IJFAC

Indonesian Journal of Fundamental and Applied Chemistry

Article http://ijfac.unsri.ac.id

Studies on the Controlled Release of Drugs from Magnetic Nanobiocomposites

Thomas Heinze[1]*, Robert Müller[2], Mengbo Zhou[1], Martin Rabel[3], Paul Warncke[3], Dagmar Fischer[3]

[1]Institute of Organic Chemistry and Macromolecular Chemistry, Friedrich Schiller University of Jena, Humboldtstraße 10, D-07743 Jena, Germany
[2]Leibniz-Institute of Photonic Technology (IPHT), P.O.B 100239, D-07702 Jena, Germany
[3]Department of Pharmaceutical Technology and Biopharmacy, Friedrich Schiller University of Jena, Lessingstrasse 8 D-07743 Jena, Germany

*Corresponding Author: Thomas.Heinze@uni-jena.de

Abstract

Magnetic nanocomposites are a class of smart materials that have attracted recent interest as drug delivery systems or as medical implants. In this study, meltable nanobiocomposites (NBC) composed of biocompatible dextran fatty acid ester and magnetic nanoparticles (MNPs) melting close to human body temperature were prepared and loaded with Rhodamine B (RhB) or green fluorescent protein (GFP) as model drugs to evaluate their potential use as drug delivery system. The release of the model drugs from the magnetic NBC investigated under the influence of a high frequent alternating magnetic field (AMF, 20 kA/m at 400 kHz) showed that on-demand release is realized applying the external AMF. The NBC showed a long-term stability (28 d) of the incorporated iron oxide particles after incubation in artificial body fluids. This work reveals the potential of the NBC as a drug carrier.

Keywords: Dextran ester; magnetic nanobiocomposite; alternating magnetic field; controlled release; biodegradation

Article Info

Received 21 December 2018
Received in revised 1 February 2019
Accepted 7 February 2019
Available online 10 February 2019

INTRODUCTION

Magnetic composites, consisting of magnetic particles and usually an organic matrix, have been used for remote controlled drug delivery (temporal and/or spatial) [1]. The organic matrices are often hydrogels composed of polymer networks containing large amount of water or biological fluids. Intensive studies have been reported on composites of thermo-responsive hydrogels, which are switched on/off through the heat generated by a magnetic stimulus [2-4]. In most cases, the drugs are released from the hydrogels above lower critical solution temperature (LCST) [5,6]. Nevertheless, magnetic hydrogels have certain shortcomings including leaking of drug, low biocompatibility, and long relaxation times [7].

Thermoplastic materials are used as an alternative matrix to overcome the limitations of hydrogels for controlled release applications. The drug release is remote controlled by increasing the temperature to induce the glass transition or the melting of the material. Examples of such systems include low molecular compounds like monoglyceride-based thermo-responsive drug delivery system [8], polymer-based implants using poly(methyl methacrylate) (PMMA) [9], or poly(D,L-lactic acid) based drug delivery systems [10]. However, PMMA is not biodegradable. Regarding poly(D,L-lactic acid), hydrolysis of polymer may lead to immunologic responses in the human body [11]. Thus, polymers without these shortcomings are highly desired for advanced drug delivery.

Dextran, a polysaccharide used in various medical applications [12], could be transferred by esterification with fatty acid into thermoplastic materials [13]. The melting temperature of fatty acid esters of dextran could be controlled by adjusting the type of fatty acid and the degree of substitution (DS). It is even possible to prepare dextran esters that melts near the human body temperature. Moreover, dextran ester yields homogeneous and transparent melts, which form stable films on a broad variety of materials. The transparency enables even the optical detection of the distribution of the loaded magnetic nanoparticles (MNPs) in µm-scale [14]. It could be demonstrated that these nanobiocomposites (NBC) can be molten with an external high frequent alternating magnetic field

(AMF), enabling the possibility for the remote-controlled release of immobilized compounds.

In a recent study, it was shown that release of green fluorescent protein (GFP) from magnetic nanobiocomposite (NBC) was accelerated by continuous exposure to AMF [14]. GFP was chosen because it is widely used as a biomarker and it is applied as an indicator for protein release, too [15,16]. Rhodamine B (RhB) has also been widely reported as model drug in controlled release applications. It was used to simulate a water-soluble drug [17,18]. In the present work, both compounds were loaded separately into dextran fatty ester-MNP forming NBC and the release of the model drugs was determined by spectroscopic methods under the influence of heat produced by simple external heating or by internal heating applying an alternating magnetic field (AMF). Additionally, the iron release from the incorporated MNPs was assessed as a measure for the stability of these composites under different physiological conditions using different artificial body fluids.

