Macromolecular Imprinting by sol-gel silica in the presence of polymer grafted carbon black

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Abstract

A novel method of producing macromolecular imprinted silica particles (MIP) in the presence of polymer grafted carbon black is presented. For the silica gel matrix, tetraethyl orthosilicate (TEOS) was used as the backbone monomer and 3-aminopropyl triethoxysilane (APS) as a functional monomer. Carbon black was added to the sol-gel process, yielding black silica particles. A total of 16 MIP's were synthetized in parallel with four variables in order to better understand and evaluate the role of key reactants in the synthesis procedure. Agglomeration tests were performed with all 16 MIP's in the presence of their template, alongside their respective controls using only PBS. Each of the MIPs were evaluated using an in house built device capable of simultaneously measuring up to four samples for near infrared transmission.

1. Introduction

Molecular imprinting has existed for longer than 20 years now, and yet challenges are encountered when imprinting larger than 1500 Da molecules. This process is hindered by multiple factors such as: size, molecule complexity and conformational structure of the template [1]. Furthermore the synthesis environment is usually too aggressive for the template, where the solvent might denature the template before an imprint is formed.

To achieve macromolecular imprinting, the synthesis of macromolecular imprinted polymers (MIPs) must occur at an environment where the template can maintain proper folding and 3D structures for sufficient time frames to allow the formation of the imprints. Finally, the process must allow recapturing the template for its reutilization. If new template must be used for each synthesis, then key advantages of MIPs such as scalability and cost effectiveness are eliminated; the process would be more expensive than using traditional antibodies [2]. There are cases, however, where the availability of MIPs available as sensors or markers trump the use of antibodies, for example in remote and low resourced settings or for defense applications.

The majority of imprinting technologies involve the synthesis and testing of MIPs in organic solvents. This is necessary as to increase hydrogen bonding interactions between template and the MIP. In aqueous solutions, hydrogen bonding is reduced as well as the binding strength of non covalent template-monomer interactions in the imprinting [3]. A MIP that is capable of template recognition at aqueous environment is of great importance as this greatly reduces materials and skills necessary for the recognition and separation of molecules of interest.

We investigated molecular imprinting using sol-gel silica process in the presence of polymer grafted carbon black. The presence of polymer bound carbon black in a silica gel matrix has been previously studied to increase solvent uptake, while hydrogen bonding is formed between carbonyl groups in grafted polymer and residual silanol groups in the silica gel network [4]. Thus carbon black macromolecular imprinted silica particles are synthetized.

Since antibodies recognize target molecules by multiple weak electrostatic, hydrophobic and hydrogen bonding interactions between the antigen binding site and the paratope of the antibody [5], functional monomers that mimic such interactions are needed. For the silica gel matrix, tetraethyl orthosilicate (TEOS) was used as the backbone monomer and 3-Aminopropyl triethoxysilane (APS) as a functional monomer, while carbon black and 2-pyrrolidone also have the role of potential functional monomers.

Since MIP synthesis occurs in aqueous media, water and its ionic content play an important role in the synthesis; and we studies those parameters here. Two water concentrations were used for each type of reaction using deionized water. The reactions where then repeated using 0.2M MES saline buffer instead of deionized water. Furthermore, it is typical for Stöber processes to be carried in water/alcohol/ ammonia mixtures. Since ethanol is not essential for our current reactions, all MIPs syntheses were carried with the use of ethanol, and all reactions were repeated in the absence of ethanol in solution.

Finally sodium dodecyl sulfate (SDS) was used as a structure-directing agent. Surfactants have been used for the synthesis of mesostructured silica materials with large porosity made of uniform mesopores [6]. The importance of mesopores in MIPs particles was seen as a way of increasing template absorption in the particle, while at the same time enhancing the template removal process and limiting permanent template encapsulation by enhancing network diffusion. To test the role of surfactants in the synthesis, all chemical reactions were carried in the presence of SDS and again repeated in the absence of such.

A total of 16 MIPs were synthetized, four variables were used: water concentration, ionic content, ethanol presence, SDS presence. Each variable had two test conditions, yielding 2^4 reactions. Human Chorionic Gonadotropin (hCG) was used as the template for all syntheses. After template removal and particle washing, agglomeration tests were performed with all 16 MIP's in the presence of their template, alongside their respective controls using only PBS. Each MIPs where evaluated using an in house built device capable of simultaneously measuring up to four samples for near infrared transmission.

