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# Biodegradable magnetic microspheres for drug targeting, temperature controlled drug release, and hyperthermia

**Abstract:** Magnetic microspheres (MMS) used for magnetic drug targeting consist of magnetic nanoparticles (MNP) and a pharmaceutical agent embedded in a polymeric matrix material. The application of MNP for drug targeting enables guiding the MMS to a target area, imaging the position of the MMS with magnetic particle imaging, and finally inducing drug release. As latter takes place by degradation of the MMS or diffusion through the matrix, an increase in temperature, e.g. through magnetic hyperthermia, leads to an accelerated drug release. Here, MMS consisting of poly(lactic-co-glycolic)acid (PLGA) with different monomer ratios were prepared by an oil-in-water emulsion evaporation method. The model drug Camptothecin (CPT) and magnetic multicore nanoparticles (MCNP) with a high specific heating rate were embedded into the microspheres. We obtained MMS in the preferred size range of 1 to 2  $\mu\text{m}$  with a concentration of MCNP of 16wt%, a drug load of about 0.5wt% and an excellent heating performance of 161 W/g<sub>MMS</sub>. Investigations of the drug release behaviour showed an accelerated drug release when increasing the temperature from 20 °C to 37 °C or 43 °C by using a water bath. In addition, an increase in drug release of about 50% through magnetic heating of the MMS up to 44 °C compared to 37 °C was observed. By this, a magnetic hyperthermia induced CPT release from PLGA MMS is demonstrated for the very first time.

**Keywords:** magnetic microspheres, drug delivery, hyperthermia, biodegradation, magnetic nanoparticles

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## 1 Introduction

The treatment of tumours with chemotherapy leads to severe side effects due to the systemic distribution of the anti-cancer drug and the resulting damage to healthy tissue. To deliver the therapeutic agent specifically to a desired area, so called drug delivery systems consisting of polymeric drug carriers are a promising approach [1]. Often, microspheres (MS) in the size range from 1 to 5  $\mu\text{m}$  are used as such carriers whereby natural polymers (e.g. proteins or polysaccharides) or synthetic ones (e.g. poly(ethylene glycol)) can be used [2]. A very commonly used synthetic polymer is poly(lactide-co-glycolide) because of its biodegradability and FDA approval [3-7].

To guide the drug carriers to their target area inside the human body, embedding magnetic nanoparticles (MNP) into the MS is an advantageous tool. By applying an external field, they can be moved inside the body while their current position can be determined by imaging via MPI or MRI. Additionally, MNP can generate heat by magnetic hyperthermia when they are exposed to an alternating magnetic field [8]. Since drugs are released out of the polymeric matrix by either degradation or diffusion, an increase in temperature can accelerate this process [9]. Those additional features can't be offered by other targeting strategies like antibody targeting [6].

In order to enable a fast and reliable temperature increase, MNP with a high specific heating power are needed. For this, magnetic multicore nanoparticles (MCNP) [10, 11], which show high magnetization values in an external field and at the same time low aggregation tendencies due to their low coercivity once the external field is removed, are used.

In the here presented study MCNP have been embedded into PLGA microspheres, prepared by an oil-in-water emulsion evaporation method [12] using different PLGA with varying monomer ratios (lactide:glycolide). As a therapeutic agent, the anticancer drug Camptothecin (CPT) was added to the microspheres. Morphology and magnetic properties of the MMS were investigated and the temperature dependent drug release was studied. To our knowledge, this is the first time the

combination of PLGA microspheres, magnetic multicore nanoparticles and Camptothecin is evaluated.

## 2 Methods

### 2.1 Preparation of microspheres

PLGA microspheres were prepared using an oil-in-water emulsion evaporation method [12]. Three different types of PLGA (purchased from Sigma Aldrich) were used to study the influence of the lactide to glycolide ratio on the drug release. Used ratios were 50:50, 65:35 and 75:25. In short, 200 mg of PLGA were dissolved in dichloromethane as an organic solvent. MCNP were coated with C12-bisphosphonate to ensure a stable suspension in organic solvents [13] and added to the PLGA solution at a ratio of 1:5 (MCNP:PLGA). CPT was dissolved in dimethylsulfoxid at a ratio of 1:100 (CPT:PLGA) and added to the PLGA/MCNP suspension to form the organic phase. The total volume of the organic phase was adjusted to 2 ml. As a water phase, 15 ml of a 2wt% aqueous polyvinylalcohol solution was used. The two phases were then homogenized by using a mechanical dispersing tool at varying velocities for 2 minutes to form micro droplets. The emulsion was then poured in a large volume (85 ml) of the aqueous phase and stirred for 6 hours under a fume hood to evaporate the organic solvent and thereby harden the micro droplets to form magnetic microspheres (MMS). Those microspheres were extracted via centrifugation or magnetic separation and washed several times with deionized water. For long term storage the MMS were freeze dried.

