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Polymeric particles with conjugated polymer: Layer on its surface as effective adsorbents of amino acids

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Abstract

In the present paper composite polymeric particles with polypyrrole (PPy) shell have been examined as reservoirs for uptake of amino acids. The particle morphology can be designed in a way that polypyrrole load on the particle surface as well as the PPy shell thickness can be varied easily by control of the pyrrole polymerization conditions, such as monomer concentration and choice of different oxidants. Three different oxidants have been used for the preparation of PPy outer-layers, namely $FeCl_3$, $Na_2S_2O_8$, and $H_3PMo_{12}O_{40}$, which give the possibility to incorporate different anions into PPy structure. L- and D-alanine uptake in the presence of obtained particles has been tested as a function of particle concentration and PPy amount on the particle surface. It has been found that composite particles can adsorb quite high amounts of alanine and even show certain enantioselectivity. The uptake efficiency and selectivity depend strongly on the properties of the PPy layer, such as chemical structure (oxidized or overoxidized state) and the nature of the dopant anion.

Keywords: Polypyrrole; Core–shell particles; Amino acid adsorption

1. Introduction

Biomolecular recognition is the underlying principle of many biological processes. In this way specialized structures such as antibodies, hormone receptors, and enzymes fit perfectly with their natural targets. It has been a long-term dream for researchers to build such structures, creating tailor-made receptors that are capable of recognizing and binding the desired molecular target with high affinity and selectivity. Such synthetic materials should be easier to produce and process, less costly, and more stable than biomacromolecules. Moreover, they should be accessible to the target molecules for which natural receptors do not exist or are difficult to obtain. One simple way of generating artificial macromolecular receptors is through the molecular imprinting of synthetic polymers. Recently molecular imprinting polymers (MIP) have been prepared in the form of thin layers on the solid support [1], polymeric hydrogels [2], polymeric beads [3,4], microgels [5] and dendrimers [6].

Conducting polymers (polypyrrole, polyaniline, etc.) can be interesting molecular recognition systems, since they have an intelligent ability to control the ion exchange dependent on redox potential and pH in solution. Additionally, it has been reported that a dopant anion determines the porosity of the polymer network (or network spacing), and this feature has been utilized for ion-selective electrodes to detect some inorganic anions [7,8]. An anion selectivity of a conducting polymer film has been studied by varying the size of the dopant anion and such layer has been used in potentiometric sensor for nitrate detection [8]. Another group reported the preparation of poly(*o*-phenylenediamine) imprinted by glucose by electropolymerization [9]. Recently Nagaoka's group reported the effective use of overoxidized polypyrrole in the form of films [10] and colloidal particles [11–13] for enantioselective uptake of amino acid. The strategy used in this research requires initially doping of polypyrrole with L-glutamate or L-lactate during PPy synthesis. Further overoxidation

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step gives a possibility to remove the amino acid and create a cavity which shows very good enantioselectivity for L-acid over the D-enantiomer. It has also been demonstrated that such templated colloids can adsorb different amino acids and the uptake efficiency is determined by the difference between molecular volume of the target molecule and volume of the templated cavity [13].

In our previous reports we describe the synthesis and characterization of composite core–shell particles with polypyrrole layer on its surface [14,15]. The growth of the PPy shell was carried out in the highly swollen hydrophilic brush-like layer which was chemically grafted onto the core particle surface. Oxidative polymerization of pyrrole in such conditions leads to the formation of well-defined conducting polymer layers on the surface of sub-micrometer monodisperse polystyrene particles. Schematically the structure of composite particles is shown in Fig. 1.

Obtained composite particles offer several advantages compared with PPy particles obtained by other working groups. First of all, preparation of such colloids does not require any additional surfactants or stabilizers. Monodisperse spherical poly(styrene-*co*-hydroxy poly(ethylene glycol) methacrylate) (PS/PEGMA) core particles possess bio-compatible surface and can be prepared within a broad size range, which gives a possibility to vary effectively the size of the composite beads. The thickness of the polypyrrole shell and the nature of the dopant anion can be precisely adjusted by changing polymerization parameters or oxidant. We believe that PPy chains on the particle surface are also well-organized due to the directed growth in the PEGMA–brush layer, and this fact in combination with extremely large particle surface area and all interesting features mentioned above make such particles interesting for application as smart devices for molecular recognition. The scope of the present study is the determination of the interaction of polypyrrole-containing core–shell particles with amino acids. As model amino acid the L- and D-alanine have been selected. Our aim was to check the amino acid uptake by such particles as a function of the particle concentration in the solution, PPy load on the particle surface and the nature of the dopant anion which was incorporated directly during pyrrole polymerization.

