Pannon Egyetem

Preparation of functional micro- and nanoparticles by spherical agglomeration and emulsion solvent evaporation methods

THESES OF DOCTORAL (PhD) DISSERTATION

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1 Introduction and aims

Today, in all areas of life, it is increasingly important and urgent to find new solutions beside existing ones, concerning the dosage of smaller quantities of active pharmaceutical ingredients in a targeted way or the more efficient use of the available energy resources. Increased efficiency can be achieved with functional particles which can be produced by microencapsulation.

The functional particles are solid, often composite particles that have an active role in their application, an effect on their environment and a complex structure appropriate to their function.

For microencapsulation, two different particle formation methods, i.e. spherical agglomeration and emulsion solvent evaporation techniques, were chosen. While spherical agglomeration can produce particles of a few hundred μm in size, which can be used for applications in pharmacy and even in building industry, the emulsion solvent evaporation method is a suitable method for the encapsulation of water-soluble and non-water-soluble materials, as well as for the creation of micro- and nanoparticles.

In my work, I aimed to develop nanostructured composite particles of human serum albumin model material with spherical agglomeration, which can be a model system for the application of drug particles in tablets. My aim was to design a suitable solvent system for the chosen model material and to produce granules with appropriate sphericity and flowability by studying the mechanism involved.

Our aim was to develop methods for the building industry to produce microcapsules containing paraffin and salt hydrate based phase change materials. My task was therefore to form a spherical core by spherical agglomeration of $KAl(SO_4)_2 \cdot 12H_2O$ salt hydrate (potash alum), which could subsequently be coated with a waterproof polymer shell to produce the microcapsules with a core-shell structure.

The aim was to prepare paraffin-type microcapsules containing hexadecane with core-shell structure using an emulsion solvent evaporation technique with ethyl cellulose and poly(methyl methacrylate) polymer. My task was to confirm the particle morphology by measurements and explain it forming during emulsion solvent evaporation and the porosity in the capsule shell based on the approaches of the literature.

I also aimed to microencapsulate interferon-beta to reduce the symptoms of multiple sclerosis with the expected result of producing nanocomposite particles with a narrow size

distribution, average size less than 200 nm and good encapsulation efficiency (>80%). Considering the drug release, they should be able to provide extended interferon-beta release in human plasma for at least one week under in vitro conditions.

2 Experimental methods

As preliminary experiments for the preparation of functional particles by spherical agglomeration, I determined the phase diagrams of the active ingredients and the encapsulating polymers in the chosen solvent systems by solubility studies. The particle size distributions of the particles produced by both spherical agglomeration and emulsion solvent evaporation techniques were determined using a Malvern 2000 based on the principle of laser diffraction and a Zetasizer Nano ZS. I used a visible light microscope and a scanning electron microscope to characterise the morphology of the particles. The physical properties of the potash alum spherical agglomerates investigated as phase change materials were characterized by thermogravimetric and differential scanning calorimetric techniques. The approximate compositions of the different spherical agglomerates were determined by FTIR and X-ray diffraction.

The morphology of the microcapsules containing hexadecane prepared by a simple emulsion method and the porosity of the microcapsule shell were modelled using surface tension measurements and the equations described by Torza and Mason[1] and Bolognesi[2] et al.

The amount of the protein in the chitosan-PSS polymer capsules containing human serum albumin was determined by the Bradford method. Bovine serum albumin and interferon-beta content and in vitro delivery kinetics of interferon-beta-loaded PLGA and PEG_PLGA nanoparticles were measured by micro BCA and ELISA assay, respectively.

[1] Torza, S.; Mason, S. G. Three-phase interactions in shear and electrical fields. *Journal of Colloid and Interface Science* (1970) **33**(1), 67-83. https://doi.org/10.1016/0021-9797(70)90073-1

[2] Bolognesi, A.; Mercogliano, C.; Yunus, S.; Civardi, M.; Comoretto, D.; Turturro, A. Self-Organization of Polystyrenes into Ordered Microstructured Films and Their Replication by Soft Lithography. Langmuir (2005) 21(8), 3480-3485. https://doi.org/10.1021/la047427u

3 New scientific results: thesis points

Theses

1) a. I have developed a non-classical spherical agglomeration method for the preparation of novel nanostructured particles suitable for the encapsulation of water-soluble protein-type drug (natural interferon) in chitosan and sodium polystyrene sulfonate polymers. The process involves the combination of antisolvent and chemical precipitation. Neither the presence of a surfactant nor the application of a temperature difference is required to execute the method.

1 b. I determined the narrow composition range described by 10–15% (v/v) ethanol and 65–70% (v/v) ethylacetate for the solvent mixture with water, where spherical agglomerates containing HSA in a polymer matrix of chitosan and sodium polystyrene sulfonate were obtained from the initial two-phase mixture by the addition of ethanol or ethanol–ethyl acetate mixture. I have experimentally demonstrated that the solvent compositions formed in the sub-steps of the process have a significant effect on the morphology of the resulting agglomerates.

2) I prepared spherical agglomerates of $KAl(SO_4)_2 \cdot 12H_2O$ by two different methods, namely spherical agglomeration and emulsion solvent diffusion.

