

Nanostructured materials for selective recognition and targeted drug delivery

O Kotrotsiou, K Kotti, E Dini, O Kammona and C Kiparissides

Chemical Engineering Department, Aristotle University of Thessaloniki and Chemical Process Engineering Research Institute, P.O. Box 472, 54006 Thessaloniki, Greece

E-mail: cypress@cperi.certh.gr

Abstract. Selective recognition requires the introduction of a molecular memory into a polymer matrix in order to make it capable of rebinding an analyte with a very high specificity. In addition, targeted drug delivery requires drug-loaded vesicles which preferentially localize to the sites of injury and avoid uptake into uninvolved tissues. The rapid evolution of nanotechnology is aiming to fulfill the goal of selective recognition and optimal drug delivery through the development of molecularly imprinted polymeric (MIP) nanoparticles, tailor-made for a diverse range of analytes (e.g., pharmaceuticals, pesticides, amino acids, etc.) and of nanostructured targeted drug carriers (e.g., liposomes and micelles) with increased circulation lifetimes. In the present study, PLGA microparticles containing multilamellar vesicles (MLVs), and MIP nanoparticles were synthesized to be employed as drug carriers and synthetic receptors respectively.

1. Introduction

The on-going discovery of novel phenomena and processes at the nanometer scale is providing science with a wide range of tools, materials, devices and systems with unique characteristics. Nanostructured materials, nanodevices and novel nanofabrication processes have created great excitement in biomedical research and technologies because of their numerous and diverse applications. The capability of synthesizing and processing nanomaterials with tailored structures and enhanced properties provide tremendous opportunities for designing novel biomaterials of exceptional promise for biomedical applications. Nanotechnology will help reach the elusive goal of selective recognition and active drug targeting to specific cells within the body by the development of miniature drug-carriers (e.g., nanoparticles, liposomes, micelles) with properly modified surfaces in order to avoid interactions with other vehicles, cells and proteins and thus with increased lifetimes [1], as well as through the synthesis of molecularly imprinted polymers (MIPs) and MIP-sensors.

Liposomes are microscopic and submicroscopic vesicles with sizes ranging from 10nm to 20 μ m. They are usually made up of phospholipids, although other amphiphiles such as nonionic surfactants can also be employed for their construction. When phospholipids are hydrated, they spontaneously form spherical lipid bilayers enclosing the aqueous medium and the solute. Liposomes offer several advantages over other delivery systems including biocompatibility, control of biological properties via modification of physical properties (e.g., lipid composition, vesicle size, lipid membrane fluidity etc.) and several modes for drug delivery to cells (e.g., absorption, fuse, endocytosis, phagocytosis).

Liposomes can be classified according to the number of the lipid bilayers as unilamellar vesicles (ULVs) and multilamellar vesicles (MLVs). Functionalized liposomes can be synthesized using peptides and oligosaccharides in order to achieve both targeting and circulation longevity. Peptides can be used in order to guide liposomes to desired receptors whereas, poly(ethylene oxide) (PEO)-grafted phospholipids are known to dramatically increase liposome survival in the circulation. A surface modified liposomal drug delivery vehicle can be developed for selective targeting by coupling an arginine-glycine- aspartic acid (RGD) peptide to the liposome through a PEO spacer [2].

Polymeric micelles are the simplest of all amphiphilic self-organizing structures. They have diameters in the range of 10-200 nm depending on the length and solvation properties of the polymer blocks and they can be used as drug delivery systems for hydrophobic drugs. PEO is commonly used as the corona-forming block aiming to optimize the surface properties of the polymeric micelle carriers, since it is a non-toxic, biocompatible, highly hydrated polymer. Additionally, PEO prevents the opsonization of drug vehicles, i.e. interactions with proteins such as immunoglobulins that identify drug vehicles as being of foreign origin. The drug loading of the micelles can be achieved either by direct addition of the drug to the micellar aqueous solution or by dissolution of the drug and the polymer in a common solvent followed by dialysis against water [3]. Functional block copolymers can be designed aiming to the synthesis of micelles with increased stability, improved drug targeting and controlled drug release.

Molecular imprinting of synthetic polymers is a process where functional and cross-linking monomers are co-polymerized in the presence of the target analyte i.e., the imprint molecule, which acts as a molecular template. The functional monomers initially form a complex with the imprint molecule, and following polymerization, their functional groups are held in position by the highly cross-linked polymeric structure. Subsequent removal of the imprint molecule reveals binding sites that are complementary in size and shape to the analyte. Three particular features have made molecularly imprinted polymers (MIPs) the target of intense investigation: i) their high affinity and selectivity, which are similar to those of natural receptors, ii) their unique stability which is superior to that demonstrated by natural biomolecules and iii) the simplicity of their preparation and the ease of adaptation to different practical applications. Molecularly imprinted polymers can be prepared in a variety of physical forms to suit the final application desired [4,5].

