A method for coating colloidal particles with molecularly imprinted silica films

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A method of coating colloidal particles with molecularly imprinted silica films of nano size is described. The imprinted film is prepared *via* a "sacrificial spacer" imprinting technique with testosterone as the template and silver colloidal particles as the core, and then characterized by transmission electron microscopy and infrared spectroscopy. Steady-state binding results show that the imprinted film has a highly selective recognition ability for template molecules. Moreover, due to its thickness in nanometres, the imprinted film presents a very fast kinetic profile for binding templates, with a saturation time less than 10 min. Furthermore, this technique offers a unique means of fine-tuning the thickness of imprinted materials on a core.

Introduction

The synthesis of materials with molecular recognition properties has become an attractive topic of extreme technological and scientific interest.[1] One of the most popular strategies for preparing synthetic receptors is molecular imprinting.[2] In the commonly used process, functional monomers initially form a complex with the template molecule through covalent or non-covalent interactions such as hydrogen bonding, electrostatic forces, van der Waals forces, or hydrophobic interactions, and then are copolymerized with cross-linking monomers in the presence of a solvent or porogen. Subsequently, the template is removed by chemical reaction or washing, so that binding sites are obtained which are complementary to the template in size, shape, and position of the functional groups.[3,4] As a result, these materials retain a "molecular memory" of the template, and thus have the ability to selectively rebind it.

Compared with natural biomolecules, molecularly imprinted materials have many outstanding advantages including predetermined recognition ability, stability in harsh environments, ease of preparation, low cost and potential application to a wide range of target molecules. Consequently, molecular imprinting has found wide applications in chemical sensing,[5–7] catalysis,[8] drug development and screening,[9] and separation.[10,11] However, up to now, molecularly imprinted materials have not been used as a real alternative or complement to biomolecules because of their inherent limitations,[12,13] such as slow mass transfer in and out of the polymer matrix, slow leaching out of trapped template molecules, and overall low binding affinity. To overcome these drawbacks, some new morphologies and manufacturing techniques have been developed.

In recent years, imprinted material films have attracted much attention due to their binding sites close to the surface of the imprinting matrix, causing several advantages, such as more accessible sites, faster mass transfer, and faster binding kinetics. Moreover, those spherical particles coated with imprinted material films are very useful in chromatography and solid-phase extraction because of their spherical shape and uniform particle size and film thickness. Most of such coatings are prepared by binding polymerizable double bonds onto the surface of the supports first, and then grafting molecularly imprinted polymers in the presence of an initiator in a monomer solution.[14,15] Some improvements are carried out by binding initiators to the support surface prior to polymerization,[16–19] including the thickness adjustments of the grafted films.[16] Another new technique was performed by polymerizing a monomer–template complex and cross-linker onto surface-modified silica spheres initially, and then removing the template *via* thermal cleavage.[20] The imprinted silica film coated onto porous silica beads was first reported by Mosbach and co-workers.[21] Recently, a new imprinted silica film coating technique was developed with the template compounds covalently immobilized on the surface of silica particles prior to polymerization.[22] However, most of the present imprinting techniques display several unsatisfactory aspects including the uncontrollable film thickness, incomplete wetting of the support surface, limited density of the grafted polymer, limited film thickness because of the obvious agglomeration and gelation of particles, or relatively low binding capacity.

In this paper, we present a method that enables the *in situ* synthesis of molecularly imprinted silica films coated on spherical particles with controllable thickness. Testosterone (Fig. 1) is one of the endogenous anabolic androgenic steroids. It is of great significance to develop new adsorbents for the separation of testosterone.[23] Therefore, testosterone was selected as the template molecule in this study. As a model, silver colloidal particles were used as supports. In addition, the "sacrificial spacer" imprinting strategy, which was first developed by Whitcombe and co-workers[24,25] using covalent imprinting techniques to assemble sites that bind the target molecules in a non-covalent fashion, combines the advantages of both covalent and non-covalent imprinting methods. Consequently, a "sacrificial spacer" imprinting technique was used herein for the preparation of the imprinted material. The obtained materials were characterized by high-resolution transmission electron