MATERIALS AND METHODS

Materials

Dextran myristic acid ester (degree of substitution, DS = 2.76, Mw = 22 000 g/mol) and dextran palmitic acid ester (DS = 2.69, Mw = 19 000 g/mol) were prepared by conversion of dextran produced by Leuconostoc mesenteroides ssp. (6000 g/mol) with fatty acid iminium chlorides, which were obtained by activation of the fatty acid with DMF/oxalyl chloride according to the procedure described in reference [13]. Magnetic nanoparticles (NMPs) were prepared from a solution of $FeCl_2x4H_2O/FeCl_3x6H_2O$ by the basic precipitation process. The MNPs were coated with oleic acid in a ball mill (Pulverisette 5, Fritsch, Germany) for 2 h and 150 rpm as described in reference [19]. Green fluorescent protein (GFP) was supplied by University of Applied Sciences of Jena (Jena, Germany, concentration = 1 mg/mL). Rhodamine B (RhB) was obtained from Sigma-Aldrich. Phosphate buffered saline (PBS, pH=7.4) was purchased from life technologies in UK. Deionized water was purchased by VWR international.

Methods

Preparation of Magnetic Nanobiocomposites (NBC)

NBC were prepared by solution casting method [14]. Briefly, 1.98 g dextran myristate dissolved in 5 mL tetrahydrofuran (THF) were mixed with 0.02 g oleic acid coated MNPs. The suspension formed was treated in an ultrasonic bath (Elma Transsonic 460/H, 35 kHz) for 20 min. It was poured into a petri dish and a film (thickness < 1 mm) was formed under airflow. The NBC was collected in form of granulates and dried in vacuum for 2 days. Dextran palmitate magnetic composite was prepared in comparable procedure. For investigations of the stability, 50 mg NBC granulate was filled into a mold and the temperature was increased to 50 °C for 20 min in an oven forming a composite disk after solidification. The disk samples had a radius of 3 mm and thickness of about 2 mm.

Preparation of NBC Containing GFP

NBC (50 mg) was filled into a blister pack with radius of 3 mm and thickness of 4 mm. The temperature was increased to 50 °C for 20 min in an oven forming a composite disk after solidification. 20 µL GFP solution (1 mg/mL) was dropped on the composite with micropipette. The GFP solution was allowed to dry under air for 2 h to form a layer on the composite surface. Subsequently, another 50 mg magnetic dextran myristic ester was added on the GFP layer and the temperature was increased to 50 °C for 20 min in an oven. After cooling, a disk shape GFP loaded NBC (radius of 3 mm and thickness of 4 mm) was formed (see Fig. S1). The composites were stored in the dark at 4 °C. The composite was kept in the blister to ensure a constant surface area to PBS medium during the controlled release experiment.

Preparation of NBC Containing RhB

Dextran ester (1.96 g) and 0.02 g RhB were dissolved in 10 mL THF and 0.02 g oleic acid coated MNPs were added. The suspension formed was treated in an ultrasonic bath (Elma Transsonic 460/H, 35 kHz) for 30 min. It was poured into a petri dish and a film (thickness < 1mm) was formed under airflow. The RhB containing NBC was collected in form of granulates and dried for 2 days in vacuum. RhB containing NBC (50 mg) was filled into a blister pack with a radius of 3 mm and thickness of 4 mm (see Fig. S1). The temperature was increased to 50 °C for 20 min in an oven forming a composite disk after solidification. The NBC were stored in the dark at 4 °C. The nanobiocomposite was kept in the blister during the controlled release experiment.

The samples for the controlled release experiments are summarized in Table 1.

Differential Scanning Calorimetry (DSC) of NBC

Magnetic myristate nanobiocomposite (10 mg) was investigated with a DSC equipment (Netzsch DSC 204 F1 Phoenix) in an aluminum pan under nitrogen environment. The heating rate was 10 °C/min and cooling rate was 20 °C/min. Samples were first cooled to -30 °C and heated up to 200 °C. The heating/cooling cycles were carried out for three times.

Biodegradation of NBC using Artificial Body Fluids

The biodegradability of the NBC was tested in different artificial body fluids simulating a physiological environment according to published procedures [21-23] in order to determine the iron release from the incorporated MNPs as a measure for the stability. Simulated body fluid (SBF, pH 7.4) and artificial lysosomal fluid (ALF, pH 4.5) were used to simulate the plasma or lysosomal compartment, respectively. The solutions were sterile filtered (0.2 µm filter) after pH adjustment. For degradation testing the composites (1% m/m MNP) were incubated in 10 mL ALF or SBF each for 28 days at 37 °C and 110 rpm in an incubation shaker (IKS 4000ic control, IKA-Werke, Germany). After 1, 2, 4, 8, 24, 48, and 72 h as well as after 7, 14, 21, and 28 days, 1 mL supernatant was collected and replaced with 1 mL of fresh ALF or SBF. The samples were stored at 4 °C for further analysis of iron content. The released iron was measured by bathophenanthroline disulfonic acid (Thermo Fisher, Germany) complexation and spectroscopic quantification (n=3) at 535 nm using a multiwell-plate reader (Spark 10M, Tecan Group, Switzerland) according to a modified method of Arbab *et al.* [21]. For measurements in SBF, solutions were acidified with an aqueous 5 mM citric acid solution for absorption stabilization. Iron concentrations were normalized in dependency on an iron (III) citrate (Sigma-Aldrich, Germany) standard curve (up to 0.06 mmol/L). The cumulatively released iron amount was calculated at each time point. The corresponding artificial body fluids (ALF, SBF) were used as blanks. The experiments were repeated once (SBF) or twice (ALF). Data are presented as the mean ± standard deviation (SD) of all experiments.

