CHAPTER 4

Interaction of metal oxide nanoparticles with water, saline and egg albumen: A physicochemical study

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INTRODUCTION

Nanoparticles possess unusual physicochemical properties due to a variety of parameters such as small size, surface structure, chemical composition, shape, solubility and agglomeration. The distribution of particles within the body is dependent on the particle's size and surface characteristics. Nanoparticles have the ability to bind and interact with biological matter changing their surface characteristics depending on the environment they are present in and resulting in an increased concern over its potential effect on human body. Thus the characterization of nanoparticles in biological system is necessary before classifying them as hazardous (Elsaesser & Howard, 2012). Surface charge and other physicochemical properties of nanoparticles cause the formation of agglomerates (micro-sized particles) thus changing their characteristics and properties. Many studies these days focus on the interaction of nanoparticles with protein for extent of agglomeration, dispersion and related properties, which can directly affect bioavailability of nanoparticles (Lynch & Dawson, 2008). Reports suggest composition to be responsible for toxicity of conventional materials, whereas physicochemical properties such as size, surface area, surface chemistry, surface roughness, medium of dispersion and ability to agglomerate are considered to be responsible for toxicity of nano materials (**Figure 4.1)** (Gatoo *et al.*, 2014).

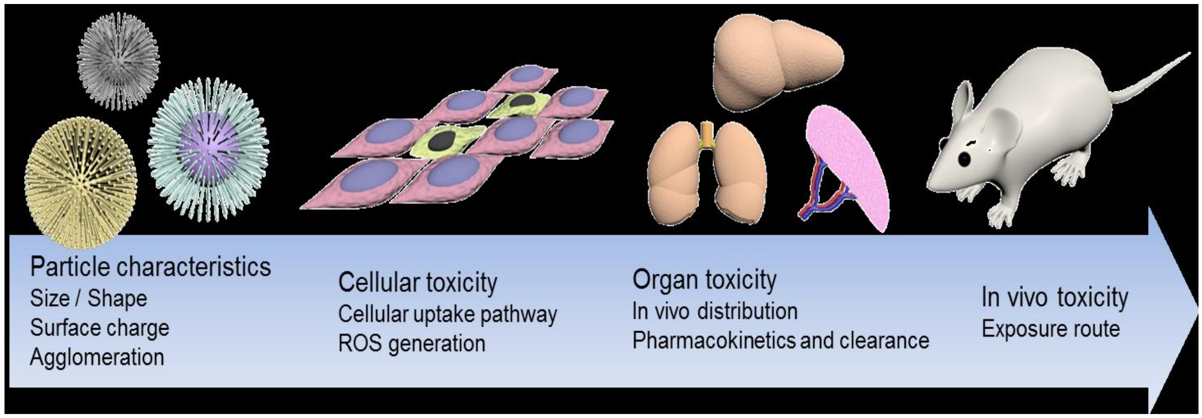


Figure 4.1: Schematic drawing of nanoparticles induced cytotoxicity. Intrinsic features of nanoparticles, such as size, surface charge, agglomeration, can significantly affect cytotoxicity. Such cytotoxicity can be affected at the levels of the cell, organ and even *in vivo* systems (Shin *et al.*, 2015).

Hence, in this study metal oxide nanoparticles (TiO_2 and Fe_2O_3) were dispersed in saline, water and egg albumen and physicochemical parameters such as density, viscosity, surface tension, friccohesity, activation energy and molecular radii were measured. Since the physicochemical properties infer the level of solubility, reactivity, binding and dispersion of nanoparticles in medium, the critical evaluation of molecular interaction can be useful. The extent of interaction with egg albumen can be correlated with the extent of protein binding and subsequent toxicity. MgO, Al_2O_3 and ZnO NPs did not show significant *in ovo* toxicity (chapter 2) and hence based on this preliminary screening, it was thought appropriate not to consider them for physicochemical studies. However, Fe_2O_3 and TiO_2 NPs showed toxicity and hence the physicochemical changes following their interaction with egg albumen, water or saline were analyzed in detail using Mansingh Survismeter (Patel *et al.*, 2016).