2. Experimental procedure

2.1. Materials

Tetraethyl orthosilicate (TEOS) and 3-aminopropyl triethoxysilane (APS) were obtained from Sigma and used as is. Human chorionic gonadotropin (hCG) was obtained lyophilized from Sigma; a solution of 20 mg/ml was prepared in PBS buffer. Sodium dodecyl sulfate was in powder from sigma and a 10 % w/v solution was prepared using ultrapure water. Hydrochloric acid (37%), ethanol, anhydrous acetic acid and methanol were acquired from Fisher Scientific. Ammonium hydroxide was obtained from sigma at 30% concentration. Polymer grafted carbon black was obtained by collecting ink from HP 33 cartridges. According to literature, average sizes of this carbon black is found to be 15 nm [7] and grafted with 2-Pyrrolidone as per MSDS. Ultrapure water was obtained from a Milli-Q Millipore unit with a water quality of at least 18.2 MΩ. 0.2M MES buffer was obtained from Fisher Scientific in 500 ml pouches.1X PBS 200 ml tablets were obtained from Sigma.

2.2. Synthesis of Imprinted Silica Particles

Imprinted silica particles were prepared by the sol-gel method. Due to the complex reaction kinetics of the silica sol-gel process, a multivariable test was performed in order to better understand the role of key reagents in the mixture. Variables for the batch process were: water content: high or low, ionic content: distilled or 0.2 MES, ethanol presence: yes or no, SDS presence: yes or no. A total of 16 parallel reactions were performed.

From previous experience, refinements to the order of mixtures and reagent quantities were performed. The order of reagent is paramount and if not properly followed, nucleation and gelation might occur prior to the addition of the template; hence no molecular imprinting will occur. Furthermore, ammonium hydroxide was used as a catalyzer and APS was limited to 172 µl. In order to minimize template exposure and to increase control in reactions, two solutions were prepared. Solution one was devised to promote gelation over nucleation in solution and to introduce carbon black and SDS if present. Since solution one can be stored without reagent consumption, two such batches were prepared beforehand with and without SDS as a variable: solution 1a was composed of 8 ml of ink, 8 ml of SDS and 6.08 ml of ammonium hydroxide. Solution 1b was composed of 8 ml of ink and 6.08 ml of ammonium hydroxide.

Solution two was devised to promote monomer nucleation, which occurs in a solution at or just below a pH of 4.7. Afterwards, functional monomer was added, which neutralizes the solution pH. Since a neutral pH does not favor nucleation, TEOS is allowed to hydrolyze prior to functional monomer addition, as the basicity and quantity of the monomer neutralizes the reaction's pH; TEOS hydrolysis is determined by the solution's return to room temperature. Once the solution achieves a neutral pH, the solution matches physiological conditions of the template. For our particular batch, pH was left as is on all solutions and HCG template was added.

Since all 16 reactions were synthesized in parallel of each other, individual solutions were prepared. In order to simplify the batch process, solutions 2 were grouped as following:

Table 1: Group 1

Solution	Water Quantity	Ionic content	Alcohol	SDS
2A	Low	Deionized	YES	YES*
2B	Low	Deionized	YES	NO†
2C	Low	Deionized	NO	YES*
2D	Low	Deionized	NO	NO†

Table 2: Group 2

Solution	Water Quantity	Ionic content	Alcohol	SDS
2E	Low	0.2M MES	YES	YES*
2F	Low	0.2M MES	YES	NO†
2G	Low	0.2M MES	NO	YES*
2H	Low	0.2M MES	NO	NO†

Table 3: Group 3

Solution	Water Quantity	Ionic content	Alcohol	SDS
2I	High	Deionized	YES	YES*
2J	High	Deionized	YES	NO†
2K	High	Deionized	NO	YES*
2L	High	Deionized	NO	NO†

Table 4: Group 4

Solution	Water Quantity	Ionic content	Alcohol	SDS
2M	High	0.2M MES	YES	YES*
2N	High	0.2M MES	YES	NO†
2O	High	0.2M MES	NO	YES*
2P	High	0.2M MES	NO	NO†

Synthesis of group 1

Solutions 2A-2D have two common variables; despite of this each solution was prepared individually. Briefly, these solutions were prepared using 1.5 ml of deionized water each. For solutions 2A and 2B, 820 µl of ethanol was added respectively. Then 6 µl of HCl was added to all solutions. All solutions were gently agitated by hand; reactions were then allowed to return to room temperature before any further reagent addition. Afterwards, 35 µl of APS was added, followed by 25 µl of HCl. It is at this stage where pH should be neutral as determined by prior experimentation.