Thermal Treatment of Samples Externally with Water Bath and Internally with Alternating Magnetic Field

Stock solution of GFP were diluted with HPLC water to a concentration between 1 and 10 ng/mL and measured in a 10 mm path-length quartz cuvette (Helma 111-QS, Germany). The emission wavelength maximum at λ = 510 nm of GFP was measured with fluorescent spectrometer (Perkin Elmer LS50-B, UK) with excitation at 400 nm.

Stock solutions of Rhodamine B were diluted with HPLC water to a concentration between 0.5 and 5 mmol/L and measured in a 10 mm path-length cuvette. The amount of RhB was determined with spectrophotometry (Lambda 950 UV/Vis/NIR spectrometer, Perkin Elmer) by measuring the absorption at 544 nm. Data is shown as mean ± SD (n = 3).

The NBC disks filled in the blister pack (samples **R4-R6**, Table 1) were placed in a 15 mL polypropylene tube containing 3 mL PBS solution (pH value of 7.4). The polypropylene tube was heated in a water bath at 42 °C for 12 min. After 1 h, the PBS solution was removed and diluted with PBS (to meet the linear fitting concentration range of calibration curve). PBS was filled into the tube and it was heated in a water bath at 42 °C for 12 min. NBC (samples **R7-R9** and **R13-R15**, Table 1) loaded with model drugs were put in the polypropylene tubes containing 0.5 mL PBS solution and placed in the middle of coil in AMF (3 turns, 5.5 cm diameter, water cooling).

Table 1. Samples of nanobiocomposites for controlled release studies (dextran myristate containing 1% magnetic nanoparticles)

Sample No.	Model drug	Heating source	Duration of release (h)	Duration of AMF within release time (min)
R1-R3	Rhodamine B	No	6	-
R4-R6	Rhodamine B	Water bath	6	-
R7-R9	Rhodamine B	Alternating magnetic field	6	36
R10-R12	Green fluorescent protein	No	8	-
R13-R15	Green fluorescent protein	Alternating magnetic field	8	36
R16-R18	Rhodamine B	No	1440	-
R19-R21	Rhodamine B	Water bath	1440	-
R22-R24	Rhodamine B	Alternating magnetic field	1440	72

AMF was turned on for 12 min for each exposure (field amplitude of 20 kA/m and frequency of 400 kHz). After 48 min PBS solution was removed and diluted with HPLC water. PBS was filled into the tube and next AMF was applied for 12 min. For studying the release of RhB for the 60 days (**R19-R21**), the tube was heated for 12 min at 42 °C externally in each cycle, in order to adjust the heating time with the samples heated with AMF (**R22-R24**). After 4 h, the sample was removed for analyzing. The temperature of the composite was determined with a fiber optical sensor (OPTOcon, Dresden, Germany). Control samples (**R1-R3**, **R10-R12** and **R16-R18**) were placed in polypropylene tubes at 25 °C without any heat treatment. Cumulative release was quantified as mass released at time t, M_t, over the total mass loaded, M_{sum}. Data are shown as mean ± SD (n = 3).

RESULT AND DISCUSSION

Preparation and Thermal Properties of Bionanocomposites (NBC)

Esterification of dextran with fatty acids is a path to convert the biopolymer into a thermoplastic material. The thermoplastic constitutes a matrix polymer for the preparation of remote-controlled release systems that can be load with magnetic nanoparticles [13,14]. It is expected that the NBC undergoes a softening/melting above human body temperature under exposure of an alternating magnetic field (AMF), leading to an accelerated release of drugs loaded.

Dextran myristic- and dextran palmitic acid ester with degree of substitution (DS) of 2.76 and 2.69, respectively, were chosen as polymer matrix, because they are solids at 37 °C and show softening (melting) above human body temperature. Above the melting temperature, the viscosity of the pure esters drops down to about 1 Pa·s at 100°C [14].

On the contrary to hydrogels, the dextran esters possess high DS of long hydrocarbon chains. Thus, they are hydrophobic and insoluble in biological matrices. The incorporation of lipophilic MNPs (with oleic acid shell) and of lipophilic drugs should easy to be carry out. The thermal behavior of the magnetic NBC of dextran myristate was studied by differential scanning calorimetry (DSC). A broad endothermic signal occurs at 40 °C, with a maximum at 52 °C in the second and third run as shown in the Supporting Information (Fig. S2). The temperature maximum corresponds to the softening observed by investigation with optical microscope. The second and third runs are reproducible, indicating that the temperature range of softening is not changed after a heating/cooling cycle.