Borosil Mansingh Survismeter

Borosil Mansingh Survismeter accurately determines physicochemical indicators for interacting and reacting molecular materials. Diagrammatic representation of Mansingh Survismeter is given in Figure 4.2. The principle of **Survismeter** is as follows:

Sur: Surface tension, interfacial tension, cohesive force, wetting coefficient, thin film & coating

Vis: Viscosity, Frictional Forces, Newtonian and Non Newtonian liquids, solvent binding, shear stress, thixotropic (property exhibited by gels or fluid that are thick (viscous) under normal conditions but flow (become thin and less viscous) when shaken or agitated)

Meter: Works on cohesive forces and Intermolecular forces of moderate strengths and liquid distribution equilibrium in closed jacket

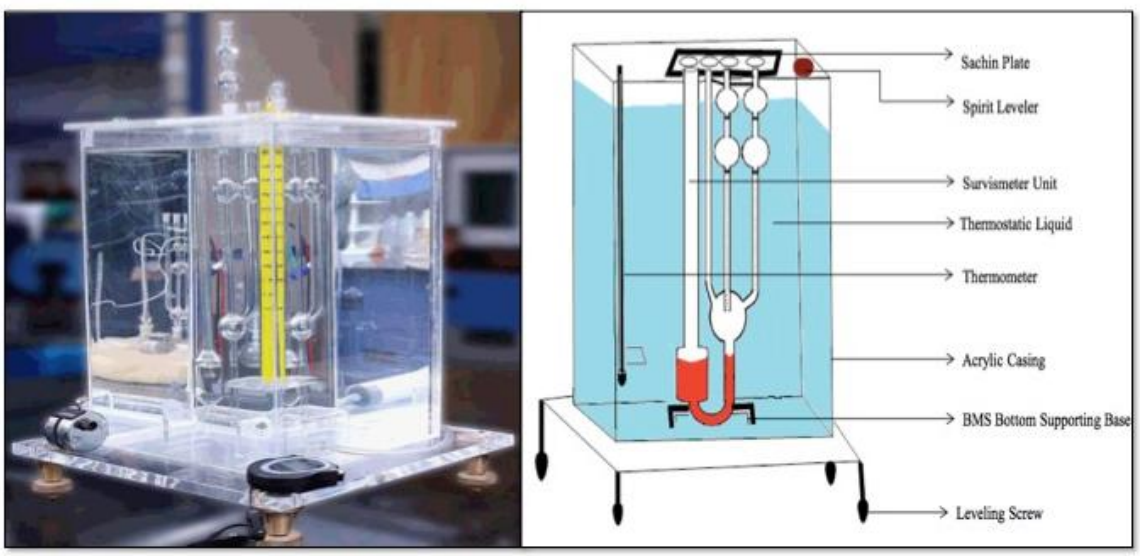


Figure 4.2: Borosil Mansingh Survismeter

Borosil Mansingh Survismeter can measure the following physicochemical parameters

1. Surface Tension of liquids
2. Viscosity of liquids
3. Interfacial tension between two immiscible liquids
4. Wetting coefficient
5. Contact angle between solid glass wall and liquid interfaces
6. Friccohesity, a dual force theory
7. Activation Energy
8. Kinetic energy of liquid flow
9. Gibbs free energy
10. Entropy
11. Surface area
12. Particle size
13. Surface excess concentration
14. Viscosity
15. Hydrodynamic volume
16. Dipole moment
17. Water/solvent binding capacity of molecules
18. Ionic and molecular impacts on evaporation of single drop method