By spherical agglomeration mechanism, less stable agglomerates, not fully spherical and identifiable as $KAl(SO_4)_2 \cdot 12H_2O$, can be produced.

By emulsion solvent diffusion mechanism, more stable and almost perfectly spherical agglomerates are formed, but they are not pure potash alum with 12 crystallization water in composition. In addition to potash alum, 4 other crystalline salts can be detected: rostite ($Al(SO_4)(OH) \cdot 5H_2O$), alunogen ($Al_2(SO_4)_3 \cdot 17H_2O$), anhydrous $KAl(SO_4)_2$ and anhydrous K_2SO_4.

The amount of heat measured during dehydration and melting of the samples with different average crystallization water and potash alum contents is proportional to the crystallization water and potash alum contents, respectively.

3) I stated that microcapsules formed with poly(methyl methacrylate) and ethyl cellulose carrier polymers containing n-hexadecane phase change material, using emulsion solvent evaporation method and polyvinyl alcohol or Tween80 emulsifiers do not reach their equilibrium morphology based on the spreading coefficients. The different surface tension conditions due to the different emulsifier solutions are revealed in the different degree of porosity in addition to the core-shell structure.

4) I successfully prepared interferon-beta containing nanoparticles of (poly(lactic acid-glycolic acid) and (poly(lactic acid-glycolic acid)-polyethylene glycol carrier with 80–90% encapsulation efficiency and volume average particle size of 160–170 nm. The nanoparticles were able to provide extended interferon-beta drug release in human blood plasma for at least one week under in vitro conditions. The interferon-beta carrying nanoparticles did not show cytotoxicity in in vitro cytotoxicity assays, however in in vivo experiments the nanoparticles formed with these two polymers induced mild toxicity in the kidneys.

4 Publications on which the thesis is based

Articles in international journals:

1. **Andrea Fodor-Kardos**, Ádám Ferenc Kiss, Katalin Monostory and Tivadar Feczkó, Sustained in vitro interferon-beta release and in vivo toxicity of PLGA and PEG-PLGA nanoparticles, RSC Advances, 10, 27, (15893-15900), (2020) doi: 10.1039/C9RA09928J, Impakt faktor: 3,049 (2019), Q1
2. **Kardos, AF**; Toth, J (Toth, Judit); Trif, L (Trif, Laszlo); Gyenis, J (Gyenis, Janos); Feczko, T (Feczko, Tivadar) Preparation of spherical agglomerates from potash alum, RSC Advances. 6 , 5466-5473 (2016) doi: 10.1039/c5ra18497e Impakt faktor: 3,228, Q1
3. **Fodor-Kardos, A**; Toth, J (Toth, Judit); Gyenis, J (Gyenis, Janos) Preparation of protein loaded chitosan microparticles by combined precipitation and spherical agglomeration. Powder Technology, 244, 16-25 (2013) DOI: 10.1016/j.powtec.2013.03.052 Impakt factor: 2,64, Q1
4. Feczko, T (Feczko, Tivadar); **Kardos, AF**; Nemeth, B (Nemeth, Bence); Trif, L (Trif, Laszlo); Gyenis, J (Gyenis, Janos), Microencapsulation of n-hexadecane phase change material by ethyl cellulose polymer. Polymer Bulletin, 71, 3289-3304 (2014) DOI: 10.1007/s00289-014-1250-y Impakt factor: 1,33, Q2.

Presentations in Hungarian with full text:

1. **Fodorné, Kardos A**; Tóth, J; Hasznosné, Nezdei M, Szférikus agglomeráció háromkomponensű oldószerelegyben, In: Nagy, Endre; Simon, Ferenc; Kiss, Katalin; Hegedűs, Imre (szerk.) Műszaki Kémiai Napok ’08 = Conference of Chemical Engineering: conference proceedings, Veszprém, Magyarország: MTA VEAB, Veszprémi Egyetemi Kiadó, (2008) pp. 245-249. , 5 p.
2. **Fodorné Kardos Andrea**, Feczkó Tivadar, Szabályozott hatóanyagleadású biokompatibilis és biológiailag lebontható interferon-béta tartalmú kompozit részecskék fejlesztése, MŰSZAKI KÉMIAI NAPOK 2017, Veszprém, 2017. április 25-27.

Posters in foreign language:

1. **Fodor-Kardos, A** ; Tóth, J ; Hasznos-Nezdei, M, Precipitation of HSA loaded microspheres in a polymeric spherical crystallization system pp. 1-4.,In: Anon (szerk.) 6th World Congress on Particle Technology, Nürnberg, Németország : Nürnberger Messe- und Austellungsgesellshaft, (2010)

Presentations in foreign language with full text:

1. **Fodor-Kardos, A** ; Feczkó, T, Preparation of interferon-beta loaded PLGA and PEG-PLGA nanoparticles ,In: 5th international symposium on reliable flow of particulate solids 2017, Skien Norway, (2017) pp. 1-6. Paper: 8A1 , 6 p.
2. **Andrea Fodor-Kardos**, János Gyenis, Preparation of potash alum PCM core by spherical agglomeration, The MacroJournals Conference on Medicine, Science, and Technology – Dubrovnik 2014, August 4-5th, 2014