2. Liposomes

In the present study, MLVs were synthesized using hydration, followed by sonication and extrusion. Various types of phospholipids (e.g., Phospholipon 80, 80H, 90 and 90H) and cholesterol were employed for the synthesis of the liposomes. Hydroquinone, a hydrophilic drug used for skin whitening was employed as the active ingredient. The morphology of the MLVs after the hydration step was examined by means of optical microscopy (Figure 1a) and their size distribution was measured by dynamic light scattering. The size of the MLVs was found to depend on the preparation method (Figure 1b) the type of the phospholipid and the pore size of the membrane used during the extrusion process. The hydroquinone-loaded MLVs were subsequently encapsulated in poly(lactic-co-glycolic acid) (PLGA) microparticles employing a complex solvent evaporation process. Simple PLGA particles containing hydroquinone were also prepared using the same technique. An aqueous solution of hydroquinone or of hydroquinone-loaded liposomes was added to a PLGA solution in dichloromethane resulting in the formation of a w/o emulsion [6]. The latter was then added to an aqueous PVA solution leading to the formation of a w/o/w emulsion. Simple and composite (Figure 1c), spherical PLGA microparticles were formed by solvent evaporation from the w/o/w emulsion at increased temperature. The release rate of hydroquinone from the composite PLGA microparticles was compared to that from the simple ones and was found to be significantly retarded (Figure 1d).

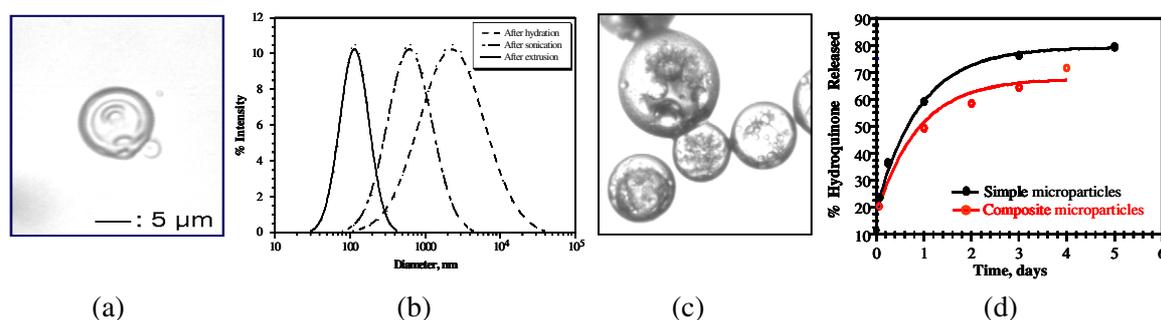


Figure 1. (a) Optical micrograph of MLVs prepared by hydration, (b) effect of preparation method on PSD, (c) optical micrograph of composite PLGA particles and (d) effect of PLGA particle type on the release rate of hydroquinone.

3. Molecularly Imprinted Polymers

In the present study, precipitation polymerization was employed for the synthesis of MIP nanoparticles to be used as synthetic receptors selective for theophylline [7,8] and simazine [9]. The template molecule was added in acetonitrile in a borosilicate glass tube equipped with a screw cap followed by the addition of the functional monomer, methacrylic acid, the crosslinker, EGDMA and the initiator, AIBN. The polymerization was induced by placing the tube in a preheated water bath. The resultant polymeric nanoparticles were collected by centrifugation and were subsequently washed (five times) with methanol containing 10% (v/v) acetic acid to remove the template species. After the fifth washing cycle the MIP particles were placed in acetonitrile for 24hrs and were then collected by centrifugation. UV spectroscopy was employed to verify the removal of the print molecules. As it was expected, no adsorption of the print molecules was observed, indicating that the template species had been removed. Non-imprinted polymeric nanoparticles were also prepared under the same conditions in order to compare their affinity to the template molecules with that of the MIP particles. The surface morphology of the polymeric particles was assessed by SEM (Figures 2 and 3a,b) and their size distribution was determined by laser diffraction (Figure 3c). As can be seen from figures 2 and 3a,b, both MIP and non-imprinted particles exhibit a rough, porous surface indicating that the presence of the template does not influence significantly the polymer morphology.