The schematic diagram of Mansingh Survismeter is shown in Figure 4.3

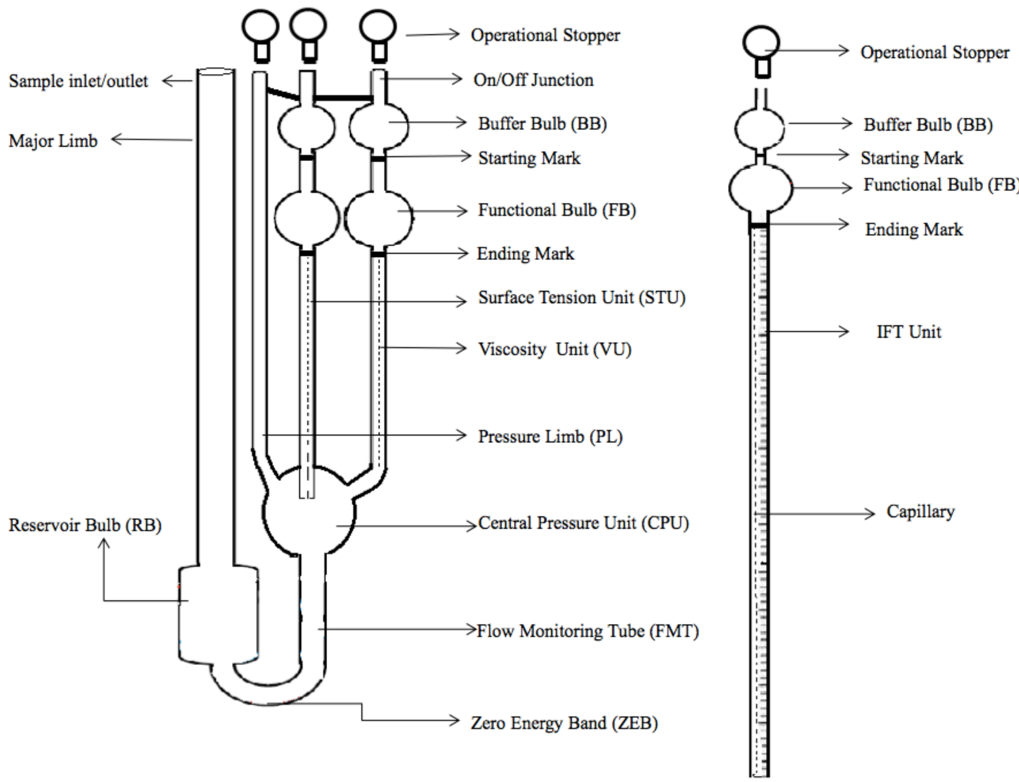


Figure 4.3: Schematic diagram of Borosil Mansingh Survismeter

MATERIALS AND METHODS

Physicochemical analysis

The physicochemical properties such as density, viscosity, surface tension, activation energy, friccohesity and molecular radii were assessed in absence or presence of TiO_2 NPs (1-100 μg/ml) or Fe_2O_3 NPs (10-500 μg/ml) in water, saline or egg albumen (freshly collected). Density of NPs in water, saline and egg albumen were determined with Anton Paar Density and Sound velocity Meter (DSA 5000 M). Density was calculated using the equation:

$$\rho = \rho^o + S_\rho m + S'_\rho m^2$$

(ρ^o at $m \rightarrow 0$ is limiting density, $S\rho$ is the 1st slope)

Viscosity was measured as viscous flow times (VFT) using Borosil Mansingh Survismeter (Patel *et al.*, 2016) at physiological temperature of 37°C (LAUDA ALPHA RA 8 thermostat) and calculated by equation:

$$\eta = \left(\frac{t}{t_\circ}\right)\left(\frac{\rho}{\rho_\circ}\right)\eta_\circ$$

(η_o is viscosity of water and t_o, t are flow times of solvent and mixtures respectively)

The η data were regressed with following equation:

$$\eta = \eta^o + S_\eta m$$

(η^o at $m \rightarrow 0$ is limiting viscosity; S_η is the 1st degree slope).

Surface tension was measured by counting pendent drop numbers (PDN) using Borosil Mansingh Survismeter and calculated by equation:

$$\gamma = \left(\frac{\eta_\circ}{\eta}\right)\left(\frac{\rho_\circ}{\rho}\right)\gamma_\circ$$

(γ_o is surface tension of water, η_o and η are pendent drop numbers of medium and solutions respectively)

The γ data were regressed for limiting values γ^o at m → 0 with following equation:

$$\gamma = \gamma^o + S_\gamma m$$

(γ^o is limiting surface tension, and S_γ is the 1st degree slope)

Friccohesity was calculated using Mansingh equation (Chandra *et al.*, 2013):

$$\sigma = \sigma_o\left[\left(\frac{t}{t_o} \pm \frac{B}{t}\right)\left(\frac{\eta}{\eta_o} \pm 0.0012(1-p)\right)\right]$$

(σ is friccohesity, t and t_o are the sample and solution viscous flow times respectively, η_o and η are the pendant drop numbers of medium and solutions respectively)

Reference friccohesity was calculated by

$$\sigma_o = \eta_o/\gamma_o$$

Where, η_o and γ_o are the viscosity and surface tension of references respectively.

For activation energy, the partial molar volume V_2 was calculated with following equation:

$$V_2 = \left[\frac{1000\,(\rho^o - \rho)}{m\,\rho^o\rho}\right] + \frac{M}{\rho}$$

(M is molar mass, ρ^o is density of water and ρ is density of solution)

The V_1 for water, saline and albumen at 37°C is calculated with equation:

$$V_1 = \frac{M}{\rho}$$

V_1 and V_2 are used for calculating activation energy by using equation:

$$\Delta\mu_1^* = RT\,\ln\left(\frac{\eta_o V_1}{hN}\right)$$

($\Delta\mu_1^*$ is activation energy of water, saline or albumen, R is gas constant, h is Planck constant and N is Avogadro number (6.023X 10^{23}). Activation energy ($\Delta\mu_2^*$ J/mol) was calculated with equation:

$$\Delta\mu_2^* = \Delta\mu_1^* - \left[\left(\frac{RT}{V_2}\right)\left((1000\eta) - (V_1 - V_2)\right)\right]$$

Molecular radii r (nm) is calculated by using the equation:

$$r = \sqrt[3]{\frac{3\phi}{4\pi Nc}}$$

(ϕ is volume fraction of water, saline and albumen entangled with NPs, N is Avogadro number, c is concentration and π is constant)

RESULTS

Physicochemical analysis of Iron oxide nanoparticles

Density

The densities of test solvents obtained herein were albumen (1.034178 Kgm^{-3}) > saline (0.998609 Kgm^{-3}) > water (0.993433 Kgm^{-3}) (Table 4.1). After addition of Fe_2O_3 NPs, the respective solvent system showed that Fe_2O_3 NPs + albumen (1.032826 Kg m^{-3}) has higher limiting density with 3.4% and 3.9% stronger intra molecular force interactions between Fe_2O_3 NPs and albumen as compared to Fe_2O_3 NPs + saline (0.998773Kg m^{-3}) and Fe_2O_3 NPs + water (0.993402 Kg m^{-3}) (Table 4.2). There was a dose dependent increment in density of Fe_2O_3 NPs + albumen (Figure 4.4A and Table 4.3).

