

Supporting Information for
A Spectroscopic Investigation on the Interaction of a Magnetic Ferrofluid with a
Model Plasma Protein: Effect on the Conformation and Activity of the Protein

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S1. Synthesis of $\text{Ni}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$ ferrofluid

Chemical synthesis of charged magnetic nanoparticles and its stable dispersion in water was first described by Massart¹⁻⁵ in the early 90s. The concept was then highly cited in the literature and was further successfully extended by Sousa et al.⁶ particularly for the spinel ferrite $M\text{Fe}_2\text{O}_4$, where M belongs to the d -block metal- Fe, Co, Cu, Ni, Zn. In the present work, we report the preparation of magnetic dispersion following the initial procedure as described by Massart as it leads directly to the formation of stable aqueous sol of charged particles peptized in acidic medium. The thermodynamic stability of the prepared sol was found to be poor by Sousa et al.,⁶ but according to Massart the stability can be achieved only in the presence of suitable counterion, like NO_3^- . The synthesized particles obtained by this procedure were found to have diameter in the range 2 – 20 nm,^{1,2} with mean value of 7 – 10 nm.^{1,2,7} The point of zero charge (PZC) for these charged magnetic nanoparticles is located at pH ~ 8.0 as described by Massart et al.^{1,2} Thus at a pH near the PZC, the surface charge could be significantly reduced but sufficient to allow the dispersion of the particles in the carrier liquid in the presence of suitable counterion (e.g., NO_3^- , in the present case).

A simplified illustration for the process of preparation of stable magnetic colloid is depicted in Scheme S1 which includes the following steps:

(i) 25 ml of aqueous metal ion solution containing 0.05 M nickel nitrate, 0.05 M zinc nitrate and 0.2 M ferric nitrate was added into 100 ml 1.0 M NaOH solution.

(ii) The temperature of the NaOH solution was strictly maintained above 90 °C while mixing as both the processes precipitation and condensation are required for the

preparation of the ferrite particles. The mixing of metal ion solution was performed as quickly as possible while maintaining vigorous stirring condition.

(iii) The mixture was then stirred for 1 h at the same temperature.

(iv) A sluggish precipitate was formed which collected and decanted several times following washing with Milli-Q water. The nature of the precipitation, high ionic strength and the presence of highly polarizing counterion (Na^+) creates a surface screening effect and makes the particles flocculating under reaction condition.⁸

(v) Finally, the precipitate was washed with acetone for several times to completely remove the excess ions.

(vi) The residue was then air dried and was added to the 0.2 M aqueous HNO_3 solutions (Step-2 in Scheme S1). The dilute acidic solution helps generate the surface positive charge ($-\text{OH}_2^+$) due to the presence of the amphoteric hydroxyl group ($-\text{OH}$) to the particle surface. This is a vital step in the preparation since the surface positive charge that is created would dictate the final stability of the colloidal particles.

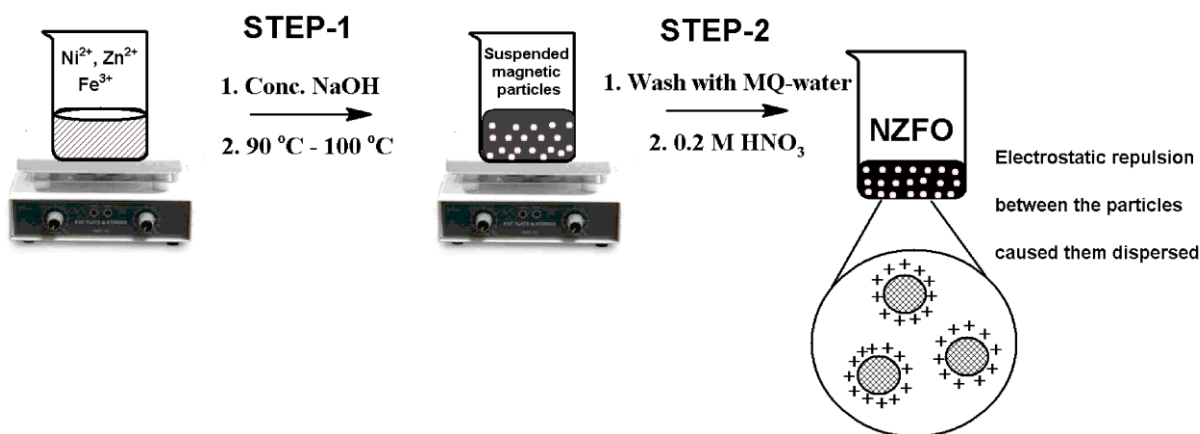
(vii) A calculated amount of this acidic ferrofluid solution was then added to calculated amount of aqueous buffer (buffer prepared in Milli-Q water)¹⁻⁵ solution of $\text{pH} = 7.4$ in order to carry out the experiments.

