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Novel polyelectrolyte multilayer micro- and nanocapsules as magnetic carriers

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Abstract

Polyelectrolyte multilayer (PEM) capsules are introduced as versatile magnetic carrier systems. Superparamagnetic magnetite is mounted to the multilayer shell itself or is a component of the capsule interior. The PEM is formed at different (decomposable) colloidal templates, e.g. melamine formaldehyde resin, glutaraldehyde fixed red blood cells, emulsion oil droplets. The results are illustrated by transmission electron microscopy and confocal laser scanning microscopy.

Keywords: Microcapsules; Polyelectrolyte multilayers; Magnetic carriers; Confocal laser scanning microscopy; Transmission electron microscopy; Erythrocyte

1. Introduction

Polyelectrolyte multilayer (PEM) micro- and nanocapsules were introduced [1,2] as an extension of PEM on flat substrate surfaces [3–5]. When decomposable colloids are used as templates they can be removed after completion of the PEM coating and "hollow" capsules or shells are obtained [6,7]. Surprisingly, they are very stable in different aqueous and organic solvents. Recently, the colloidal PEM were combined with superparamagnetic magnetite [8–10]. Colloidal PEM are easily fabricated in a layer-by-layer procedure [2,8] which is algorithmic and iterative that means it can be carried out continuously with a high degree of automatization. More effective "single step" formulations based on complex precipitation are applied now and can be used alone or in combination with the layer-by-layer approach [11,12]. One of the advantages of PEM capsules is their versatility of physical and chemical properties by variation of composition and fabrication as well as application conditions. Several dozen of natural and synthetic polyelectrolytes or other charged substances are suitable as layer components, e.g. chitosan, chitosansulfate, poly(L-lysine), poly(acrylic acid), poly(diallyldimethylammonium chloride),

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dextransulfate, and proteins [13]. Many of them can be functionalized to provide special surface structures of biological or technical relevance. Also inorganic [14] and organic nanoparticles, multivalent ions [15] or surfactants, e.g. lipids [16], are selected materials for capsule layer construction. Consequently, PEM microcapsules can be designed with interesting optical, electrical, magnetic, catalytic, biological, and mechanical peculiarities. They are applied as containers for inorganic and organic matter, e.g. drugs, or macromolecules [15,17]. Either the colloidal matter itself intended to be encapsulated has served as template during the PEM formation, or the hollow capsules are filled afterwards out of solution by precipitation [18] or crystallization [17] into the capsule lumen, or by polymer synthesis inside the capsule [19], or by solvent exchange [20]. The release properties are controllable by capsule composition and after-treatment.

This publication presents both the direct incorporation of superparamagnetic magnetite into the PEM itself as a "layer" as well as its adsorption at the template particle surface. It is shown, for the first time, that single step PEM coating of the oil phase of an unprotected unstable oil-in-water emulsion results in a stable emulsion. The coating has to be done out of an aqueous salt solution. The oil phase contains finely dispersed superparamagnetic magnetite nanoparticles. The characterization of the capsules is obtained by transmission electron microscopy and confocal laser scanning microscopy. Possible applications and further directions of research are discussed with respect to the PEM capsule family.

2. Materials and methods

Materials: The polyelectrolytes sodium poly(styrenesulfonate), PSS, M_W ~70 000 and poly(allylamine hydrochloride), PAH, M_W ~50 000–65 000, were obtained from Aldrich and used without further purification. The water purification (USF Seral, Germany) yields water of better than 18.2 MΩ cm. NaCl (ACS), rhodamine B (chloride), sodium fluorescein, glutaraldehyde, HCl (ACS) and NaOH (ACS) were obtained from SIGMA.

Templates: Acid decomposable melamine formaldehyde resin latex of a diameter of about 2.7 μm was obtained from microparticles GmbH, Berlin, Germany. Glutaraldehyde fixed human red blood cells were prepared as described in Ref. [21]. Superparamagnetic magnetite dispersion in iso-octane (45 mT–11% v/v) stabilized by 9-octadecenoic acid was obtained from Mediport Kardiotechnik GmbH, Berlin, Germany.

Membrane filtration equipment: Millipore/Amicon ultrafiltration cell 8200 was used together with Millipore membrane filters.

Emulsification: Dispersion of the magnetite loaded octane was made by an Ultra Turrax T 25 basic from IKA Werke, Staufen, Germany.