Viscosity

An increase in viscosity was observed in water, saline and albumen (0.696, 0.7280 & 2.5583 mPa·s, Table 4.1) respectively. On interaction with Fe_2O_3 NPs there was significant increase recorded in limiting viscosities, which were found to be 2.296269 mPa·s and 0.744932 mPa·s for Fe_2O_3 NPs + albumen and Fe_2O_3 NPs + saline respectively (Table 4.2). Stronger interactions of Fe_2O_3 NPs with albumen resulted in an increase in viscosity of albumen (Figure 4.4B and Table 4.3).

Surface tension

Surface tension of water (73.35 mNm^{-1}) was found to be higher than that of saline (71.36 mNm^{-1}) and albumen (71.139 mNm^{-1}, Table 4.1). Higher dose of Fe_2O_3 NPs (200µg/ml) had recorded a significant increment in surface tension of Fe_2O_3 NPs + saline (Table 4.3) Indices of surface tension showed a dose dependent increment in surface tension of Fe_2O_3 NPs + albumen (Figure 4.4C and Table 4.3)

Friccohesity

The limiting friccohesity was found to be higher in saline (0.010433 s.m.$^{-1}$) as compared to Fe_2O_3 NPs + albumen (0.001732 s.m.$^{-1}$, Table 4.4). In the present study, saline witnessed 0.04% increase at 200 µg/ml followed by a 0.08% decrement at 500 µg/ml whereas, albumen recorded a steady decrement of 0.01% (Figure 4.4D and Table 4.3). Higher activation energy was recorded at 200µg/ml in Fe_2O_3 NPs + saline and Fe_2O_3 NPs + albumen (Figure 4.4D and Table 4.3)

Activation energy

A dose dependent decline in activation energy was recorded from 1-200 µg/ml doses with -56.18 KJ.mole^{-1} as the highest value and -66.20 KJ.mole^{-1} as the lowest value respectively (Figure 4.4E and Table 4.3)

Molecular radii

In our study, we found that Fe_2O_3 NPs + albumen showed low molecular radii as compared to Fe_2O_3 NPs + water. Observations of molecular radii of Fe_2O_3 NPs + saline were similar to albumen at all the lower doses (10-100 µg/ml) but showed an increment at 200µg/ml (Figure 4.4F and Table 4.3)

Physicochemical analysis of Titanium dioxide nanoparticles

Density

After addition of TiO_2 NPs, the respective solvent system showed that TiO_2 NPs + albumen (1.031606 Kg m^{-3}) has higher limiting density with 3.3% and 3.8% stronger intra molecular force interactions between TiO_2 NPs and albumen as compared to TiO_2 NPs + saline (0.998296 Kg m^{-3}) and TiO_2 NPs + water (0.993300 Kg m^{-3}) (Table 4.5). There was a decrement in density (1.031064 kg.m^{-3}) of TiO_2 NPs + albumen at 10 µg/ml, whereas, 25, 50 and 100 µg/ml recorded steadily ascending values (1.031567, 1.031979 and 1.032092 $kg.m^{-3}$ respectively) (Figure 4.5A and Table 4.6).

Viscosity

On interaction with TiO_2 NPs there was significant increase recorded in limiting viscosities, which were found to be 2.558895 mPa·s and 0.758464 mPa·s for TiO_2 NPs + albumen and TiO_2 NPs + saline respectively (Table 4.5). Lower concentrations of TiO_2 NPs (1 and 5 µg/ml) recorded higher viscosity indices (2.57 and 2.60 mPa.s). The viscosity indices of 10, 25 and 50 µg/ml doses were comparable to each other (2.47, 2.49 and 2.45 mPa.s) but, 100 µg/ml dose accounted for a decline in viscosity (2.35 mPa.s) (Figure 4.5B and Table 4.6)

Surface tension

TiO_2 NPs recorded a significant decrement in surface tension of TiO_2 NPs + saline (Figure 4.5C and Table 4.5) Indices of surface tension showed an increase at 10 µg/ml concentration of TiO_2 NPs (65.98 $mN.m^{-1}$) as compared to 1 and 5 µg/ml concentrations (65.45 and 65.44 $mN.m^{-1}$ respectively). However, 25, 50 and 100 µg/ml doses recorded a steady increment in surface tension (66.01, 66.64 and 66.65 $mN.m^{-1}$) (Figure 4.5C Table 4.6)