2. Experimental

2.1. Materials

Styrene (ST, from Fluka) and pyrrole (Py, from Aldrich) were distilled under vacuum and stored in a refrigerator before use. ω-Hydroxy poly(ethylene glycol)methacrylate (PEGMA, from Aldrich) with average $M_w = 526$ g/mol was used as supplied. Sodium peroxydisulphate (SPDS), iron(III) chloride ($FeCl_3$), and phosphomolybdic acid ($H_3PMo_{12}O_{40}\cdot 29H_2O$) were received from Aldrich and used as commercially available. Distilled water was employed as polymerization medium. Amino acids used were of reagent grade (Aldrich), and used as supplied.

2.2. Particle synthesis

2.2.1. Synthesis of PS/PEGMA core particles

Detailed information about the synthesis of PS/PEGMA particles has been reported elsewhere [14]. Appropriate amount of PEGMA was dissolved in water and then the monomer was added to the stirred solution. Obtained pre-emulsion was stirred for 1 h. Double-wall glass reactor equipped with stirrer and reflux condenser was purged with nitrogen. Pre-emulsion was placed into the reactor and the water solution of initiator was added under continuous stirring. Temperature was increased to 70 °C to start the polymerization process. Latexes were prepared at ca. 10% solid content.

2.2.2. Synthesis of core–shell particles

Diluted PS/PEGMA dispersions were placed into the stirred reactor. Appropriate pyrrole amounts were injected by syringe and the mixture was stirred for 15 min under nitrogen flow at 25 °C. Water solution of oxidant ($FeCl_3$, SPDS or $H_3PMo_{12}O_{40}\cdot 29H_2O$) was added dropwise to start pyrrole polymerization. After 5–10 min, dispersion became coloured (blue or dark red colour) indicating that pyrrole polymerization started. After 24 h, formed composite particles were removed from the reaction vessel and cleaned by dialysis to remove non-reacted pyrrole and all by-products.

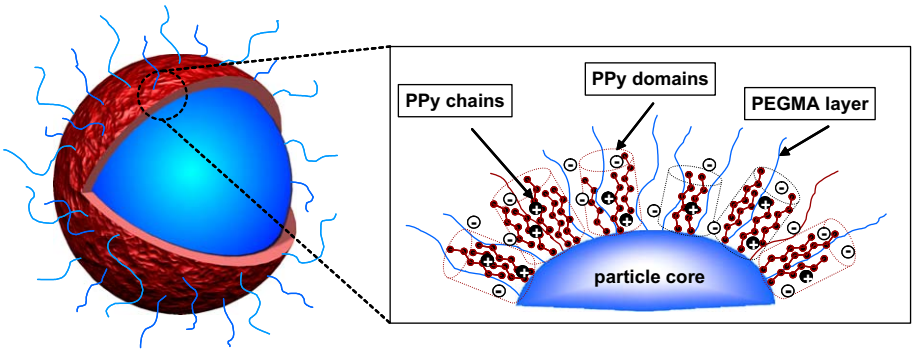


Fig. 1. Schematic representation of the composite particles with polypyrrole shell.

2.3. Base treatment

Cleaned composite particles have been dried in the oven overnight at 40 °C and then were dispersed in aqueous 0.1 M NaOH with an ultrasonic homogenizer. The suspension was stirred overnight under anaerobic condition at room temperature and then purified by centrifugation at 8000 r/min for 15 min. The resulting sediment was rinsed several times with water.

2.4. Amino acid uptake

The uptake of amino acid by the composite particles was determined as follows: a 5 ml aqueous solution (pH value was adjusted by the addition of HCl) containing 0.0125 mmol amino acid and 0.01 g of particles was shaken vigorously for 24 h and the colloidal particle was then removed by a membrane filter (0.45 μm). The pH of the filtered solution was adjusted to 7 with buffer solution. Two milliliters of final solution was added to 0.4 ml of ninhydrine reagent (0.1 g ninhydrine in 10 ml ethanol with water for 100 ml) and the mixture was heated in boiling water bath for 40 min. The solution shows typical blue colour which gives special UV adsorbance at $\lambda = 570$ nm. The peak intensity corresponds to the concentration of non-adsorbed amino acid which can be calculated from the calibration curve. The calibration curves have been determined separately for different amino acids. The amino acid uptake by particles is given as the ratio between adsorbed amino acid and initial amino acid concentration in solution: $(c_{ads}/c_{in}) \times 100\%$.

