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# Iron oxide nanoparticles with supramolecular ureidopyrimidinone coating

<https://doi.org/10.1515/cdbme-2022-1004>

**Abstract:** Superparamagnetic iron oxide nanoparticles are a promising material in nanomedicine, especially for generating magnetically controlled drug delivery systems. We developed an innovative ureidopyrimidinone (UPy) coating based on supramolecular hydrogen bonding units. The synthesized nanoparticles possess a positively charged surface with a hydrodynamic diameter of 177 nm at pH 7 and magnetization of 31 emu g<sup>-1</sup>. The system has the potential to be modified by drugs or bioactive molecules altered with UPy units.

**Keywords:** Iron oxide nanoparticles, ureidopyrimidinone, cationic coating, supramolecular system

## 1 Introduction

In nanomedicine, specially designed nano-devices or nanostructures are used to offer new solutions to biomedical problems. The focus of research are mainly the four big multidisciplinary areas of diagnostics, therapeutics, drug delivery systems, and regenerative medicine [1]. Iron oxide nanoparticles (IONs) are biocompatible, can be fast and cost-efficient synthesized by co-precipitation, have a high specific surface area with the potential to bind a considerable amount of drug, and have the possibility of external guidance by a magnetic field because of their superparamagnetic character [2]. The IONs can be used as T2 contrast agents for magnetic resonance imaging and in cancer therapy through hyperthermia [1]. An external alternating magnetic field can be applied to generate heat, leading to cell death by lysosomal permeability [3].

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Furthermore, the IONs can be used for magnetically guiding stem cells for tissue engineering [1, 4]. Magnetically controlled drug delivery allows carrying the drug directly to cancer cells and generates a high local concentration [5]. This accumulation can lead to an overall drug reduction and efficient treatment by avoiding the drug's toxicity to healthy cells [5, 6]. Organic or inorganic coatings are often applied to improve the particle properties of bare IONs (BIONs). Specially designed layers generate a biocompatible core-shell structure and protect the BIONs from uncontrolled oxidation and aggregation [2, 7].

Supramolecular systems based on the fourfold hydrogen bonding ureido-pyrimidinone moieties can be applied as coating materials. The amphiphilic supramolecular molecule is based on a UPy unit combined with a urea moiety, a hydrophobic alkyl spacer connected to an amine-functionalized, water-soluble oligo ethylene glycol group by a urethane unit. [8]. The UPy molecules can assemble into fibrous aggregates by hydrogen bonding protected by hydrophobic pockets composed of additional hydrogen bonding of the urethane groups [9]. UPy-moieties can be designed to form different biomaterials like hydrogels, elastomers, or self-healing polymers. Dankers et al. have analyzed eleven different UPy-compounds regarding their cytotoxicity. They showed that the UPy-based materials did not affect the cell viability and did not show immunogenic or mutagenic behavior. The in vitro experiments have shown good biocompatibility for the UPy-based biomaterials. A large library of differently covalently functionalized UPy units exists [10]. For example, a drug bound to a UPy moiety can be easily mixed in to form a multi-component functional supramolecular system [11, 12] UPy-coated IONs have the potential to be used for magnetically controlled drug delivery with flexible added UPy functionalized drugs.

In this work, UPy-coated IONs are synthesized by imine formation of polyglutaraldehyde functionalized IONs with UPy-NH<sub>2</sub> (C<sub>50</sub>H<sub>97</sub>CIN<sub>8</sub>O<sub>16</sub>, UPy-C6-Urea-C12-Urethane-PEG12-C2-NH<sub>3</sub>Cl, 1101.82 g/mol) The ION@UPy-NH<sub>2</sub> are characterized by Fourier-transform infrared spectra (FT-IR), X-ray diffraction (XRD), and thermogravimetric analysis (TGA) regarding their composition. The magnetization is analyzed by the superconducting quantum interference device (SQUID). The surface properties are determined with zeta potential measurements. The formation of hydrophobic

pockets was analyzed with the dye Nile Red, which is strongly fluorescent when encapsulated in a hydrophobic compartment. The hydrodynamic diameters are compared at different pH values in the last step.

