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Co-entrapment of Sorafenib and Cisplatin in Poly[ε-Caprolactone-Co-(12-Hydroxystearate)] Copolymer for Dual Drug Delivery Application †

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Abstract: Drug-loaded nanocarriers have overcome various challenges compared with the bare chemo-drug, such as limited bioavailability, multiple drug resistance, poor patient compliance, adverse drug reactions, particularly side effects of chemotherapy and offer advantages such as protection from degradation in the blood stream, better drug solubility and improved drug stability. One promising group of controlled and targeted drug delivery systems is the polymer-based nanoparticles which can sustain release of active agent by diffusion and their degradation. Sorafenib is the only drug which is capable to prolong the life of patients suffered from hepatocellular carcinoma. Cisplatin remains one of the most widely used broad spectrum anticancer drug for the treatment of a variety of solid tumors. Nanoformulations can exert synergistic effect by entrapping two drugs with different mode of action, such as sorafenib and cisplatin. In our study, we prepared polymeric nanoparticles by optimised double emulsion solvent evaporation method with a good production yield by a novel biocatalytically synthetized 12-hydroxystearic acid ε-caprolactone copolymer (12CL) which is biocompatible and biodegradable carrier for the co-entrapment of sorafenib and cisplatin in nanotherapeutics in order to investigate the synergistic effect of sorafenib in combination with cisplatin. The active agents were encapsulated and also cross-linked with carbodiimide to increase the encapsulation efficiency. To improve the drug encapsulation efficiency, bovine serum albumin (BSA) was also incorporated as a protein capable of complexation with the cisplatin.

Keywords: polymeric nanoparticles; drug encapsulation; drug delivery; sorafenib; cisplatin

1. Introduction

Nanomedicine is one of the most growing fields of pharmaceutics and can be defined as nanotechnology that uses materials between 1 and 100 nm, applied to health and medicine [1]. Current problems in treating cancer include low specificity, rapid drug clearance and biodegradation, and limited targeting [2] (Sinha et al. 2006). The properties of nanocarriers, including their nanoscale sizes,

high surface-to-volume ratios, favourable drug release profiles, and targeting modifications, can allow them to better reach target tumor tissue and release drugs in a stable, controlled manner [1].

Nanocarriers have a large spectrum of composition, various morphologies, furthermore their chemistry was modified to improve the loading efficiency and delivery capacity [3].

Among the most prominent development there is a broad range of engineered and functional nanoparticles for site-specific targeting of therapeutic agents to the diseased area. Significant outcomes in clinical investigations have been recorded, especially for some types of cancer and microbial infection treatments [4]. Currently there are several clinically approved nanoscale materials that are promising nanoparticle drug delivery systems that have shown reduced side effects and effective therapeutic levels [5].

Sorafenib is an orally active multikinase inhibitor drug. Beside hepatocelullar carcinoma sorafenib is used for the treatment of kidney cancer and thyroid cancer [6]. The most common drug related side effects are diarrhoea and hand-foot skin reaction. [7].

Cisplatin is administered intravenously and interferes with DNA replication, which kills the cancer cells. Today cisplatin remains one of the most widely used and effective anticancer agent for the treatment of a variety of solid tumors, including sarcomas, carcinomas, liver, lung, ovarian, cervical cancer [8]. Its use is associated with serious side-effects like: kidney and hearing problems, nerve damages [9].

Disadvantages of these active agents are that they are quickly recognized and removed by the immune system of the body before they reach their target and because they are not targeted in the body during their administration, their dose must be increased in order to reach the therapeutic level, causing serious side effects. Nanoscale drug carriers offer many advantages compared with the bare chemo-drug, such as protection from degradation in the blood stream, better drug solubility and improved drug stability.

With controlled and targeted drug delivery the drug toxicity, the adverse effects and the administrated doses can be reduced. One of the controlled and targeted drug delivery systems is polymer-based nanoparticles which can sustain release of active agent by diffusion and their degradation [10].

The aim of this work was to prepare anticancer drug-loaded polymeric nanoparticles from biodegradable and biocompatible polymer co-encapsulating sorafenib and cisplatin that can become effective sustained and targeted drug delivery devices.

2. Materials and methods

2.1. Materials

ε-caprolactone-12-hydroxy stearic acid copolymer (ECL-12HSA) polyester was synthesized biocatalytically in our previous work [11]. Polyvinyl alcohol (PVA, Mw = 30,000–70,000 g/mol, 87–90% hydrolyzed), dichloromethane (DCM), acetone, dimethyl sulfoxide (DMSO), 1-ethyl-3(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxy succinimide (NHS), N,N-dicyclohexylcarbodiimide (DCC) were obtained from Sigma Aldrich (St. Louis, MO, USA). Sorafenib (free base) was purchased from Active Biochem (Hong Kong, China) and cis-Platin 99.99% (trace metals basis) was purchased from Acros Organics.

2.2. Preparation of Nanoparticles by Double Emulsion Solvent Evaporation Method

The dual drug-loaded nanoparticles were prepared by the double emulsion solvent evaporation method.

