A statistical approach to control particle size of poly(acrylic acid) stabilized iron oxide nanoparticles

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ABSTRACT

Poly(acrylic acid) coated superparamagnetic iron oxide nanoparticles were prepared in aqueous solutions of iron salts with in situ coating of poly(acrylic acid). Influence of reaction variables, namely, iron concentration, reactive(COOH)/iron mole ratio, base (NH_4OH) amount and polymer molecular weight, primarily on hydrodynamic size and stability of the particles was investigated. Also, size and stability of washed particles (no excess coating material) and magnetization as a function of these variables were studied. In order to design best set of experiments and correlate the results to variables, statistics programs Design Expert 7.0 and Minitab14 were used. Results will be discussed in this paper.

Keywords: magnetic nanoparticles, iron oxide, poly(acrylic acid), size control

1 INTRODUCTION

Superparamagnetic iron oxide nanoparticles are interest of wide range of applications from magnetic recording to biomedical applications. Each application requires a specific size and surface in addition to stability. Therefore, controlling particle size and size distribution, providing functional surfaces and preventing particle aggregation are key issues in the field.

Nanoparticles having smaller size and larger surface area exhibit different physical and chemical properties from those of relatively larger nanoparticles. Particle size also dictates the biodistribution of the nanoparticles in the in vivo applications. Particles larger than 100 nanometers are realized by macrophages and cleared from blood rapidly preventing targeting organs other than those that constitute the RES system, namely liver and spleen [1]. Thus, ability to control particle size is a key issue that would enable the use of nanoparticles for target specific applications as well as broader in vivo use.

Large hydrodynamic size of nanoparticles actually is a result of aggregation. Coating nanocrystals with polymeric materials is one of the commonly used methods to prevent such aggregation. Polyvinyl alcohol (PVA), poly(N-vinylpyrollidone) (PVP) and polyethylene glycol (PEG) are widely used coating materials for in vivo applications due to water solubility and biocompatability [2]. Such polymer-coated nanoparticles have been used in different fields, e.g. ferrofluid, magnetic resonance imaging contrast enhancement, targeted drug delivery, cell labeling and magnetic cell separation [3-4]. In our study, polyacrylic acid sodium salt was used as a polymeric stabilizer for the magnetic cores. Polyacrylic acid offers multiple functional groups (carboxylic acid) for adsorption to the crystal surface as well as providing functional surface (carboxylic acids) to the coated particles.

Two major methods to prepare polymer-coated nanoparticles: physical adsorption of polymer on the particle and emulsion polymerization in the presence of nanoparticles [5-6]. Later approach is more efficient in providing large aggregates.

Here, we focus on the in situ coating of the iron oxide nanoparticles with polyacrylic acid sodium salt to prevent aggregation during synthesis and control crystal size and size distribution. This was achieved by treating iron salts with ammonium hydroxide in water in the presence of PAA. There are number of variables that may control the particle size (core and hydrodynamic), size distribution and stability (resistance to agglomeration). PAA molecular weight, iron and base concentrations and (COOH)/iron mole ratio are the four factors chosen to control these responses (Table 1). The aim of this paper is to determine the most effective factors determining primarily the particle size and find a mathematical equation that predicts size using these effective parameters. Also, we are interested in identifying the factors effective on particle stabilization and magnetization. Design Expert 7.0 and Minitab14 Release statistical programs, two-level full factorial design was used to determine best set of experiments and find the possible correlations between these four factors and responses.

2 EXPERIMENTAL

$FeCl_3.6H_2O$ and $FeCl_2.4\ H_2O$ were used as purchased from Fluka. Ammonium hydroxide (26% NH_3 in water, w/w) was purchased from Riedel-de Haen and used as supplied. Two different molecular weights (Mw ~ 5100 and Mw~15000) of polyacrylic acid sodium salt was purchased from Aldrich. MilliQ water was used for all preparations and work-up.

2.1 Synthesis of Iron Oxide Nanoparticles in the Presence of PAA

PAA sodium salt was dissolved in required amount of water, transferred into a 100ml three necked round bottomed flask fitted with a mechanical stirrer, condenser

and nitrogen inlet. After the polymer solution was deoxygenated for 30 minutes, iron salts (Fe^{3+} / Fe^{2+} mole ratio of 2) were added to the flask and stirred at 400rpm under nitrogen for about 15 min. Reaction flask placed into an oil bath at 85°C. After 10min of mixing, ammonium hydroxide was injected to the flask with vigorous stirring at 800rpm and reaction allowed to continue for 30 minutes. After the colloidal suspension cooled to room temperature, it was transferred to a glass bottle and sit atop a handheld magnet (0.3Tesla) overnight. Any precipitate that might occur was removed.