## 2 Materials and methods

### 2.1 Synthesis of ION@PGA@UPy-NH<sub>2</sub>

BIONs are synthesized by co-precipitation via the Massart process [13]. To prepare ION@APTS, 2.00 g BIONs are dispersed in 600 mL absolute ethanol and ultrasonicated for one hour. After adding 12.0 mL (3-Aminopropyl)triethoxysilan (APTS, Sigma-Aldrich), the particles are ultrasonicated for one hour. Via magnetic decantation, the ION@APTS are washed with ethanol (4x) and water (3x) until the conductivity drops below 150  $\mu\text{S}/\text{cm}$ .

400 mg ION@APTS are dispersed in 400 mL of a 2% glutaraldehyde water solution. The pH is adjusted to pH 11 NaOH solution, and the dispersion is stirred for one hour. The ION@APTS@PGA are washed multiple times with water (10x), 0.50 M NaCl (2x), water (10x), and 0.50 M NaCl (2x), and finally three times with water.

20.0 mg of ION@APTS@PGA are dispersed in 1.00 mL 20.0 mM Na<sub>3</sub>PO<sub>4</sub> and 1.00 M NaCl buffer (pH 6.8). Before adding 6.70 mg UPy-NH<sub>2</sub>, the particles are ultrasonicated for 15 min. The suspension is again ultrasonicated for 5 min and incubated at 25 °C, 1000 rpm overnight, washed with buffer (5x), and water (2x) by magnetic decantation (conductivity <150  $\mu\text{S}/\text{cm}$ ).

### 2.2 Characterizations

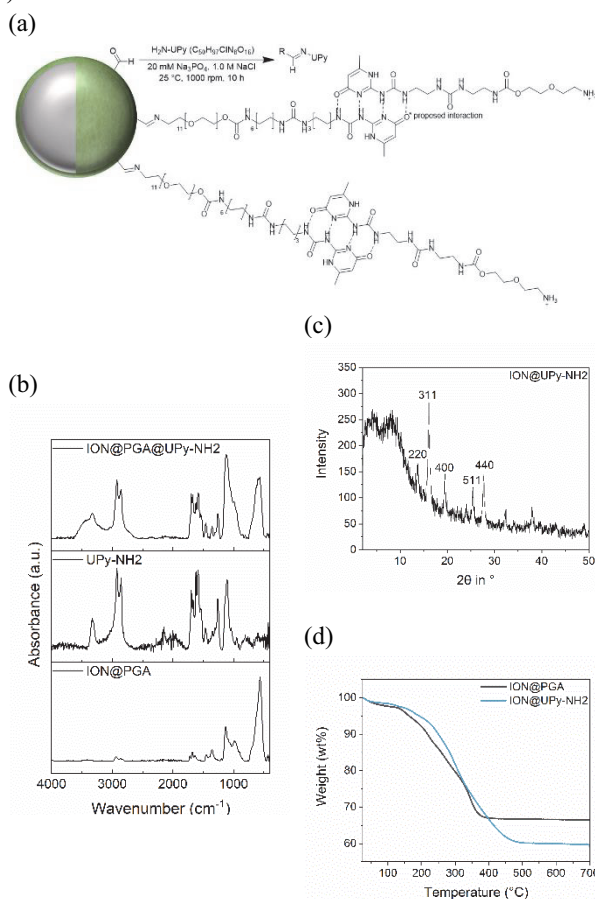
Zeta potential and dynamic light scattering (DLS) experiments are performed with the Malvern Zetasizer Ultra of 1.00 g/L ION solutions. For DLS, the samples are measured in triplicates (disposable 10x10 plastic cell) and zeta potential (disposable, folded capillary cell) in quintuplets with an equilibration time of 120 s at 25 °C. A Bruker ALPHA II spectrometer obtains FT-IR spectra (24 scans, 4000-400  $\text{cm}^{-1}$ ). Background subtraction is done with a concave rubber band method (OPUS 8.1). The magnetization is measured with the SQUID magnetometer MPMS XL-7 (Quantum Design) at 300 K and a magnetic field variation between  $\pm 3979$  kA/m (10 mg sample). The amount of bound UPy-NH<sub>2</sub> is analyzed by TGA of freeze-dried IONs (50  $\mu\text{L}$  aluminum oxide jar) with the STA 449C Jupiter between 25-700 °C.