Friccohesity

The limiting friccohesity was found to be higher in saline (0.01064 $s.m.^{-1}$) as compared to TiO_2 NPs + albumen (0.002093 $s.m.^{-1}$, Table 4.7). Friccohesity indices showed a decline at 10 µg/ml TiO_2 NPs (0.002009 $s.m.^{-1}$) as compared to 1 and 5 µg/ml concentrations (0.002113 and 0.002136 $s.m.^{-1}$ respectively). A steady decline in friccohesity (0.002027, 0.001974 and 0.001899 s.m.$^{-1}$ at 25, 50 and 100 µg/ml doses respectively) was also observed in this study (Figure 4.5D and Table 4.6)

Activation energy

A dose dependent decline in activation energy was recorded from 1-100 µg/ml doses with -57.81 KJ.mole^{-1} as the highest value and -61.26 KJ.mole^{-1} as the lowest value respectively (Figure 4.5E and Table 4.6)

Molecular radii

An increase in molecular radii (5-11.53 nm) was observed at 1-10 µg/ml TiO_2 NPs. Further, a steady increase in molecular radii (15.69, 19.66 and 24.45 nm) was observed at 25, 50 and 100 µg/ml doses respectively (Figure 4.5F and Table 4.6)

Table 4.1: Density ($\rho \pm 10^{-3}$kg m^{-3}), viscosity ($\eta \pm 10^{-5}$mPa·s) and surface tension ($\gamma \pm 10^{-2}$mN m^{-1}) of water, saline and albumen at 37 °C

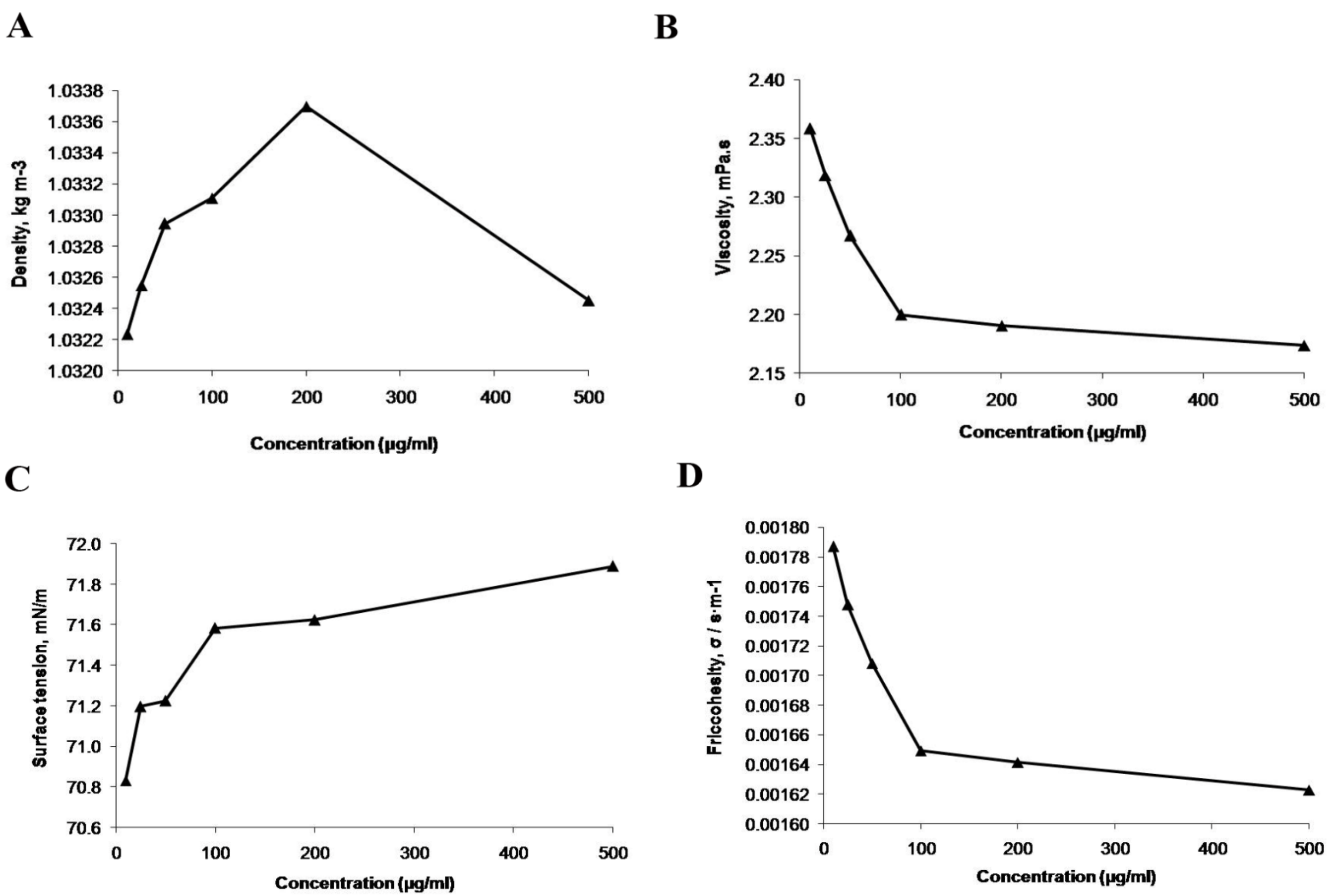
Pure solvents	**ρ**	**H**	**ϒ**
Water	0.993433	0.696	73.35
Saline	0.998609	0.7280945	71.36
Albumen	1.034178	2.5583631	71.139

