amount of extracted metoprolol [μg] PPy vs. PTh			Amount of extracted oxprenolol [μg] PPy vs. PTh			Amount of extracted mexiletine [μg] PPy vs. PTh			Amount of extracted propranolol [μg] PPy vs. PTh			Amount of extracted propaphenon [μg] PPy vs. PTh		
	4.9[a]	7.0[a]	8.6[a]	4.9	7.0	8.6	4.9	7.0	8.6	4.9	7.0	8.6	4.9	7.0	8.6
5	1.64/1.34	1.71/2.22	3.30/3.21	2.30/1.73	1.93/2.52	2.46/2.47	1.94/2.00	2.22/2.00	2.83/2.46	1.47/1.37	1.49/1.54	2.62/1.94	4.43/4.16	4.68/6.16	7.26/7.50
7	2.30/2.46	3.27/4.26	5.20/6.21	2.41/2.01	2.37/3.00	3.44/4.41	2.37/2.55	2.73/3.08	3.92/4.55	2.36/1.83	2.52/2.24	4.27/3.34	6.36/5.63	6.53/7.80	10.65/9.82
10	2.35/2.54	3.54/3.67	5.96/6.65	2.37/2.46	2.41/3.52	3.81/6.04	3.41/2.71	3.16/3.21	7.06/5.85	3.39/2.44	2.67/2.93	2.67/5.22	6.15/6.87	7.41/8.91	9.91/14.78
15	2.74/2.93	4.52/3.96	11.16/8.78	2.51/2.62	2.72/3.88	7.58/7.14	2.83/2.96	3.52/3.73	8.44/7.21	2.56/3.24	2.98/3.71	5.64/6.58	7.06/7.84	9.67/10.72	16.40/18.15
20	3.08/4.67	5.90/4.77	11.24/8.00	2.69/2.73	2.77/4.47	8.13/9.29	3.34/4.04	3.92/4.04	9.39/7.64	2.53/3.37	3.19/3.97	6.31/7.53	6.83/8.25	9.69/12.08	16.87/20.40
30	4.54/3.75	5.18/5.90	15.70/11.24	2.61/2.35	2.78/5.62	10.38/11.64	3.65/2.62	3.90/5.09	10.07/11.96	2.44/3.79	3.49/4.42	7.63/12.12	8.85/9.38	11.59/12.75	20.05/27.66

[a] pH.

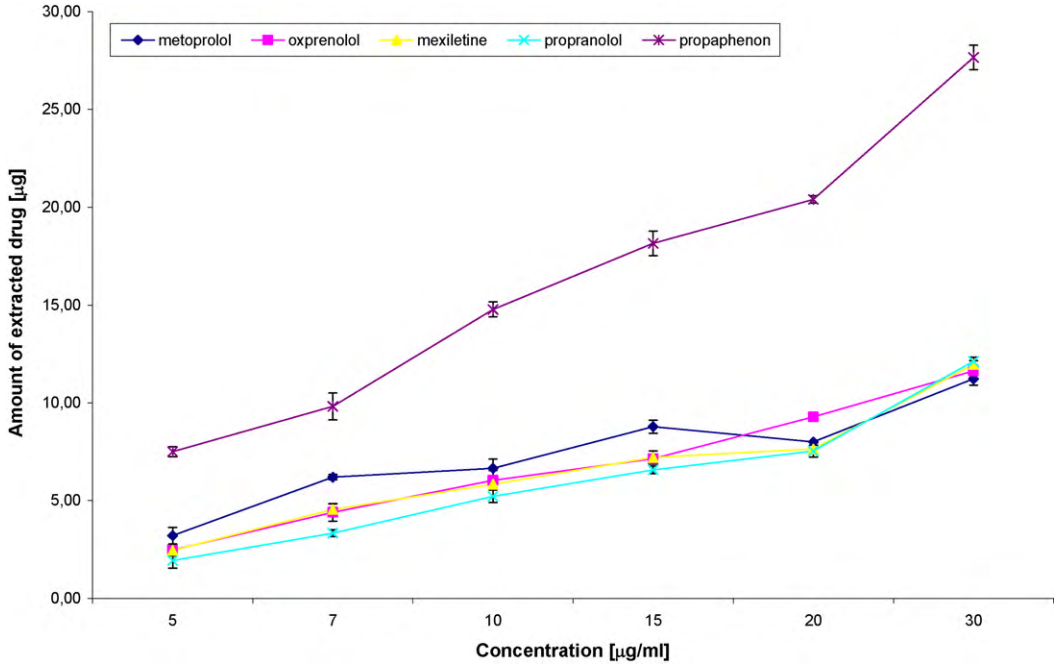

Fig. 6. Extraction of adrenolytic drugs with use of polythiophene fibers and methanol solutions at pH 8.6.

in Table 6. In case of polypyrrole fibers the highest correlation coefficients (R^2) were calculated for desorption at pH 4.9 (0.9382–0.9692). Beside extraction efficiencies were the highest in pH = 8.6 but this condition did not allow for obtaining as good correlation coefficients (R^2) like in others pH values. In this condition (pH = 8.6) R^2 was in range 0.8886–0.9399. Relative standard deviations (RSD) calculated for all drugs in all pH conditions are similar and in range since 1.0–2.8%.