### 2.2 Characterization

To evaluate the size and size distribution of the MMS, static light scattering (Mastersizer 2000, Malvern Panalytical, Almelo, Netherlands) was used.

Size and size distribution was also investigated with scanning electron microscopy (SEM; FEI Helios NanoLap G3 UC, Hillsboro, Oregon, USA) which also allows statements on the morphology of the MMS. By enhancing the contrast for heavy elements and preparing cross sections with a focused ion beam the MCNP can be located. Additionally X-Ray spectroscopy (EDX) for carbon, oxygen and iron with  $E_0 = 3 \text{ keV}$  was used to determine the composition on the microspheres surface and at the centre of the cross section.

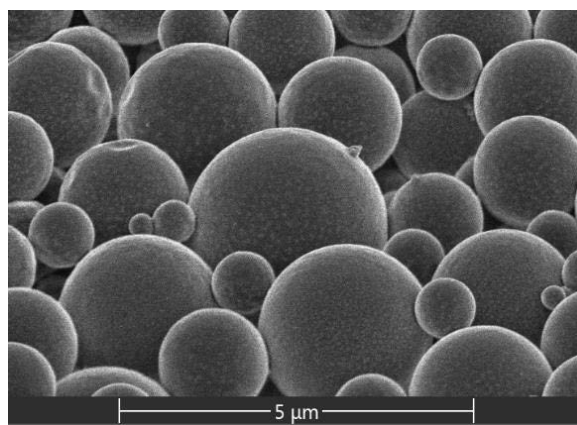
For magnetic characterisation of the MCNP and the MMS a vibrating sample magnetometer was used (VSM; MicroMag 3900, Princeton Measurements, Princeton, USA).

Heating performance (specific absorption rate; SAR) of the MMS was evaluated by using calorimetric measurements as described before [14]. For this, MMS were dispersed in water at different concentrations and a magnetic field of 24 kA/m and 410 kHz was applied. The temperature rise was measured with a fiberoptic probe (FOTEMP2, Optocon, Dresden, Germany).

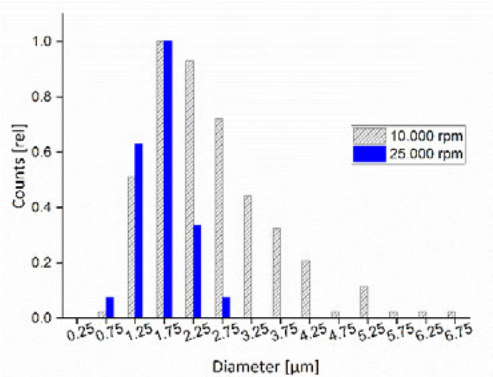
To study the temperature dependent drug release, aliquots of 2 mg CPT loaded MMS were dispersed in 2 ml PBS ( $\text{pH} = 7.4$ ) and stored at room temperature or in water baths with 37 °C and 43 °C. The remaining drug content in the MMS was determined at certain time points by dissolving the MMS in an organic solvent. CPT concentration in this solution was measured via UV/VIS spectroscopy at the absorption maximum for Camptothecin at 364 nm using a calibration curve. It was made sure that no other involved chemical agent showed a significant absorption at this measurement wavelength. To show the acceleration of drug release through magnetic heating, aliquots of 10 mg CPT loaded MMS in 0.5 ml PBS were stored at 37 °C or heated up to 44 °C magnetically for one hour. Remaining drug content was measured as described above.

## 3 Results and Discussion

SEM images of the MMS showed a perfectly round morphology for all microspheres loaded with CPT and MCNP (see **Figure 1**). The mean size of the MMS was in the desired range with values of 1.3 to 1.8  $\mu\text{m}$  measured with the mastersizer. Homogenization speed had no significant influence on the mean size taking into account the invariable peaks of the size distributions of the MMS derived from SEM images. However, looking at the width of the size distribution, an increasing homogenization speed leads to a narrower distribution without altering the peak (see **Figure 2**).



**Figure 1:** SEM image of MMS



**Figure 2:** Size distribution derived from SEM images for two different homogenization velocities. Intervals cover the stated value  $\pm 0,25 \mu\text{m}$