Table 4.2: Limiting density ($\rho°$, kg m^{-3}), and its 1st slope ($S\rho$, kg^{2}m^{-3}mol^{-1}), Limiting viscosity ($\eta°$, mPa·s), and its 1st slope ($S\eta$, mPa·s kg m^{-1}), Limiting surface tension ($\gamma°$, mN m^{-1}), and its 1st slope ($S\gamma$, mN kgmol^{-1} m^{-1}) of Fe_2O_3 NPs

System	**Density**		**Viscosity**		**Surface tension**	
	ρ^o	S_ρ	η^o	S_η	γ^o	S_γ
Fe_2O_3 NPs + Water	0.993402	0	0.694179	0	73.35	0.0005
Fe_2O_3 NPs + Saline	0.998773	0	0.744932	-0.000020	71.36	0.006
Fe_2O_3 NPs + Albumen	1.032826	0	2.296269	-0.000300	71.13	0.00171

Table 4.3: Microgram/microlitre (µg/ml), density ($\rho \pm 10^{-3}$ kg m^{-3}), viscosity ($\eta \pm 10^{-5}$ mPa·s), surface tension ($\gamma \pm 10-2$ mN m^{-1}), friccohesity (σ, s.m.$^{-1}$), activation energy ($\Delta\mu2^{*} \pm 10^{-2}$ kJmol^{-1}), molecular radii (r, nm) at 37°C of Fe_2O_3 NPs

µg/ml	ρ	η	γ	σ	$\Delta\mu_2^{*}$	r
Fe_2O_3 NPs + Water						
10	0.993428	0.702902	72.91	0.00964	-66.82	21.56
25	0.993430	0.6949087	73.52	0.00945	-67.23	29.15
50	0.993404	0.6929255	73.52	0.00942	-73.63	36.70
100	0.993476	0.6904715	73.53	0.00939	-73.87	46.18
200	0.993558	0.6900007	73.53	0.00938	18.07	58.18
500	0.993771	0.7022573	73.55	0.00955	-2.10	79.43
Fe_2O_3 NPs + Saline						
10	0.998678	0.7179489	70.95	0.01012	-54.42	11.68
25	0.998744	0.7381678	71.56	0.01031	-53.82	16.01
50	0.998850	0.7427258	71.56	0.01038	-53.49	20.21
100	0.998900	0.7543984	71.57	0.01054	-50.94	25.60
200	0.998924	0.7784446	73.93	0.01053	-57.35	60.56
500	0.998985	0.7151111	73.94	0.00967	-56.81	79.91
Fe_2O_3 NPs + Albumen						
10	1.032233	2.358320	70.83	0.00179	-56.18	11.35
25	1.032547	2.318580	71.20	0.00175	-56.55	15.31
50	1.032945	2.267059	71.22	0.00171	-57.40	19.15
100	1.033107	2.199850	71.58	0.00165	-58.99	23.89
200	1.033697	2.190523	71.62	0.00164	-66.20	30.06
500	1.03245	2.173649	71.89	0.00162	-63.73	40.69



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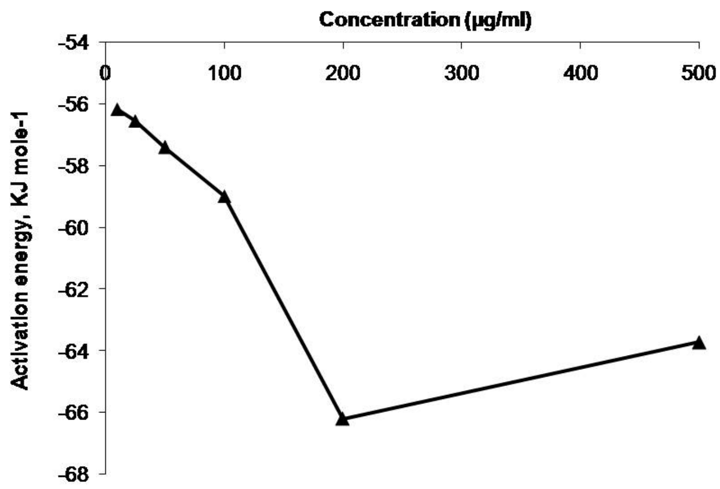