2.5. Analytical methods

The dynamic light scattering measurements were obtained with DLS 700 (Otsuka, Japan) at different scattering angles. In dynamic LS, the Laplace inversion of a measured intensity–time correlation function $G^{(2)}(t,q)$ in the self-beating mode can result in a line-width distribution $G(\Gamma)$ [10]. For a pure diffusive relaxation, Γ is related to the translational diffusion coefficient D by $\Gamma/q^2 = D$ at $q \to 0$ and $c \to 0$, or a hydrodynamic radius R_h by $R_h = k_B T/(6\pi\eta D)$ with k_B, T and η being the Boltzmann's constant, absolute temperature, and solvent viscosity, respectively.

SEM images were taken with Gemini microscope (Zeiss, Germany). Samples were prepared in the following manner. Dispersions were diluted with deionized water, dropped onto aluminium support and dried at room temperature. Pictures were taken at a voltage of 4 kV.

3. Results and discussion

3.1. Composite particles

Table 1 shows a summary of most important properties of composite particles used in the present study. Selected PS/PEGMA latex sample (particle diameter 482 nm) was used for the preparation of core–shell particles. Composites have been prepared with different oxidants and PPy amount on the particle surface was varied. The polypyrrole load was determined by elementary analysis. Table 1 shows that in the presence of different oxidants the determined polypyrrole load is lower as expected. This can be explained by the partial pyrrole polymerization in aqueous solution and by the formation of secondary particles. Such secondary PPy particles were not connected to the core surface and have been removed during cleaning procedure therefore final PPy load in composites is lower as expected. In cases where phosphomolybdic acid was used as oxidant very low PPy yields have been detected. In this case probably the low oxidation efficiency of phosphomolybdic acid is the reason for such effect leading to high amount of unreacted pyrrole which was removed by dialysis.

Table 1
Composite particles used in the present study (polymerization pH = 2)

Sample	Oxidant	PPy^T (%)	PPy^P (%)
1	$Na_2S_2O_8$	2.5	2.28 ± 0.2
2	$Na_2S_2O_8$	5	4.5 ± 0.34
3	$Na_2S_2O_8$	7.5[a]	6.41 ± 0.42
4	$Na_2S_2O_8$	10	9.38 ± 0.51
5	$Na_2S_2O_8$	12.5	13.4 ± 0.56
6	Mo	2.5	0.84 ± 0.01
7	Mo	5	1.2 ± 0.03
8	Mo	10	2.44 ± 0.2
9	Mo	15	4.02 ± 0.31
10	Mo	20	5.36 ± 0.34
11	$FeCl_3$	2.5	1.91 ± 0.1
12	$FeCl_3$	5	3.49 ± 0.26
13	$FeCl_3$	10	6.32 ± 0.44
14	$FeCl_3$	15	11.63 ± 0.44
15	$FeCl_3$	20	13.78 ± 0.50

[a] PPy load at reaction pH = 4 and pH = 6 is 7.17 and 7.46, respectively; Mo – $H_3PMo_{12}O_{40} \cdot 29H_2O$; T and P – theoretical and practical load, respectively.

Fig. 2 shows the SEM images of PS/PEGMA particles and composite particles after polypyrrole deposition.

Deposition of conjugated polymer leads to the formation of the shell around polystyrene core and increases the surface roughness considerably. In our previous paper we demonstrated that no secondary PPy particle formation in the aqueous medium was observed up to 25 wt% PPy deposition.

It can be expected that the structure of the polypyrrole chains on the particle surface would influence considerably the interactions of amino acids with composite particles. Scheme 1 shows that pyrrole polymerization can be performed at pH = 2 leading to oxidized state of PPy shell. In this case PPy chains possess some positive charge which is counterbalanced by incorporated anions from the oxidant. If pyrrole polymerization is performed at pH = 6 it is possible to obtain overoxidized PPy [13]. In this case the conjugated structure of PPy is destroyed as a result of carbonyl group introduction.

By NaOH treatment oxidized particles can be transformed into reduced state, the mechanism of which has been first reported by Beck et al. [16]. This reversible process leads to neutral state of PPy chains and rejection of anions into water phase. In case of overoxidized particles no strong changes in the PPy structure can be expected after the treatment with base. Fig. 3 presents SEM images of composite particles after

