Determination of the properties and loading efficiency of encapsulated BSA-FITC and dexamethasone for drug delivery systems

E Chudinova¹, M Surmeneva¹, A Koptyug², V Sokolova³, O Prymak³, S Bouckercha³, M Epple³, R Surmenev¹

¹Physical Materials Science and Composite Materials Centre, National Research Tomsk Polytechnic University, 30 Lenin Avenue, Tomsk 634050, Russian Federation
²Department of Mechanical Engineering and Quality Technology, SportsTech Research Centre, Mid Sweden University, Akademigatan 1, SE 831 25, Östersund, Sweden
³Inorganic Chemistry and Center for Nanointegration Duisburg-Essen (CeNIDE), University of Duisburg-Essen, Essen 45117, Germany

¹E-mail: rsurmenev@mail.ru

Abstract. In this work porous microparticles of calcium carbonate were synthesized with bovine serum albumin - fluorescein isothiocyanate conjugate (BSA-FITC) and dexamethasone, and then used for encapsulation in polymer microcapsules by means of layer-by-layer assembly (LbL). The properties of the obtained microcapsules were characterized by scanning electron microscopy, dynamic light scattering, infrared-, ultraviolet- and visible spectroscopy. According to the performed DLS measurements, an average hydrodynamic diameter ranged from 4 to 8 µm and zeta-potential for all types of capsules was determined as -18 and -21 mV. BSA-FITC was encapsulated using this approach yielded a loading efficiency of 49 % protein. This value calculated for dexamethasone was of 38%. The microcapsules filled with an encapsulated drug may find applications in the field of biotechnology, biochemistry, and medicine.

1. Introduction
Encapsulation and release of pharmaceutically active substances are increasingly important tasks in biomedical research. Microencapsulation provides the means to alter colloidal and surface properties, provide environmental protection and control over the release kinetics of the encapsulated drugs [1]. The primary reason for microencapsulation is found to be either for sustained or prolonged drug release [2].

A promising type of drug delivery systems is represented by hollow polyelectrolyte capsules [3]. Their fabrication presents the layer-by-layer (LbL) self-assembly of polyelectrolyte thin films and yields hollow capsules that have a diameter in the range of micrometers or even below [4, 5]. Briefly explained, the LbL technique is based on the alternating adsorption of charged species onto an oppositely charged substrate, using electrostatic interactions as the driving force. The main advantage of the LbL technique is the ease of manipulation and the unmet degree of multifunctionality [6], allowing one to tailor the surface with different kinds of functional groups [7], lipids [8], nanoparticles [9].

In case of this method, materials are entrapped within or adsorbed to sacrificial template particles prior to LbL coating. Here, inorganic calcium carbonate (CaCO₃) microparticles represent a valuable category of a template for loading, as they provide an excellent loading capacity for biological materials
together with rather “biofriendly” processing techniques [10, 11]. These inorganic components will provide lower-risk materials in various practical applications.

As the materials for loading can be used also proteins, drug microcrystals, enzymes and other biomolecules. Thus, for the synthesis of microcapsules and determination of the amount of a loaded component as model protein bovine serum albumin – fluorescein isothiocyanate conjugate (BSA-FITC) molecules can be chosen for encapsulation into the calcium carbonate microcapsules in the process of the capsule preparation [12-14]. Besides, dexamethasone can be used for loading in capsules as a model drug because of its potential use in specialized delivery systems in treating peritumoral edema associated with brain tumors and for treating and preventing retinal diseases. Dexamethasone is a glucocorticoid that is clinically used for its anti-inflammatory and immunosuppressive effects [15, 16].

Therefore, the main goal of this work was the synthesis of microcapsules with BSA-FITC and dexamethasone and determination of their composition, loading efficiency for the further possibility to determine the release of the encapsulated substance from the capsule.

