University of Pannonia
Chemical Engineering and Material Sciences Doctoral School

Microencapsulation of traditional chemotherapeutic agents and synthetic anthocyanidin anticancer active substances with polymers produced by biocatalysis

SUMMARY OF PHD DISSERTATION

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2023

Introduction and aims of the research

Nanoformulations have a significant potential to advance anticancer drug therapies. Compared to pure chemotherapeutics, drug-loaded nanocarriers have been able to overcome many challenges, such as limited bioavailability, multiple drug resistance and adverse effects. Polymer-based nanoparticles are a potential class of targeted and controlled drug delivery devices, because they can release the active agent through diffusion and their degradation. In recent years, a green approach of producing biodegradable synthetic polymers with a variety of uses, including nanoparticle-sized carriers for drug administration, has been developed. Polyesteramides were significantly less investigated in this area, although having the potential benefit of higher mechanical and thermal resistance given by amide moiety.

The aim of my PhD work was to prepare anticancer drug-loaded nanoparticles that can become effective sustained and targeted drug delivery devices. An important aspect was not only to find the active agents used, but also the selection of suitable carrier materials. Therefore, my aim was to explore a synthetically

produced, naturally-based anthocyanidin active ingredient in addition to employing the widely used sorafenib and broad-spectrum cisplatin. The antioxidant and anticancer effects of anthocyanidins have already been well known; however, the nanoscale use of these compounds, especially for targeted cancer therapy, is still relatively new. In order to find suitable biodegradable and biocompatible carrier polymers, I investigated new types of carriers that were produced in a green way with biocatalysis, and had not been used for drug carrier purposes until now.

The main goal of my work was to investigate the microencapsulation and surface modification possibilities of ε-caprolactone-12-hydroxystearic acid and ε-caprolactam-16-hydroxyhexadecanoic acid copolymers with the active ingredients sorafenib and cisplatin, as well as the production and testing of possible co-delivery systems. I aimed to use the iRGD tumor penetration peptide together with the nanoparticles by physical and/or chemical bonding. I also studied the molecular encapsulation of new types of anticancer anthocyanidins with cyclodextrin derivatives and the microencapsulation

of the resulting complexes with the selected polymers. My goal was to optimize the nanoparticle preparation method in order to achieve the appropriate particle size and improve the encapsulation efficiency, as well as to test the nanoparticles in drug release and cytotoxicity tests, preparing them for possible *in vivo* studies.

Experimental methods

The synthesized biocompatible and biodegradable ε-caprolactone-12-hydroxystearic acid and ε-caprolactam-16-hydroxyhexadecanoic acid copolymers were used as new encapsulating agents to produce polymeric nanoparticles containing sorafenib, cisplatin or anthocyanidin. The nanoparticles were prepared by using the emulsion-solvent evaporation method, optimizing the methods for particle size and encapsulation efficiency The formed nanoparticles demonstrated a promising drug release profile and *in vitro* cytotoxic efficacy on HepG2 hepatocellular cells. During my work, in the expectation of a synergistic effect, I also produced polymer nanoparticles with dual active ingredients, using an improved double emulsion-solvent evaporation method,

achieving a good production yield. To increase the incorporation of cisplatin, the bovine serum albumin (BSA) model compound was used, which was successfully replaced with the iRGD tumor penetration peptide that can provide the targeting function of the nanoparticles. The efficacy of the dual drug system was demonstrated by cytotoxicity test on HepG2 cell line. The potential cytostatic properties of the novel anthocyanidin derivatives against HCT116 and HepG2 cancer cells were examined *in vitro*. Additionally, the halochromic properties of the two most active compounds were investigated, that may affect the biological activity. In order to enhance their solubility in water and bioavailability, these two substances were molecularly enclosed in β-cyclodextrin derivatives.

During the investigation of the nanoparticle properties, the hydrodynamic size of the particles was measured by dynamic light scattering method, and their stability was determined by laser-Doppler-electrophoresis method, using a Zetasizer Nano ZS instrument. The morphology of nanoparticles was investigated by scanning and transmission electron microscopy. To measure the

encapsulation efficiency, UV-vis spectrophotometer and inductively coupled plasma optical emission spectrometer (ICP-OES) was used. The production yield was determined gravimetrically. *In vitro* drug release test was performed in blood plasma (pH = 7.4) and acidic buffer solution (Na-acetate buffer, pH = 5.5), modeling the tumor microenvironment. *In vitro* cytotoxicity was tested with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) viability test on HepG2 and HCT116 human cancer cell lines.