Afterwards 65 µl of hCG template was added. A short amount of time is allowed for template adsorption at nucleating sites of the silica sol; solutions are gently agitated by hand during this process. Finally, 135 µl of APS was added followed by the appropriate solution one; for solutions 2A and 2C solution 1a and solutions 2B and 2D solution 1b were used. All solutions were topped off by adding of 40 ml of deionized water.

Synthesis of group 2

Solutions 2E - 2H were synthesized as 2A - 2D with the exception of using 0.2 M MES rather than deionized water.

Synthesis of group 3

Solutions 2I – 2L prepared using 2.4 ml of deionized water each. For solutions 2I and 2J, 820 µl of ethanol was added followed by 6 µl of HCl. All solutions were gently agitated by hand; reactions were then allowed to return to room temperature before any further reagent addition. Afterwards, 56 µl of APS was added, followed by 25 µl of HCl. Afterwards 100 µl of hCG template was added. A short amount of time is allowed for

template adsorption at nucleating sites of the silica sol; solutions are gently agitated by hand during this process. Finally, 115 µl of APS was added followed by the appropriate solution one; for solutions 2I and 2K solution 1a and solutions 2J and 2L solution 1b was used.

Synthesis of group 4

Solutions 2M – 2P were prepared as 2I-2L, with the exception of using 0.2 M MES buffer instead of deionized water.

2.6. Template Removal

To remove unreacted monomers, resulting molecular imprinted particles were centrifuged at 4000 RPM for 10 minutes, rinsed with 40 ml of deionized water in triplicate. In previous papers imprinted particles were allowed to dry and grinded against a stainless steel mortar until large agglomerates were no longer observed. This was not done because the carbon black limits the aggregate size and prevents large agglomerations and most of the imprints are located at the surface of the particles. In short particle grinding is not necessary for particles that are easily suspended in water and remain colloidal for longer than 5 minutes.

After triplicate rinsing, particles were washed in 40 ml of elution solution consisting of 50% v/v mixture of glacial acetic acid and methanol at room temperature under sonication for 10 minutes. Particles were then centrifuged at 4000 RPM for 10 minutes, 2 ml of supernatant was collected per solution. Particles were then rinsed in triplicate with 40 ml of deionized water. Absorbance of the elution supernatant was measured by uv-vis spectrometry at 280 nm. If absorbance was measured in the supernatant, this indicated unbinding of the template. If absorbance values were larger than .040, solutions were washed again in 40 ml of elution solution and rinsed in triplicate. Finally, particles were centrifuged at 4000 RPM for 10 minutes and suspended in a 1x PBS solution and stored at room temperature until needed. Particle concentrations were found to be at 58±18 mg/ml.

3. Results

After synthesis of all MIP, data was obtained as explained below.

3.1. Imprinted Particles Color Intensity

After template removal, black color intensity was observed different from groups 1 and 2 when compared against groups 3 and 4. In order to quantify color intensity, images were recorded and black saturation percentage measured using Adobe Photoshop CS5.1. Results are summarized in table 5.

Table 5: Black Color Intensity

	Group 1	Group 2	Group 3	Group 4
Particles	Color %	Color %	Color %	Color %
A|E|I|M	95	95	80	90
B|F|J|N	95	95	80	85
C|G|K|O	95	95	90	85
D|H|L|P	95	95	80	85

3.2. Reagent Retention and Template Removal

During template removal process, batch groups 1 and 2 where particularly difficult to process. These set of particles better retained reagents and SDS as well. Because of this, group 1 and 2 particles required an extra washing procedure when compared to groups 3 and 4. These samples also showed darker color intensity. Thus, we conclude that higher carbon black concentrations enhances solvent uptake, but at the same time this suppresses washing effectiveness probably by increasing network crosslinking.