Magnetite particles: All magnetite particles were obtained from Mediport Kardiotechnik GmbH, Berlin, Germany. They were prepared by co-precipitation of ferrous and ferric salts solution by concentrated ammonium hydroxide and stabilized by monolayers of cis 9-octadecenoic acid or dodecanoic acid [22]. The octadecenoic acid stabilized magnetite was homogeneously dispersed in iso-octane and results in a 11% v/v ferrofluid. The dodecanoic acid stabilized particles were further coated by an ethoxylated C_{12} alcohol with 9 mol/mol ethoxy groups. These particles were dispersed in water of pH 7. This dispersion was purified and surplus surfactant removed [23]. The water base ferrofluid included about 5% v/v magnetite (sized 20–30 nm), and the coated magnetite particles provided a (stabilizing) negative Zeta-potential.

Coating by membrane filtration: The details of the method are given in Ref. [8]. Incubation and washing cycles were alternated in a membrane filtration equipment. The incubation medium consisted of 1 g/l polyelectrolyte in 0.5 M NaCl or 0.2% v/v magnetite dispersion in 0.25 M NaCl. The incubation period was always longer than 5 minutes. Each incubation was followed by 2 washing cycles minimum.

Coating of the melamine formaldehyde resin (MF) latex: The negatively charged magnetite nanoparticles were incubated with the bare positively charged MF particles. This was followed by adsorption of 7 layers PAH and PSS. By exposing the coated system to 0.1 M HCl the MF templates were

decomposed into parts which were released from the capsules. TEM images of the capsules were taken.

Coating of glutaraldehyde fixed human red blood cells (RBC): Four layers PSS and PAH were adsorbed to the RBC starting with PSS. The latter was found empirically to result in a better performance of the fabrication procedure and quality of the product. After that the incubation in 0.2% v/v magnetite suspension was carried out. Four layers of PAH and PSS followed to enclose the magnetite into the shell itself. The outermost layer is PSS.

Coating of magnetite-in-octane-in-water emulsion: To 10 ml of 2.5 g/l PSS and 2.0 g/l PAH in 0.1% w/w NaOH (0.1 M NaCl) 2 ml of the magnetite-octane suspension was added. The system was emulsified by the Ultra Turrax at low intensity for about 1 min and then stopped. Under slight shaking there was added continuously 0.1% w/w HCl to neutralize the suspension during about 1 minute. This procedure resulted into a stable emulsion.

Confocal Laser Scanning Microscopy: Images were obtained by the Leica Confocal Laser Scanning Microscope TCS SP using $100\times$ oil immersion Aristoplan and $40\times$ C Plan objectives.

Transmission electron microscopy (TEM): The samples were dried on a carbon coated copper grid and the images taken with the Philips CM-12 microscope at an acceleration voltage of 120 kV.

3. Results

The results obtained by the stepwise layer-by-layer PAH/PSS/magnetite coating are presented in Figs. 1 and 2. In Figs. 3 and 4 the single step surface precipitation of PAH/PSS onto the magnetite loaded octane emulsion droplets is illustrated.

In Fig. 1, fluorescence images of sodium fluorescein labeled PAH/PSS capsules (eight layers) on glutaraldehyde fixed human red blood cells are shown. There are two populations visible which are different with respect to their fluorescence light intensities. The brighter ones are doped with superparamagnetic magnetite particles as a separate layer between fourth and fifth PAH layers. The darker ones are not magnetized. The situation is vice versa with respect to labeling by rhodamine B chloride (not shown here). By inspection of Figs. 1a–1d the motions of the brighter capsules, in contrast to the stationary darker ones, are clearly visible. They respond to the variation of the magnetic field applied to the system.

In Fig. 2, the transmission electron microscopy images of PAH/PSS capsules (seven layers) are presented. MF latex of 2.7 μm diameter was used as template. Negatively charged 9-octadecenoic acid protected magnetite nanoparticles are adsorbed to the latex. Then seven PAH/PSS layers are consecutively adsorbed and effectively confine the magnetite. After that the core is decomposed by 0.1 M HCl. In Figs. 2a and b large clusters of magnetite nanoparticles are seen. In Fig. 2b also smaller patches can be recognized. The system is still superparamagnetic because the capsules separate if the applied magnetic field is switched off. The same result was obtained for capsules prepared from the system presented in Fig. 1 by decomposition of the fixed human red blood cell core.