UV spectroscopy was employed to measure the affinity and selectivity of the MIP particles. A small amount of nanoparticles (e.g., 0.1gr) were incubated overnight at room temperature in print molecule/acetonitrile solutions of known concentrations (e.g., 1.4 μmole of theophylline/ml of solvent and 0.8 μmole of simazine/ml of solvent). The binding capacity of the MIP particles was compared to that of the non-imprinted polymeric nanoparticles. It was shown that the nanoparticles which were imprinted with theophylline adsorb 14.57 μmole of theophylline per 1gr of polymer whereas, those imprinted with simazine were found to adsorb 2.2 μmole of simazine per 1gr of polymer. When non-imprinted polymeric nanoparticles were used, 3.12 μmole of theophylline per 1gr of polymer were adsorbed, whereas, in the case of simazine, minimal binding of the target analyte was observed. Competitive analysis was also performed employing caffeine, an analyte which is chemically-related to theophylline, in order to examine the selectivity of the theophylline imprinted polymeric receptors. It was shown that the MIP nanoparticles adsorb 1.79 μmole of caffeine per 1gr of polymer thus, proving the selectivity of the artificial receptors towards the template molecule.

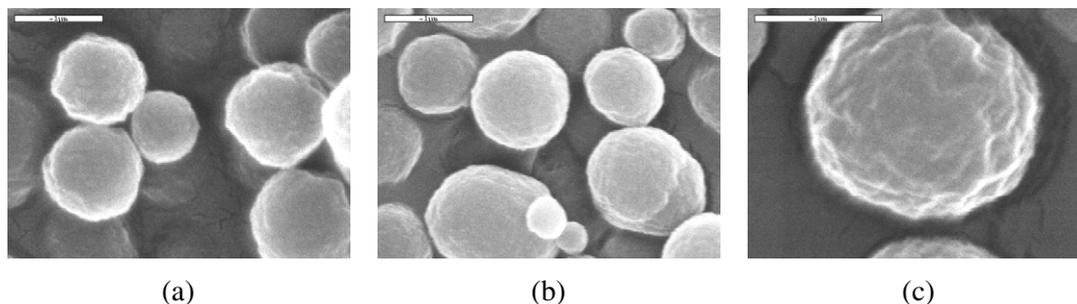


Figure 2. SEM photomicrographs of the MIP nanoparticles against (a) theophylline and (b,c) simazine.

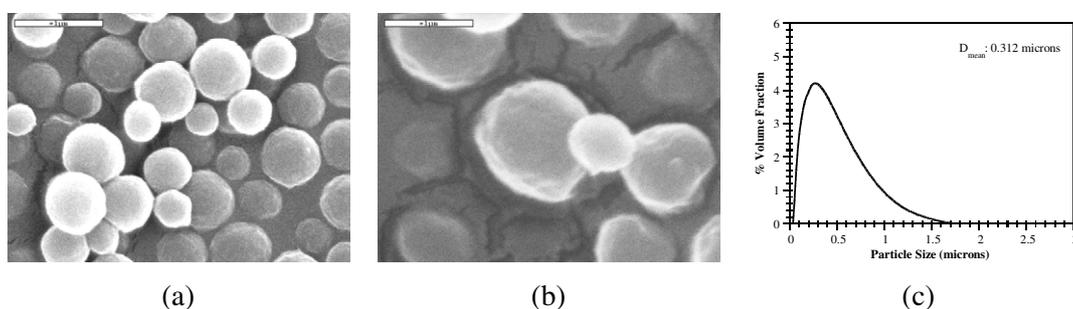


Figure 3. (a, b) SEM photomicrograph and (c) PSD of the non-imprinted polymeric nanoparticles.

4. References

- [1] Alexandridou S and Kiparissides C, *Proc. of the EC-NSF Workshop on Nanotechnology-Revolutionary Opportunities and Societal Implications* (Lecce, Italy, January 31-February 1, 2002)
- [2] Lestini B J, Sagnella S M, Xu Z, Shive M S, Richter N J, Jayaseharan J, Case A J, Kottke-Marchant K, Anderson J M and Marchant R E 2002 *J. Controlled Release* **78** 235-47
- [3] Jones M C and Leroux J C 1999 *Eur. J. Pharm. Biopharm.* **48** 101-11
- [4] Byrne M E, Park K and Peppas N A 2002 *Adv. Drug Delivery Rev.* **54** 149-61
- [5] Allender C J, Richardson C, Woodhouse B, Heard C M and Brain K R 2000 *Int. J. Pharm.* **195** 39-43
- [6] Hans M C and Lowman A M 2002 *Current Opinion in Solid State and Materials Science* **6**, 319-327
- [7] Ye L, Weiss R and Mosbach K 2000 *Macromolecules* **33**, 8239-45
- [8] Ciardelli G, Cioni B, Cristallini C, Barbani N, Silvestri D and Giusti P 2004 *Biosens. Bioelectron* (in press)
- [9] Matsui J, Miyoshi Y, Doblhoff-Dier O and Takeuchi T 1995 *Anal. Chem.* **67** 4404-08