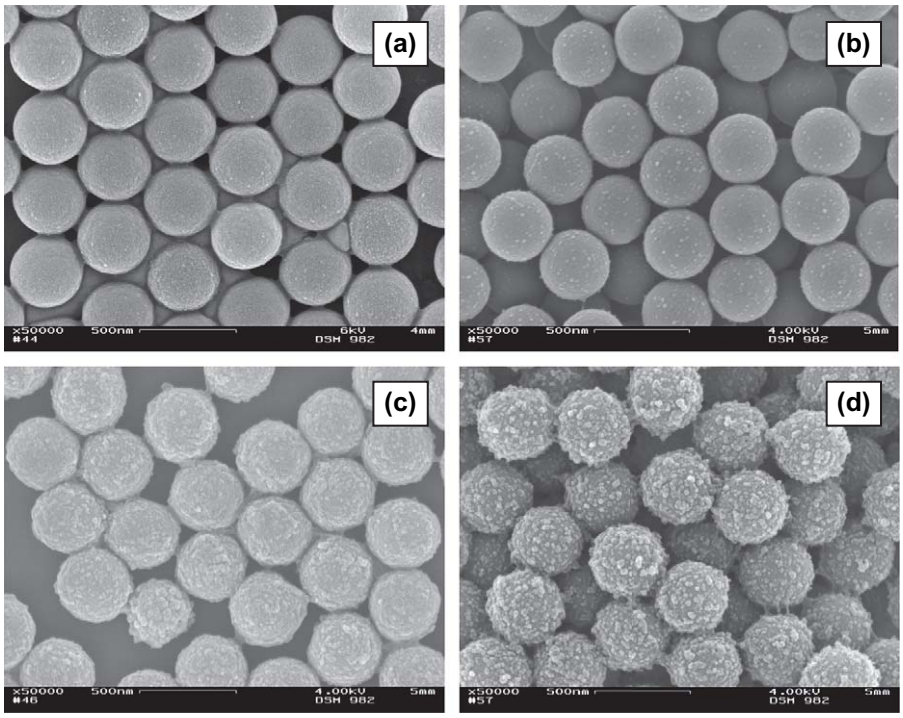
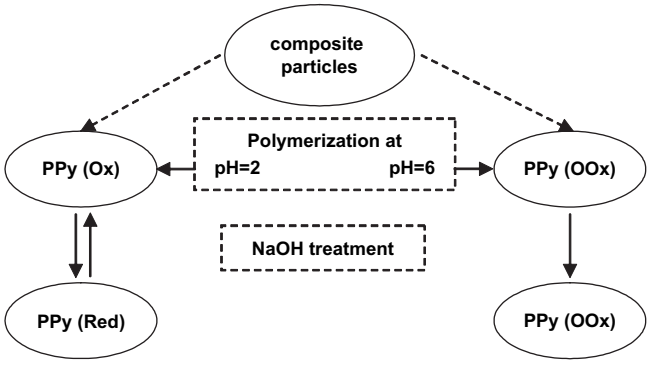


Fig. 2. SEM of core particles (a) and composite particles with different PPy contents: 2.28% (b), 4.5% (c), and 6.41% (d).

treatment with NaOH. On comparing Fig. 3 with Fig. 2d it can be observed that the treatment with NaOH does not change the core–shell morphology of composite particles.



Scheme 1. Different states of PPy layer in composite particles.

It is quite important to define the pH value for the investigation of the amino acid uptake by core–shell particles. In this case it is necessary to consider both the ampholyte behaviour of amino acids and the structure of PPy layer on the particle surface.

Scheme 2 indicates the acid/base equilibrium of alanine and it is clear that at $pH < 2$ these molecules are positively charged, in the pH range 3–9 they are amphoteric and negatively charged in basic medium.

From the acid/base equilibrium of alanine with pH, we can expect different possible interactions of amino acid and PPy in the system dependent on the pH of surrounding medium. Scheme 3 shows the overoxidation mechanism of polypyrrole presented by Beck et al. [16]. From Scheme 3, we can see that oxidized particles (positively charged) show repulsive forces to positively charged amino acids at uptake $pH = 1$. Contrarily, at values of $pH > 4$ some attractive forces can be

