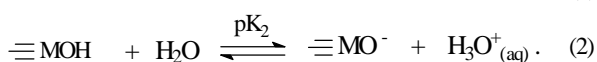
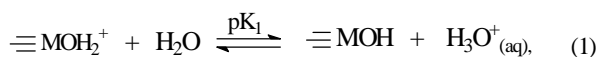


A THEORETICAL APPROACH OF SUCCIMER COMPLEXATION AT THE SURFACE OF FERRITE NANOPARTICLES IN ORDER TO IMPROVE THE SYNTHESIS OF BIOCOMPATIBLE MAGNETIC FLUIDS

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INTRODUCTION: Currently, magnetic carrier technology is widely used for biological and biomedical purposes such as cell sorting, drug vectoring, contrast agents in magnetic resonance imaging (MRI), and oncological treatment. Many applications of these materials are based on specific receptor-effector complexes where the latter is coupled with coated iron oxide particles. The receptor is located on the outer monolayer of the membrane and the effector is bonded to the particle surface through non-toxic chelating agents like meso-2,3-dimercaptosuccinic acid (DMSA). Moreover succimer is also a polyfunctional Brønsted acid leading to five species, including amphoteric ones, whose amounts vary strongly with pH. It is therefore surprising that until now, the speciation diagram of DMSA has not been taken into account in the nanoparticle surface complexation mechanism of spinel ferrite-based magnetic fluid. On the other hand, it has been shown very recently [1] that magnetic fluids behave as a mixture of a strong acid (bulk dispersion) and a weak diprotic acid (nanoparticle surface). In this model, the charge of the particle surface, responsible for the colloidal stability of electric double layered magnetic fluids (EDL-MF), is obtained through the aquation reaction of metal ions and the following hydrolysis reactions:



Such equilibria lead to three kinds of superficial sites where most of them are MOH_2^+ in strong acidic medium, MO^- in strong basic medium, with MOH being the intermediate amphoteric sites (where M = superficial metal). $\text{p}K_1$ and $\text{p}K_2$ are the thermodynamic constants of each respective chemical equilibrium. Then, the surface charge density σ_0 results from a proton transfer mechanism between the particle surface and the bulk dispersion. Therefore, the analysis of equilibria (1) and (2) allows the determination of the pH dependence of the surface charge density, and σ_0 is given by [1]:

$$\sigma_0 = \frac{F}{A} V \left(\frac{10^{-2\text{pH}} - 10^{-(\text{p}K_1 + \text{p}K_2)}}{10^{-2\text{pH}} + 10^{-(\text{pH} + \text{p}K_1)} + 10^{-(\text{p}K_1 + \text{p}K_2)}} \right) C_T, \quad (3)$$

where F is the Faraday constant, A is the total surface area of particles, and V is the volume of the dispersion. The total concentration of surface sites C_T and the dissociation constants can be easily obtained using simultaneous potentiometric and conductimetric titrations.

The aim of the present work is to explore the surface – DMSA complexation in order to improve the synthesis of biocompatible magnetic fluids. In this context, using our formalism of Brønsted complex equilibria involving amphoteric species, we first obtain the quantitative pH dependence of the superficial density of charge for the case of EDL-MF based on cobalt ferrite nanoparticles. Then, the speciation diagram for charged sites of particle surface is coupled with that of DMSA in order to analyse the mechanism of surface – DMSA complexation. Finally, we propose a surface reaction which leads to the maximum complexation rate.

METHODS: The elaboration of EDL-MF sample was carried out using the usual procedure. CoFe_2O_4 oxide nanoparticles were prepared through hydrothermal coprecipitation of aqueous solutions of $\text{Co}(\text{NO}_3)_2$ - FeCl_3 in alkaline medium. Then the particles were peptized in acidic medium by adjustment of the ionic strength, resulting in stable sols of high quality. The mean nanoparticle size was determined by X-ray diffraction recorded from dry powder samples, obtained by evaporation of the liquid carrier, and was found to equal 12.0 nm. Simultaneous potentiometric and conductimetric titrations of 40 mL of the ferrofluid dispersion (volume fraction $\Phi = 1.46\%$, corresponding to 1.7×10^{22} particles per m^3) were performed using a titrant solution of 0.106 mol L^{-1} sodium hydroxide. All titrations were repeated two times. The potentiometric readings were obtained with a pH meter (Metrohm 713) using a glass double-junction electrode, while the conductivity was measured with a conductometer (Metrohm 712). Both apparatus were gauged using appropriate templates. From the simultaneous titration curves, the Henderson-Hasselbalch equation was used to determine the $\text{p}K$'s corresponding to both equilibria (1) and (2). Moreover C_T was calculated using the mass balance. Then, the speciation diagram of surface sites was obtained. In the case of DMSA, the molar ratio for each species was determined using $\text{p}K$ values from the literature [2].

