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New magnetically responsive polydicarbazole-magnetite nanoparticles†

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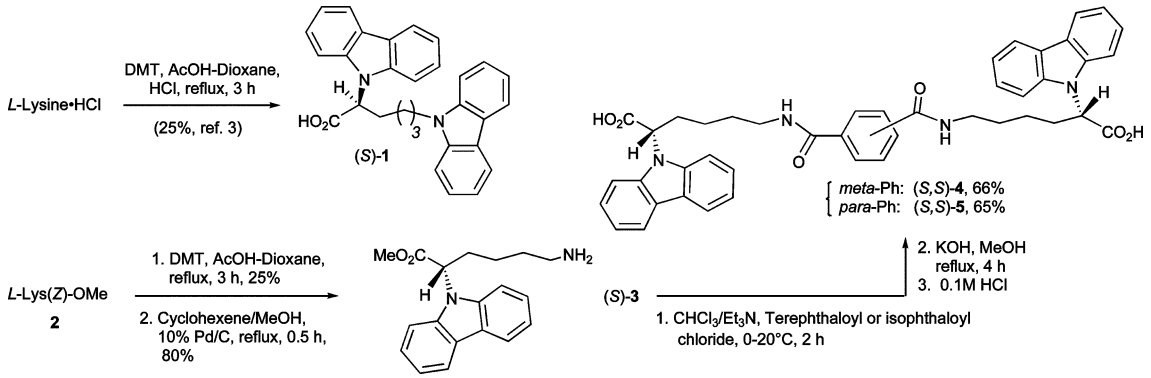
Magnetically responsive COOH-polydicarbazole-magnetite nanocomposites have been prepared by chemical oxidation of three COOH-dicarbazole monomers 1 and 4–5 in the presence of magnetite nanoparticles. These functionalized nanoparticles have been tested for DNA hybridization experiments.

Because of their magnetic susceptibility, magnetically responsive microspheres and composite particles are of great interest for biomedical applications such as cell separations, magnetic field-guided delivery of drugs, relaxation and contrast enhancements in magnetic resonance imaging of tissues, and magnetic fluid hyperthermia for cancer therapy.[1] Conducting polymer nanocomposites (NCs) where inorganic paramagnetic nanosized maghemite (γ-Fe_2O_3) or magnetite (Fe_3O_4) cores are covered by thin layers of conducting polymers (CPs) are quite new core-shell materials of underestimated potential. Colloidal stable polypyrrole-magnetite-silica NCs with diameters in the range 100–580 nm have been described by the Armes' group.[2] They were produced by the chemical oxidation of *nonfunctional pyrrole* around magnetically responsive colloidal silica-magnetite particles suspended together with two aqueous oxidants H_2O_2/Fe^{3+}/HCl and $(NH_4)_2S_2O_8$. Obviously, the full potential of paramagnetic CPs-maghemite/magnetite nanoparticles has not yet been exploited regarding monomers that possess oxidizable heterocycles different from pyrrole, and chemical functions allowing the *post-polymerization* covalent attachment of biological species. Our group recently described the electrosynthesis of stable polydicarbazole (pDC) films onto microelectrodes from activated esters of the chiral dicarbazole monomer (*S*)-**1** (ee ~ 98%) prepared from L-lysine using a modified Clauson-Kaas reaction (Scheme 1).[3] Covalent attachment of polyphenol and glucose oxidases onto these pDC-films was successfully performed toward biosensor constructs.[4] Interestingly, this starting chiral dicarbazole monomer contained two linked carbazole units that afforded highly cross-linked stable pDC-conducting films during electropolymerization. This last result was remarkable since *N*-substituted monocarbazole monomers do not produce electrochemically stable polycarbazole films, but rather short tetrameric oligomers that are soluble in the electrochemical medium.[5]

Here, we report that the chiral *bis*-heterocyclic COOH-dicarbazole monomers (*S*)-**1** and (*S*,*S*)-**4**/(*S*,*S*)-**5** *can be chemically oxidized in the presence of nanosized magnetite nanoparticles* toward magnetically responsive nanosized pDC-magnetite particles with potential use in DNA testing. The two chiral COOH-dicarbazole monomers (*S*,*S*)-**4** and (*S*,*S*)-**5** were readily synthesized using a *m*- or a *p*-benzoic acid linker connecting twofold the intermediate amine-linked carbazole methyl ester (*S*)-**3** (modified Clauson-Kaas reaction of L-lys(*Z*)-OMe with 2,5-dimethoxytetrahydrofurane DMT,[3] and Z-deprotection; 20% yield, ee ~ 97%, Scheme 1). By design, chirality and functionality features present in compound (*S*)-**1** were retained in these novel dicarbazole monomers ((*S*,*S*)-**4** and (*S*,*S*)-**5**: 4 synthetic steps, 13.2 and 13.0% respectively, ee ~ 97% confirmed by the non detection of *meso* diastereoisomers in the last amidation step by high-field ^{13}C-/^{1}H-NMR).