Table 1: Reaction variables:

	Factors	Low	High
Fe conc.[a]	0.03	0.3	0.03
Reactive/Fe[b]	0.3	4	0.3
Base ratio[c]	1	3	1
Mw of PAA[d]	5100	15000	5100

[a] Total iron concentration (M): (mole Fe(II) + mole Fe (III))/Volume

[b] Reactive/Fe : mole COONa/ mole Fe

[c] Base ratio : mole base/[COONa mole + (2.5 mole Fe)]

[d]Molecular weight of PAA: g/mol

Eighteen experiments were created by the statistic programs based on four factor two-level full factorial design with two centerpoints per block within the given range of each variable (Table2). These ranges were chosen based on our previous trials and literature values to obtain small size particles.

2.2 Measurement of particle size distribution

Hydrodynamic size (D_h) of PAA coated iron oxide nanoparticles were measured by Malvern Zetasizer Dynamic Light Scattering (DLS). Hydrodynamic sizes of particles were measured both before and after washing off excess polymer via ultrafiltration. Reported sizes are 1/100 dilution of original reaction concentrations (stock solution) in order to eliminate the viscosity effect on particle size measurements. Zeta potentials were measured by Brookhaven ZetaPals Zeta Potential Analyzer.

3 RESULTS AND DISCUSSION

At the end of the reaction pH of all reactions were 9-10 All particles have zeta potential around -100mV.

3.1 Evaluation of variables and responses

Data was analyzed by the statistics programs by using log normal transformation. Table 2 shows experimental parameters and the hydrodynamic size that was measured after synthesis.

Factors that are significant in affecting particle size were found as iron concentration, reactive/ iron mole ratio, molecular weight of PAA and interaction between iron concentration and reactive/iron mole ratio (Figure 1).

Table 2: Design space and hydrodynamic size

Mw of PAA (g/mol)	Reactive/ Fe	Base ratio	Fe conc. (M)	Size (D_h) (nm)
5100	2.15	2	0.165	60.0
15000	0.3	3	0.03	110.0
15000	0.3	1	0.3	150.0
15000	4	3	0.3	150.0
5100	0.3	1	0.03	90.0
5100	0.3	3	0.3	150.0
15000	4	1	0.03	25.0
5100	4	1	0.3	80.0
5100	4	3	0.03	17.0
15000	2.15	2	0.165	200.0
5100	0.3	3	0.03	150.0
15000	0.3	3	0.3	180.0
15000	4	3	0.03	25.0
5100	4	3	0.3	60.0
15000	4	1	0.3	200.0
5100	0.3	1	0.3	90.0
15000	0.3	1	0.03	150.0
5100	4	1	0.03	7.0

Base ratio was not significant. Minitab14 Release program was used to evaluate the four factors by using 'Main Effects Chart'. This data imply that nanoparticle size increases with increasing iron concentration and molecular weight of PAA and decreasing reactive/Fe mole ratio (Figure 1).

Design Expert 7.0 fit a quantitative relation between these significant factors and the hydrodynamic size (S). Equation (1) was created for PAA molecular weight 5100 and (2) for molecular weight 15000.

Eqn. 1:

$$\ln(S) = 4.73927 - 0.11358\times F - 0.63233\times R + 1.8986\times F\times R$$

Eqn. 2:

$$\ln(S) = 5.2235 - 0.11358\times F - 0.63233\times R + 1.89860\times F\times R$$

Stability upon dilution, stability after removal of excess coating material and magnetization are also very important for applications of nanoparticles. Stability means resistance to aggregation so, if particles do not precipitate but size increases then it is evaluated as unstable as well. Statistical evaluation indicates that reactive/iron ratio is important for stability. For lower molecular weight PAA, concentrated solutions with high reactive/iron ratio are stable and in case of higher molecular weigh PAA reactions higher iron concentrations with lower reactive/Fe ratio is desired for stability. High reactive/Fe ratios and higher molecular weight encourages aggregation as we have seen from size

analysis. This may also indicate that longer chains might adsorb on multiple crystals causing less compact coating of the particle, less attachment of the single chain per crystal leading to poorer stability. In case of magnetization, high reactive/Fe ratio along with low Fe concentration produces non-magnetic materials (Figure2). Although we do not have the XRD data yet on these particles, this observation might originate from having too much coating material and not allowing crystal growth.