RESULTS & DISCUSSION: Fig.1 shows the pH dependence of the surface charge density for the cobalt ferrite EDL-MF sample. The saturation value is equal to 0.326 C m^{-2} which corresponds to approximately one charge per 0.5 nm^2 or about 905 sites per particle. This is in excellent agreement with reported values [3]. At pH values of around 3.5 and 10.5, σ_0 reaches the maximum positive and negative values, respectively, and the particle surface is charge saturated. This agrees with ultrastable magnetic sols obtained experimentally in acidic or basic media. However, for extreme values of pH, the high ionic strength does not allow stable ferrofluid dispersions, although the particle surface is charge saturated.

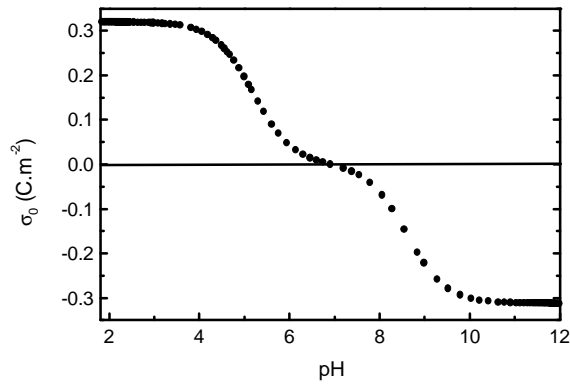


Fig. 1: pH dependence of the superficial density of charge for EDL-MF sample.

In the pH range corresponding to physiological medium, the superficial charge density approaches zero and the ferrofluid dispersion flocculates showing that uncoated particles cannot be used for biological purposes.

Table 1 lists the experimentally determined thermodynamic constants associated with the equilibria between the particle surface and the bulk dispersion. The molar ratio values of particle surface sites can then be calculated and plotted as function of pH as shown in Fig. 2. This speciation diagram gives a quantitative description of the particle surface illustrating the domains of protonation of surface sites. At low (high) pH values in acidic (basic) medium (typically $\text{pH} \leq 3.5$ and $\text{pH} \geq 10.5$), the particle surface is charge saturated. For small pH ranges around the pK values, Fig. 2 shows the coexistence of charged and neutral sites.

Table 1. pK_1 and pK_2 values and the modulus of the maximum superficial density of charge for the EDL-MF sample.

pK_1	pK_2	$ \sigma_0^{\text{MAX}} (\text{C m}^{-2})$
5.2	8.6	0.326

Close to the neutral region (physiological medium), the molar ratio of charged sites strongly decreases, and, contrary to the expected result, the cobalt ferrite ferrofluid solution does not present any point of zero charge since the molar ratio of the amphoteric sites does not reach the unity value. However, an isoelectric point is found and is readily related to the equilibrium constants for the surface sites species.

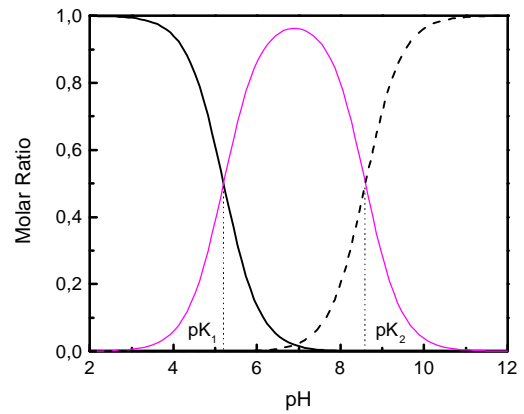


Fig. 2: Speciation Diagram of particle surface sites.