(viii) In a parallel experiment, the same precursor solutions (with same concentrations) were prepared and reacted with 1 M hot NaOH solution to obtain the precipitate. The precipitated product was then washed (with excess water and acetone) and dried well and stored safely for the phase analysis study.

The preparation of particles, ferrofluids and magnetorheological fluids has been the subject of numerous patents and research publications. A comprehensive bibliography

of this information can be found updated in each of Proceedings of the International Conference on Magnetic Fluids (ICMF), under the following authors Zahn et al.,⁹ Charles et al.,¹⁰ Kamiyama et al.,¹¹ Blums et al.,¹² Cabuil et al.,¹³ Bhatnagar et al.,¹⁴ Vekas et al.¹⁵ Reviews of the subject have been given by Rosensweig,¹⁶⁻¹⁸ Charles et al.,¹⁹ Martinet²⁰ and Scholten.²¹

Scheme S1: Simplified illustration for the synthesis procedure of NZFO



The transmission electron microscopy (TEM) images of the acidic ferrofluid sample provide an efficient conduit to assess the size, shape and distribution of the particles in solution. Therefore, the technique can be exploited to extract information about the stability of the particles and uniformity of distribution in the solution. The striking conformity of the TEM images of the freshly prepared particles with the one week-aged ones ensures a stable distribution of the particles in solution (Figure S1 and S2).

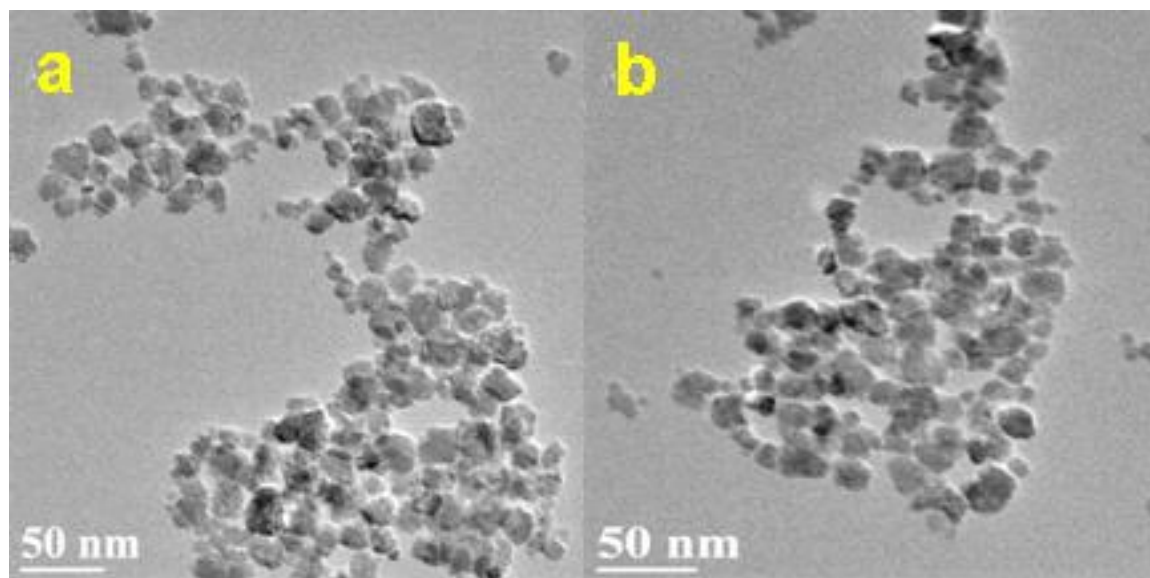


Figure S1: *TEM images of colloidal NZFO with the freshly prepared solution (a) and after 1 week (b).*

Below in Figure S2 is shown the well resolved HRTEM images of the nanoparticles aged for one week. The Selected Area Electron Diffraction (SAED) pattern of the sample displays the polycrystalline nature of the sample.

