Magnetic Polymer Nanospheres for Anticancer Drug Targeting

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Abstract. Poly(D,L-lactide-co-glycolide) polymer (PLGA) nanospheres loaded with biocompatible magnetic fluid as a magnetic carrier and anticancer drug Taxol were prepared by the modified nanoprecipitation method with size of 200–250 nm in diameter. The PLGA polymer was utilized as a capsulation material due to its biodegradability and biocompatibility. Taxol as an important anticancer drug was chosen for its significant role against a wide range of tumours. Thermal properties of the drug-polymer system were characterized using thermal analysis methods. It was determined the solubility of Taxol in PLGA nanospheres. Magnetic properties investigated using SQUID magnetometry showed superparamagnetism of the prepared magnetic polymer nanospheres.

1. Introduction

Drug targeting is defined as selective drug delivery to specific physiological sites, organs, tissues or cells where drug’s pharmacological activities are required. In principle, a drug distributes in the whole body when it is injected in the blood, and the drug that is distributed to sites other than the therapeutic sites may cause toxic side effects. Magnetically controlled drug targeting is one of the various possibilities of drug targeting. This technique is based on binding a selected anticancer drug with magnetic fluids into a pharmacologically stable formulation. The drug can be dissolved, entrapped, encapsulated or attached to nanoparticles and depending upon the method of preparation, nanoparticles, nanospheres (NPs) or nanocapsules can be obtained. Magnetic polymer NPs, made from organic and inorganic components, have unique characteristics due to the specific properties of the blend. The constituents of a magnetic polymer NPs play different roles: the polymer matrix acts as a shell, reservoir, and vehicle for the active component, whereas magnetite is the component that makes targeting possible by external magnetic field manipulation. The polymer matrix of the NPs must fulfil several requirements such as biocompatibility, biodegradability, mechanical strength and ease of processing. The best known class of biodegradable materials extensively used in controlled drug delivery systems are poly(lactide-co-glycolide) (PLGA) polymers. Their main advantage is that they are broken down into biologically acceptable molecules that are metabolized and removed from the body via normal metabolic pathways. Paclitaxel (Taxol®, TAX), an important anticancer drug, has been chosen for encapsulation to the polymer for its significant role against a wide range of tumours (breast cancer, ovarian carcinoma, lung cancer, head and neck carcinomas) [1]. Taxol
is not affordable from the nature. The other limitation of Taxol is its high insolubility in water and most pharmaceutical solvents. Adjuvants such as Cremophor EL are used to make Taxol injectable. In order to eliminate the side effects of the adjuvants (hypersensitivity reactions) and improve the therapeutic efficacy of the drug, alternative dosage forms have been suggested, including liposomes, microspheres and polymeric nanoparticles.

Thermal analysis methods are very useful tool in the characterization NPs and interactions in polymer–drug systems [2]. Differential scanning calorimetry (DSC) can be used to calculate the solubility of the drug in the polymer, an important parameter which influences the drug encapsulation and release from NPs [3, 4]. Thermogravimetry (TGA) is a good method for the determination the presence or absence of residual components in NPs. It is based on the monitoring the mass loss of individual components. The aim of the work was to prepare Taxol loaded magnetic and non–magnetic polymer NPs and to apply thermoanalytical methods to characterize thermal properties and determine the drug solubility drug in prepared PLGA NPs.

2. Materials and experimental methods
Taxol was obtained from Indena company. PLGA polymer with D,L-lactide to glycolide ratio of 85:15, molecular weight of 50–75 kg.mol\(^{-1}\) and the glass transition temperature of 50°C, and Pluronic F68 used as a stabilizing agent were purchased from SIGMA company. Poly(ethylene glycol) (PEG) with molecular weight of 1 kg.mol\(^{-1}\) was supplied by Merck. Magnetite (Fe\(_3\)O\(_4\)) was prepared by co-precipitation of ferric and ferrous salts in an alkali aqueous medium [5]. To prepare stable colloidal suspension of magnetic particles, sodium oleate as a first surfactant was used to prevent their agglomeration. PEG as a second surfactant was added to the system magnetite–sodium oleate to improve biocompatibility of magnetic material.

The modified nanoprecipitation method [6] used for entrapment magnetic fluid (MFPEG) and anticancer drug Taxol into polymer nanospheres was described in more details in [7]. After the sphere formulation, the thermal behaviour of the prepared NPs was characterized by DSC and TGA methods and SQUID magnetometry at room temperature. DSC measurements were performed using Perkin Elmer DSC 7 calorimeter at the rate of 10°C/min up to 250°C in the flowing nitrogen atmosphere. We intended to determine the solid-state solubility of the drug in the polymer at the drug melting temperature by measuring the enthalpy change of melting of the free drug (not incorporated into the polymer) in dried NPs with variable Taxol loading. The thermogravimetric investigations were carried out using a SETARAM thermobalance model TGDTA92 at the heating rate of 10°C/min from 30°C to 600°C in the argon atmosphere.