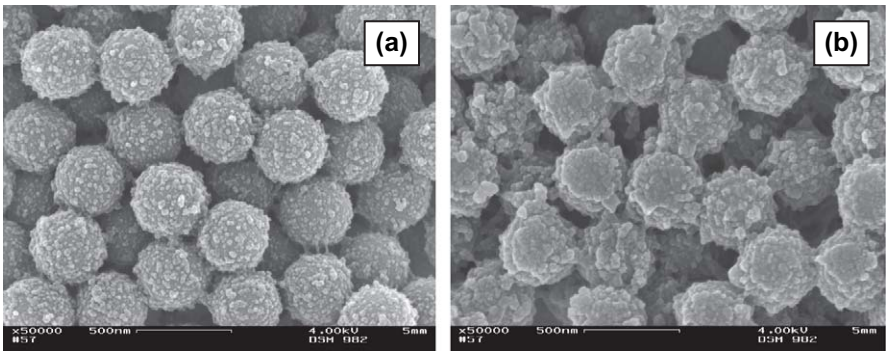
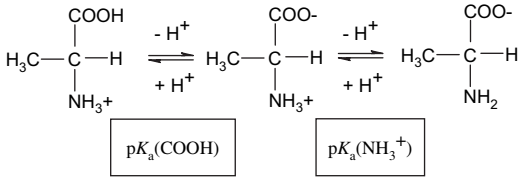
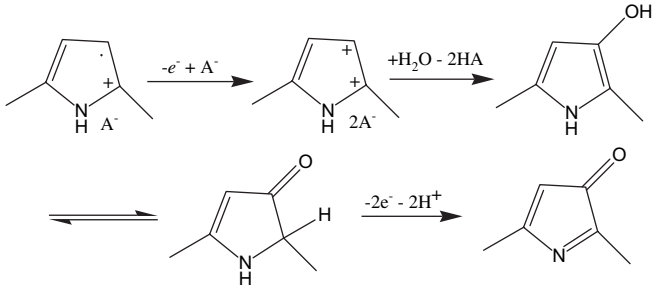


Fig. 3. Composite particles containing 6.41% PPy after treatment with NaOH.



Scheme 2. Acid/base equilibrium of alanine.



Scheme 3. Overoxidation mechanism of polypyrrole.

expected due to the ampholyte behaviour of amino acid (positive and negative charges). Overoxidized particles possess some carbonyl and carboxylic functions and much less counteranions and interactions with amino acids at pH = 1 can be due to hydrogen bond formation (low pH) and electrostatic attraction with positively charged amino acids in case of strong acidity of groups formed during overoxidation. Oxidized particles can be reversibly transferred to the reduced state by the treatment with NaOH. This causes the removal of counterions from PPy and more or less neutral charge of the polymer backbone. In this case at pH = 1 we can expect strong concurrence of Cl^- ions which can dope PPy chains and convert them into oxidized state and uptake positively charged amino acids. Uptake at pH > 4 will promote interaction of amino acid with more or less neutral PPy. Overoxidized particles treated with NaOH can lose some anions which were incorporated into PPy, but no strong change of the structure can be expected.

Fig. 4 shows the uptake of L-alanine by core–shell particles which was performed at different pH values. These investigations have been made for composite particles prepared at pH = 2. Fig. 4 indicates that L-alanine uptake has minimal value in the pH range when amino acid becomes an ampholyte (pH = 4). From the acid/base equilibrium of aniline we can see that the first pK_a is equal to pH = 2.3, which means that when pH is higher than 2.3 aniline will be partly negatively charged. And higher the pH value is the more negative charges aniline has until the second pK_a is reached. From this point of view, the strong increase in adsorption at pH > 4 is associated with interaction between negatively charged COO^- groups and PPy (partial positive charge) in oxidized state (polymerization performed at pH = 2). It is also obvious that particles treated with NaOH (PPy in reduced state) show much higher L-alanine uptake in similar condition as non-treated particles (oxidized state). Table 2 shows the L-alanine uptake values determined at pH = 1 and pH = 5 (indicated with arrows in Fig. 4) for composite particles prepared at different pH values.

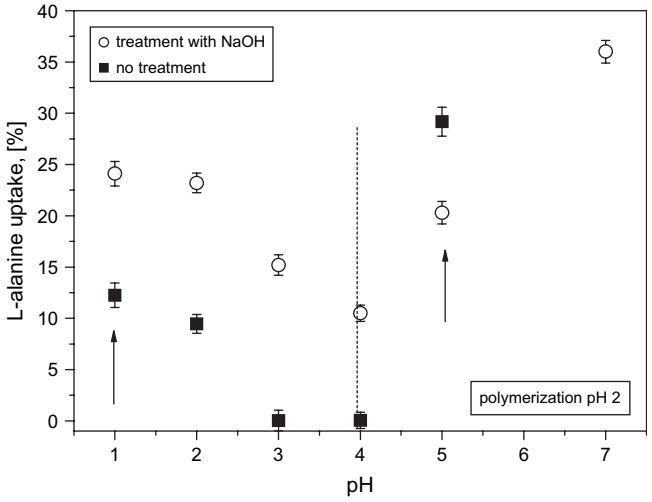


Fig. 4. Uptake of L-alanine at different pH values ($c_{particles}$ = 2 g/l; PPy content 6.41% (SO_4^{2-}); $c_{L\text{-alanine}} = 2.5 \times 10^{-3}$ M).