In Figs. 3 and 4, the situation after the single step surface precipitation of polyelectrolytes PAH and PSS on the magnetized octane droplets is illustrated. The fluorescence images with rhodamine B chloride (Fig. 3a) and sodium fluorescein (Fig. 3b) point to rather thick and inhomogeneous surface layers. This is supported by Fig. 4 which makes the surface layer detectable under transmission light conditions even without fluorescence.

In Fig. 3c, an applied magnetic field is collecting the droplets into chains which are oriented in parallel to the field direction. The droplets undergo no coalescence process even after prolongation of the field action to several minutes. After switching off the field the chains fall apart and the droplets separate from each other. The beginning of this process is illustrated in Fig. 3d. The emulsion is stable for a time period of approximately 1 week at 4°C.

4. Discussion

The magnetization of the microcapsules can be done at different sites. One can form intrinsic magnetite layers inbetween the polyelectrolyte layers or one can coat the template with magnetite first be-

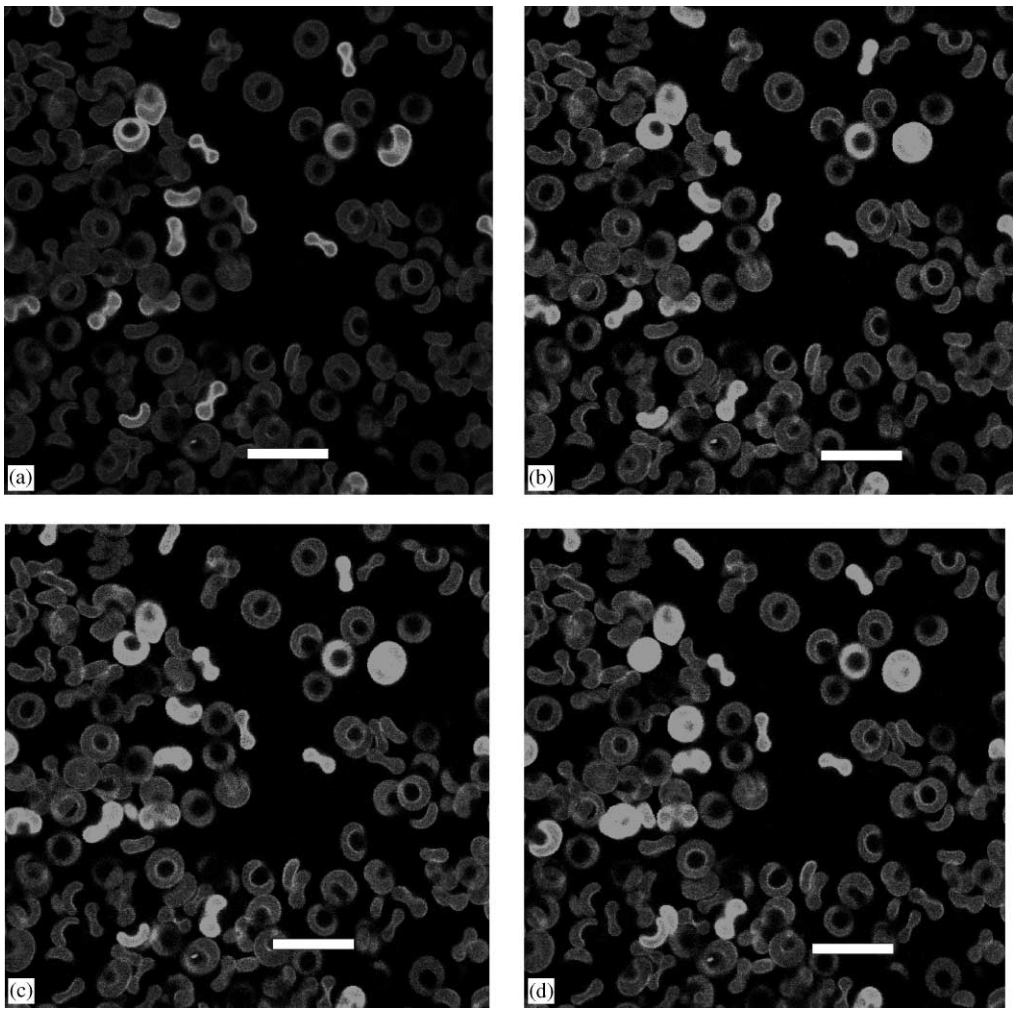

Fig. 1. Fluorescence image of fluorescein (sodium salt) labeled PAH/PSS multilayer capsules (eight layers) on fixed human red blood cells. Brighter capsules contain superparamagnetic magnetite particles as separate layer between fourth and fifth PAH layers. The scalebar corresponds to 12 μm. Figs. 1a–d illustrate different positions and/or orientations of magnetite containing capsules caused by variation of an applied magnetic field. The darker capsules without magnetic properties are stationary.