The organic phase is composed of 10 mg polymer containing 2 mg SOR in 1 mL DCM. The 200 µl inner water phase consist of BSA (0.5–1 mg) or CIS (0.5–1 mg).

In the first step the inner water phase was added to the organic phase and the two phases were emulsified by sonication using Hielscher Ultrasonic Processor UP200St (Hielscher Ultrasonics GmbH)

at an amplitude of 30% for 30 s. Then, this first emulsion was transferred to the outer water phase that is 5 mL aqueous solution of PVA (1%), and homogenized again with sonication. The organic solvent was evaporated by stirrig the mixture magnetically 1 h at room temperature and under atmospheric pressure. Nanoparticles were centrifuged by a Hermle Z216 MK microcentrifuge (Gosheim, Germany) with 15,000 rpm for 20 min, washed thrice and redispersed in MilliQ water.

2.3. Characterization of Nanoparticles

2.3.1. Particle Size Analysis

The size distribution of the nanoparticles was determined by Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) operated upon photon correlation spectroscopy. The particles were characterized by their intensity mean diameter and polydispersity index.

2.3.2. Yield and Encapsulation Efficiency

The yield of the produced nanoparticles was determined by gravimetric measurement after washing and drying to a constant mass of 0.5 mL of nanoparticle suspension. The encapsulation efficiency of SOR was investigated by dissolving the pellet resulted from the centrifugation of 0.5 mL nanoparticles suspension in 1 mL DMSO, and the solution was diluted to be detectable in the linear calibration range (1.25–25µg/mL). For sorafenib, the absorbance of the solutions was measured by Unicam UV 500 UV/VIS spectrophotometer (Unicam Instruments Ltd., Cambridge, UK) at the absorbance maximum (270 nm).

The encapsulated cisplatin was measured as platinum content by inductively coupled plasma optical emission spectrometric (ICP-OES) method using a Spectro Genesis ICP-OES (Kleve, Germany) equipment and diluting samples to be in a detectable range.

The encapsulation efficiency (EE) of the active agents was calculated as follows:

Encapsulation efficiency (%) = (mass of drug in nanoparticles/mass of total drug)×100

3. Results and Discussion

The double emulsion solvent evaporation method was optimised by encapsulating BSA in polymeric nanoparticles. The optimisation of the method was carried out by varying the preparation parameters like oil-in-water ratio, polymer concentration, polyvinyl alcohol concentration and the amount of BSA.

Among the experiments presented in Table 1, the sample number 12CLBSA6 was proved to be the most desirable. The one-to-five oil-in-water ratio with 0.5 mg BSA gave an acceptable size, but the size distribution was too wide which can be seen from the PDI value.

In order to decrease the polydispersity of the system we tried to couple the BSA in the polymer matrix with EDC carbodiimide, but introducing this carbodiimide did not improve the polydispesity index of the nanopaticles emulsion.

Table 1. Optimisation of the nanoparticle preparation method by BSA encapsulation.

Sample	Z-avg [nm] (Before Washing)	PDI (Before Washing)	o/w Ratio	Polymer [%]	PVA [%]	BSA [mg]	EDC [mg]
12CLBSA1	476.6	0.450	1/2	1	0.5	1	-
12CLBSA2	241.3	0.341	1/3	1	1	1	-
12CLBSA3	481.9	0.692	1/3	2	1	1	-
12CLBSA4	232.3	0.360	1/3	1	1	0.5	-
12CLBSA5	269.9	0.355	1/5	1	1	1	-
12CLBSA6	245.5	0.275	1/5	1	1	0.5	-

12CLBSA7	301.2	0.461	1/4	1	1	1	-
12CLBSA8	338.8	0.568	1/4	1	1	0.5	-
12CLBSA9	438.5	0.810	1/5	1	1	1	1
12CLBSA10	250.7	0.353	1/5	1	1	0.5	1
12CLBSA10*	271.6	0.414	1/5	1	1	1	1

The behaviour of the double emulsion system was investigated in the presence of active agents SOR and CIS (Table 2). The active agents were encapsulated and also cross-linked with carbodiimide. The samples had wide size distribution, except two samples (12CLBSASORCIS2 and 12CLBSACIS3) where we obtained a particle size about 200 nm and a very narrow size distribution.

However, in these samples the forming polymer suspension yield was too low, we suppose that the carbodiimide made the particles water soluble, hence the particle yield became very low. In this case the size measurement was not reliable.

Table 2. Encapsulation of active agents SOR and CIS.

Scheme	Z-avg [nm] (Before Washing)	PDI (Before Washing)	BSA [mg]	SOR [mg]	CIS [mg]	EDC [mg]
12CLBSASOR1	223.4±2.69	0.243±0.01	0.5	1	-	-
12CLBSASOR2	274.4±9.62	0.582±0.02	0.5	1	-	1
12CLBSASOR3	212.3±0.90	0.258±0.03	0.5	1	-	1
12CLBSASORCIS1	239.6±4.13	0.378±0.02	0.5	0.5	0.5	-
12CLBSASORCIS2	196.1±1.86	0.081±0.02	0.5	0.5	0.5	1
12CLBSASORCIS3	249.3±9.07	0.362±0.02	0.5	0.5	0.5	1
12CLBSACIS1	252.2±15.02	0.413±0.02	0.5	-	1	-
12CLBSACIS2	246.3±30.37	0.397±0.08	0.5	-	1	1
12CLBSACIS3	188.9±2.27	0.091±0.01	0.5	-	1	1

To prevent the water solubility of the cross-linked polymer particles which was caused by EDC carbodiimide, dicyclohexyl-carbodiimide (DCC), a water insoluble crosslinker was used.