Figs. 7 and 8 illustrate the comparison between the extraction efficiencies obtained applying PTh-SPME and PPy-SPME fibers from human plasma samples spiked with adrenolytic drugs (5 µg/ml). For both kind of fiber (PTh and PPy), utilized pH 8.6 has led to the best results, but metoprolol exhibited the highest extraction efficiency from all of the studied adrenolytic drugs. Additionally, PTh-SPME fibers presented extraction abilities higher than PPy-SPME fibers.

Correlation coefficients calculated for experiments performed with the use of polythiophene fibers in plasma are inversely than this prepared in polypyrrole case – higher in pH 7.0 and 8.6. For metoprolol and oxprenolol the most linear dependence was observed in pH 7.0–0.9706 and 0.9854, respectively. For mexiletine, propranolol and propaphenon the most optimal conditions were pH 8.6–0.9506, 0.9762 and 0.9671, respectively. Relative standard deviation (RSD) for all drugs and conditions was in range 1.0–2.6%.

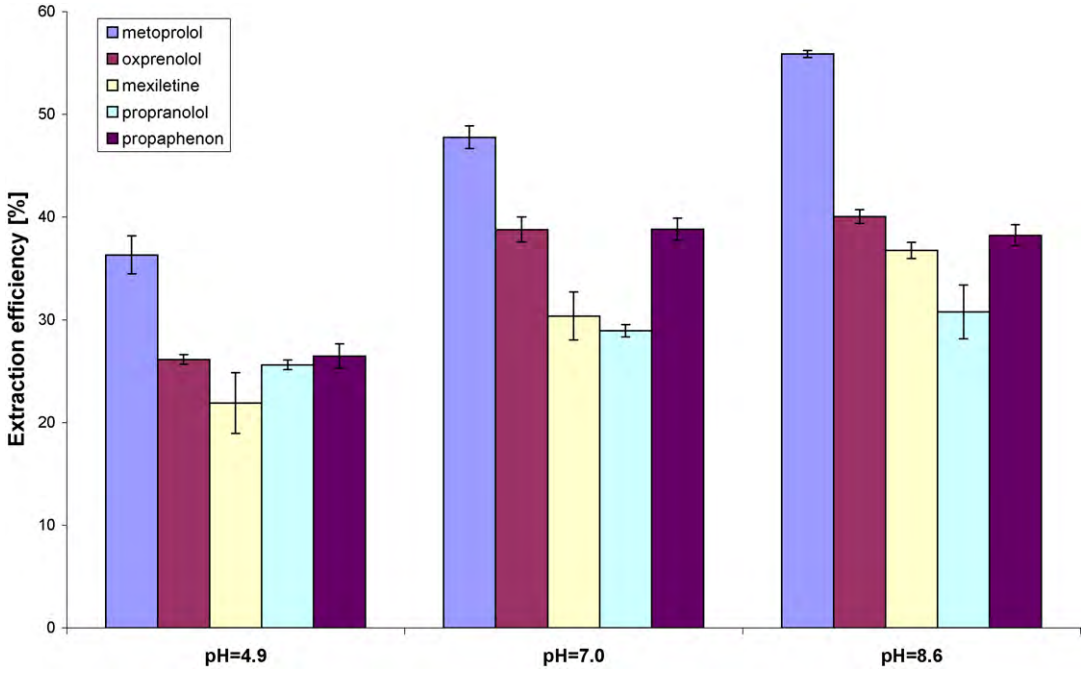

Fig. 7. Effect of the pH values on the PTh-SPME on the extraction efficiency of analytes from plasma samples (5 µg/ml).

Table 6
Extraction of adrenolytic drugs from plasma with use of different desorption conditions for polypyrrole and polythiophene fibers ($n = 3$).

Drug	Parameter	Slope			Intercept			R^2			RSD [%]		
	Fiber	4.9[a]	7.0[a]	8.6[a]	4.9	7.0	8.6	4.9	7.0	8.6	4.9	7.0	8.6
Metoprolol	PPy	1.5459	1.9916	4.8401	7.7017	19.563	0.8969	0.9572	0.8904	0.8969	2.5	2.0	2.3
	PTh	1.4578	1.7119	3.495	11.498	25.294	40.963	0.9318	0.9706	0.9367	1.8	2.6	2.1
Oxprenolol	PPy	0.5322	1.0991	12.422	43.497	35.737	3.3727	0.9671	0.9613	0.9345	2.8	1.8	2.5
	PTh	1.0316	4.4543	13.133	32.412	37.863	25.804	0.9132	0.9854	0.9563	1.2	1.6	2.2
Mexiletine	PPy	1.846	2.0862	9.1964	25.745	31.875	49.361	0.9382	0.8858	0.8975	1.6	1.4	1.9
	PTh	0.8658	3.446	10.929	30.106	21.291	13.187	0.9108	0.9158	0.9506	1.8	1.0	1.8
Propranolol	PPy	0.9949	4.5111	14.013	106.72	76.073	78.843	0.9475	0.9661	0.9399	1.7	2.1	1.3
	PTh	6.5895	7.6749	26.721	35.188	59.322	6.1382	0.9229	0.9518	0.9762	1.9	2.0	1.2
Propaphenon	PPy	5.446	8.2156	16.141	121.29	119.5	174.75	0.9692	0.8899	0.8886	1.0	1.5	1.5
	PTh	5.07	8.3047	25.038	102.85	165.9	138.38	0.9107	0.9102	0.9671	1.4	1.8	1.6

[a] pH.