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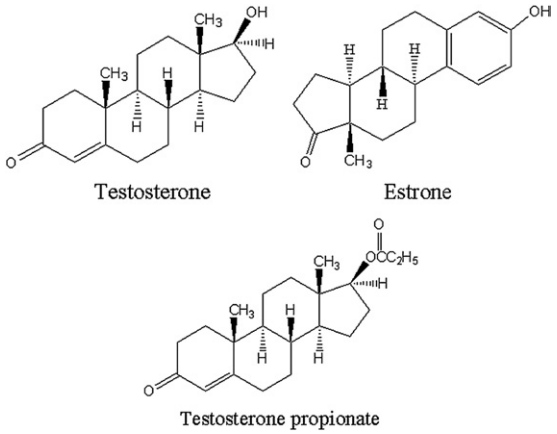

Fig. 1 Chemical structures of testosterone and its analogues.

microscopy (HRTEM) and infrared (IR) spectra. The effect of repeated gelation reactions on the thickness of the silica films was also studied. Moreover, the specific recognition ability of the imprinted particles for testosterone was investigated by steady-state binding experiments using testosterone propionate and estrone (Fig. 1) as its analogues, and their binding kinetics to testosterone were also studied.

Experimental

Chemicals

Testosterone and estrone were obtained from Acros (99%, New Jersey, USA). Testosterone propionate was bought from Hangzhou Yumei Biological Health Care Co. Ltd. (≥98%, Hangzhou, China). 3-(Triethoxysilyl)propyl isocyanate was purchased from Tokyo Kasei Kogyo Co. Ltd (Tokyo, Japan). Tetraethoxysilane (TEOS) was obtained from Aldrich (98%, Steinheim, Germany). Silver nitrate of analytical grade was acquired from Beijing Chemicals Plant (Beijing, China). 3-Aminopropyltriethoxysilane was bought from Acros (99%). L-Ascorbic acid and poly (vinylpyrrolidone) (PVP-30) with an average molar mass of 30 kg mol^{-1} were obtained from Beijing Chemical Reagents Company (Beijing, China). Gum arabic powder was bought from Tianjin Chemical Reagents No. 6 Plant (Tianjin, China). Tetrahydrofuran (THF) and chloroform were purified by a standard method prior to use. Analytical grade ethanol and Milli-Q water were used in all preparations. Other chemicals were used as received without further purification.

Preparation of TestSi

The template–monomer complex (TestSi) was synthesized *via* the reaction of 3-(triethoxysilyl)propyl isocyanate with testosterone according to our previous report.[26]

Preparation and characterization of molecularly imprinted silica films

A "sacrificial spacer" imprinting technique was used to prepare the testosterone–imprinted silica as shown in Fig. 2.

The silver particles, about 350 nm in diameter, used here were synthesized by a previously reported method.[27] The solution

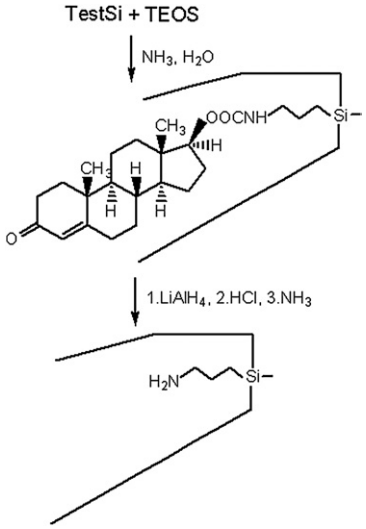

Fig. 2 Schematic synthesis route for testosterone–imprinted silica films.

containing 6.552 g ascorbic acid, 0.20 g gum arabic and 56 mL Milli-Q water was put into a round-bottom flask of 250 mL, and stirred with a variable-speed magnetic stirrer (1100 rpm). Then, the solution containing 1.571 g silver nitrate, 0.20 g gum arabic and 56 mL Milli-Q water was quickly added to the vigorously stirred acid solution. The resulting mixture was stirred for up to 24 h at room temperature under the exclusion of light. After that, the silver particles obtained were centrifuged and washed three times with water (3000 rpm for 20 min), and then kept in water for further use. The morphologies of the silver colloidal particles were characterized by scanning electron microscopy (SEM) on an AMRAY 1910FE microscope (AMRAY, USA).