In addition of being required for molecular imprinting, excessive concentrations of polymer grafted carbon black will slower the gelation process which is necessary for molecular imprinting to occur. Thus, we conclude that there is a best concentration range of carbon black approximately between 0.5 and 1.5 %. This is because carbon black must render enough material to assist in molecular imprinting while at the same time the effect of reagent uptake and the suppression of the gelation process must be limited.

3.4. Rate of Precipitation

In order to quantify precipitation rates, a device was built with 4 sensors capable of reading infrared transmission changes per time in a standard disposable cuvette. The device is capable of collecting changes from full dispersion up to almost full precipitation with a resolution of 1024 bits. The device was programed to collect values of all sensors simultaneously every 2 seconds and values where recorded and transferred to a spread sheet for processing and evaluation.

For rate of precipitation tests, 2 mg of all batch particles where collected in solutions individually. Then 400 µg of hCG was added to each. Finally the solutions where suspended in a total volume of 3 ml in 1xPBS solution. The same process was repeated but with the absence of hCG as a control value of rate of precipitation per solution. Tests were performed in pairs of solutions with their respective template and control per particle type.

Figure 1 shows the near IR transmission through a cuvette in the presence of dispersed beads for each pair as function of time. As can be seen from the figure, all solutions block nearly all transmission initially, but become more transparent as the particles settle due to gravity and agglomeration. The rate of precipitation is determined by the slope of the curves As each batch will have a slightly different precipitation rate, the ratio of the rates when hCG was added to the rates in solvent alone were calculated. A successful imprint was determined if a ratio equal or greater than 2 was observed, meaning that the rate of precipitation for the template containing solution must be at least twice as fast as the rate of precipitation of its own control. Table 6 summarizes the precipitation rates for the 16 samples.

Table 6: Ratio of Template Over Control Precipitation Slopes.

	Group 1	Group 2	Group 3	Group 4
Particles	Ratio	Ratio	Ratio	Ratio
A|E|I|M	1	2	4.15	5.08
B|F|J|N	1	1	4.22	1
C|G|K|O	1	1	1	1
D|H|L|P	1	1	2.83	N/A

N/A: Estimated values from prior observations. Particles were not available for testing with device.

For group 1, there was no successful imprinting in any of the particles A to D. In group 2 only particles 2E were successful in rebinding their template but had the smallest slope ratio of all other successful imprints. In group 3, particles 2I, 2J and 2L would rebind their target, whereas 3L had the least slope ratio from the group and second smallest from the batch. In group 4, particles 2M and 2P rebound to their template and particles 2M showed the greatest measured slope ratio from all other particles in the batch. Even though particles 2P were not measured by the device as the particles were exhausted prior to the device being ready for use, prior observational experiments would point to a very high ratio of precipitation slopes.

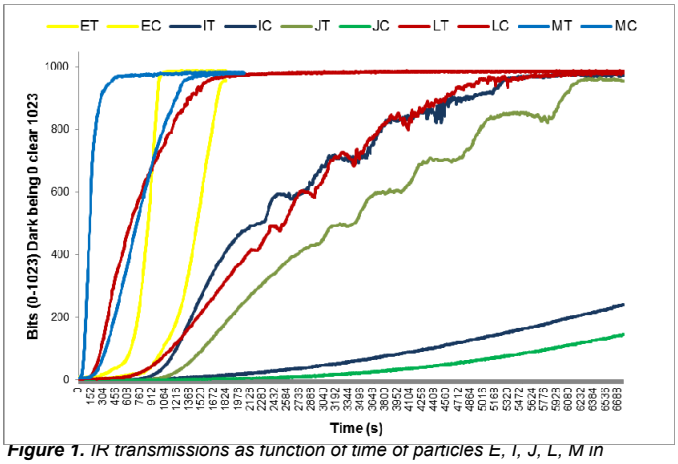

Figure 1. *IR transmissions as function of time of particles E, I, J, L, M in presence and absence of their template (hCG).*

3.4. Discussion

From the graphs in Figure 1, it can be seen that groups 3 and 4 precipitated faster than particles in group 2. These results are in agreement with the previous reasoning that an optimal carbon black ratio must be found in order to achieve good rebinding and good particle dispersion. If control particles remain for an indefinite amount of time under dispersion, then it will be difficult to achieve precipitation of template loaded particles since dispersion is widely favored by the 2-Pyrrolidone.