Nanosized magnetite particles were prepared by a modification of the method of Sugimoto[6] (oxidative hydrolysis of iron sulfate in an alkaline medium under nitrogen, mean particle size of 20–40 nm by TEM analysis, FT-IR peaks characteristic for magnetite†, *v*: 410, 590 cm^{-1}).[7] Polymerization parameters, that were specifically examined and screened, included different (i) polymerization time, (ii) oxidants ($Fe(NO_3)_3$, $Fe(ClO_4)_3$, $H_2O_2/FeCl_3$/0.1 M HCl, $CuCl_2$, $K_2Cr_2O_7$, $K_2S_2O_8$, $(NH_4)_2S_2O_8$, CAN: $(NH_4)_2Ce(NO_3)_6$), (iii) molar/weight ratio of dicarbazole monomer/magnetite nanoparticles, (iv) polymerization solvent (alcohols, alcohols–H_2O mixtures, CH_3COCH_3, CH_3CN, $CHCl_3$), (v) medium temperature (in the range of 30–55 °C *with or without ultrasonic irradiation* by an ultrasonic cleaner Bransonic at full 42 KHz power), and, (vi) magnetite concentration (0.5–5.0% w/v range at constant experiment volume). Resulting carboxylated pDC-magnetite NCs pDC(**1**) and pDC(**4**/**5**) were analyzed by TGA between 200–750 °C, and FT-IR analyses to confirm successful polymerization. This selection process rapidly evolved to the discovery of an optimal

† Electronic supplementary information (ESI) available: experimental details including analyses. See http://www.rsc.org/suppdata/cc/b3/b309375a/



Scheme 1 Preparation of chiral dicarbazole monomers (*S*)-**1** and (*S*,*S*)-**4**/(*S*,*S*)-**5**.

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reproducible set of polymerization conditions for the dicarbazole COOH-monomers (*S*)-**1** and (*S*,*S*)-**4**/(*S*,*S*)-**5** in the presence of magnetite nanoparticles.

A representative experiment is detailed herein. Freshly prepared magnetite nanoparticles (189.0 mg, 3.5% w/v for a total volume of 6.0 mL CH_3COCH_3) are suspended in CH_3COCH_3 (5.0 mL, 20 °C). The dicarbazole COOH-monomer (*S*)-**1** or (*S*,*S*)-**4**/(*S*,*S*)-**5** (0.224 mmol, 1.0 mL CH_3COCH_3), and, finally, the CAN oxidant (0.224 mmol, 120.0 mg) were added. The fine black suspension is ultrasonicated for 5 h during which the medium temperature raised to 55 °C (ultrasound-mediated constant stirring). The resulting brown-black NC[7] is magnetically-decanted with the help of a powerful external magnet. It is serially washed by 4 × 10 mL of each of the indicated solvents/buffers: de-ionized H_2O, CH_3COCH_3, 0.1 M MES buffer (pH 5.0), neutral PBS buffer, TNET buffer (pH 7.5, each washing operated at 65 °C for 5 min), and, finally, again with de-ionized H_2O and neutral PBS buffer to eliminate soluble by-products (inorganic salts, excess oxidants, unreacted monomers, and short colored carbazole oligomers). (*Reagents*: Data relevant to MES, PBS and TNET buffers as well as to washing solutions have been described in the ESI section.†) Resulting magnetically responsive carboxylated pDC-magnetite nanoparticles[7] are suspended in a neutral PBS buffer at a 1% w/v concentration and stored at 4 °C. These colloidal suspensions were observed to be stable during at least 3 months in these storage conditions.

