Optical sensing

DOI: 10.1002/anie.201107644

Cubic Molecularly Imprinted Polymer Nanoparticles with a Fluorescent Core**

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Molecular recognition is a fundamental process for all living systems. Applications of the phenomenon have exploited antibodies as the selective components of immunoassays, affinity phases and biosensors and as therapeutic agents. Molecularly imprinted polymers (MIPs) are one of the leading contenders for alternatives to antibodies.[1] MIPs generally are cross-linked polymers synthesized in the presence of the target compound (the template). After the removal of the template, cavities with complementary size and shape to the template are created, which can recognize and selectively rebind the template.[1] MIPs have been successfully applied to solid-phase extraction[2] and in biosensors.[3] The synthesis of MIPs with architectures which are well defined on the molecular level is a future priority.

MIP nanoparticles (NPs) can be prepared by a number of methods.[4] Each of these have some disadvantages including: production of particles with irregular shapes and sizes, heterogeneous binding sites, decreased affinity due to use of surfactants and high polymerization temperatures, long polymerization time, relatively low yield, etc. Here, we have developed a new core–shell approach.

In order to detect binding, fluorescence is a promising technique, since fluorescent labeling can be a powerful tool for probing the local environment, as well as for biological imaging.[5] As a site for placement of a fluorescent label, the NP core is a promising location, since it is isolated from the bulk solution by the surrounding polymer shell. There are currently two reported approaches for the incorporation of fluorescent species in the core of MIP NPs. One example involved the creation of a MIP layer over inorganic quantum dots.[6] In the second case two layers were created over silica-coated magnetic particles; the first consisting of a fluorescently labeled polymer and the second, outer layer, was the MIP.[7]

In our approach dendrimers were selected as the core from which to graft a MIP shell to form imprinted NPs. Dendrimers are biocompatible, soluble macromolecules possessing highly defined regularly branched structures, a well defined shape, size and number of peripheral functionalities.[8] Using dendrimers as the core in a controlled or "living" polymerization would ensure precise grafting at the molecular level. An earlier example of dendrimer-based MIP NPs was reported by Zimmerman.[9] The monomolecular imprinted NPs formed had only one binding site at the centre of the particles and involved a long and tedious synthesis. The use of dendrimer-based macroinitiators for the synthesis of NPs using atom transfer living radical,[10] nitroxide-initiated[11] and microemulsion[12] polymerizations have been reported. Unfortunately the synthesis conditions are mostly incompatible with the imprinting process (high temperature, presence of ions, etc.). Much more favorable conditions would be associated with use of iniferters (initiator, chain transfer agent, terminator).[13]

Here we describe the synthesis of NPs in solution without the use of surfactant and no requirement for high temperatures in ≤ 120 s polymerization time. The NPs are dispersible in both aqueous and organic solvents. A unique, versatile and highly controlled technique combining dendrimer-based macroiniferters, living polymerization, nanotechnology, molecular imprinting and novel fluorescent sensing technique is demonstrated.

In order to first characterize the properties of dendrimer-core NPs, a non-fluorescent core was used. Iniferter units were attached on the periphery of polyamidoamine (PAMAM) dendrimers, generation 4, creating a soluble macroiniferter. This was used as core for the synthesis of NPs (Scheme 1). As iniferter *S*-(carboxypropyl)-*N*,*N*-diethyldithiocarbamic acid (CNDDA) was used and coupled to the peripheral primary amino groups of the dendrimers (Supporting Information).

The polymerization was initiated simultaneously from potentially 48 points per dendrimer moiety. Dendrimer-core MIP-shell NPs were synthesized in just two minutes by UV irradiation. The use of UV-initiated living radical polymerization, besides imparting better control over the size distribution, has another fundamental advantage. The polymerization process can be re-initiated by UV exposure, where the already synthesized NPs will act as a macroinitiator,[13] allowing many possibilities for further modification of the particle surface properties.