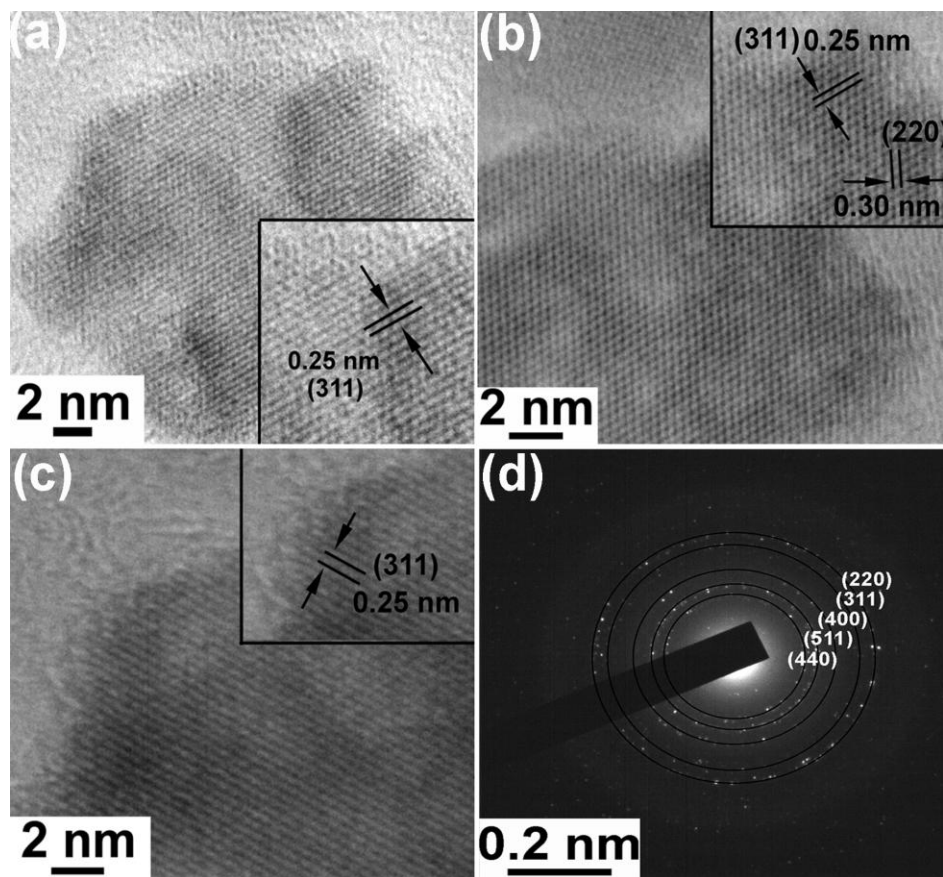


Figure S2: HRTEM images (a–c) and SAED image (d) of colloidal NZFO nanoparticles aged for 1 week.

S2. X-Ray Diffraction (XRD) analysis of the powder sample

Before peptization in the carrier liquid (aqueous acidic solution) the powder obtained in the co-precipitation reaction was subjected to XRD analysis to find out the phase and average crystallite size. Figure S3 shows the XRD pattern of the powder sample which can be indexed for pure spinel-like Ni-Zn ferrite, showing that the product is absolutely phase pure. A comparison of the observed and standard (JCPDF PDF# 080234) d -values for different crystallographic planes is given in Table S1. The average crystallite size (d) value was calculated from the most intense line (311) in the XRD patterns, using Scherrer's equation i.e.,

$$d = 0.9\lambda/\beta \cos \theta \quad (\text{S1})$$

where, λ is the wavelength of X-ray, β is the full width at half maxima (FWHM) and θ is the Bragg angle. Lattice parameter (a) was calculated using the following equation:

$$a = \frac{\lambda}{2 \sin \theta} \sqrt{h^2 + k^2 + l^2} \quad (\text{S2})$$

The Lattice parameter (a) is found to be 0.8407 nm for the particles.