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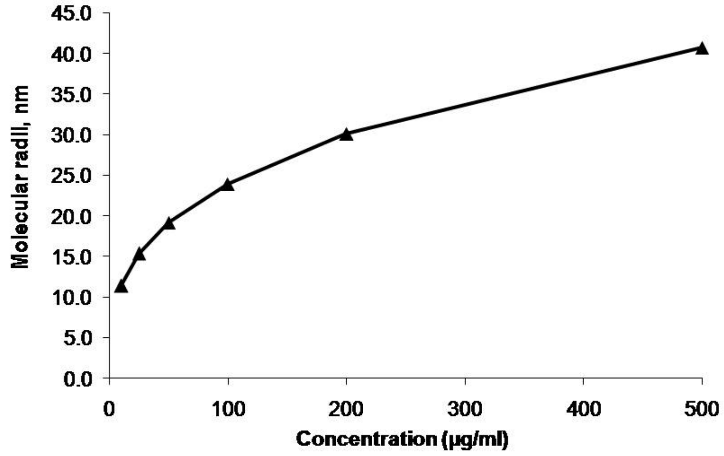


Figure 4.4: Density, Viscosity, Surface tension, Friccohesity, Activation energy and Molecular radii (A-F) of Fe_2O_3 NPs in egg albumen

Table 4.4: Limiting friccohesity (σ^0, s.m.$^{-1}$), and its 1st slope (S_σ, s.m.$^{-1}$ kg^2 mol^2), Limiting activation energy ($\Delta\mu_2^{*0}$, kJ mol^{-1}), and its 1st slope ($S\Delta\mu_2^*$, kJ L/mM^2), Limiting molecular radii (r^0, nm), and its 1st slope (S_r, nm) of Fe_2O_3 NPs

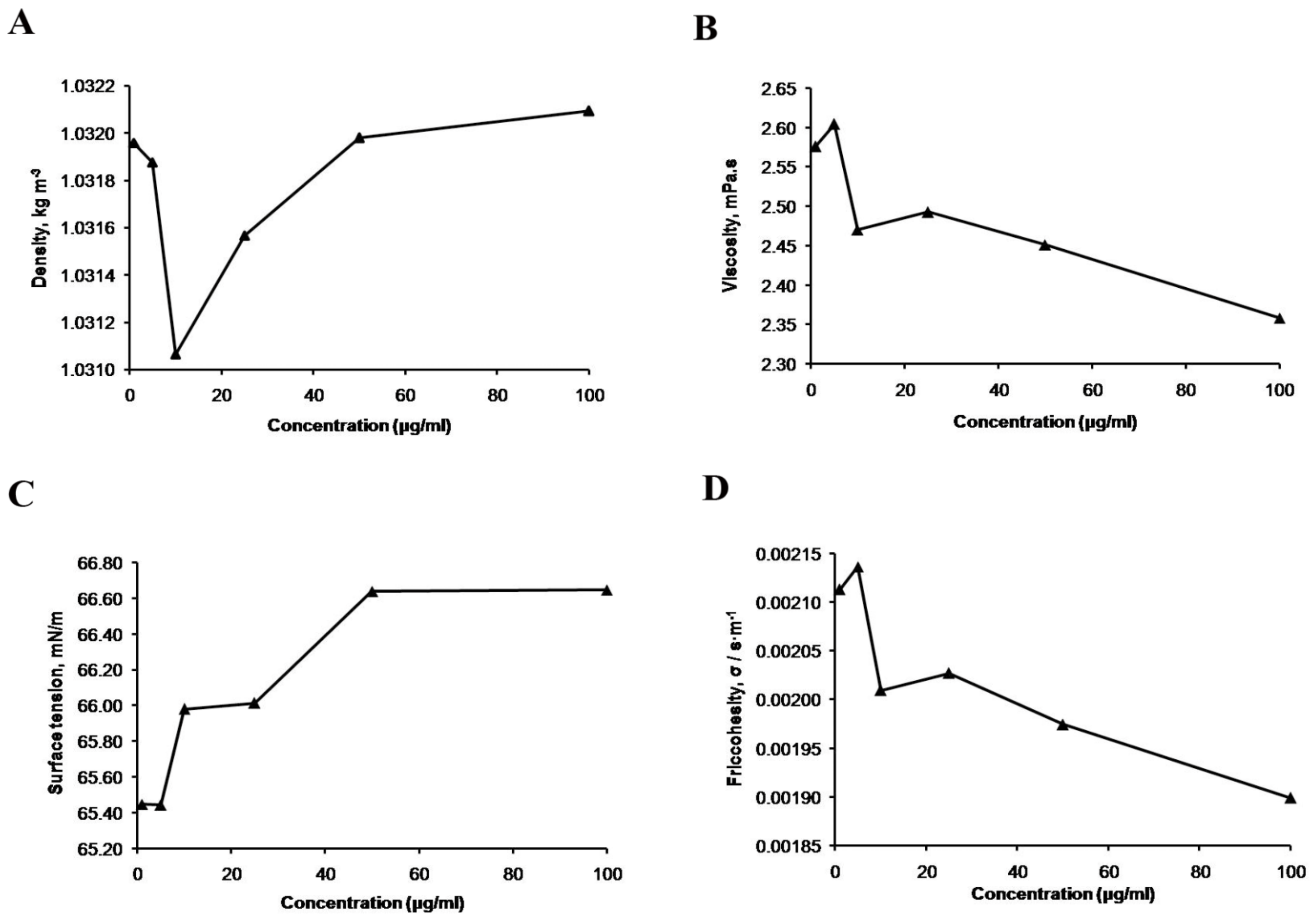
System	**Friccohesity**		**Activation energy**		**Molecular radii**	
	σ^0	S_σ	$\Delta\mu_2{*}^0$	$S\Delta_{\mu 2}{*}$	r^0	S_r
Fe_2O_3 NPs + Water	0.09464	0.000000	-68.45	0.16	29.19	0.11
Fe_2O_3 NPs + Saline	0.010433	-0.000010	-53.34	-0.007	14.66	0.14
Fe_2O_3 NPs + Albumen	0.001732	-0.000000	-57.37	-0.0167	15.31	0.06