In order to study the influence of the kind of heating (AMF or by simple water bath) of the NBC with respect to heating rate and accessible temperature, heating tests under certain “geometrical” arrangements like sample size, volume of water bath and position of sensor were carried out. It turned out that the temperature of an NBC disk floating in 0.5 mL water can be easily increased by AFM (Fig. 1).

The temperature reached surpassed the melting range of the NBC after about 6 min. The AMF heating time was set as 12 min in order to melt the NBC. After cooling, the NBC solidified, and it could be molten again by increasing the temperature applying AFM.

Stability of magnetic NBC in artificial body fluids

The stability of magnetic NBC was assessed in two different artificial body fluids by measuring the iron release. Simulated body fluid (SBF) was used for the simulation of a neutral body environment (pH 7.4) that can be found in the blood stream, the extracellular matrix, or in the cytoplasm.

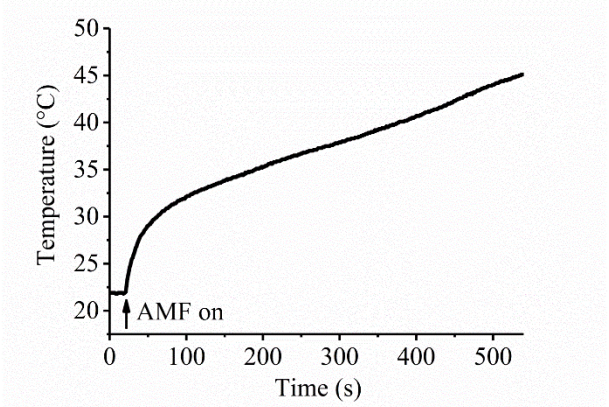

Figure 1. Temperature increase (measured with a fiber optical sensor) of a disk of the nanobiocomposite (1 wt.% magnetic nanoparticles) surrounded by 0.5 mL water subjected to continuous alternating magnetic field (AMF, strength of 20 kA/m and frequency of 400 kHz).

It is well known that iron oxide nanoparticles are degradable in lysosomes after systemic application by acidic disintegration mainly by iron chelating agents such as citrate [23,24]. To simulate the degradation of potentially released iron oxide particles from the NBC and to assess the shielding effect of the dextran fatty acid ester in acidic environments, artificial lysosomal fluid (ALF) as a complex artificial fluid was used [20]. Iron release from the incorporated MNPs was determined using bathophenantroline complexation to follow the degradation process quantitatively (Fig. 2). NBC stored in SBF released only 0.31% (dextran palmitate) or 0.19% (dextran myristate) of the total iron content indicating the stability of the MNPs in the NBC. The release of iron from NBC incubated in ALF over time was higher; a cumulative amount of 5.4% (dextran palmitate) or 2.17% (dextran myristate) was mobilized after 28 d at 37 °C.

Thus, the pH value of the system influences the stability of NBC, which is in accordance with the results of Gutiérrez *et al.* [24] studying the dependency of the degradation behavior of MNPs on the pH value. Under similar pH conditions (pH value of 4.5), a considerable effect of the coating was revealed as well. It may assume that the hydrophobicity of the matrix prevents degradation by limiting the penetration of the aqueous simulation media into deeper regions of the composites and therefore only superficial areas or MNPs located on the surface of the composites degraded. This indicates a sufficient stability of the composites even at acidic conditions. Therefore, if applied as implants, the novel NBC could be used as

remote-controlled drug delivery systems. Moreover, their use for the induction of hyperthermia [25] over a long period since they show no relevant signs of degradation at neutral conditions seems to be possible. Obviously, the dextran fatty acid esters are able to protect the incorporated MNPs or possibly drugs from degradation even at acidic conditions (ALF). There were no significant differences regarding the stability of the composites between the two investigated polymers, it was decided to choose the dextran myristic ester for further experiments since it displays a lower softening/melting temperature (40 °C, myristic acid ester compared to 45 °C, palmitic acid ester). The lower temperature is beneficial since many drugs, as for example proteins, are thermos-sensitive and release of the drug could be achieved at lower temperatures.

