Size Control of hydrophilic polycentric Ferrofluids for Locoregional Tumor Therapy by Pulse Etching during Synthesis

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Hydrophilic magnetic nanoparticles with bound drug or radiation target are promising tools for locoregional therapy of tumors [1,2]. Those magnetic targeting methods depend on the availability of biocompatible nanoparticles free of large particles or aggregates, which can cause embolic problems. Nevertheless the nanoparticles for medical targeting applications have to depict a high specific magnetic moment to overcome the blood flow upon magnetic immobilization at the tumor site. Thus the particles have to be a compromise in size and magnetic moment.

We introduced an access to medium-sized magnetic nanoparticles by synthesis of ferrofluid precipitates from iron-citrate mixtures and subsequent modification by pulse etching and size separation by fractionated sedimentation [3]. The method yields medium sized nanoparticles of 20-100 nm size, which consist of smaller spherical core particles of 6-8 nm size embedded in a bound cluster. The nanoparticles are stabilized by a citrate shell, which can subsequently exchanged by phosphodextrane (MW 10,000). The final polymer shell is the acidic binding site for the basic drug load (Mitoxanthrone), which is added in the last step.

The critical step of our ferrofluid preparation is the selective crushing of the primar large particle aggregates (µm-sized) by pulse etching. This is done by rapid mixing of a crude buffered ferrofluid suspension, pre-adjusted in pH, and a specific pulse of citric acid (using a stopped flow device, or manually for preliminary experiments). The time coarse of the process was investigated by time resolved pH-estimation, dynamic light scattering TR-DLS *) and time resolved electron microscopy TR-EM in the time range of 2s – 1 week. Furthermore the acid addition was varied and investigated with time resolution (2D-kinetics). The main effect occurred during 5 min while some slower reaction proceeded up to 2 days. The etching was terminated by addition of a Tris-Citrate stop buffer, pH 7.5 or dilution with 0.1 M sodium citrate.

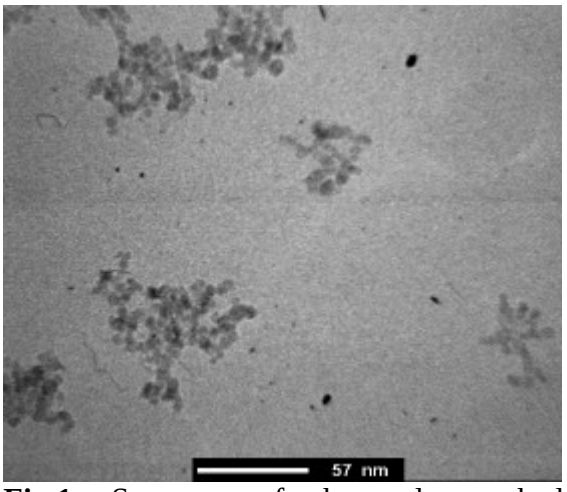

Fig.1: Structure of the pulse etched polycentric ferrofluid after size separation depicted by iron electron microscopy .

The obtained citrate shelled ferrofluid was washed and isolated in 0.1 M sodium citrate buffer, pH 7.1. Finally the size distribution was limited by fractionated sedimentation. The analysis of a typical main product fraction “m” (medium size) by direct electron microscopy (iron imaging, no stain) and dynamic light scattering of the concentrated original sample (50mg/ml ferrofluid) is shown in Fig.1 and 2. The electron micrograph depicts the structure of the polycentric ferrofluid of spherical core particles in clusters of 50 nm average size with a content of 100 nm particles sufficient for

medical application. The DLS demonstrates the particle size distribution of a main population of 20-100 nm size, a minor fraction of 6-10 nm (free core particles) and traces of larger particles, which can be removed later by filtration. The estimation of the macroscopic magnetic moment with a magnetic balance revealed 50% of the effect of a commercial magnetite sample (3 µm particle size).

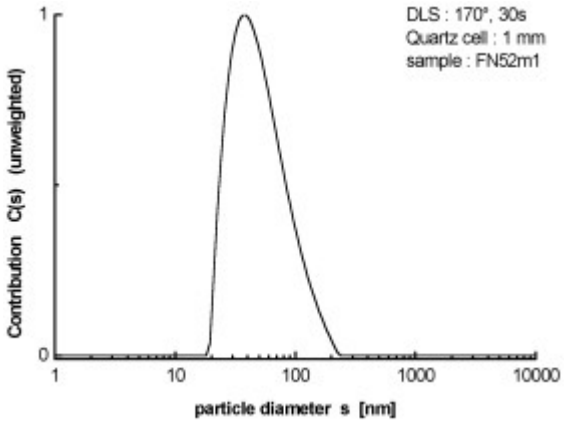

Fig.2: Particle size distribution of the pulse etched polycentric ferrofluid obtained by DLS of the concentrated original sample (back scattering, 170°).

The structure of a hydrophilic polycentric ferrofluid after exchange of the primar citrate shell by phosphodextrane (M = 10,000; 10% sugar phosphorylation) is shown in Fig.3. The image obtained by direct EM (iron imaging, no stain) demonstrates that the shown synthesis and modification procedure yields medium sized ferrofluid particles comparable to the Chemicell products and well defined size. The next steps will be the variation of the phosphodextrane shell and drug loading.
The size distribution of several Chemicell commercial ferrofluid products will be discussed. A study with silica-embeded magnetite nanoparticles from Degussa will start after finishing the formal regulation by the TUM central administration.

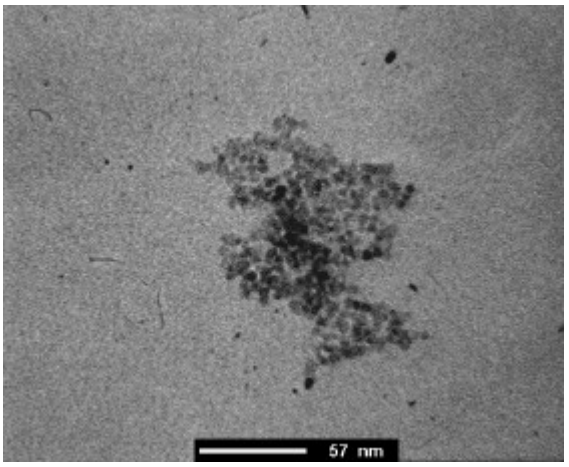

Fig.3: Structure of polycentric ferrofluid after shell exchange by phosphodextrane depicted by iron electron microscopy .

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[1] Alexiou, C., Arnold, W., Klein, R. J., Parak, F. G., Hulin, P., Bergemann, C., Erhardt, W., Wagenpfeil, S. and Lübbe, A. S., Locoregional cancer treatment with magnetic drug targeting. *Cancer Research* **60** (2000) pp. 6641-6648.

[2] Alexiou, C., Arnold, W., Hulin, P., Klein, R. J., Renz, H., Parak, F. G., Bergemann, C. and Lübbe, A. S., Magnetic mitoxantrone nanoparticle detection by histology, X-ray and MRI after magnetic tumor targeting. *J. of Magnetism and Magnetic Materials* **225** (2001) pp. 187-193.

[3] Nawroth, T., Rusp, M. Parak, F. G., Structure hierarchy of citrate shelled polycentric hydrophilic ferrofluids – Synthesis and characterization by Electron Microscopy and Dynamic Light Scattering DLS (2004) in preparation.