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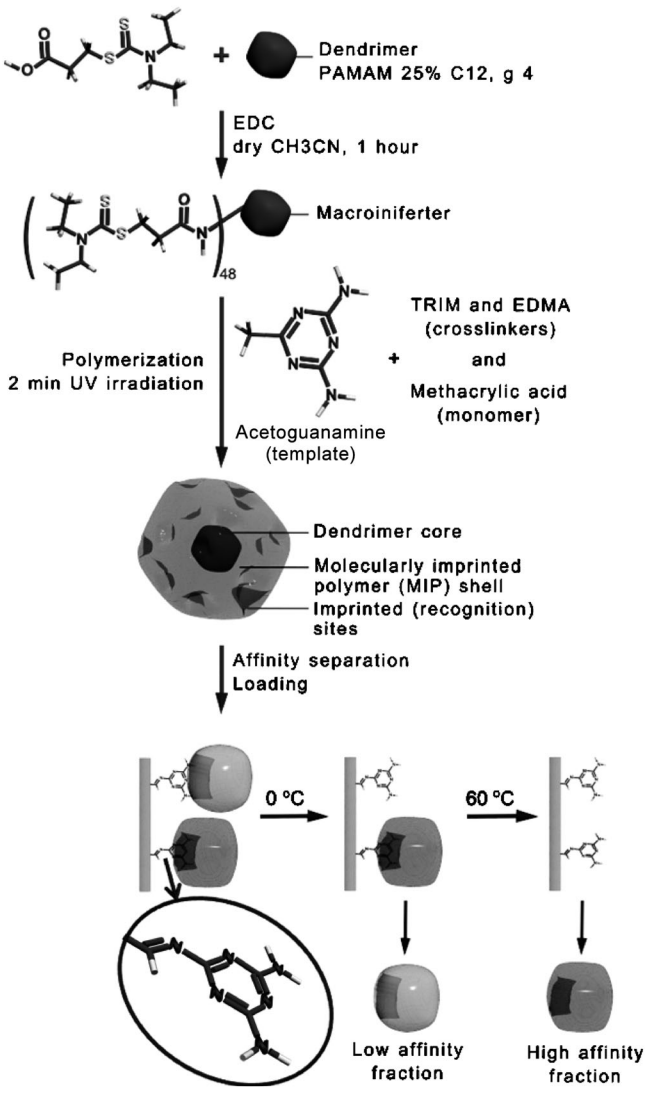
[**] The authors thank the European Union for funding this work under the Framework 7 programme, CP-FP 226524 Water treatment by molecularly imprinted materials (WATERMIM), and Malvern Instruments Ltd. for the zeta potential measurements.

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201107644.



Scheme 1. Schematic representation of the synthesis of the NPs. EDC = 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride, TRIM = trimethylolpropane trimethacrylate, EDMA = ethyleneglycol dimethacrylate.