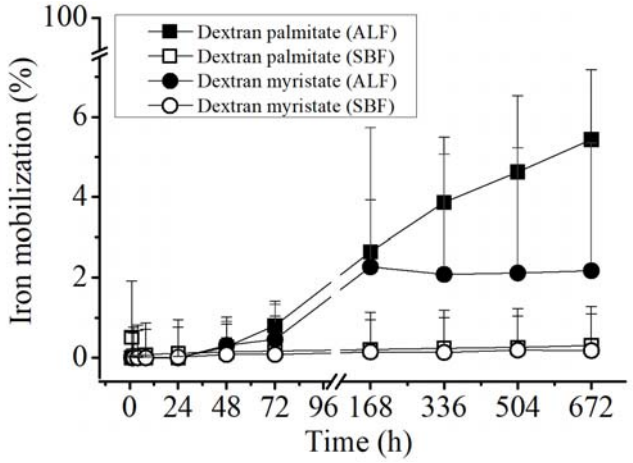

Figure 2. Iron release from magnetic nanobiocomposites quantified by bathophenanthroline complexation and spectroscopic determination at 535 nm (n=3). Experiments were run at 37 °C, 110 rpm over 28 d. Results are shown as the mean of two (SBF) or three (ALF) independent measurements ± SD.

Release of GFP and RhB in PBS Solution

GFP was used as model drug with a large molecular weight compared to RhB. Moreover, GFP can be easily detected by fluorescence spectroscopy down to ng/mL. NBC containing GFP was obtained by the entrapment trapped in the NBC as dumpling-like structure without any external stimulus.

Fig. 3 shows pulsatile release profiles of GFP from magnetic NBC (samples **R13-15**) induced by AMF compared to control samples (**R10-12**). There was no release of GFP at t_0 and t_1. After treating the sample by AMF for 12 min and for 48 min without AMF treatment, 5% of the GFP loaded was released from the NBC after 2 h. The release of the GFP was achieved after internal heating the NBC by AMF. After 3, 3.5 and 4 h total running time without additional exposure to AMF, a release of GFP could be observed (squares, Fig. 3) that was low compared to the amount released under exposure of AMF after 2 h. By applying repeated treatments with AMF, the amount of GFP released could be increased (at 4.5 and 6.5 h). Therefore, the dumpling-like structure was open after first melting process and GFP can diffuse into the medium. The release starts after the sample was treated with AMF because GFP cannot penetrate the tightly fitted polymer layer due to its thickness of about 1 mm [14]. The advantage of the magnetic system is that leaking of drug can be avoided compared to a hydrogel system, where a drug release could be achieved by changing of water absorption resulting in a swelling/shrinking of matrix structure [6].

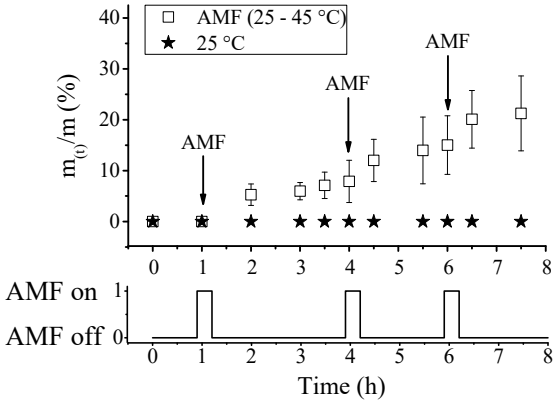

Figure 3. Cumulative of GFP release from nanobiocomposite (released mass at time t over the total mass of GFP, $m_{(t)}/m$) placed in phosphate buffered saline (PBS) solution (samples **R13-R15**). The samples were heated applying water bath (42 °C) or by alternating magnetic field (AMF) for 12 min interval. Control samples were kept at 25°C

Disks of the NBC containing RhB prepared by the casting method as discussed were treated by AMF or by external heating using a water bath applying same duration and cycles of heating (samples R4-6, R7-9, R19-21, R22-24, see Table 1). The starting of the heating cycles in case of short term experiments (hours) are indicated in Fig. 4, for long term experiments (days) they are after 1, 2, 3, 7, 14, 30, and 60 days shown in Fig. 5. As mentioned, different to the external heating in water bath, AMF generates a heating inside of the NBC. Release from the NBC containing RhB obtained under the different kind of heating shows a steady increase of released RhB at 42°C (temperature above melting) by external heating, on one hand (Fig. 4). On the other hand, internal heating with AMF yield comparable amount of RhB released (about 2%) within two hours. Further AMF

treatment yield an increased amount of RhB released. It turned out that externally heating using a water bath yields a slightly higher amount of RhB released of up to 6 wt.% compared to AMF. At 25°C a release of RhB was observed as well; after six hours about 2 wt.% are released.