fore polyelectrolyte layer formation starts. The latter results in a magnetic innermost layer of the capsule shell after template removal. It is also possible to encapsulate core particles which are already magnetized inside their volume. These results are all demonstrated.

We present, for the first time, the encapsulation of magnetic oil droplets by surface precipitation of polyelectrolytes out of aquous salt solutions. Despite of no further fixation of the capsules, (e.g., crosslinking) the obtained stability with respect to coalescence in the presence of the magnetic field is surprisingly high. The droplets aggregate under the action of the field but do not fuse. If the magnetic oil droplets are protected by surfactants only the applied magnetic field leads to remarkable phase separation within a few minutes.

Another way of coating the oil microdroplets is given by the layer-by-layer procedure (in contrast to the presented one-step one). It also leads to

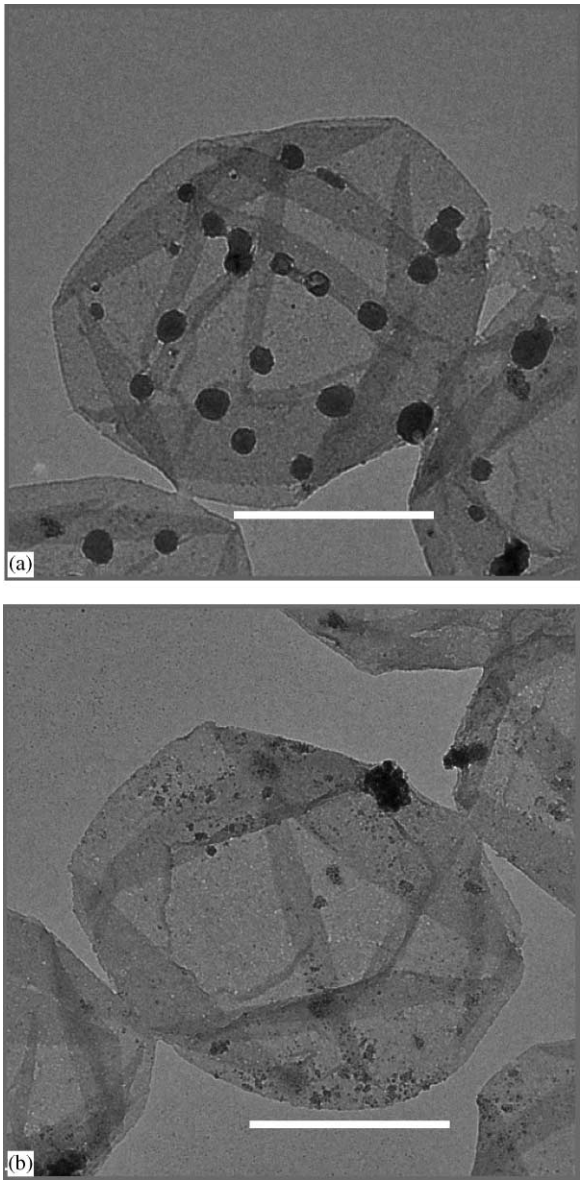

Fig. 2. Transmission electron microscopy images of PAH/PSS multilayer capsules are (seven layers) originally formed on melamine formaldehyde resin latex as spherical template of 2.7 μm diameter. The core was removed by acid decomposition. Fig. 2a shows large clusters and Fig. 2b, in addition, also smaller clusters of magnetite particles. The capsules are still superparamagnetic. The scalebar corresponds to 1.2 μm.

a very stable emulsion. In this case one has to start the coating procedure with PSS (or another more amphiphilic polyelectrolyte). Then one adds further layer-forming polyelectrolytes one after the other into the weakly stirred system with or without intermediate separation and washing steps (results not shown).