2. Materials and methods
The CaCO₃ microparticles were synthesized to serve as a core of the capsules; BSA-FITC and dexamethasone were also encapsulated in the core of the capsules. Briefly, 0.615 mL of CaCl₂ solution (1 M) was added to a 2.5 mL of distilled water, after that 0.615 mL of Na₂CO₃ solution (1 M) was rapidly added under vigorous stirring (1100 rpm). This suspension was mixed during 25 minutes in order to form spherical CaCO₃ microparticles. The synthesis with BSA-FITC (Sigma, A9771, 4 mg mL⁻¹) and dexamethasone (KRKA, 4 mg mL⁻¹) was performed in the same conditions; they were added instead of the water. Then, the CaCO₃ microparticles were suspended in an aqueous solution of PAH (2 mg mL⁻¹) containing 0.15 M NaCl. Polymers poly(styrenesulfonate) (PSS) and poly(allylamine hydrochloride) (PAH) were used for formation of layers. The PAH was allowed to adsorb onto three types of microparticles for 10 min using a shaker with 180 rpm of rate. After that, the dispersion was centrifuged and washed three times with deionized water to remove the non-adsorbed polyelectrolyte. Next, the deposition of PSS was carried out on the PAH-coated three type of CaCO₃ particles using the same manipulation. Such a procedure of PAH/PSS deposition was repeated six times in order to form a core-shell structure. Afterwards, the particles were centrifuged and washed three times in deionized water.

The hydrodynamic diameter (HDD) of 3 different types of capsules was measured by Dynamic Light Scattering (DLS) using a Malvern Zetasizer Nano ZS and Nanoparticle Tracking Analysis (NTA) using a NanoSight LN 10. Zeta-potential of the obtained microparticles was also measured by DLS. The morphology of the microparticles was estimated using an ESEM Quanta 400 FEG instrument operating in a high vacuum with gold/palladium-sputtered samples. Ultraviolet and visible (UV-Vis) spectroscopy (Cary 300 Bio UV-Vis-spectrophotometer, Varian) was used to calculate the amount of the encapsulated microparticles in the obtained hybrid capsules. The chemical structure of the capsules was examined by Infrared (IR) spectroscopy (Vertex 70, Bruker). For that, the capsules were dried in an oven at 55 degrees for 12 hours, then 2 mg of capsules and 200 mg of KBr were taken, everything was thoroughly mixed in a mortar and the tablets were formed using a hydraulic system.

3. Results and discussion
SEM images of the synthesized capsules with BSA-FITC and dexamethasone are shown in Figure 1. It should be noted that morphology of the prepared capsules is close to spherical. According to the obtained micrographs, synthesized microparticles have a consistent size, with an average diameter for both type of capsules around 2 μm and pronounced roughness of their outer surface. These structural and morphological characteristics of CaCO₃ microparticles are directly determined by the mechanism of their formation from supersaturated solutions. It was found earlier [17, 18] that the growth of CaCO₃ microparticles is a kind of colloidal aggregation of primary CaCO₃ nanoparticles, instantaneously formed with mixing of calcium chloride and sodium carbonate solutions, into spherical micron-sized microparticles, which further slowly recrystallize in water into rhombohedral calcite crystals.
Results of DLS analysis presented in Table 1 showed that an average HDD of synthesized microcapsules was in the range from 4 to 8 µm (Table 1).

Since the outer layer of the capsules was negatively charged PSS, the zeta-potential of the control CaCO$_3$ microcapsules, microcapsules with BSA-FITC and dexamethasone was -18.1, -20.9 and -20.5 mV, respectively (Table 1).

| Type of microcapsules      | HDD (µm) | Zeta-potential (mV) |
|----------------------------|----------|---------------------|
| Control CaCO$_3$ capsules  | 3.8±0.2  | -18.1±1.0           |
| Capsules with BSA-FITC     | 4.6±0.2  | -20.9±0.9           |
| Capsules with dexamethasone| 8.4±0.3  | -20.5±2.5           |

To determine the absorption maxima for PSS, BSA-FITC and dexamethasone, a series of standard solutions were prepared. Initially, the firstly-prepared solution containing capsules was taken. Then the series of 50% dilutions were prepared from it.

According to the obtained data, the absorbance maximum of PSS (control), BSA-FITC and dexamethasone was observed at 226 nm (Figure 2a), 280 nm and 486 nm (Figure 2b) and 241 nm (Figure 2c), respectively. These values of maximum correspond to the references [19-21].
Figure 2. The absorbion spectra of the control CaCO$_3$ microcapsules (a), BSA-FITC (b), dexamethasone (c), recorded as different concentrations, respectively, and their IR spectra (d).

After that, the concentrations of the components were determined using the standard calibration curves. According to the calibration curve calculations, the concentration of BSA-FITC in the initial solution was determined to be 4.79 mg/mL. While the concentration of this component in the supernatant after the synthesis of the core was 1.89 mg/mL. Thus, the concentration difference was 2.10 mg/mL, which was 48% of the effective load. This value means how much protein was loaded into the core.