The general procedure for coating silver colloids with molecularly imprinted silica films consists of two steps: growth of the silica incorporating the bound testosterone and removal of the testosterone by reduction with $LiAlH_4$. The first step followed a modified process as shown in Fig. 3.[28] Typically, 100 mL of the silver colloidal solution containing 4.72 g L^{-1} silver particles

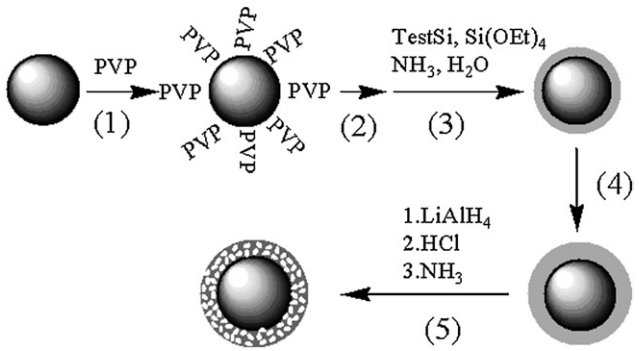

Fig. 3 Diagram of the general procedure for the coating of silver colloids with imprinted silica film. (1) PVP is adsorbed onto the colloidal particles. (2) The stabilized particles are transferred into a solution of ammonia in ethanol. (3) The template-incorporated silica shell is grown by addition of TEOS and TestSi. (4) Thicker silica shells are grown by repeating steps (2) and (3). (5) The template molecules are removed by reduction with $LiAlH_4$ and sequential treatment with HCl and NH_3 solutions.

(about 350 nm in diameter) was put into a 500 mL round-bottom flask, which was cleaned previously with hydrofluoric acid (8 vol%) and subsequently rinsed several times with water. Then, 174 mL PVP-30 solution with a concentration of 9.5 mg mL^{-1} was added to the flask and mixed by magnetically stirring (600 rpm) for 24 h at room temperature. The silver particles coated with PVP-30 were collected by centrifugation, and quickly redispersed in 173 mL of a solution of ammonia in ethanol [4.2 vol% ammonia (29.3 wt% NH_3 in water) in ethanol]. After that, 1.74 mL of a solution containing 10 vol% TEOS and 0.0211 g (0.04 mmol) TestSi in ethanol was added immediately, and the reaction was carried out under stirring (600 rpm) for 12 h. The silver particles coated with testosterone-incorporated silica films were acquired by centrifugation (3000 rpm for 20 min) and then rinsed three times with ethanol. The thickness of the testosterone-incorporated silica films can be increased by repeating the gelation reaction.

The removal of testosterone was carried out by a reported procedure.[29] Simply, the silver particles coated with testosterone-incorporated silica were dried at 60 °C, dispersed in anhydrous THF, treated with 70 mM $LiAlH_4$ under nitrogen at 60 °C for 24 h, and then sequentially rinsed with anhydrous THF, 0.1 M HCl, 0.1 M NH_3 and water over a period of 48 h. The silver particles coated with testosterone-removed silica were dried at 120 °C for 24 h for further use. Thus prepared composite materials were named as testosterone-imprinted Ag–Si particles.

These particles were then characterized by HRTEM and IR on a Hitachi H9000 NAR apparatus (Japan) operating at 300 kV and a Nicolet Magna-IR 750 FT-IR spectrometer (USA), respectively.

The control Ag–Si particles were prepared in almost the same manner as for the testosterone-imprinted Ag–Si particles except that 3-aminopropyltriethoxysilane was used in place of TestSi. All reaction steps for the preparation of Ag–Si particles were carried out under the exclusion of light.

Binding experiments

In steady-state binding experiments, imprinted or control Ag–Si particles (30 mg, prepared with a reaction time of 24 h) were added to the solutions of testosterone, testosterone propionate, or estrone in chloroform (5 mL) at various concentrations (0.5 mM, 1.0 mM, and 1.5 mM). After incubating for 24 h at room temperature, the Ag–Si particles were isolated by centrifugation. The filtrate was concentrated to dryness by evaporation of the solvent, dissolved by acetonitrile, and then analyzed by a HPLC method. The HPLC analyses were performed on an Agilent 1100 HPLC system including a quaternary pump and a variable wavelength UV detector (Agilent, USA). The instrument control and data processing were carried out by an Agilent ChemStation software. An Agilent Zorbax Eclipse XDB-C8 (5 μm, 150 mm × 4.6 mm i. d.) analytical column was used for the determination of analytes. The eluent was acetonitrile–H_2O (50/50, v/v) at a flow-rate of 1.0 mL min^{-1}. The injection volume was 20 μL, and the column effluent was monitored at 245 nm.

