Characterization of Adhesion Between Chemical Robots and Biological Substrates

Tokarova V., Ullrich M., Haufova P., Pittermannova A., *Stepanek F.
Inst Chem Technol Prague, Czech Republic (frantisek.stepanek@vscht.cz)

INTRODUCTION AND OBJECTIVE

Composite microparticles for targeted delivery of active substances are of both practical and fundamental interest. The purpose of such delivery vehicles is to store the active compound at a high concentration, transport it through an environment and eventually anchor to the target site and release the encapsulated payload either spontaneously or upon an external stimulus. For the design a targeted delivery system, not only the release kinetics of the encapsulated compound but also the specific interactions of the microcapsules with the target point of controlled release – their adhesion properties – are crucial.

This work describes the synthesis, composition and characterization of two types of such composite particles – chemical robots – consisting of a hydrogel core and a mesoporous silica shell. The first particle type is prepared by the InkJet technology (Dohnal 2010) and consists of alginate hydrogel with immobilised phospholipid vesicles (liposomes) inside the gel. A silica coating of the alginate body is prepared by the sol-gel or layer-by-layer deposition processes (Haufova 2011). The second particle type consists of the thermoresponsive hydrogel PNIPAM and a silica nanoparticle shell prepared by the Pickering emulsion polymerization (Tokarova 2012).

The adhesion properties of the composite particles were investigated using a microfluidic flow cell which simulates flow conditions in the human body. Silica nanospheres which formed the outer shell of the chemical robots were used for specific adhesion based on antigen-antibody interactions. The surface of SiO_2 is modified with antibody fragment (M75). Antibody used in this work specifically bind tumour-associated antigen (*CAIX*) which is a trans-membrane protein and gives us the possibility for target binding. The specific antigen-antibody interaction will be proved and adhesion properties will be compared to unspecific ones in the present work. The adhesion properties inside the microfluidic cell and the effect of volumetric flow rate on the overall particle adhesion will be also presented.

MATERIALS AND METHODS

Alginate/liposome/silica particles
The first step of particle preparation was the synthesis of liposomes by the Bangham method. Liposomes act as internal compartments for the storage or active components of their precursors and their role in the chemical robots is similar to that of vacuoles in a living cell. Liposomes can be selectively opened by the application of radiofrequency heating. Then, sodium alginate solution (usually 2% w/w) was mixed with a suspension of liposomes purified by gel chromatography and stirred for 15 minutes. The obtained mixture was then dripped into a solution of 2% (w/w) calcium chloride, where the alginate chains were cross linked due to divalent calcium ions and formed a gel (Dohnal 2010).

The core-shell capsules were synthesized as follows. First, the alginate gel cores (with or without liposomes) in the size range of 40 to 90 μm were prepared via the drop-on-demand InkJet technique. A silica shell has then been formed by a sol-gel process using alkoxysilane precursors (TMOS and APTrMOS). Since APTrMOS has positively charged groups, it can interact with the anionic alginate polymer through electrostatic force. Consequently the hydrolysis and polycondensation of methoxy functions of both precursors occurs and silica precipitates around the alginate core (Haufova 2011).

PNIPAM/SPION/silica particles
PNIPAM/SPION/SiO_2 composite microcapsules were prepared by the Pickering emulsion polymerization. Dried SiO_2 nanospheres prepared according to the Stober method (Stober 1968) was disperesed in toluene phase. The water phase consists of monomer NIPAM, crosslinker BIS, initiator of the polymerization reaction APS and superparamagnetic iron oxide nanoparticles (SPION). The o/w mixture was then heated to 70°C in order to initiate the polymerization reaction (Cejkova 2010).

Adhesion studies
The adhesion properties of the particles were studied in a custom-designed adhesion cell where it is possible to measure the ratio of adhered capsules with controlled volumetric flow rate, and which enables rapid exchange of the tested substrates (Tokarova 2012).

RESULTS AND DISCUSSION

Adhesion experiments were carried out with the composite PNIPAM/SPION/SiO_2 microcapsules using a range of substrate materials. Their selection was based so as to simulate various surfaces of both natural (cholesterol, palm oil, gelatin) and man-made

(teflon, paraffin, polystyrene, rubber, glass) origin that could potentially be encountered during targeted delivery applications.

The fraction of adhered microcapsules decreased with increasing flow rate for all the investigated model surfaces (Fig. 1). However, it can be seen that both the strength of the decrease and the actual fraction of adhered microcapsules for a given flow rate varied widely among the investigated materials.

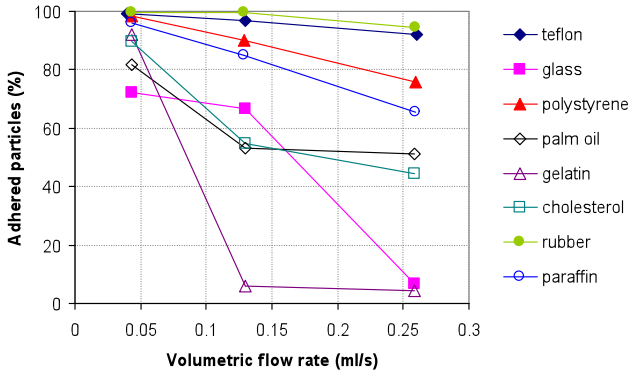

Figure 1: Dependance of adhesion of composite particles on volumetric flow rate.

Specific adhesion of antibody modified silica nanospheres (SiO2-IgG_M75) with tumour associated antigen CAIX were proved by the ELISA test (Fig. 2). The fluorescent responses of 3 types of particle in 4 different dilutions were compared. The difference between specific (SiO2-IgG_M75) interaction and unspecific (SiO2-IgG_irrelevant) or unmodified (SiO2) silica spheres is considerable. The test was repeated in time sequence and no antibody deactivation was observed.

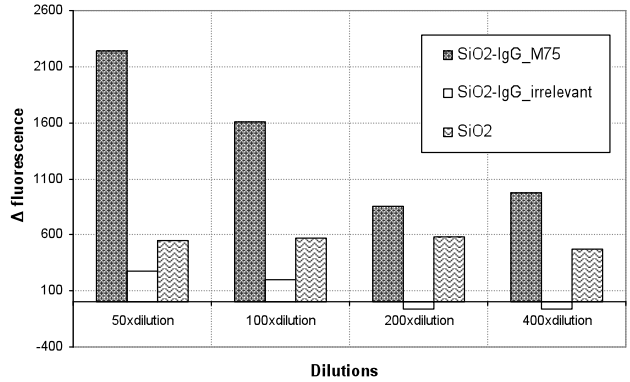

Figure 2: ELISA test of specific antibody-antigen interactions.

CONCLUSION

This work described synthesis of two types of so called chemical robots. These structured microparticles are used as carriers of active substances for controlled delivery. The adhesion character of composite PNIPAM/SPION/silica particles was tested inside the microfluidic cell. The phase transition of the underlying PNIPAM, which is hydrophilic below its LCST and hydrophobic above its LCST, does not affect the adhesion force of the structured microcapsules. The surface character formed by the SiO_2 nanoparticles directed the adhesion of microparticles which provides opportunities for their functionalization to tailor the adhesion towards specific substrates. Antibody modified silica was proved to specifically bind with antigen and is a suitable way to target delivery. Our future work will focus on adhesion experiments with biological substances like human cells and tissues to emphasize pharmaceutical application of chemical robots.

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