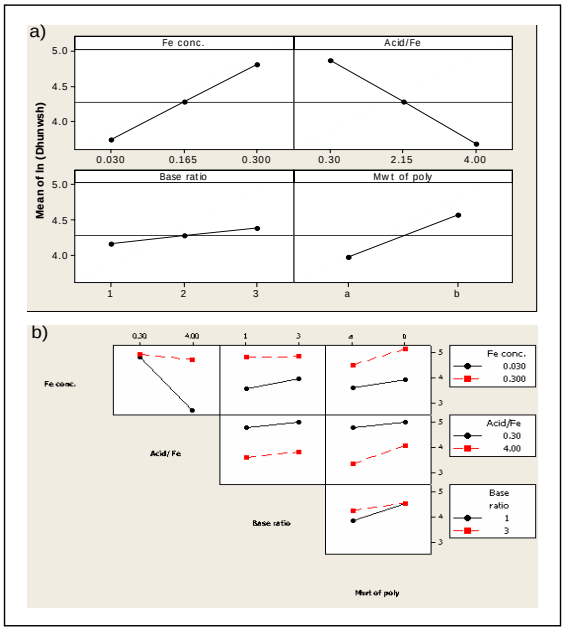

Figure 1: a) Main effects plot, b) Interaction plot for hydrodynamic size of the particles.

In order to control the efficiency of equations (1) and (2), test experiments were performed in the chosen area. Hydrodynamic sizes obtained and predicted are shown in Table 3. Experimental results deviated from predicted by about ±10% which is quite good for hydrodynamic size measurements.

Table3: Trial experiments

Fe conc. (M)	Reactive/Fe (mol/mol)	Base ratio	Mw of PAA (g/mol)	Size[1] (nm)	Size[2] (nm)
0.18	2.8	2	5100	45	49.7
0.15	3	2	5100	25	39.6
0.1	3	2	5100	35	30.0
0.03	4	1.47	5100	11	11.4

[1] experimental hydrodynamic size
[2] theoretical hydrodynamic size

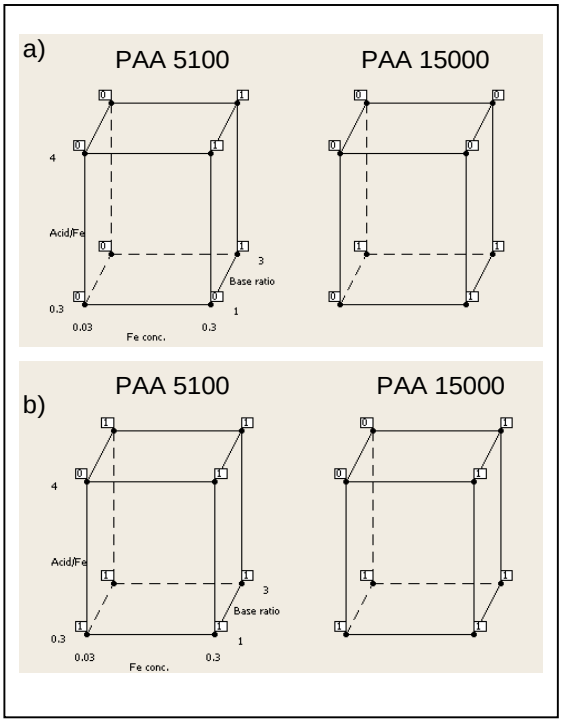

Figure 2 : Cube plots for a) stability upon dilution of unwashed particles (1:stable, 0: unstable); b) magnetization (1:magnetic, 0:non-magnetic).

4 CONCLUSION

Poly(acrylic acid) coated iron oxide nanoparticles were synthesized in aqueous solutions with varying experimental parameters. Iron concentration, reactive/Fe molar ratio, molecular weight of PAA and Fe concentration*Reactive/Fe interaction were found significant in affecting the hydrodynamic size of the particles. Design Expert 7.0 provided a quantitative relation between particle size and significant factors. Test runs showed quite a good agreement between experimental and predicted values. Stability upon dilution and magnetization of nanoparticles increased with increasing Fe concentration and decreasing reactive/Fe ratio.

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