In kinetic binding experiments, the imprinted Ag–Si particles (125 mg, prepared with a reaction time of 24 h) were added to the solution of testosterone in chloroform (25 mL) with a concentration of 1.5 mM and mixed by magnetic stirring. Then a certain volume of the mixture was withdrawn at regular time intervals and filtrated by ultrafiltration immediately. The target molecule in the filtrate was determined by the above HPLC method.

Results and discussion

Preparation of imprinted materials

To get highly specific sites and nanoscale films, a "sacrificial spacer" imprinting technique and a previously reported coating method[28] were jointly used to prepare the testosterone-imprinted silica films coated on colloidal particles (Fig. 2 and 3).

First of all, a suitable type of colloidal particles should be selected as the support. Recently, colloidal metal particles have been of great fundamental and industrial interest.[27] There have been several methods developed for the synthesis of these particles.[30–32] However, most of these methods were developed to produce small metal particles (with radii of less than 100 nm). Only recently, large silver particles (with radii up to 1.2 μm) and gold particles (with radii up to 2 μm) were synthesized by controlled aggregation of nanoparticles in the presence of steric stabilizers.[27,30] Compared with gold particles, silver colloidal particles were more low-cost and convenient to be prepared in a wide range of sizes and with a narrow size distribution. Therefore, silver colloidal particles were selected as a model support in this study. The silver particles used here were synthesized by reducing silver nitrate with ascorbic acid in an aqueous medium.[27] Moreover, in order to separate the absorbent from the filtrate more easily, only silver particles with radii of 300–400 nm were used here. Fig. 4 shows SEM photographs of spherical and relatively monodisperse silver particles with a narrow size distribution and average radius of about 350 nm.

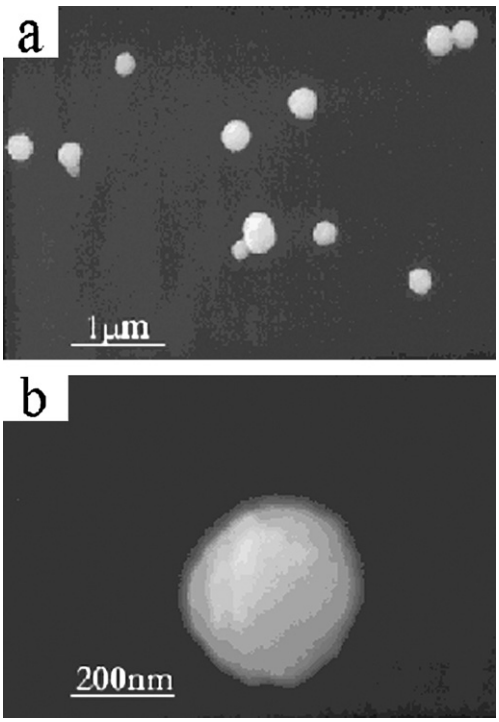

Fig. 4 SEM photographs of (a) monodisperse silver particles and (b) a single silver particle.

These characteristics are very beneficial to the preparation of testosterone-imprinted composite particles with uniform radius and film thickness.

To prepare the testosterone-imprinted silica, a monomer–template complex TestSi, synthesized according to our previous report, was chosen here.

As shown in Fig. 3, before coating with imprinted silica, the silver particles were treated with PVP-30 solution to adsorb sufficient PVP molecules, which can achieve both stabilization of the silver colloids during the film growth and a higher affinity of the silver surface to the silica.[28] After that, TestSi together with tetraethoxysilane (TEOS) was gelated on the silver particles in ethanol in the presence of aqueous NH_3, forming a testosterone-incorporated silica film (Fig. 2 and 3). Thicker silica films could be grown by repeating the gelation reaction (Fig. 3).