In contrast to most precipitation processes the presence of salt is essential for the formation of polyelectrolyte multilayers of appropriate material density and amount. Frequently [25], and also in the studied case, the surface layer exhibits gel-like behavior. This is similar to the polyelectrolyte couple PSS/poly(vinylbenzyltrimethylammonium chloride) (PVBTAC) in the ternary solvent acetone/sodium bromide/water we have recently studied with respect to surface precipitation on solid colloids [11]. The latter system could not be successfully transferred to the studied octane system. In both solvents, the alkaline aqueous salt solution and the ternary acetone/sodium bromide/water, the polyelectrolytes are not reacting with each other but their reaction can be induced by changing the solvent quality. There is a difference in the two systems which has to be explored further. Probably, the hydrophobic interaction of the polyelectrolytes with the oil/water interface is strongly modified by the presence of acetone.

The extension of the longevity of the studied emulsion can be obtained by hardening and fixation of the surface gel, e.g. by glutaraldehyde which is reacting with the amino-groups of the PAH and is forming intra- and intermolecular bridges. Other procedures usually done for gel stabilization are also of use [24].

The layer-by-layer approach adds stable and strong magnetic properties. The amount of magnetite coupled to the capsules exceeds in weight the amount of polyelectrolytes themselves. This is already obtained forming one layer of magnetite. Meanwhile, we have formed capsules with three layers of magnetite each separated from each other by three layers of polyelectrolyte (data not shown). As we expected, the presented procedure without new aspects was sufficient for successful formation of this more complex capsule type.

A very interesting new effect with respect to fluorescence modification by magnetite incorporation is demonstrated. The magnetized and non-magnetized capsules are identical with respect to their volume and surface properties. The magnetization