Particles produced in the presence of SDS and ethanol yielded a total of 3 out of 4 successful imprints, whereas particles produced in the absence of SDS and ethanol produced a total of 2 out 4 successful imprints. For particles produced in the presence of ethanol and the absence of SDS, only particle J imprinted successfully. No particles where successfully imprinted for those in the presence of SDS and the absence of ethanol.

Based on these observations, it is clear that SDS is not a required reagent as thought of before, but it does play a role in the reaction increasing the chance of a successful imprint. It is then believed that an optimal SDS quantity must be used for templates that are able to tolerate low doses of SDS, whereas if a delicate template is used SDS could be be avoided. The role of ethanol is yet unclear, it seems to be more important when SDS is also used, potentially it acts as a dispersant od SDS.

Water content was found to be particularly important for the material yield of the synthesis. Where group 1 and 2 had the least material and group 3 and 4 produced the most material. Furthermore ionic content role was found more difficult to interpret since deionized water produced the same amount of successful imprints as those produced in 0.2 MES. Despite of this 0.2 MES is believed to be the best alternative as 2 of its 3 successful imprints produced the largest slope ratios from the batch. Furthermore all particles produced under 0.2 MES produced straight slopes whereas particles produced with deionized water had steps in each of the template containing solutions with successful imprinted particles.

Finally, a new imprinting method is proposed for this particular sol-gel process, where molecular imprinting occurs at the gelation process and not in the nucleation of the monomers; hence produced MIPs are not bulk particles but rather a new type of MIP were template imprinting occurs at the aggregation sites between primary particles. This imprinting method could be thought of as a new type of surface imprinting.

4. Conclusions

The effect of water, quantity, ionic content, SDS and ethanol in the reaction environment to synthesize of molecularly imprinted particles was investigated. The ability of the MIPs to rebind their target was evaluated by measuring sedimentation rates. While no clear rules can be derived yet, the amount of water is critical, the amount of carbon black should be below 1.5%, SDS and alcohol should either both be present or absent and it is preferable to use MEP over deionized water. Resulting MIPs are surface imprinted and can rebind their target. Under the best reaction conditions tested to date, the MIPs will precipitate by a factor of 5 times faster in the presence of their template that without it. When sealed from the environment, the MIPs are stable for months, which may make them very attractive to be used as sensors in low resource or defense applications.

References

[1] David R. Kryscio and Nicholas A. Peppas, "Critical review and perspective of macromolecularly imprinted polymers," Acta Biomaterialia, vol. 8, pp. 461-473, November 2012.

[2] Jenna L. Bowen, Panagiotis Manesiotis, and Chris J. Allender, "Twenty years since "antibody mimics" by molecular imprinting were first proposed: A critical perspective," Molecular Imprinting, vol. 1, pp. 35-40, February 2013.

[3] Daniel M. Hawkins, Derek Stevenson, and Subrayal M. Reddy, "Investigation of protein imprinting in hydrogel-based molecularly imprinted polymers (HydroMIPs) ," Analytica Chimica Acta, vol. 542, pp. 61-65, March 2005.

[4] Kazuhiro Fujiki, Tomoaki Ogasawara, and Norio Tsubokawa, "Preparation of a silica gel-carbon black composite by the sol-gel process in the presence of polymer-grafted carbon black," Journal of Materials Science , vol. 33, pp. 1871-1879, December 1998.

[5] Yu Hoshino et al., "Recognition, Neutralization, and Clearance of Target Peptides in the Bloodstream of Living Mice by Molecularly Imprinted Polymer Nanoparticles: A Plastic Antibody," Journal American Chemical Society, vol. 132, pp. 6644-6645, March 2010.

[6] Patricia Valle-Vigon, Marta Sevilla, and Antonio B. Fuertes, "Mesostructured silica-carbon composities synthesized by employing surfactants as carbon source," Microporous and Mesoporous Materials, vol. 134, pp. 165-174, June 2010.

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