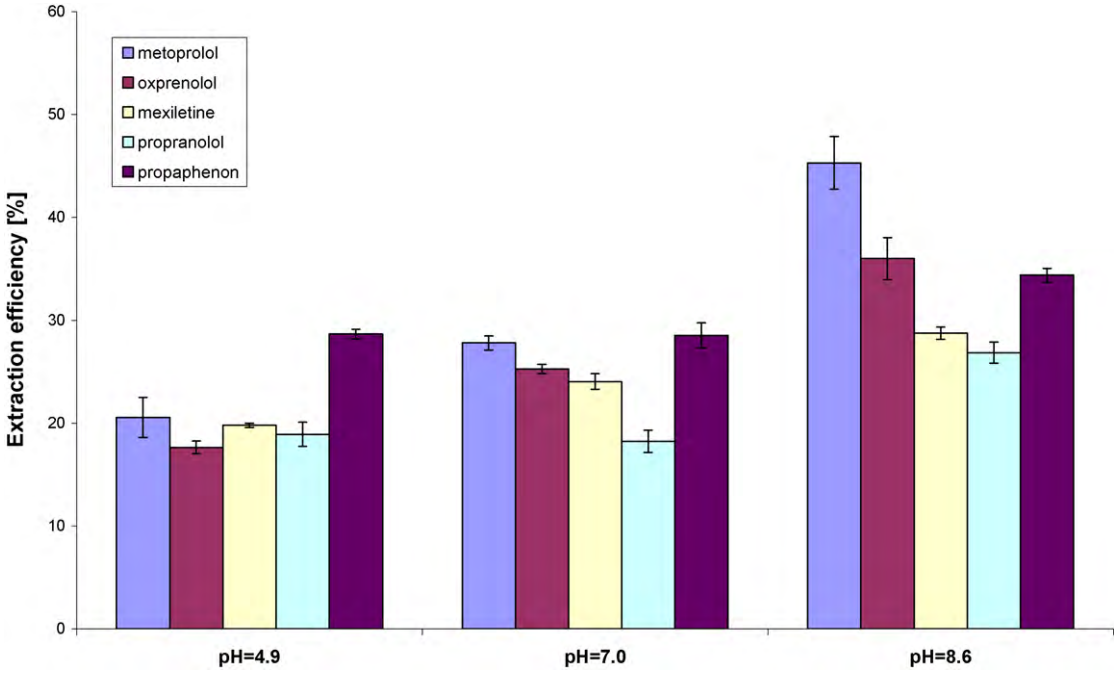

Fig. 8. Effect of the pH values on the PPy-SPME on the extraction efficiency of analytes from plasma samples (5 μg/ml).

The reproducibility of the extraction using polypyrrole and polythiophene fibers was confirmed by variation coefficients (CV) lower than 8% and 3%, respectively. This experiment was evaluated in case of the electrochemical PPy and PTh coating procedure.

4. Conclusions

In this work possible application of polypyrrole and polythiophene as an adsorbent in solid-phase microextraction sampling of five adrenolytic drugs were checked. In experiments aqueous solutions and human plasma samples were applied. Proposed SPME-HPLC/UV method rather provides an easy, rapid and sensitive powerful analytical device, characterized by small biological sample volumes, for the determination of adrenolytic drug therapy in the treatment of serious diseases, such as hypertension or chronic atrial fibrillation, which need to be monitored directly in the circulating blood human system.

Performed kinetics measurements showed that an optimal adsorption and desorption times were 10 and 5 min, respectively. Additionally, the optimized desorption solution was found at pH 8.6. In spite of that in polypyrrole case, correlation coefficient was higher in pH = 4.9. Inflected situation was observed in case of polythiophene fibers, where the best correlation coefficients were obtained after desorption in pH = 7.0 (metoprolol and oxprenolol) and in pH = 8.6 (mexiletine, propranolol and propaphenon), respectively. The polypyrrole and polythiophene SPME coatings displayed high extraction capacity (selectivity and sensitivity) towards the target compounds. Hence, presented method with use of home-made PPy and PTh fibers can be useful for the determination of the beta-blockers studied in human plasma samples from patients. The method may be also used in the evaluation of whole blood levels in adrenolytic drugs as a promising tool for the selective extraction of them in clinical analysis. Nevertheless, in the more advanced form and some modification may be successfully utilized for therapeutic drug monitoring (TDM) application in biomedical and pharmaceutical fields.

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