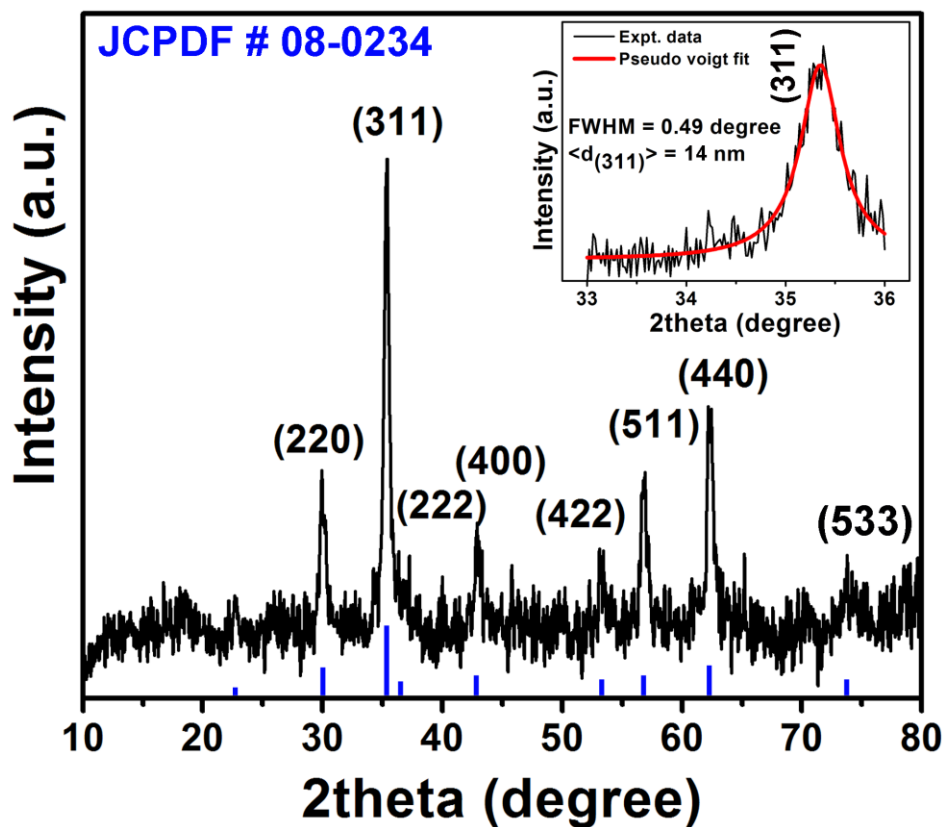


Figure S3: X-ray diffraction profile for the powder product (NZFO) obtained from co-precipitation at 90 °C. The inset shows the pseudo Voigt fit of the major peak corresponding to (311) plane which yields the Full-Width at Half Maximum (FWHM). Comparison of the obtained XRD data with the standard JCPDF file (JCPDF# 08-0234) is also shown (blue lines).

Table S1: Comparison of d-values obtained from XRD with JCPDF

Phase: $\text{Ni}_{0.5}\text{Zn}_{0.5}\text{Fe}_2\text{O}_4$; Lattice parameter (a): 0.8407 nm; Crystallite size: 14 nm							
Plane	220	311	222	400	422	511	440
$d_{\text{XRD}} (\text{Å})$	2.98	2.53	2.46	2.1	1.72	1.61	1.48
$d_{\text{JCPDS}} (\text{Å})$ (PDF# 080234)	2.96	2.53	2.42	2.1	1.71	1.61	1.48

S3. Atomic Absorption Spectral (AAS) data analysis

Atomic absorption spectroscopy analysis (AAS) is an effective tool for the determination of ion(s) concentration present in a diluted aqueous solution (on the order of ppm). Thus in the present study we have performed the AAS analysis of the ionic ferrofluid solution in order to determine the ion concentrations of the dispersed particles which in turn gives us the information of the composition of the particles.