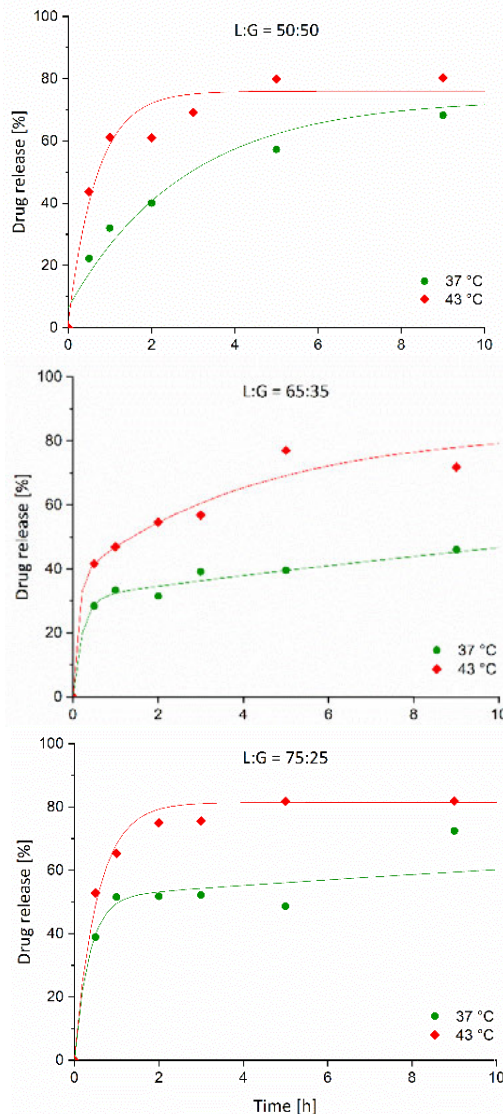
Concerning the location of the MCNP inside the MMS the FIB cross sections revealed the particles to be mostly located on the surface of the MMS. X-Ray spectra confirmed these findings by showing a larger intensity for iron than for carbon on the surface, whereas the cross section through the microspheres has a very low Fe concentration.

Drug loaded MS showed an initial amount of CPT up to 0.5wt%. Drug release studies at room temperature, 37 °C and 43 °C done by water bath heating revealed a clear dependency between temperature and drug release kinetics. In general, a burst type drug release was observed especially for 37 and 43 °C, with fast release of CPT in the first hours followed by a plateau phase, as expected from the literature [5, 12]. After 196 h, about 80% of the initial drug load was released for 37 °C and 43 °C whereas at room temperature a maximum of 50% was released. The main difference between 37 and 43 °C is the significantly faster drug release that can be seen in the first hours (see **Figure 3**). The influence of increasing temperature on the drug release matches with literature [9]. In contrast, the PLGA type has a minor influence on the drug release behaviour and from the fast drug release within hours, a diffusion based release mechanism can be deduced since polymer degradation happens in weeks to months.

VSM data show a coercivity of 3.7 kA/m for the MMS, which matches the values measured for the pure MCNP, and indicates a ferrimagnetic behaviour. From the saturation magnetization of 11.6 Am<sup>2</sup>/kg for the MMS, a concentration of 16wt% is determined for the MCNP inside the MMS, which relates to an encapsulation efficiency of nearly 97%.

SAR measurements showed a very promising heating power of the MMS (see **Figure 4**). At a concentration of 2.5wt% MMS in water, which is a realistic value for application, temperature increased by 10 K in 20 seconds and an SAR of 161 W/g<sub>MMS</sub> was calculated.

As a proof of principle, accelerated drug release through magnetic heating was demonstrated. MMS heated up to 44 °C

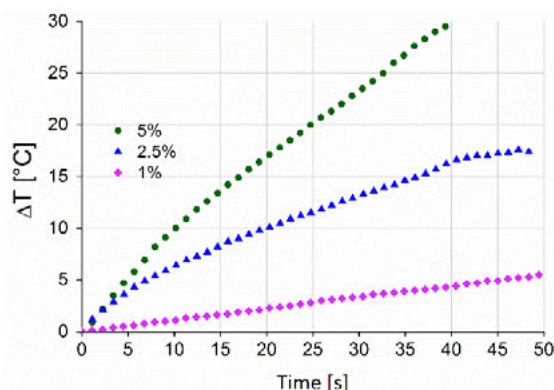


**Figure 3:** Drug release kinetics for different PLGA types in the first 10 hours. Lines only serve as a guide to the eye

for one hour in a magnetic field, significantly ( $p=0.0001$ ) released 48,7% more drug than MMS stored at 37 °C.

## 4 Conclusion and Outlook

In this study, we prepared perfectly spherically shaped PLGA microspheres with a main size of 1 to 2  $\mu\text{m}$ , loaded with Camptothecin and magnetic multicore nanoparticles. MCNP are mainly located at the surface of the MMS and enable heating the MMS with an external magnetic field, whereby a SAR of 161 W/g<sub>MMS</sub> is obtained. Drug release kinetics show a clear dependency on the temperature by an acceleration and a higher overall release when temperature is increased. By heating the MMS magnetically up to 44 °C for one hour, 50%



**Figure 4:** Temperature curves for different concentrations of MMS in water

more drug is released comparing to MMS stored at 37 °C. First attempts to manipulate and move the MMS with a magnetic field gradient or a rotating magnetic field within an MPI scanner showed promising results. In following studies, the targeting efficiency has to be characterized quantitatively. Our findings confirm the concept of using polymeric microspheres for drug delivery and controlling the drug release via magnetic heating.

#### Author Statement

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