An analysis of the selected literary sources over the past three years has revealed an active interest in using BSA-FITC as a model protein. The release of BSA-FITC from the polyelectrolyte microcapsules was also investigated elsewhere [22]. The protein yield was obtained within 38% at a temperature of 22°C. The authors noted that that value depended on the thickness of the shell of the capsules, the method of encapsulation, as well as the composition of the salt, pH and temperature. Similar studies were also presented in the paper of Bolzinger M. A. et al., where the loading efficiency is correlated with the values obtained in this experiment. However, the authors provide ways to increase efficiency by reducing the volume of internal and external phases [23].

According to similar calculations for dexamethasone and PSS (control capsules) using a calibration curve, the loading efficiency was of 36 and 51%, respectively.

It is known in literature that the efficiency of dexamethasone loading is about 51% [24], 77% [25], when similar method of encapsulation - layer-by-layer adsorption is used.

The IR spectra of the control CaCO$_3$ microcapsules, BSA-FITC and dexamethasone are shown in figure 2d. As a result of this method of analysis the following chemical bonds such as C – H, C – O, O – H, O – C – O, C = O are detected.
The IR spectra of the BSA-FITC- and dexamethasone-loaded CaCO$_3$ microcapsules reflect the characteristic absorption bands typical for BSA-FITC, dexamethasone and calcium carbonate without any new bands, which indicates physical adsorption of CaCO$_3$ microcapsules [26-29].

4. Conclusion
In this study, calcium carbonate spherical capsules with loaded BSA-FITC- and dexamethasone have been synthesized and studied. According to the DLS results, the diameter of the prepared microcapsules with BSA-FITC, dexamethasone and control CaCO$_3$ microcapsules was 4.6 ± 0.2, 8.4 ± 0.3 and 3.8 ± 0.2 µm, respectively. Zeta-potential of each type of capsules was determined, the determined values were in the range from -18 to -21 mV. The concentration of the encapsulated components was measured using UV spectroscopy, thus, the loading efficiency of BSA-FITC, dexamethasone and PSS (control capsules) was 48, 36 and 51 %, respectively, which corresponded well to the literature data for capsules prepared using the same method. The chemical bonds C – H, C – O, O – H, O – C – O, C = O were determined using infrared spectroscopy. The future experiments are aimed to deposit the microcapsules on the surface of titanium alloy scaffolds and to perform in vivo and antibacterial experiments.

Acknowledgments
The work was supported by Russian Science Foundation (No. 15-13-00043). Ekaterina Chudinova also acknowledges the personal support from the German-Russian Interdisciplinary Research Center (G-RISC). We acknowledge Professor G.B. Sukhorukov for fruitful discussions.

References
[1] Agnihotri N, Mishra R, Goda C and Arora M 2012 Microencapsulation—a novel approach in drug delivery: a review Indo Global J. of Pharm. Sci. 2/1 1
[2] Bansode S S, Banarjee S K, Gaikwad D D, Jadhav S L and Thorat R M 2010 Microencapsulation: a review Int. J. Pharm. Sci. Res 1/2 38
[3] Vilela C, Figueiredo A R, Silvestre A J and Freire C S 2017 Multilayered materials based on biopolymers as drug delivery systems Expert opinion on drug delivery 14/2 189
[4] Guzman E, Mateos-Maroto A, Ruano M, Ortega F and Rubio R G 2017 Layer-by-Layer polyelectrolyte assemblies for encapsulation and release of active compounds Adv. Colloid Interface Sci. 249 290
[5] Richardson J J, Cui J, Bjornmalm M, Braunger J A, Ejima H and Caruso F 2016 Innovation in layer-by-layer assembly Chem. Rev. 116/23 14828
[6] Angelatou S A, Katagiri K and Caruso F 2006 Bioinspired colloidal systems via layer-by-layer assembly Soft Matter 2/1 18
[7] De Geest B G, Jonas A M, Demeester J and De Smedt S C 2006 Glucose-responsive polyelectrolyte capsules Langmuir 22/11 5070
[8] De Geest B G, Stubbe B G, Jonas A M, Van Thielen T, Hinrichs W L, Demeester J and De Smedt S C 2006 Self-exploding lipid-coated microgels Biomacromolecules 7/1 373
[9] Shchukin D G, Ustinovich E A, Sukhorukov G B, Möhwald H and Sviridov D V 2005 Metallized polyelectrolyte microcapsules Adv. Mater. 17/4 468
[10] Nayar S, Sinha M, Basu D and Sinha A 2006 Synthesis and sintering of biomimetic hydroxyapatite nanoparticles for biomedical applications J. Mater. Sci. : Mater. Med. 17/11 1063
[11] Vallet-Regi M and González-Calbet J M 2004 Calcium phosphates as substitution of bone tissues Prog. Solid State Chem. 32/1-2 1
[12] Appel E A, Tibbitt M W, Webber M J, Mattix B A, Veiseh O and Langer R 2015 Self-assembled hydrogels utilizing polymer–nanoparticle interactions Nat. Commun. 6 6295
[13] Jalani G, Naccache R, Rosenzweig D H, Haglund L, Vetrone F and Cerruti M 2016 Photocleavable hydrogel-coated upconverting nanoparticles: a multifunctional theranostic platform for NIR imaging and on-demand macromolecular delivery J. Am. Chem. Soc. 138/3 1078
[14] Kim B S, Oh J M, Kim K S, Seo K S, Cho J S, Khang G, Lee H B, Park K and Kim M S 2009 BSA-FITC-loaded microcapsules for in vivo delivery Biomaterials 30/5 902
[15] Correia C R, Pirraco R P, Cerqueira M T, Marques A P, Reis R L and Mano J F 2016 Semipermeable capsules wrapping a multifunctional and self-regulated co-culture microenvironment for osteogenic differentiation Sci. Rep. 6 21883