First, 100 μl of ferrofluid solution (the same solution used for the spectroscopic study) was added to the 400 μl of conc. HCl solution and ultrasonically treated for 1 h to ensure the complete dissolution of the particles. Under this condition it is expected that all the particles are destroyed to construct the pool of constituting ions e.g. Ni^{2+} , Zn^{2+} , Fe^{3+} .

Second, 250 μl of the as-prepared solution was dissolved in 25 ml of deionized water in a volumetric flask to yield a diluted stock solution of the respective ions.

Third, this diluted stock solution was then used for the AAS analysis and the results are comprised in Table S2.

Table S2: Atomic Absorption Spectral (AAS) data analysis chart

Information of Standard Samples					
Standard conc. used for Nickel 2 M, 4 M, 6 M		Standard conc. used for Zinc 0.5 M, 1 M, 1.5 M		Standard conc. used for Iron 1 M, 3 M, 5 M	
Information of Samples ($0.25 \text{ ml} \times S_1 = 25 \text{ ml} \times S_2$)					
S ₂ for Nickel (ppm)		S ₂ for Zinc (ppm)		S ₂ for Iron (ppm)	
0.695	0.682	0.858	0.869	2.460	2.437
0.680		0.885		2.427	
0.670		0.864		2.425	

From the value of S₂, the value of S₁ is calculated as follows:

for nickel = 2.897×10^{-5} M,

for zinc = 3.323×10^{-5} M,

for iron = 10.912×10^{-5} M.

Therefore, the molar ratio of the metal ion is = 2.897 : 3.323 : 10.912,

i.e., 1 : 1.14 : 3.766.

From the AAS data the ratio of metal ions extracted from the particles can now be estimated. The most accurate composition would be: Ni(1): Zn(1.14): Fe(3.766).

So, the molecular formula of one unit cell of the sample should be: Ni_{0.5}Zn_{0.57}Fe_{1.883}O₄.

S4. Dynamic Light Scattering (DLS) measurement

Polydispersity (δ):

In dynamic light scattering (DLS) measurement, polydispersity (δ) is a parameter often used to describe the width of the particle size distribution. In a nut-shell, the polydispersity (δ) parameter in DLS experiments is derived from a Cumulants analysis of the DLS measured intensity autocorrelation function.²² In the Cumulants analysis,²² a single particle size is assumed and a single exponential fit is applied to the autocorrelation function.

Under the assumption of a purely monomodal particle size distribution (i.e., single size population) following a Gaussian distribution, the polydispersity (δ) is described to be related to the standard deviation (σ) of the hypothetical Gaussian distribution in the fashion shown below:²³⁻²⁶

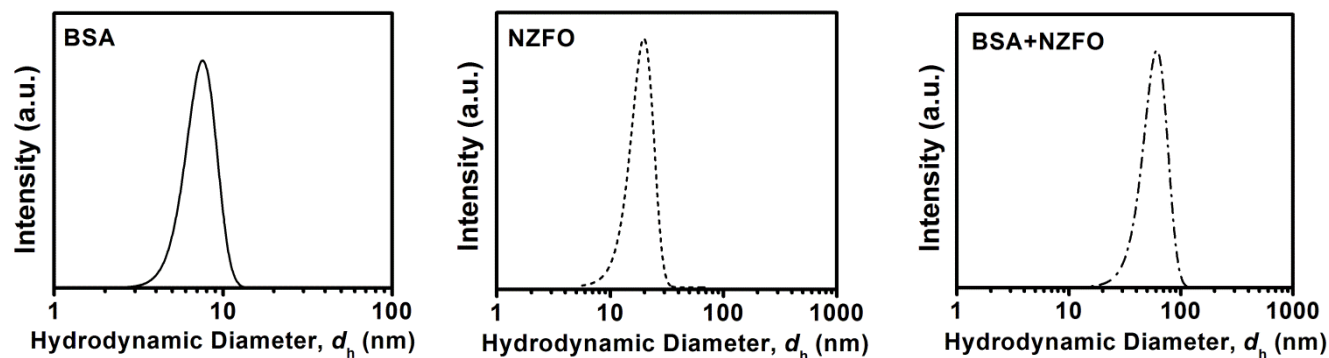
$$\text{Polydispersity, } \delta = \frac{\sigma}{d_h} \quad (\text{S3})$$