New scientific results

1. a) For the first time, I prepared new types of potential drug carrier nanoparticles from the biodegradable and biocompatible ε-caprolactone-12-hydroxystearic acid copolymer (12CL) produced by biocatalysis using optimized single emulsion-solvent evaporation method, with a suitable size for injectable drug format (219 ± 5.2 nm, hydrodynamic size) and monodisperse (polydispersity index: 0.168 ± 0.018) size distribution.

b) Sorafenib (SOR) was microencapsulated with high encapsulation efficiency (72.7%) by the biodegradable and biocompatible copolymer. The nanoparticles produced in this way were highly effective against HepG2 HCC cells and showed promising prolonged drug delivery in an in vitro model environment. (S1)

2. a) I produced polymer nanoparticles containing sorafenib and cisplatin from 12CL copolymers for the first time, using optimized double emulsion-solvent evaporation method using N,N'-dicyclohexylcarbodiimide (DCC) coupling and iRGD tumor penetration peptide. DCC significantly improved the encapsulation efficiency and the stability of the carrier system.

b) The effectiveness of the dual drug delivery system was confirmed by cytotoxicity test. The polymeric nanoparticles containing two anticancer agents were statistically proven to exhibit significantly higher cytotoxicity on HepG2 cell lines than the pure active ingredients. The polymeric nanoparticles provided sustained release in Na-acetate buffer (pH=5.5) for sorafenib compared to that found in blood plasma. (S2)

3. a) I prepared new types of potential drug carrier nanoparticles from the biodegradable and biocompatible 16-hydroxyhexadecanoic acid-ε-caprolactam copolymer (16CM) for the first time produced by biocatalysis using optimized simple emulsion-solvent evaporation method, with a suitable size for injectable drug format (180–200 nm, hydrodynamic size) and monodisperse (PDI: 0.116–0.135) size distribution.

b) I successfully microencapsulated sorafenib (SOR) into the biodegradable and biocompatible polyesteramide copolymer with adequate encapsulation efficiency (55%) and production yield (71%). The drug release was investigated in in vitro release tests. In acidic media, the initial drug release was reduced by 8% using DCC crosslinking agent or Span20 emulsifier, and PEGylation of particles improved the initial burst effect by 11% in blood plasma. (S4)

4. I was the first who produced inclusion complexes with β-cyclodextrin derivatives from new types of biocatalytically synthesized anthocyanidin compounds (6-Methoxy-4′-hydroxy-3′-methoxyflavylium hydrogensulfate marked as CN2.1 and 8-Methoxy-4′-hydroxy-3′-

methoxyflavylium hydrogensulfate marked as CR1.1). I successfully increased the water solubility of anthocyanidins (CN2.1: 6.3 mg/ml → 9.2 mg/ml, CR1.1: 2.3 mg/ml → 9.4 mg/ml) by complexation. The compounds complexed with cyclodextrins retained their cytotoxic effects on HepG2 cancer cells. The inclusion complex of CN2.1 with sulfobutylether-β-cyclodextrin was microencapsulated in 12CL and 16CM polymers with appropriate size and encapsulation efficiency. (S3)

Publication list

S1. Kántor, I.; Aparaschivei, D.; Todea, A.; Biró, E.; Babos, Gy.; Szerényi, D.; Kakasi, B.; Péter, F.; Şişu, E.; Feczkó, T. Biocatalytic synthesis of poly[ε-caprolactone-*co*-(12-hydroxystearate)] copolymer for sorafenib nanoformulation useful in drug delivery. *Catal. Today* **2021**, 366, 195–201. https://doi.org/10.1016/j.cattod.2020.05.005. Q1, IF: 6,562.

S2. Kántor, I.; Dreavă, D.; Todea, A.; Péter, F.; May, Z.; Biró, E.; Babos, Gy.; Feczkó, T. Co-Entrapment of Sorafenib and Cisplatin Drugs and iRGD Tumour

Homing Peptide by Poly[ε-caprolactone-co-(12-hydroxystearate)] Copolymer. *Biomedicines* **2022**, 10, 43. https://doi.org/10.3390/biomedicines10010043. Q1, IF 4,7.

S3. Păuşescu, I.; Kántor, I.; Babos, Gy.; May, Z.; Fodor-Kardos, A.; Miskolczy, Zs.; Biczók, L.; Péter, F.; Medeleanu, M.; Feczkó, T. Halochromic Behavior and Anticancer Effect of New Synthetic Anthocyanidins Complexed with β-Cyclodextrin Derivatives. *Int. J. Mol. Sci*. **2022**, 23, 8103. https://doi.org/10.3390/ijms23158103. Q1, IF 5,6.

S4. Benea, I.C.; Kántor, I.; Todea, A.; Pellis, A.; Bîtcan, I.; Nagy, L.; Kéki, S.; Dreavă, D.M.; Péter, F.; Feczkó, T. Biocatalytic synthesis of new polyesteramides from ε-caprolactam and hydroxy acids: Structural characterization, biodegradability, and suitability as drug nanocarriers. *React. Funct. Polym.* **2023**, 191, 105702. https://doi.org/10.1016/j.reactfunctpolym.2023.105702. Q1, IF: 5,1.

Conference presentation:

Kántor, I.; Aparaschivei, D.; Todea, A.; Péter, F.; May, Z.; Biró, E.; Feczkó, T. Co-entrapment of sorafenib and cisplatin in poly[ε-caprolactone-co-(12-hydroxystearate)] copolymer for dual drug delivery application. The 1st International Electronic Conference on Biomedicine, 2021.06.01-26., online.