Table 2
Uptake of L-alanine at pH = 1 and pH = 5 by particles prepared at different pH values

Polymerization pH	Uptake pH = 1		Uptake pH = 5	
	a	b	c	d
2	12.34 ± 0.84	24.1 ± 0.11	29.17 ± 1.2	36 ± 0.84
4	11.89 ± 10.72	14.25 ± 0.14	40.86 ± 1.5	34.85 ± 1.5
6	19.33 ± 0.91	22.56 ± 1.1	33.75 ± 1.1	38.51 ± 1.4

$c_{particles}$ = 2 g/l; PPy content 6.41% (SO_4^{2-}); $c_{L\text{-alanine}} = 2.5 \times 10^{-3}$ M; a, c – no base treatment, b, d – treatment with base.

Experimental data from Table 2 indicate that L-alanine uptake at pH = 5 is always higher and independent of the polymerization pH and treatment with base. Uptake at pH = 1 shows clear improvement of amino acid uptake by oxidized particles after treatment with NaOH, and no strong improvement in case of composite particles prepared at pH = 6 (overoxidized state). This correlates with our predictions summarized above. Further uptake investigations have been performed at pH = 1 to avoid amphoteric character of amino acids and to simplify the investigated system. In this case, amino acid is always positively charged and its interaction with PPy layers in oxidized, reduced or overoxidized state on the particle surface can be better explained.

3.2. Uptake of alanine as a function of particle concentration in the mixture

In this set of investigations the particle concentration in the system was varied and the uptake of L- and D-alanine by composite particles has been determined. Fig. 5 indicates that in all the cases increase of the particle concentration leads to higher amino acid uptake. As expected, overoxidized particles (polymerization pH = 6) show much higher amino acid uptake compared with composite particles modified with PPy in oxidized state. Fig. 5 shows also that composite particles exhibit certain stereoselectivity of the amino acid uptake. It is interesting to

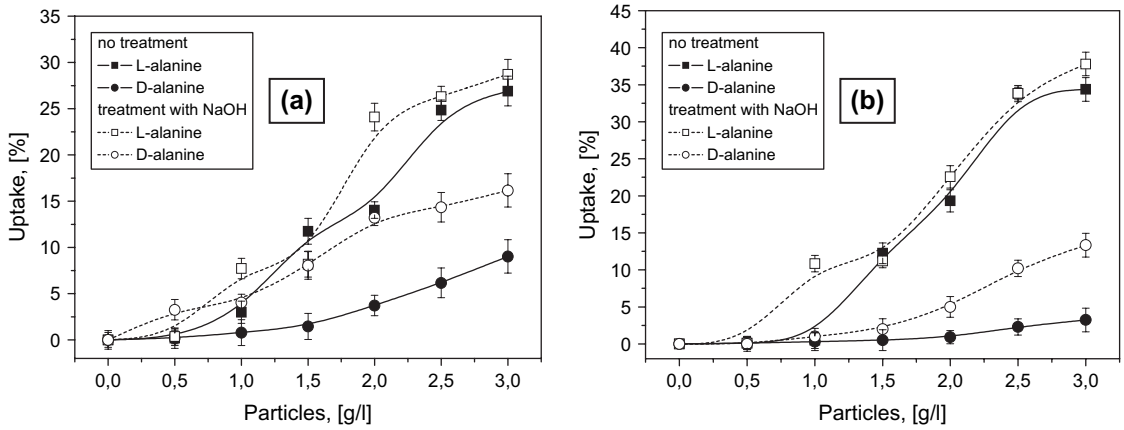


Fig. 5. Uptake of L- and D-alanine by composite particles prepared at different pH values (a: pH = 2, b: pH = 6; PPy content 6.41% (SO_4^{2-}); $c_{\text{L-alanine}} = 2.5 \times 10^{-3}$ M; uptake pH = 1).

note that particles treated with base show higher amino acid uptake and this effect is much stronger in case of D-alanine.

This fact reduces strongly the selectivity of the amino acid uptake (ratio between L- and D-stereoisomer uptake) which is summarized in Table 3.

Results summarized in Table 3 also indicate that uptake of alanine by oxidized particles is partly stereoselective (selectivity is much lower compared with overoxidized particles) and treatment with NaOH increases the D-alanine uptake and reduces selectivity of the process. As we know, the enantioselectivity normally arises only if PPy chains on the PS/PEGMA particle surface are optically active, but the difficulty of detection PPy chains by circular dichroism spectroscopy makes us not clear for this. Further study must be done in this direction to understand the mechanism.

3.3. Uptake of alanine as a function of PPy content and dopant anion

Since composite particles used in the present study possess a PPy shell of defined thickness which is actually responsible for amino acid uptake it is possible to check the influence of the PPy amount on the surface on uptake behaviour. In this case it is also important to consider the nature and the size of the dopant anion, since it will determine to some extent the spacing of PPy network on the particle surface. The uptake of alanine by composite particles prepared with different oxidants (and doped by corresponding anions) has been investigated and experimental results are presented in Fig. 6.