in which d_h represents the intensity weighted mean hydrodynamic size (Cumulants mean) of the ensemble collection of particles.

Thus the parameter polydispersity (δ) describes, in essence, the standard deviation (σ) or the width of the as-obtained size distribution profile from a given DLS measurement. Usually samples with $\delta < 0.2$ (or in other term, $\% \delta < 20 \%$) are accepted to be reasonably monodispersed²³⁻²⁶ (see also: <http://www.che.ccu.edu.tw/~rheology/DLS/outline3.htm>).

Below is shown the size distribution graphs for the presently investigated solutions as obtained from DLS measurements.

(a)



(b)

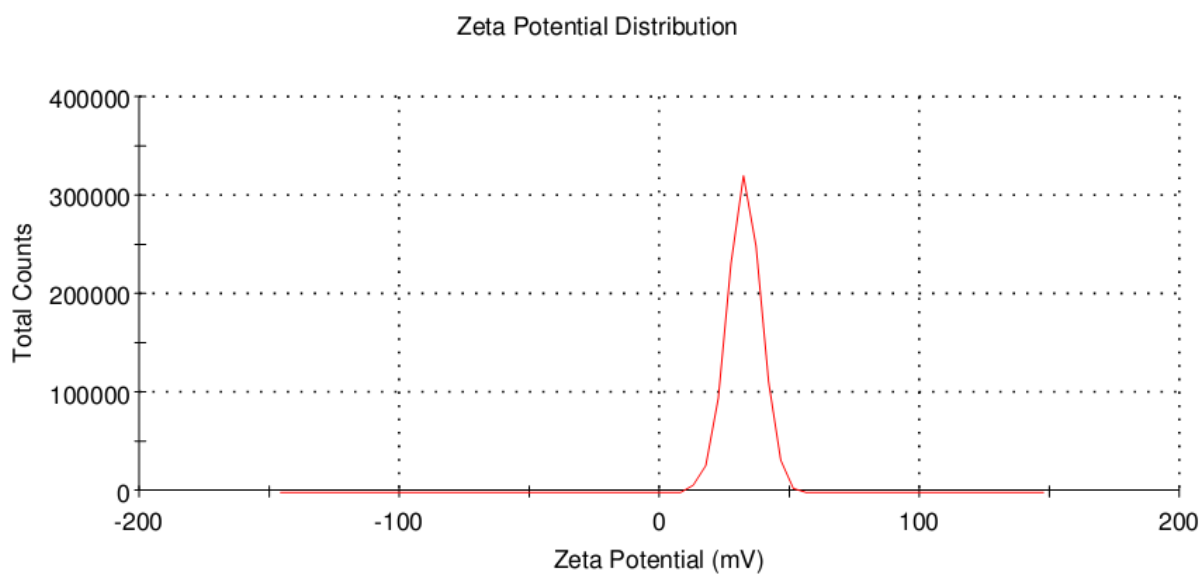


Figure S4: (a) Representative size distribution graph (in terms of intensity distribution of hydrodynamic diameter, d_h in nm) for BSA alone (—; $d_h = 7.0 \pm 0.5$ nm, $\delta = 0.07$), NZFO alone (⋯; $d_h = 19.0 \pm 2$ nm, $\delta = 0.11$) and BSA in the presence of NZFO (— · —; $d_h = 56.0 \pm 8$ nm, $\delta = 0.15$) as obtained from Dynamic Light Scattering (DLS) measurements. (b) The zeta potential distribution profile for NZFO.

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