[16] Roila F, Ruggeri B, Ballatori E, Del Favero A and Tonato M 2013 Aprepitant versus dexamethasone for preventing chemotherapy-induced delayed emesis in patients with breast cancer: a randomized double-blind study J. Clin. Oncol. 32/2 101

[17] Volodkin D V, Petrov A I, Prevot M and Sukhorukov G B 2004 Matrix polyelectrolyte microcapsules: new system for macromolecule encapsulation Langmuir 20/8 3398

[18] Wang G, Siggers K, Jiang H, Xu Z, Zernicke R F, Matyas J and Uludağ H 2008 Preparation of BMP-2 containing bovine serum albumin (BSA) nanoparticles stabilized by polymer coating Pharm. Res. 25/12 2896

[19] Friedrich R B, Ravanello A, Cichota L C, Rolim C M B and Beck R C R 2009 Validation of a simple and rapid UV spectrophotometric method for dexamethasone assay in tablets Quim. Nova 32/4 1052

[20] Mundargi R C, Potroz M G, Park J H, Seo J, Tan E-L, Lee J H and Cho N-J 2016 Eco-friendly streamlined process for sporopollenin exine capsule extraction Sci. Rep. 6 19960

[21] Wang W, Liu S, Li C, Wang Y and Yan C 2018 Dual-target recognition sandwich assay based on core-shell magnetic mesoporous silica nanoparticles for sensitive detection of breast cancer cells Talanta 182 306

[22] Freitas S, Merkle H P and Gander B 2005 Microencapsulation by solvent extraction/evaporation: reviewing the state of the art of microsphere preparation process technology J. Controlled Release 102/2 313

[23] Bolzinger M, Bordes C, Gauvrit J and Briançon S 2007 Improvement of a bovine serum albumin microencapsulation process by screening design Int. J. Pharm. 344/1-2 16

[24] Joshi A B and Srivastava R 2009 Polyelectrolyte coated calcium carbonate microparticles as templates for enzyme encapsulation Adv. Sci. Lett. 2/3 329

[25] Jayant R, McShane M and Srivastava R 2009 Polyelectrolyte-coated alginate microspheres as drug delivery carriers for dexamethasone release Drug delivery 16/6 331

[26] Chen W, Qian C, Zhou K-G and Yu H-Q 2018 Molecular spectroscopic characterization of membrane fouling: a Critical Review Chem 4/7 1492

[27] Facchinetto S E, Bortolotto T, Neumann G E, Vieira J C, Menezes B B d, Giacomelli C and Schmidt V 2017 Synthesis of Submicrometer Calcium Carbonate Particles from Inorganic Salts Using Linear Polymers as Crystallization Modifiers J. Braz. Chem. Soc. 28/4 547

[28] Wang C, He C, Tong Z, Liu X, Ren B and Zeng F 2006 Combination of adsorption by porous CaCO3 microparticles and encapsulation by polyelectrolyte multilayer films for sustained drug delivery Int. J. Pharm. 308/1-2 160

[29] Zhang J, Liu Y, Luo R, Chen S, Li X, Yuan S, Wang J and Huang N 2015 In vitro hemocompatibility and cytocompatibility of dexamethasone-eluting PLGA stent coatings Appl. Surf. Sci. 328 154