## 3 Results and discussion

The cationic supramolecular moiety UPy-NH<sub>2</sub> was successfully coupled to polyglutaraldehyde functionalized IONs (ION@PGA) via imine formation by reaction of the aldehyde group and the amine of the UPy (Figure 1 a) [14]. The IONs@UPy-NH<sub>2</sub> are characterized by their particle composition, surface properties, magnetization, and hydrodynamic diameters.

### 3.1 Particle composition

XRD, FT-IR, TGA, and SQUID are used to analyze the particle composition of the IONs@UPy-NH<sub>2</sub>. The effective UPy coating is visualized by FT-IR measurements (Figure 1 b).



**Figure 1:** Schematic illustration of ION@UPy-NH<sub>2</sub> with the PGA coating in green and the imine formation. The Hydrogen bonding is proposed (a). FT-IR Spectra of UPy-NH<sub>2</sub>, ION@PGA, and ION@UPy-NH<sub>2</sub> (b), XRD spectra of ION@UPy-NH<sub>2</sub> (Mo source, (c)), TGA curve of the both particle species (d).

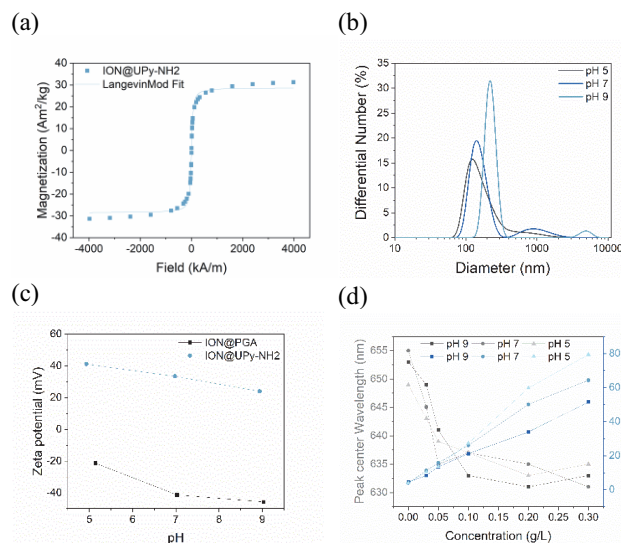
The iron oxide core is represented by its characteristic Fe-O stretching vibration at 574  $\text{cm}^{-1}$  [13]. Typical peaks of UPy are visible at 2853  $\text{cm}^{-1}$  and 2923  $\text{cm}^{-1}$  for C-H stretching vibrations, 1700  $\text{cm}^{-1}$  and 1669  $\text{cm}^{-1}$  for C=N and C=O

stretching vibrations, and at  $1262\text{ cm}^{-1}$  and  $1123\text{ cm}^{-1}$  for C-N and C-O stretching vibrations [15]. The typical iron oxide reflexes (220), (311), (400), (511), and (440) for magnetite are visible in the X-ray diffractogram (Figure 1 c) [13]. The amorphous coating generates a brought reflex between  $2^\circ$  and  $10^\circ$ . Similar behavior was already seen by amorphous silica coatings [16]. The UPy coating does not influence the crystal structure of the magnetite core. The amount of bound UPy-NH<sub>2</sub> was determined by TGA (Figure 1 d). The weight difference between ION@UPy-NH<sub>2</sub> and its precursor ION@PGA is 7.23wt%; this is the UPy-NH<sub>2</sub> bound to the nanoparticle surface. SQUID measurements of ION@UPy-NH<sub>2</sub> show the characteristic sigmoidal curve of superparamagnetic nanoparticles, which is only a slightly deviating to the simulated curve by the LangevinMod Fit (Figure 2 a). The UPy coated particles show a magnetization of  $\pm 31.3\text{ Am}^2/\text{kg}$ . As the particles contain additional nonmagnetic material as coating the magnetization is lower than the one of BIONs at around  $60\text{ Am}^2/\text{kg}$  [12]. The analytics show that ION@UPy-NH<sub>2</sub> comprises a superparamagnetic, crystalline iron oxide core and a UPy coating. The influence of the coating on the surface properties and the particle size is analyzed by dynamic light scattering (DLS), zeta potential, and Nile Red assay.