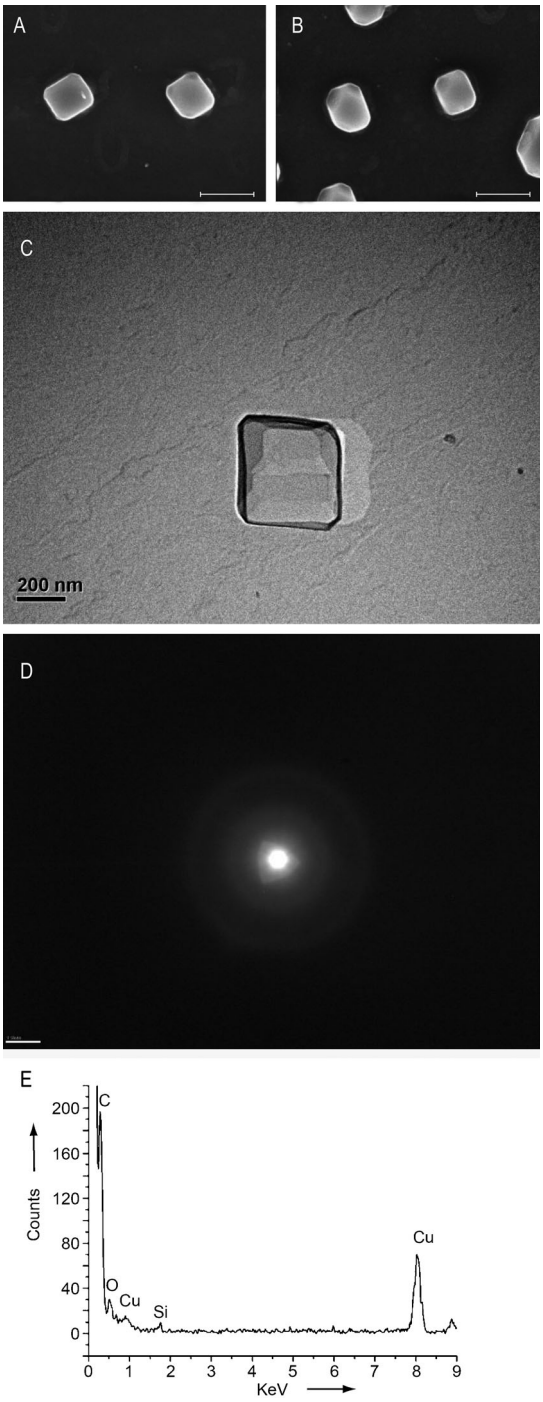


Figure 1. A,B) SEM photographs of MIP NPs (A) and blank NPs (B). C) TEM of blank NPs. D) Electron diffraction pattern of a cube-like NP, showing it to be amorphous in nature; E) EDX spectrum of a cubic NP suggested it is rich in both C and O.

As a model system we chose to imprint a methacrylic acid-based polymer shell with the triazine derivative 2,4-diamino-6-methyl-1,3,5-triazine (acetoguanamine), which is a close structural analogue of melamine. Triazines have been successfully imprinted before, using similar monomer compositions.[14,15] Control (blank) polymers were prepared following the same protocol but in the absence of template. The NPs produced were characterized by dynamic light scattering (DLS) measurements (Supporting Information, Table S3, Figure S6). Grafting from the surface of the dendrimer macroiniferter was achieved in homogeneous solution without the use of surfactants. The NPs were separated by affinity purification to isolate a fraction with high affinity for the template.[14]

MIP and blank NPs were analyzed by scanning electron microscopy (SEM) (Figure 1 A and B). To our surprise, the analyzed samples were found to contain NPs that were cubic in shape, with sizes between 200 and 300 nm. Reducing the irradiation time for formation of the imprinted shell to 1 min resulted in the formation of 100 nm, and to 30 s of 60 nm, NPs. The shape however was consistent in all cases (Figure S7). Additional images and electron diffraction patterns of blank NPs were recorded using transmission electron microscopy (TEM) which are consistent with the SEM images (Figure 1 C). Electron diffraction patterns of the NPs showed that

they were amorphous (Figure 1D). Chemical analysis using energy-dispersive X-rays (EDX) showed major peaks for carbon and oxygen, with a weak peak due to silicon (Figure 1E). Peaks assigned to copper came from the support grid. No other elements were observed to be present. The weak silicon peak may come from the detector.[16] Thus the organic nature of the cubic-shaped NPs could be unambiguously confirmed. To our knowledge, this is the first report of the formation of cube-like organic NPs and appears to be a result of polymerization from a dendrimer core.

The ability of the synthesized NPs to bind to the target compound was demonstrated by surface plasmon resonance (SPR) (Figure 2). The apparent dissociation constant, K_d, was determined to be 2.9×10^{-14} M for MIP, and 6.3×10^{-13} M for blank NPs on the specific surface (Supporting Information). The apparent K_d calculated for MIP NPs was 8.5×10^{-12} M and for blank 6.2×10^{-11} M on the non-specific surface.

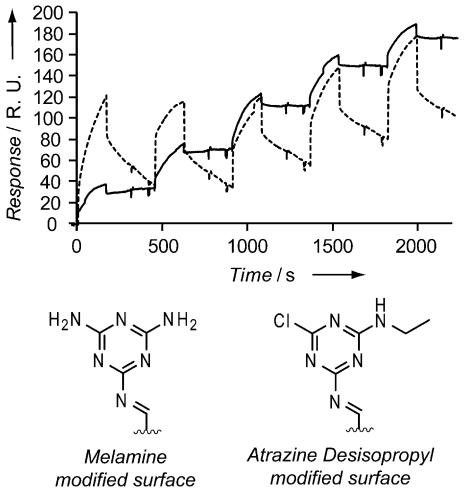


Figure 2. Above: Sensogram showing binding of MIP NPs to the specific ligand (melamine, solid line) and to a non-specific analogue (atrazine desisopropyl, dashed line) immobilized on Biacore sensor chips. Concentration of surface-accessible imprinted binding sites estimated to be present in the undiluted dispersion of MIP NPs in PBS was 2.19 μM. Injections were made in order of increasing concentration using dilutions of: 1:10000, 1:1000, 1:100, 1:10, and 1:1 with respect to the stock NP dispersion. Below: structures of the two ligand species.