$\frac{3}{4} \text{MOH}_2^+$; $\frac{3}{4} \text{MOH}$; $--- \text{MO}^-$

Table 2 lists the pK values corresponding to the equilibria involving the five DMSA species often abbreviated HL , H_2L , HL^{2-} , HL^{3-} and L^{4-} . The first and second steps in the dissociation process involve successive loss of protons from the two carboxylic groups; the third and fourth steps involve dissociation of the protonated thiol groups.

Table 2. Values of equilibrium constants of meso 2,3 - dimercaptosuccinic acid [2].

pK_{C1}	pK_{C2}	pK_{S1}	pK_{S2}
2.71	3.43	9.65	12.05

The eight curves plotted as a function of pH in Fig. 3 show the five molar ratios for DMSA containing species coupled with the three curves for nanoparticle superficial sites. Since the first two constants of DMSA are of the same order of magnitude, it suggests that the first two protons dissociate from opposite ends of the molecule. As a consequence of their physical separation, the negative charge created by the first dissociation does not greatly affect the removal of the second proton. Thus, around pH=3 the three first DMSA species coexist. The dissociation of the other two protons, relative to the thiol groups, is more difficult since they are much closer to the negatively charged carboxylate ions created by the initial dissociation.

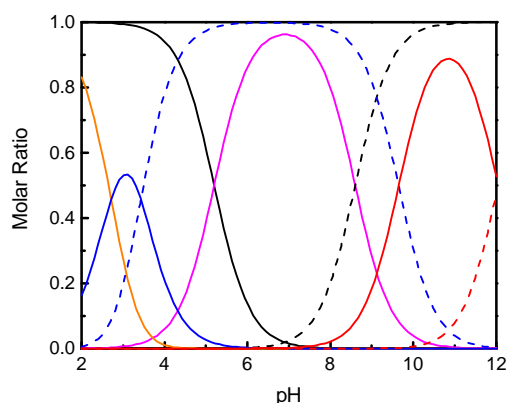
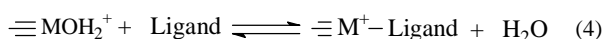


Fig. 3: Coupled speciation Diagrams of superficial sites and DMSA

$\frac{3}{4}$ MOH_2^+ ; $\frac{3}{4}$ MOH ; --- MO^-

$\frac{3}{4}$ H_4L ; $\frac{3}{4}$ H_3L ; --- H_2L^{2-} ; $\frac{3}{4}$ HL^{3-} ; --- L^{4-}

Moreover, it is well known that carboxylate acid groups form remarkably stable chelates with many metal ions in solution [4]. In this context, the elaboration of DMSA-coated magnetic nanoparticles by complexation of superficial metal ions should be controlled by the pH which also monitor the superficial density of charge. Then, it is therefore necessary to analyze the coupled speciation diagrams of particle superficial sites and DMSA. Based on the free metal ion complexation equilibrium, the most probable surface reaction can be written as:



since the exchange of ligand for OH superficial groups is thermodynamically less favored [4]. In alkaline medium, both particles and DMSA ions are negatively charged (see Fig. 3) and no complexation would occur. From these

considerations, one can easily conclude that the best pH to coat magnetic nanoparticles by DMSA is around 3. Although the pH range of the maximum molar ratio for MOH_2^+ superficial sites goes from strong acid medium to pH=3, H_3L^- and H_2L^{2-} only reach their maximum values for this pH. Once the DMSA is coated to the particle surface, the stability of the magnetic sol in biological buffers is obtained through the H_2L^{2-} species whose one end is bonded to the surface and the other ensures the charge.

CONCLUSIONS: Magnetic fluids behave as a mixture of a strong acid and a weak diprotic acid. The thermodynamic constants of the chemical equilibria involved between the bulk dispersion and the nanoparticle surface are experimentally determined. Then, the pH dependence of the nanoparticle surface charge density of EDL-MF based on cobalt nanoparticles is obtained. From the coupled speciation diagrams of both particle surface and DMSA one can predict the optimal pH for DMSA surface complexation and colloidal stability in physiological medium.

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