Testosterone molecules incorporated in the silica films were removed by reduction with $LiAlH_4$ and sequential treatment with HCl and NH_3 solutions (Fig. 2 and 3). After that, -NH_2 as a functional group was introduced inside the cavities, which were caused by the removal of the template molecules. As a result, the imprinted silica films were obtained with binding sites complementary to the template in size, shape, and position of the functional groups. The final particles acquired here were named as testosterone-imprinted Ag–Si particles. The control Ag–Si particles were prepared in the almost same way except that TestSi was replaced by 3-aminopropyltriethoxysilane.

Characterization of imprinted materials

The testosterone-imprinted Ag–Si particles were characterized by HRTEM and IR spectra. HRTEM images show that the monodisperse silver particles coated with imprinted silica have a uniform size (Fig. 5). Moreover, the imprinted silica films, prepared with gelation reactions once, twice, thrice or four times, had a uniform thickness in nanometres which would facilitate the mass transfer of template molecules in binding experiments. The thicknesses of the silica films are about 20, 50, 90, and 120 nm, respectively. The film thickness increases linearly with the time of the repeat reaction. The linear regression equation and correlated coefficient are $y = -15 + 34x$ ($r = 0.998$), where y is the average thickness of the imprinted silica film with units of nanometres, and x is the time of repeat reaction. Therefore, the thickness of the imprinted silica films can be easily controlled by repeating the gelation reaction.

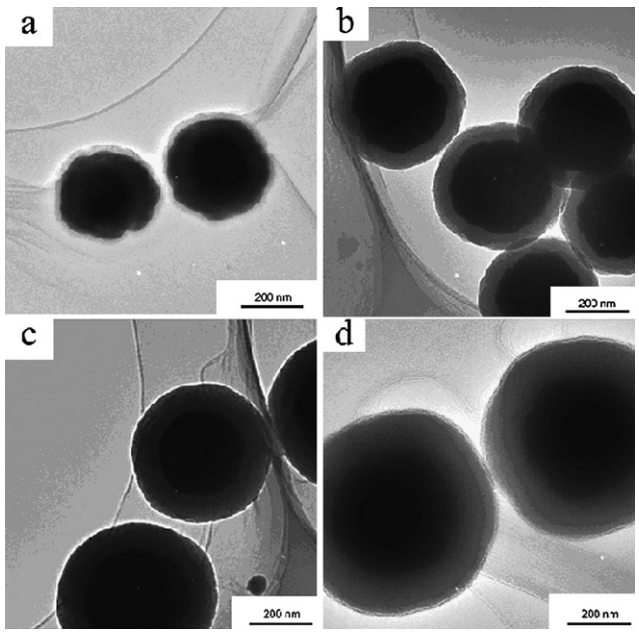

Fig. 5 HRTEM images of the silver particles coated with testosterone-imprinted silica films synthesized (a) with a reaction time of 12 h, (b) repeating reaction twice, (c) repeating reaction thrice, and (d) repeating reaction four times.

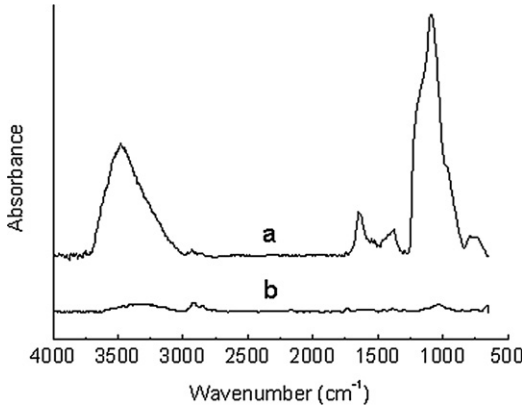

Fig. 6 FT-IR spectra of (a) the silver particles coated with testosterone-imprinted silica film and (b) the blank silver particles.

IR spectra also confirmed the formation of the imprinted silica films. As shown in Fig. 6, there is no obvious peak in the spectrum of blank silver particles, while some characteristic peaks appear in the spectrum of the imprinted Ag–Si particles. The strong peak at 1103 cm^{-1} is attributed to the stretch of Si–O–Si, indicating the formation of a silica film. The characteristic peaks of -NH_2 at 3477 cm^{-1} (ν_{N-H})[33] and 788–743 cm^{-1} (δ_{N-H}) verified the successful introduction of the functional groups in the imprinted cavities.