### 3.2 Surface properties and particle size

Zeta potential and Nile Red assay are used to determine the surface properties of the ION@UPy-NH<sub>2</sub>. Contrary to ION@PGA, ION@UPy-NH<sub>2</sub> particles have a positive surface charge for pH 5, 7, and 9 (Figure 2 c). It is hypothesized that the hydrogen bonding between the UPy-NH<sub>2</sub> moieties builds fibrous networks with a positive charge on the outside (Figure 1 a). The zeta potential rises with lower pH indicating a stabilization from moderate to good stability [17]. This effect can also be seen in DLS measurements. The hydrodynamic diameters in water at pH 5 and 7 ( $168 \pm 44.1\text{ nm}$ ,  $177 \pm 32.0\text{ nm}$ ) are 0.5 times smaller than at pH 9 ( $345 \pm 61.5\text{ nm}$ , Figure 2 a). The ION@UPy-NH<sub>2</sub> fit into the range of other nanoparticles synthesized for drug delivery of around 60 to 400 nm [18]. The Nile Red (NR) assay is used to determine the formation of hydrophobic pockets that form in the lateral direction of the UPy-network [9]. It becomes strongly fluorescent if the NR is encapsulated into the hydrophobic pockets. The fluorescent dye's red color in water shifts to a lower wavelength (blue shift) in a hydrophobic environment [19]. The more UPy-aggregates, the higher the fluorescence of the NR [20]. A shift to lower wavelength with higher particle concentrations because of more hydrophobic pockets available and a slightly increasing peak intensity with decreasing pH (Figure 2 d). It is proposed that covalently bound UPy-units can interact with free molecules by H-bonding and form

supramolecular structures. This behavior could also explain the better stability at more acidic pH values.



**Figure 2:** Magnetization of ION@UPy-NH<sub>2</sub> with LangevinMod fit (a), hydrodynamic diameters in water (b) zeta potential of ION@UPy-NH<sub>2</sub> in comparison with ION@PGA (c), at pH 5, 7, and 9, and Nile Red Assay of ION@UPy-NH<sub>2</sub> (d),

## 4 Conclusion

The IONs are successfully functionalized with UPy-NH<sub>2</sub>. The UPy shell generates a positively charged layer surrounding the superparamagnetic crystalline magnetite core. It is proposed that hydrophobic pockets form by hydrogen bonding of UPy units. The stability of the particles is slightly influenced by pH, generating smaller aggregates at pH 5 with higher zeta potential. The work shows that the free UPy units are self-assembled around the IONs by hydrogen bonding to the covalently bound ones. This supramolecular interaction allows adding UPy-drug molecules and generating an innovative magnetically controlled drug delivery system.

### Author Statement

**Research funding:** We appreciate the support of the Technical University of Munich International Graduate School of Science and Engineering (IGSSE). We are thankful for the support of Dutch Antimicrobial Resistance Technology development and Biofilm Assessment Consortium (Dartbac), and Regenerative Medicine Crossing Borders (RegMed). We appreciate the support from the German Research Foundation (DFG) and the Technical University of Munich (TUM) in the framework of the Open-Access Publishing Program. The funders had no role in the study's design, the collection, analysis, and interpretation of data, the manuscript's writing, or the decision to publish the results. **Conflict of interest:** The authors state no conflict of interest. **Informed consent:**

Informed consent has been obtained from all individuals included in this study. Ethical approval: The conducted research is not related to either human or animals use. Acknowledgments: We thank Dr. Matthias Opel for the support with SQUID measurements and Riccardo Bellan for the help with the Nile Red Assay. Data availability: The datasets of this study are available from the corresponding author on reasonable request.

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