The determined uptake of L- and D-alanine is plotted vs. loaded PPy amount on the particle surface. Uptake of amino acid increases linearly with increase of polypyrrole content independent of the anion nature. This effect shows the attractive possibility to influence the amino acid uptake not only by particle concentration in the mixture, but also by the thickness of the PPy shell on the particle surface. All composite particles show higher affinity to L-alanine, but experimental data in Fig. 6 indicate that the amount of adsorbed amino acid and selectivity of the L- and D-alanine uptake are quite different for the three dopant anions used in this study. The calculated selectivity data are shown in Table 4.

Table 3
Selectivity of alanine uptake by composite particles

	Polymerization pH = 2		Polymerization pH = 6	
	a	b	c	d
Selectivity	4.3	2.1	17.5	7.3

a, c – No base treatment; b, d – treatment with base.

It is obvious that composite particles prepared with phosphomolybdate show extremely high selectivity compared with particles doped by chloride or sulphate. In all the cases treatment with NaOH decreases selectivity of the process. If we compare the uptake values of L-alanine by different particles plotted vs. PPy amount on the particle surface (Fig. 7) it is clear that uptake depends clearly on the size of the dopant anion. It is obvious to see that particles prepared with phosphomolybdate exhibit much higher alanine uptake compared with their chloride and sulphate doped analogues with similar PPy content.

From this we can conclude that indeed anion size plays an important role in the formation of the PPy layer on the particle surface and this influences the uptake of amino acid. The open question is still the origin of this interaction forces between composite particles and alanine. In this experiment all particles have been prepared at pH = 2, so they should be in oxidized state. The reason for this is that it is not possible to use phosphomolybdate as oxidant at pH = 6, so no comparison with other oxidants can be made. As it was mentioned before, oxidized PPy layer should bear some positive charges and interaction with positively charged amino acids at pH = 1 should be rather weak. But our experimental results indicate that there is sufficient alanine uptake and partly high selectivity to L- and D-isomers. An attempt was made to test the uptake of other amino acids such as glycine and serine by composite particles doped by sulphate. Some experimental data are summarized in Table 5.

Data in Table 5 indicate that overoxidized composite particles show no serine uptake and particles treated with NaOH are not sensitive to glycine. So, it seems that prepared