The samples were prepared using the same preparation parameters. Using DCC the water solubility of the particles was avoided, furthermore, the encapsulation efficiency was high (Table 3).

Table 3. Encapsulation of BSA by polymer activation with DCC cross-linker

Sample	Z-avg [nm] Before Washing	PDI (Before Washing)	BSA [mg]	DDC [mg]	EE [%]	Yield [%]
12CLBSADCC1	217.6±2.27	0.129±0.02	0.5	1	71	-
12CLBSADCC2	229.9±5.88	0.332±0.01	0.5	1	-	-
12CLBSADCC3	225.9±2.47	0.115±0.02	0.5	2	73	46
12CLBSADCC4	219.7±1.87	0.121±0.01	1	2	82	46
12CLBSADCCBlank	233.4±1.51	0.136±0.02	-	2	-	47

From the dynamic light scattering measurement, suitable particle size and a narrow size distribution was obtained (Figure 1). The mean hydrodynamic size of the particles was between 220 and 230 nm.

Encapsulating the active agents with BSA and using DCC (Table 4) at sample 12CLBSACISH5, a better production yield and encapsulation efficiency were obtained compared with the sample 12CLBSACISH4, which was prepared without DCC. The sample CISH5 was also more stable than sample CISH4.

So, it can be concluded that the presence of active agents improves the particle size and using DCC with active agents improves the encapsulation efficiency and the system stability.

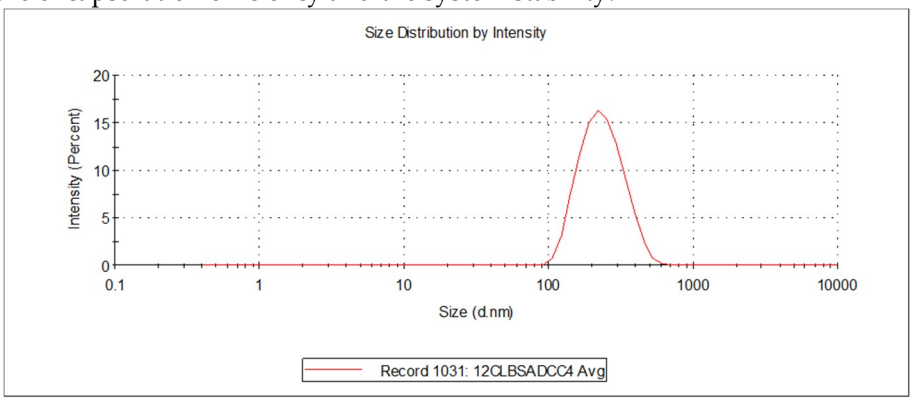

Figure 1. Particle size distribution of BSA encapsulated nanoparticles using DCC (2 mg) as cross-linker.

Table 4. Encapsulation of BSA with active agents.

Sample	Z-avg [nm] Before Washing	PDI (Before Washing)	Yield [%]	EE CIS [%]	Loading Capacity SOR [%]	CIS [mg]	DCC [mg]	o/w ratio	Polymer [%]	PVA [%]
12CLBSACISH4	204.6	0.250	40	24	2.0	0.5	-	1/5	1	1
12CLBSACISH5 Blank	212.9	0.171	39	-	-	-	2	1/5	1	1
12CLBSACISH5	209.8	0.209	61	28	0.7	0.5	2	1/5	1	1

4. Conclusions

The double emulsion-solvent evaporation method for the preparation of sorafenib and cisplatin loaded polymer nanoparticles was optimised. It can be concluded that one-to-five oil-in-water ratio with 0.5 mg BSA gave an acceptable size, but the size distribution was too wide which can be seen from the PDI value. The BSA was encapsulated and cross-linked successfully by using DCC. The cisplatin and sorafenib was co-encapsulated successfully by double emulsion method. The presence of active agents decreases the particle size and using DCC with active agents improves the encapsulation efficiency and the system stability.

5. Patents

Author Contributions: conceptualization, Izolda Kántor, Anamaria Todea and Tivadar Feczkó; methodology, Izolda Kántor and Anamaria Todea.; validation, Zoltán May, Emese Biró and Tivadar Feczkó; formal analysis, Tivadar Feczkó; investigation, Izolda Kántor Diana Aparaschivei and Emese Biró; resources, Tivadar Feczkó and Francisc Péter; writing-original draft preparation, Izolda Kántor; writing-review and editing, Emese Biró, May Zoltán and Tivadar Feczkó.; visualization, Izolda Kántor and Emese Biró; supervision, Tivadar Feczkó; project administration, Tivadar Feczkó and Francisc Péter; funding acquisition, Tivadar Feczkó and Francisc Péter.

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