The calculated apparent dissociation constants are much lower than the range of values normally determined by equilibrium binding experiments with soluble ligands, e.g. a K_d of 6.6×10^{-8} M was calculated in an earlier work for nanoparticles imprinted with the same template, immobilized on a polymer support.[14] A possible explanation could involve multiple binding interactions between functional groups on the particle surface and ligands immobilized on the surface of the sensor chip. The reported K_ds therefore are useful as a guide only and the figures should be considered as "apparent" rather than "absolute" dissociation constants.

The MIP and blank NPs bind with similar K_d values (differing only by a factor of 7) to the non-specific surface. The difference in K_d values for binding to the specific surface is greater (factor of 21 in favor of the MIP). This is despite the fraction of blank NPs used having been selected by affinity chromatography for their ability to bind to the same ligand (melamine on glass beads). Hoshino et al.[17] have shown that affinity chromatography can be used to separate a fraction of nanoparticles with high affinity for a peptide target (mellitin) from a random pool of non-imprinted particles in a similar process. Imprinting therefore has improved the strength of binding for the MIP.[18]

In order to investigate how NPs made by this approach would perform as fluorescent sensors, a fluorescent label (dansyl chloride) was covalently attached to 50% of the core dendrimer peripheral amine groups before polymerization of the shell. The remaining amino groups were modified with iniferter residues, as described above. MIP shells were created over the dendrimer core by irradiation for 1 min using a polymerization mixture four times more dilute than previously used in order to create a thinner polymer shell. Cubic NPs with the dimensions of 50 nm were created in this way (Figure S8). The method allows for the formation of NPs with a precisely controlled quantity of fluorescent label. Covalent attachment of fluorophores to the core prevents their migration within the polymer shell and shields them from the environment. Exposing the fluorescent-core NPs to solutions containing different concentrations of the template for just 10 min resulted in a concentration-dependant increase in the NPs fluorescence (Figure 3). The local environment of

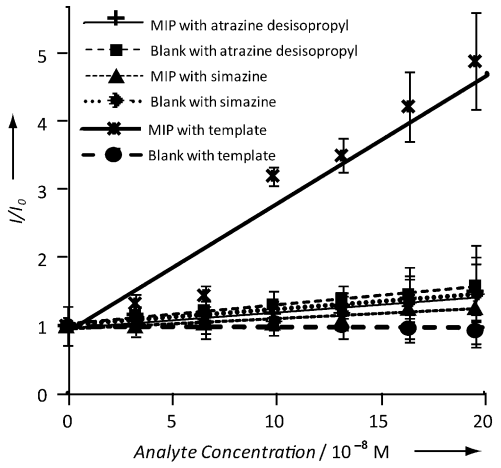


Figure 3. Intensity of the fluorescent response of MIP and blank NPs to different concentrations of the template and close analogues (atrazine desisopropyl and simazine), in acetonitrile after 10 min incubation. Error bars represent ±1 standard deviation ($n=3$).

the dansyl group, including the hydrophilic/hydrophobic nature of the solvent, determines its fluorescence properties.[19] Binding of template to the imprinted polymer shell will lead to expulsion of solvent molecules and changes in conformation of the polymer shell which have an indirect effect on the fluorophores located at the core–shell boundary. In our case this led to an observed increase in fluorescent intensity; other examples of increased fluorescence of dansyl-labeled NPs on interaction with for example, hemoglobin

have been reported.[20] Almost no change in the level of fluorescence was seen under the same conditions with blank NPs, or with MIP or blank NPs in the presence of analogues of the template (atrazine desisopropyl or simazine). The limit of detection for acetoguanamine was calculated to be 3×10^{-8} M.

In conclusion, we have demonstrated the synthesis of organic NPs with regular and reproducible shape and size for both MIP and blank NPs. For the first time cubic organic NPs are reported. Cubic NPs were an unexpected result and the origin of this morphology could be the subject of future study, however this shape may be an advantage where close-packing of NP-based material is required, such as in dense coatings. We demonstrated very good specificity and selectivity of the synthesized material. Fluorescent-core cubic MIP NPs were prepared in an innovative concept. They demonstrated excellent selectivity and affinity after just 10 min incubation time. Advantages such as precise control of the number of the fluorescent labels per particle and its polymer shielding are reported for the first time as a new technique. NPs prepared in this way are promising materials to replace antibodies in sensors and immunoassays and in drug delivery and diagnostics.

Received: October 30, 2011
Revised: March 9, 2012
Published online: April 13, 2012

Keywords: biosensors · core–shell structures · dansyl group · living polymerization · surface plasmon resonance

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