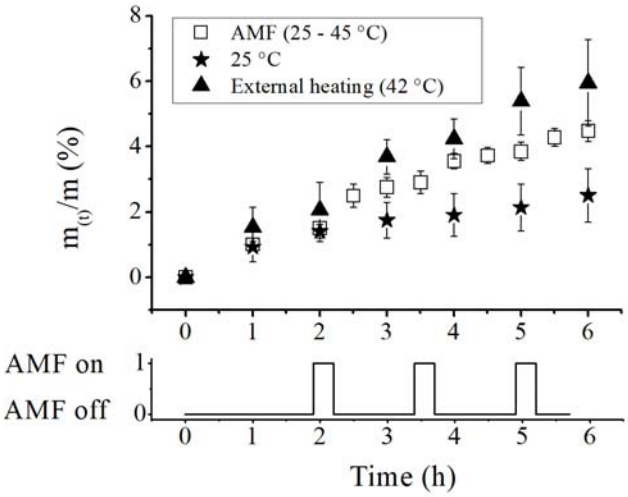

Figure 4. Cumulative mass of Rhodamine B released from nanobiocomposites over 6 h (square, 1 wt.% MNP under exposure to alternating magnetic field for 12 min duration for one heating cycle; triangle, 1 wt.% MNP, heated at 42 °C; star, 1 wt.% MNP, control sample at 25 °C) in phosphate buffered saline, $m_{(t)}$ represents cumulative mass released at time *t*, m represents the total mass loaded.

In long-term experiments, the cumulative RhB released is almost twice the amount compared to that of control samples treated at 25 °C after 60 days as shown in the Fig. 5. The amount of RhB released is comparable for samples stimulated by AMF and external heating by a water bath, considering the same heating cycles in both cases (12 min heating time per each cycle). It is occurred to the shorter effective time above the melting temperature in case of AMF heating. (see 3.3). The release rates of both heated samples (AMF-25° and Ex-25°) in Fig. 6 were calculated as the difference of mass released between the sample (**R19-21**, **R22-24**) and the control sample (25°C, **R16-18**) over the cumulative heating time, which is considered as the effect of heating. The release rates of the heated samples are higher than that of the control sample, which means that the heating increased the diffusion rate. The cumulative release rate of the control sample over the total time is given in Fig. 6 as well. Interestingly, the rates are different at short release times of the samples heated by AMF and externally, i.e., surface effects might be dominant. The local heat distribution in the samples might be different. Applying an external heating (water bath), the NBC start to melt on the surface and, thus, the drug is released fast from the outer layers, whereas the melting starts inside the samples heated by AMF and due to heat dissipation the melting of the surface starts later providing homogeneous heating in samples with homogeneously distributed MNPs occurs. Additionally, local fluctuations of the MNP concentration in the µm-scale can lead to temperature gradients. Local overheating near particle clusters can't be excluded. At long release times the time intervals between the heating cycles are bigger and the heating comes less important that can be seen on the parallel curves in Fig. 5 and the similar release rates of both heated samples in Fig. 6. Influences of the RhB concentration gradients on the release behavior over time might be possible however since the total released amount is quite small (<16%) the effect should be weak.

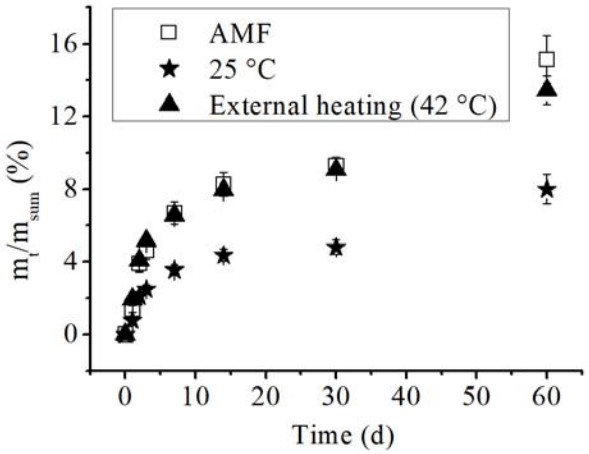

Figure 5. Cumulative mass released from Rhodamine B nanobiocomposite over 60 days (square, 1 wt.% MNP under exposure to alternating magnetic field for 12 min duration for one heating cycle; triangle, 1 wt.% MNP, heated externally at 42 °C; star, 1 wt.% MNP, control sample at 25 °C) in phosphate buffered saline (pH 7.4), m_t, represents cumulative mass released at time *t*, m_{sum} represents the total mass loaded.

Comparison of the cumulative release of the two model drugs showed that the release of GFP from a container-like sample geometry (20%) was more efficient than that of RhB from a homogeneously loaded material (6%) in 6 days despite of the much higher molecular mass of GFP. The reason could be that melting of GFP NBC caused abrupt structure collapse of the sample container and resulted in a fast release of GFP, however, the design of RhB NBC results a homogeneous distribution of RhB. Nevertheless, even after 8 hours GFP was not completely released in PBS and about 80% of GFP may be still trapped in the polymer. The diffusivity is

largely influenced by the environmental temperature and concentration in medium. It seems that a pulsatile diffusion of loaded GFP can be switched on/off, while RhB composite shows a sustained release behavior.