3. Results and discussion
Magnetic and non–magnetic PLGA NPs of the spherical shape and the mean diameter of 200–250 nm loaded with different amounts of encapsulated Taxol were prepared. Their morphology and size distribution are described in more details in [7].

Typical DSC curves of pure Taxol, physical mixture of Taxol and magnetic PLGA NPs, Taxol loaded magnetic PLGA NPs (TAX/MFPEG/PLGA NPs), blank PLGA NPs and magnetic particles loaded PLGA NPs (MFPEG/PLGA NPs) are in Fig. 1. As can be seen we observed no significant peak in the temperature range of 70–250°C for blank PLGA NPs as well as for MFPEG/PLGA NPs and TAX/MFPEG/PLGA NPs. The endothermic melting peak of pure Taxol (228°C) is only observed in the thermogram of the physical mixture and it is slightly shifted to a lower temperature (224°C). Therefore, it could be concluded that Taxol in NPs was in an amorphous or disordered crystalline phase of molecular dispersion or a solid solution state in the polymer matrix after the production [2]. The glass transition temperature \(T_g\) of PLGA polymer was not influenced by the preparation procedure.

Figures 2 and 3 show DSC traces of the dried non-magnetic and magnetic PLGA NPs, respectively, with variable amounts of the added drug up to 37.5 wt\% \((m_{TAX}/(m_{TAX} + m_{PLGA}))\).
In these thermograms, endothermic peak I (around 55°C) refers to $T_g$ of PLGA. Endothermic peak III (around 110°C) appeared in Taxol loaded magnetic NPs is associated with used MFPEG encapsulated to polymer NPs. At the melting temperature of Taxol, the observed endothermic process (peak II around 225°C) corresponds to the melting of the residual undissolved drug. Values of melting enthalpy $\Delta H_m$ for both types of NPs were plotted as a function of Taxol loading percentage $m_{TAX}/(m_{TAX} + m_{PLGA})$ in Fig. 4. The X-axis intercept provided by linear regression of the data, yielded a value of TAX in PLGA at 14.9 wt% for the non-magnetic NPs. That means, when the drug was bound by the NPs, it was possible to bind a maximal amount of about 17 mg of Taxol into 100 mg of the used polymer without excess observed by DSC. However, there was a limitation by the colloidal stability. The collected data for the magnetic NPs samples gave higher ability of comprising Taxol. If we took the assumption that TAX was incorporated in PLGA and only these two components have to be taken into account, the data analysis gives a result of $m_{TAX}/(m_{TAX} + m_{PLGA}) = 17.5$ wt%, what is 21.2 mg TAX/100 mg PLGA. This higher value in comparison with non-magnetic NPs might be a result of the presence of the surfactants (PEG, sodium oleate) in the used magnetic fluid.

Figure 5 shows TGA thermograms of the dried PEG-coated magnetite particles, magnetite loaded NPs and Taxol loaded magnetic NPs. The TGA residue for MFPEG at 600°C was 53.31 wt%, for MFPEG/PLGA was 36.2 wt% and for Taxol loaded magnetic NPs was 35.8 wt%.
Figure 5. TGA traces of pure MFPEG and MFPEG and TAX/MFPEG encapsulated in PLGA polymer.

Figure 6. Hysteresis cycles of pure MFPEG and MFPEG and TAX/MFPEG encapsulated in PLGA polymer at 300 K.

The 17.1 wt% difference between up and down thermograms was associated with the PLGA and Taxol presence. Compared with the amount of Taxol added (theoretical Taxol loading: 0.6 wt%/wt), it was shown that most of the Taxol was encapsulated into PLGA polymer matrix.

In Fig. 6 there are results obtained from SQUID measurements referred to the same mass of the samples. All types of samples exhibit similar overall superparamagnetic behaviour at room temperature. The presence of the nonmagnetic shell is evident by the fact that saturation magnetizations of TAX/MFPEG/PLGA and MFPEG/PLGA NPs ($I_S = 1.4$ mT and 1.6 mT, respectively) are about one half of value $I_S$ of the used pure MFPEG (3.4 mT). The calculated values of coating for PLGA 15.5 wt% and for Taxol 0.5 wt% are in a good agreement with PLGA and Taxol concentrations obtained from TGA measurements (Fig. 5).

In conclusion, non–magnetic and magnetic PLGA nanospheres with variable loadings of Taxol were succesfully prepared and characterized. DSC measurements of non-magnetic NPs gave an estimation of Taxol loading capacity at 17 mg of Taxol into 100 mg of PLGA, although such dispersions were not stable colloids. Higher loadings without excess drug might be achievable in Taxol loaded magnetic NPs dispersions that were more stable. They were found to be superparamagnetic with saturation magnetization of 1.4 mT. The results confirmed incorporation of magnetic particles and drug in the PLGA polymer and the opportunity that the proposed Taxol loaded magnetic NPs could be effective in magnetic drug targeting.

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