Binding properties of imprinted materials

The testosterone recognition ability of the imprinted Ag–Si particles was investigated by a steady-state binding method. As shown in Fig. 7, the imprinted Ag–Si particles had a much higher recognition ability than the control Ag–Si particles. In the testosterone solution with a free concentration of 0.145 mM, the imprinted Ag–Si particles bound 71% of the analyte, while the control Ag–Si particles bound only about 17%. We also investigated the specific recognition ability of the imprinted Ag–Si particles for testosterone propionate and estrone (Fig. 1), two structural analogues of testosterone, under the same conditions. The imprinted Ag–Si particles showed a much higher specific recognition ability for testosterone than both testosterone propionate and estrone (Fig. 7), indicating that the new imprinting method used in this study is successful.

The success of the imprinting technique was further assessed by measuring the affinities of analytes to imprinted Ag–Si particles *versus* those of control Ag–Si particles. When you know the masses of the solution and the Ag–Si particles, a partition coefficient K, could be calculated as follow:

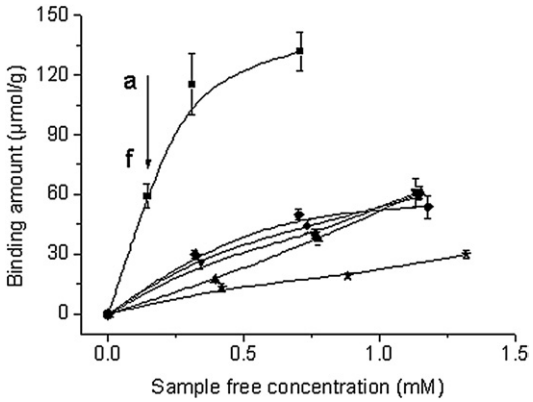

Fig. 7 Amount of bound testosterone by (a) imprinted Ag–Si and (c) control Ag–Si particles; amount of bound testosterone propionate by (d) imprinted Ag–Si and (e) control Ag–Si particles; amount of bound estrone by (b) imprinted Ag–Si and (f) control Ag–Si particles. The particles used here were all prepared with a reaction time of 24 h. The mean of three replicates was used as each binding amount with error bars indicating the standard deviation.

$$K = \frac{\left(\frac{\text{moles of the tested analyte bound to Ag-Si particles}}{\text{mass of Ag-Si particles}}\right)}{\left(\frac{\text{moles of the tested analyte remaining in solution}}{\text{mass of solution}}\right)} \quad (1)$$

Additionally, a comparison between the imprinted and control material was accomplished by calculating the imprinting factor:

$$\text{Imprinting factor (IF)} = \frac{K_i}{K_c} \quad (2)$$

where K_i and K_c are the partition coefficients of analyte for the imprinted and control Ag–Si particles, respectively.

Table 1 lists the partition coefficients of testosterone and its analogues for the imprinted and control materials. It shows that, at each of the tested sample concentrations (0.5 mM, 1.0 mM and 1.5 mM), the partition coefficients of testosterone for the imprinted particles are much higher than those of estrone and testosterone propionate, meaning that the template molecule has a relatively higher affinity for the imprinted material than its analogues. Moreover, the imprinting factors of testosterone are also much higher than those of its structural analogues. These results further verified the satisfactory imprinting efficiency of the present method.

The different affinities of the tested analytes to the imprinted materials could be attributed to their structural differences. As shown in Fig. 1, estrone has the same functional groups, a hydroxyl and a carbonyl group, as testosterone except it has a different molecular skeleton. The lower binding ability of estrone indicates that the complementarity of cavities in imprinted materials to the template in size and shape is very important for its binding ability. On the other hand, testosterone propionate possesses almost the same molecular skeleton as testosterone, and has a carbonyl group, but does not have a hydroxyl group. The still lower binding ability of testosterone propionate implies that the hydrogen bond interactions between the -OH moiety in testosterone and the -NH_2 group in imprinted materials is another significant factor for template binding. The much higher affinity of testosterone to the imprinted material should result from the synergic effect of recognition by size and shape and that by hydrogen bonding.