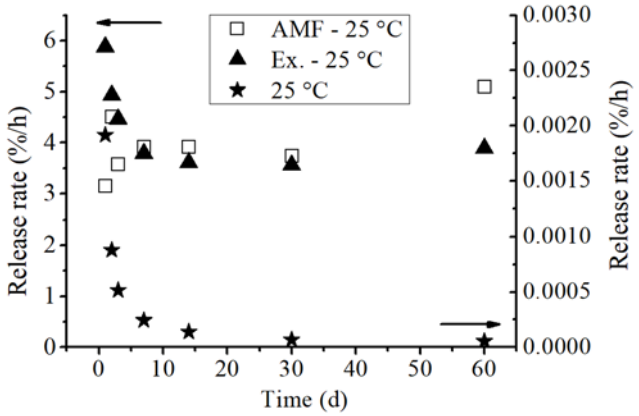

Figure 6. Release rate of samples heated by alternating magnetic field (AMF - 25°C), calculated by difference of released mass between AMF heating and control sample over the cumulative heating time. Release rate of samples heated externally (Ex - 25 °C), calculated by difference of released mass between external heating sample and control sample over the cumulative heating time.

CONCLUSION

Meltable polysaccharide-based biopolymers can be applied to design magnetically remote-controlled systems for drug release. The thermoplasticity of the magnetic nanobiocomposites enables the fabrication of carriers by melt casting without using solvents to design different geometries. The dextran esters protect the MNPs incorporated from degradation in artificial body fluids displaying a long-term stability (up to 28 days). GFP, i.e. compound of rather high molar mass can be immobilized in the NBC without leaking when no external thermal stimulus is applied compared to the hydrogel system. Increasing of temperature by AFM yield may accelerated diffusion of model drugs (GFP and RhB). This meltable and bio-based magnetic composite will be studied for the immobilization of drug and their controlled release by applying.

ACKNOWLEDGMENT

The financial support of the German Science Foundation (SPP1681, projects HE2054/14-2 and MU2382/5-1) is gratefully acknowledged. The authors thank M. Schmidt (University of Applied Science, Jena) for providing GFP. This work was financially supported by the Federal Ministry of Education and Research (BMBF, project 03XP0003).

REFERENCES

[1] K. Hauser, R. J. Wydra, N. A. Stocke, K. W. Anderson, J. Z. Hilt, “Magnetic nanoparticles and nanocomposites for remote controlled therapies,” *J. Control. Release,* vol. *219*, pp. 76-94, 2015.

[2] S. Brulé, Michael Levy, C. Wilhelm, D. Letourneur, F. Gazeau, C. Ménager, C. Le Visage, "Doxorubicin Release Triggered by Alginate Embedded Magnetic Nanoheaters: A Combined Therapy," *Adv. Mater.*, vol. *23,* no. 6, pp. 787-790, 2011.

[3] HM. Yun, SJ. Ahn, KR. Park, MJ. Kim, JJ. Kim, GZ. Jin, HW. Kim, EC. Kim, "Magnetic nanocomposite scaffolds combined with static magnetic field in the stimulation of osteoblastic differentiation and bone formation," *Biomaterials*, vol. *85*, pp. 88-98, 2016.

[4] J. Thevenot, H. Oliveira, O. Sandre, S. Lecommandoux, “Magnetic responsive polymer composite materials,” *Chem. Soc. Rev.*, vol. *42*, pp. 7099-7116, 2013.

[5] N. S. Satarkar, D. Biswal and J. Z. Hilt, “Hydrogel nanocomposites: a review of applications as remote controlled biomaterials,” *Soft Matter*, vol. *6*, pp. 2364-2371, 2010.

[6] T. Hoare, B. P. Timko, J. Santamaria, G. F. Goya, S. Irusta, S. Lau, C. F. Stefanescu, D. Lin, R. Langer, D. S. Kohane, “Magnetically Triggered Nanocomposite Membranes: A Versatile Platform for Triggered Drug Release,” *Nano Lett.*, vol. *11,* no. *3*, pp. 1395-1400, 2011.

[7] S. B. Campbell, M. Patenaude, T. Hoare, “Injectable Superparamagnets: Highly Elastic and Degradable Poly(N-isopropylacrylamide)–Superparamagnetic Iron Oxide Nanoparticle (SPION) Composite Hydrogels,” *Biomacromolecules*, vol. *14,* no. *3*, pp. 644-653, 2013.

[8] A. E. Mengesha, R. J. Wydra, J. Z. Hilt, P, M. Bummer, "Binary blend of glyceryl monooleate and glyceryl monostearate for magnetically induced thermo-responsive local drug delivery system,“ *Pharm Res.*, vol. *30,* no. *12*, pp. 3214-3224, 2013.

[9] S. A. Rovers, R. Hoogenboom, M. F. Kemmere, J. T. F. Keurentjes, “Repetitive on-demand drug release by magnetic heating of iron oxide containing polymeric implants,” *Soft Matter*, vol. *8*, pp. 1623-1627, 2012.

[10] H. Yin, S. Yu, P. S. Casey, G. M. Chow, “Synthesis and properties of poly(d,l-lactide) drug carrier with maghemite nanoparticles,”

Mater. Sci. Eng. C., vol. *30,* no. *4*, pp. 618-623, 2010.