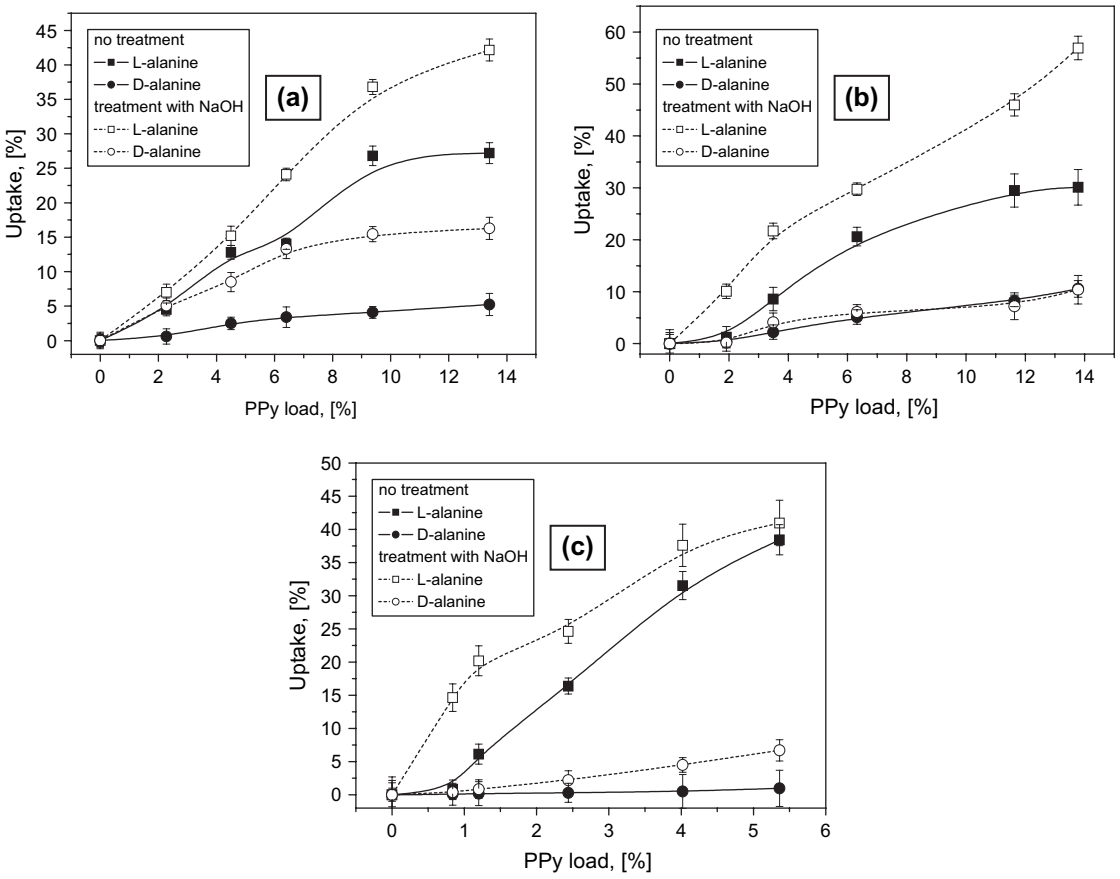


Fig. 6. Uptake of L-alanine as a function of PPy content on the particle surface: (a) dopant sulphate, (b) dopant chloride, and (c) dopant molybdate (particles prepared at pH = 2; $c_{\text{particles}} = 2$ g/l; $c_{\text{L-alanine}} = 2.5 \times 10^{-3}$ M; uptake pH = 1).

Table 4
Selectivity of alanine uptake by composite particles

	Cl^-		SO_4^{2-}		Mo	
	a	b	a	b	a	b
Selectivity	6.1	5.8	6	2	50	11

a – No base treatment; b – treatment with base.

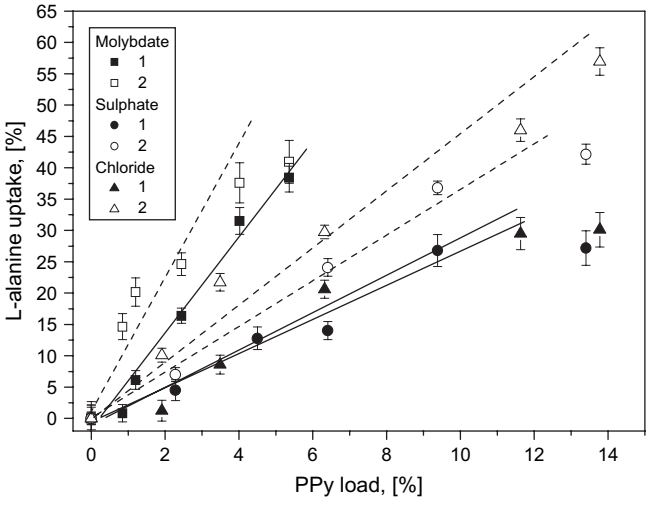


Fig. 7. Uptake of L-alanine as a function of PPy content on the particle surface: (1) no treatment and (2) treatment with NaOH ($c_{\text{particles}} = 2$ g/l; $c_{\text{L-alanine}} = 2.5 \times 10^{-3}$ M; uptake pH = 1).

Table 5
Uptake of different amino acids by composite particles

Amino acid	Polymerization pH = 2		Polymerization pH = 6	
	a	b	a	b
Glycine	6.66×10^{-7}	0	3.19×10^{-6}	0
L-Alanine	1.76×10^{-6}	3.01×10^{-6}	2.42×10^{-6}	2.82×10^{-6}
L-Serine	5.23×10^{-6}	4.23×10^{-6}	0	0

a – No base treatment; b – treatment with base.

composite particles cannot be treated as universally effective adsorbers of different amino acids and the system should be better optimized and better investigated in future.

4. Conclusions

In the present study composite polymeric particles with polypyrrole layer on its surface have been tested as adsorbents of amino acids. It has been shown that composite particles can adsorb quite high amounts of alanine and even show certain enantioselectivity. It has been found that the alanine uptake can be controlled by the particle concentration in the mixture as well as by the PPy amount on the particle surface. The uptake of alanine can be influenced by the size of the dopant anion and the maximum value was detected for the particles doped with phosphomolybdate. It has been established that

the treatment of composite particles with NaOH (reduction of PPy shell) increases the alanine uptake but decreases the enantioselectivity. It is believed that such composite particles can be effective tool for amino acid separation/purification. They offer numerous advantages such as adjustable size, controlled amount of polypyrrole on the surface, and defined design of the surface layer. Particles used in the present study possess PPy layer prepared without any template molecules, which can probably improve the selectivity and uptake of different amino acids. These investigations should be performed in future to use maximally the potential of such composite colloids.

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