The repeatability and reproducibility of the imprinted materials were also investigated by using three batches of the imprinted beads prepared on different days. The particles, prepared with a reaction time of 24 h, was added to testosterone solution in 5 mL chloroform with a concentration of 1.0 mM. After incubation for 24 h, the binding amount was measured as listed in Table 2. Results showed that the repeatabilities of the three batches of particles were all satisfactory with a relative standard deviation less than 15% (13.3%, 5.5% and 13.1%, respectively). Moreover, the different batches of particles showed good reproducibility in binding template with a relative standard deviation of 11.4%.

Table 1 Partition coefficients for testosterone-imprinted and control Ag–Si particles[a]

	T			E			TP		
Conc./mM	K_i	K_c	IF	K_i	K_c	IF	K_i	K_c	IF
0.5	408	89	4.6	94	32	2.9	77	44	1.7
1.0	373	60	6.2	71	22	3.2	53	49	1.1
1.5	186	51	3.6	46	23	2.0	54	51	1.1

[a] T, E and TP represent testosterone, estrone and testosterone propionate, respectively. K_i and K_c are the partition coefficients of analytes on imprinted and control Ag–Si particles, respectively. The IF is the imprinting factor of analytes, defined as K_i/K_c. The values of K_i and K_c were calculated using the average binding amount of three replicates with relative standard deviation of less than 15% as shown in Fig. 7.

Binding kinetics of imprinted materials

The general kinetic profile of binding testosterone to molecularly imprinted Ag–Si particles was also investigated (Fig. 8). The imprinted Ag–Si particles with a silica film of thickness 50 nm were used here. It can be seen that the imprinted silica film coated on silver particles shows a very fast uptake profile with a saturation time of less than 10 min, which is much faster than those of traditional molecularly imprinted materials (more than several hours).[34–36] This phenomenon might be attributed to the film thickness in nanometres, which would greatly facilitate mass transfer of the analyte in and out of the silica matrix. This unique characteristic makes the imprinted material very suitable as a recognition element for template molecules with promising performance.

Table 2 Repeatability and reproducibility of the testosterone-imprinted particles[a]

Batch	1	2	3	Average
Binding amount/μmol g^{-1}	115.2 (13.3%)	93.7 (5.5%)	96.7 (13.1%)	101.9 (11.4%)

[a] The mean of three replicates was used for each binding amount with the relative standard deviation shown in parentheses. 30 mg of the imprinted particles were incubated in testosterone solution (1.0 mM) in 5.0 mL chloroform.

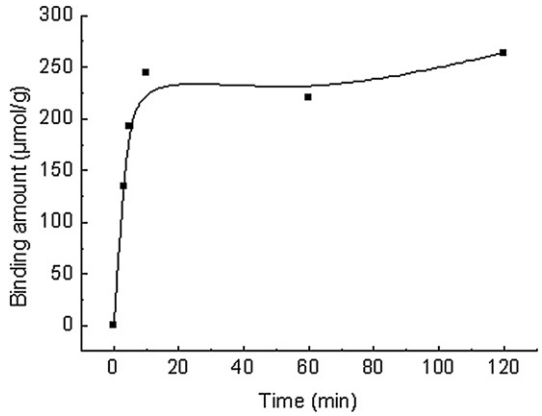

Fig. 8 Kinetic binding profile of testosterone binding to the imprinted Ag–Si particles. The imprinted particles used here were prepared with a reaction time of 24 h.

Conclusions

A method was developed for coating colloidal particles with molecularly imprinted silica films *via* a "sacrificial spacer" imprinting technique. As a model, testosterone was selected as a template molecule and silver colloidal particles as the core in this study. The method involved the *in situ* gelation of functional and cross-linking monomers in the presence of silver colloidal particles and aqueous ammonia, and the subsequent removal of the template by reduction with $LiAlH_4$. The imprinted material was obtained as spherical and monodisperse particles with a narrow size distribution, coated by molecularly imprinted silica with uniform and controlled film thickness in nanometres. Thus imprinted particles showed much higher molecular recognition ability for the template than its analogues and had very fast kinetics of binding the template, indicating the success of the imprinting technique used in this study. Moreover, due to the versatility of the coating technique used in this study,[28] the method developed here can be potentially used to prepare molecularly imprinted silica films coated on various particles with nano-, submicro- or micrometre diameters.

Acknowledgements

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