[11] Y. Lee, J. Kwon, G. Khang, D. Lee, "Reduction of inflammatory responses and enhancement of extracellular matrix formation by vanillin-incorporated poly(lactic-co-glycolic acid) scaffolds," *Tissue Eng. Part A.*, vol. *18,* no. *19-20*, pp. 1967-1978, 2012.

[12] T. Liebert, J. Wotschadlo, P. Laudeley, T. Heinze, "Meltable Dextran Esters As Biocompatible and Functional Coating Materials," *Biomacromolecules*, vol. *12,* no. *8*, pp. 3107-3113, 2011.

[13] M. Zhou, T. Liebert, R. Müller, A. Dellith, C. Gräfe, J. H. Clement, T. Heinze, "Magnetic Biocomposites for Remote Melting," *Biomacromolecules*, vol. *16,* no. *8*, pp. 2308-2315, 2015.

[14] R. Müller, M. Zhou, A. Dellith, T. Liebert, T. Heinze, "Meltable magnetic biocomposites for controlled release," *J. Magn. Magn. Mater.*, vol. *431*, pp. 289-293, 2017.

[15] D. M. Sagar, S. Aoudjane, M. Gaudet, G. Aeppli, P. A. Dalby, "Optically induced thermal gradients for protein characterization in nanolitre-scale samples in microfluidic devices," *Sci. Rep.*, vol. *3*, pp. 2130, 2013.

[16] A. Cao, Z. Ye, Z. Cai, E. Dong, X. Yang, G. Liu, X. Deng, Y. Wang, S.-T. Yang, H. Wang, M. Wu, Y. Liu, "A facile method to encapsulate proteins in silica nanoparticles: encapsulated green fluorescent protein as a robust fluorescence probe," *Angew. Chem. Int. Ed.*, vol. *49,* no. *17*, pp. 3022-3025, 2010.

[17] A. Berkland, M. King, A. Cox, K. Kim, D. W. Pack, "Precise control of PLG microsphere size provides enhanced control of drug release rate," *J. Control. Release*, vol. *82,* no. *1*, pp. 137-147, 2002.

[18] N. Csaba, A. Sánchez, M.J. Alonso, "PLGA:poloxamer and PLGA:poloxamine blend nanostructures as carriers for nasal gene delivery," *J. Control. Release*, vol. *113,* no. *2*, pp. 164-172, 2006.

[19] R. Müller, H. Steinmetz, M. Zeisberger, Ch. Schmidt, S. Dutz, R. Hergt, W. Gawalek, "Precipitated Iron Oxide Particles by Cyclic Growth," Zeitschrift für Phys. Chemie, vol. 220, no. 1, pp. 51–57, Jan. 2006.

[20] M. R. Marques, R. Loebenberg, M. Almukainzi, "Simulated Biological Fluids with Possible Application in Dissolution Testing," *Dissolution Technol.*, vol. *18*, pp. 15-28, 2011.

[21] A. S. Arbab, L. B. Wilson, P. Ashari, E. K. Jordan, B. K. Lewis, J. A. Frank, "A model of lysosomal metabolism of dextran coated superparamagnetic iron oxide (SPIO) nanoparticles: implications for cellular magnetic resonance imaging," *NMR Biomed.*, vol. *18*, no. 6, pp. 383-389, 2005.

[22] N. Feliu, D. Docter, M. Heinze, P. del Pino, S. Ashraf, J. Kolosnjaj-Tabi, P. Macchiarini, P. Nielsen, D. Alloyeau, F. Gazeau, "In vivo degeneration and the fate of inorganic nanoparticles," *Chem. Soc. Rev.*, vol. *45*, pp. 2440-2457, 2016.

[23] M. Lévy, F. Lagarde, V.-A. Maraloiu, M.-G. Blanchin, F. Gendron, C. Wilhelm, F. Gazeau, "Degradability of superparamagnetic nanoparticles in a model of intracellular environment: follow-up of magnetic, structural and chemical properties," *Nanotechnology*, vol. *21*, no. 39, id. 395103, 2010.

[24] L. Gutiérrez, S. Romero, G. B. da Silva, R. Costo, M. D. Vargas, C. M. Ronconi, C. J. Serna, S. Veintemillas-Verdaguer, M. del Puerto Morales, "Degradation of magnetic nanoparticles mimicking lysosomal conditions followed by AC susceptibility," *Biomed. Tech. (Berlin)*, vol. *60*, no. 5, pp. 417-426, 2015.

[25] Hilger, "In vivo applications of magnetic nanoparticle hyperthermia," *Int. J. Hyperthermia*, vol. *29*, no. 8, pp. 828-834, 2013.

[26] A. Saeed, S. S. Ashraf, "Denaturation studies reveal significant differences between GFP and blue fluorescent protein," *Int. J. Biol. Macromol.*, vol. *45*, no. 3, pp. 236-241, 2009.