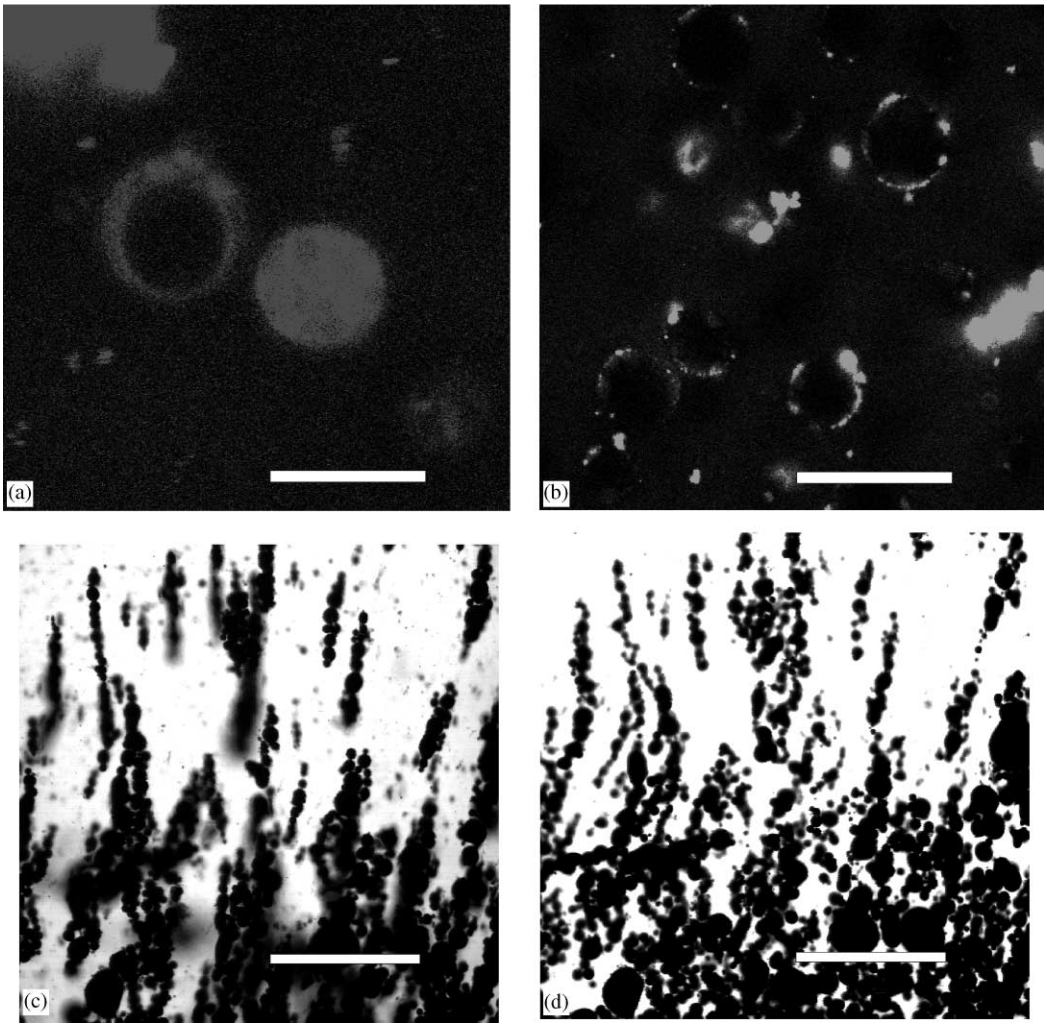

Fig. 3. Light microscopy images of PAH/PSS stabilized octane-in-water-emulsion. 9-octadecenoic acid protected magnetite is dispersed in the octane phase. Fig. 3a shows the fluorescence image of rhodamine B chloride labeled capsules coating the oil droplets and Fig. 3b that of the capsules labeled by sodium fluorescein. The scalebars in (a) and (b) correspond to 5 μm. Fig. 3c illustrates the emulsion under the influence of the magnetic field applied from above in the pictures plane. In Fig. 3d, the same system is shown about 2 s after switching off the magnetic field. The droplet chains break and the droplets start to separate. The scalebars in (c) and (d) correspond to 50 μm.

is not seen at the surface of the capsule which was recently demonstrated by scanning force microscopy measurements (not shown). Nevertheless, the magnetized capsules are brighter in fluorescein fluorescence and darker in rhodamine fluorescence than the comparable non-magnetized ones. Consequently, one recognizes the magnetized capsules by inspection of their fluorescence properties alone. Magnetic and fluorescence properties are highly correlated with each other. The reason for this favorable combination is not clear but may be caused by defects that the magnetite nanoparticles add to the polyelectrolyte network. This could lead to exposure of additional binding sites for negatively charged fluorescein and repulsive interaction with positively charged rhodamine B. We expect that this phenomenon can be exploited for (immunological) sensoring and detection tests and for

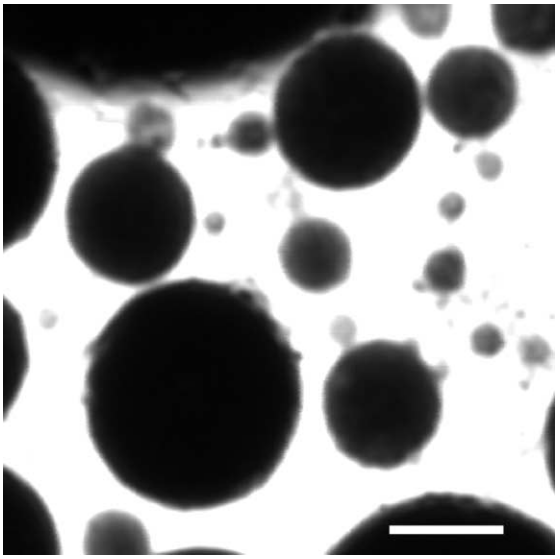

Fig. 4. Light microscopy image of the coated emulsion of Fig. 3. The thick surface coat of PAH/PSS gel-like precipitate around the droplets is visible. The scalebar corresponds to 2 μm.

studying interactions and packing architecture of particles.

The red blood cell templates are decomposed by the deproteinizer NaOCl. This is done under alkaline conditions. Strong alkaline conditions are also applied to keep the polyelectrolyte couple PSS/PAH not reacting with each other before coating. These transient alkaline conditions normally destroy the magnetite. The experimental results, however, do not show such disadvantageous effect. This can be caused by effective shielding of the magnetite from the medium. The protective layers as well as the oil environment in the case of emulsion drops constitute, presumably, a barrier to the chemical attack. We assume further that magnetite cluster formation and interaction with the polyelectrolyte multilayer are protecting sufficient magnetic material and preventing its destruction during the 30-min incubation period with the deproteinizer.

The connection of magnetic properties with colloidal PEM leads to a promising magnetic carrier system. Like a "Lego" system all imaginable combinations of polyelectrolyte, nanoparticles, dyes and other interesting substances, e. g. antibodies, can be combined to result in very complex (on the nanometer scale) or very simple PEM capsules. They can be adapted to different demands, e. g. medium conditions. Their stability, longevity, release properties and biocompatibility are controllable.

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