COOH-pDC-magnetite NCs pDC(**1**) and pDC(**4–5**) have been characterized by TGA† and FT-IR spectroscopy† (KBr pellets) to check the formation of pDC-polymers in the presence of magnetite nanoparticles. TGA curves showed quite similar two-step profiles regarding weight losses in the range 200–750 °C.† Gravimetric measurements showed that they possess 76, 69 and 71% w/w magnetite/maghemite[7] respectively. Elemental analyses compared to similar bulk pDC-polymers synthesized in absence of magnetite were found quite consistent with TGA data (pDC-NCs **1** and **4–5**, C: 75, 68 and 70%). NCs FT-IR spectra were found very similar.† They included mixed absorption peaks characteristic of magnetite (ν: 1021–1024, 1083–1093, and 568–578 cm^{-1}), and of the pDC-polymeric system {ν: 2850–2964 ($\delta_{Csp3\text{-}H}$), 1650–1750 ($\nu_{C=O}$, carboxyl), 1457–1558 ($\nu_{Csp2\text{–}Csp2}$, polycarbazole aromatics), 800–808 ($\delta_{Csp2\text{–}H}$, out of plane stretching) cm^{-1}}.† Bulk magnetization *versus* applied magnetic field experiments using dried magnetic NCs pDC(**1**), and pDC(**4–5**) afforded the respective saturation magnetizations M_s and coercivities H_c {M_s (emu g^{-1})/H_c (Oe): 35/132, 52/35 and 51/55 (300 K)}.† Interestingly, transmission electron microscopy (TEM) studies† indicated that our pDC-NCs fabrication process neither altered the cubic sheet-like morphology nor the average size (20–40 nm) that characterized the starting magnetite.[6] Additionally, mixed structures of the type discrete pDC particulates-magnetite nanoparticles could not be detected.[8] These observations are consistent with the relatively low quantity of pDC-polymers deposited onto magnetite nanoparticles independently of dicarbazole monomers (~24–31% w).

The magnetic NCs pDC(**1**) and pDC(**4–5**) have been tested for DNA covalent immobilization and hybridization (Fig. 1).† The amine-modified 20-*mer* oligonucleotide **DNA 1** H_2N–$(CH_2)_{12}$-5′GCACTGGGAGCATTGAGGCT, that characterizes the 20210 mutation in the Human Factor II gene,[9] has been covalently attached onto the three pDC-NCs after carboxylate activation in a 0.4M MES buffer (pH 5.0) by the water soluble carbodiimide EDC (0.15M MES buffer, 2 h incubation at 20 °C), and hybridized (3 min, 60 °C) with the fluoresceine-labeled *anti-sense* 20-*mer* oligonucleotide **FITC-DNA 2** (Fluoresceine-5′AGCCT-CAATGCTCCCAGTGC). After addition of an *anti*-FITC HRP-labeled mouse monoclonal antibody and incubation (10 min, 20 °C), the HRP substrate TMB (3,3′,5,5′-tetramethylbenzidine, Aldrich) was added and reacted with the immobilized amplifying enzymatic construct during 5 min at the same temperature before visible reading at 620 nm (Elisa Plate Reader Anthos ht II). Similarly, omission of the **FITC-DNA 2** during parallel experiments allowed us to evaluate the nonspecific binding (NSB) characteristic of the assay, that means the affinity of tested pDC-NCs to physically adsorb the reporter *anti*-FITC HRP-labeled antibody. Preliminary data indicated that NSB for hybridization/detection assays† in the above format was found very low. The **FITC-DNA 2** sequence could be reliably and reproducibly detected at sensitivities of 10^{-12} M for pDC(**4**), and 10^{-13} M for pDC(**1**)/pDC(**5**) for a signal/noise ratio in the range 2.7–3.4. For comparison needs, COOH-Dynabeads® M-270 (Ø 2.8 μm, Dynal AS, Oslo, Norway), that are routinely used for magnetism-driven suspension assays, were found less effective by a factor 10 and 100 respectively. Importantly, this low-range detection level was achieved *without using passivating steps (Egg albumin, BSA, PEG_{1000} or dextran) commonly used in the diagnostic field.*

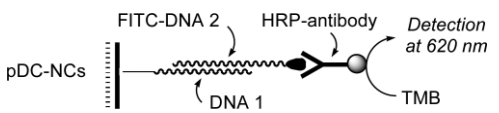

Fig. 1 DNA covalent attachment onto pDC-NCs and hybridization.

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8 NCs pDC(**1**) and pDC(**4–5**) were also analyzed by high-resolution TEM (HR-TEM) and Energy-Dispersive X-Ray Microanalysis (EDAX). HR-TEM micrographs of the nanocomposite pDC(**5**) clearly showed two superposed magnetite/maghemite crystals covered by an amorphous 7.0–7.5 nm thick polydicarbazole layer.† This morphology has been confirmed by EDAX elemental analyses for the *three* pDC nanocomposites, that showed iron-poor carbon-enriched crystal edges *versus* centers.†.

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