Table 4.5: Limiting density (ρ°, kg m^{-3}), and its 1st slope (Sρ, kg^{2}m^{-3}mol^{-1}), Limiting viscosity (η°, mPa·s), and its 1st slope (Sη, mPa·s kg m^{-1}), Limiting surface tension (γ°, mN m^{-1}), and its 1st slope (Sγ, mN kgmol^{-1} m^{-1}) of TiO_2 NPs

System	Density		Viscosity		Surface tension	
	ρ^o	S_ρ	η^o	S_η	γ^o	S_γ
TiO_2 NPs + Water	0.993300	0.000001	0.704443	0.000054	73.52	0
TiO_2 NPs + Saline	0.998296	0.000001	0.758464	0.000422	71.28	0
TiO_2 NPs + Albumen	1.031606	0.000005	2.558895	0.002093	65.64	0

Table 4.6: Microgram/microlitre (µg/ml), density ($\rho \pm 10^{-3}$ kg m^{-3}), viscosity ($\eta \pm 10^{-5}$ mPa·s), surface tension ($\gamma \pm 10{-}2$ mN m^{-1}), friccohesity (σ, s.m.$^{-1}$), activation energy ($\Delta\mu 2^{*} \pm 10^{-2}$ kJmol^{-1}), molecular radii (r, nm) at 37°C of TiO_2 NPs

µg/ml	ρ	η	γ	σ	$\Delta\mu_2^{*}$	r
TiO_2 NPs + Water						
1	0.993356	0.710711	73.52368	0.009667	-58.0122	12.65763
5	0.993296	0.705368	73.51923	0.009594	-58.455	21.59023
10	0.993314	0.703203	73.5206	0.009565	-59.2951	27.17413
25	0.993293	0.697575	73.51905	0.009488	-60.7382	36.7823
50	0.993491	0.696546	73.5337	0.009473	-60.11	46.32
100	0.993334	0.703027	73.52208	0.009562	-67.7572	58.53998
TiO_2 NPs + Saline						
1	0.998130	0.7799	71.51819	0.010905	-57.783	5.577241
5	0.998265	0.763618	71.52786	0.010676	-58.0512	9.470113
10	0.998440	0.736208	70.93922	0.010378	-58.9138	11.78709
25	0.998448	0.736078	70.93982	0.010376	-60.576	15.9966
50	0.998260	0.730522	70.92643	0.0103	-60.3571	20.10362
100	0.998334	0.72389	70.93172	0.010206	-63.2651	25.25208
TiO_2 NPs + Albumen						
1	1.031959	2.576379	65.45	0.002113	-57.8093	5.425946
5	1.031876	2.604565	65.44	0.002136	-57.9546	9.311949
10	1.031064	2.470162	65.98	0.00201	-58.0253	11.52694
25	1.031567	2.492927	66.01	0.002027	-58.5362	15.69238
50	1.031979	2.451386	66.64	0.001975	-59.536	19.66072
100	1.032092	2.358271	66.65	0.001899	-61.2601	24.45326



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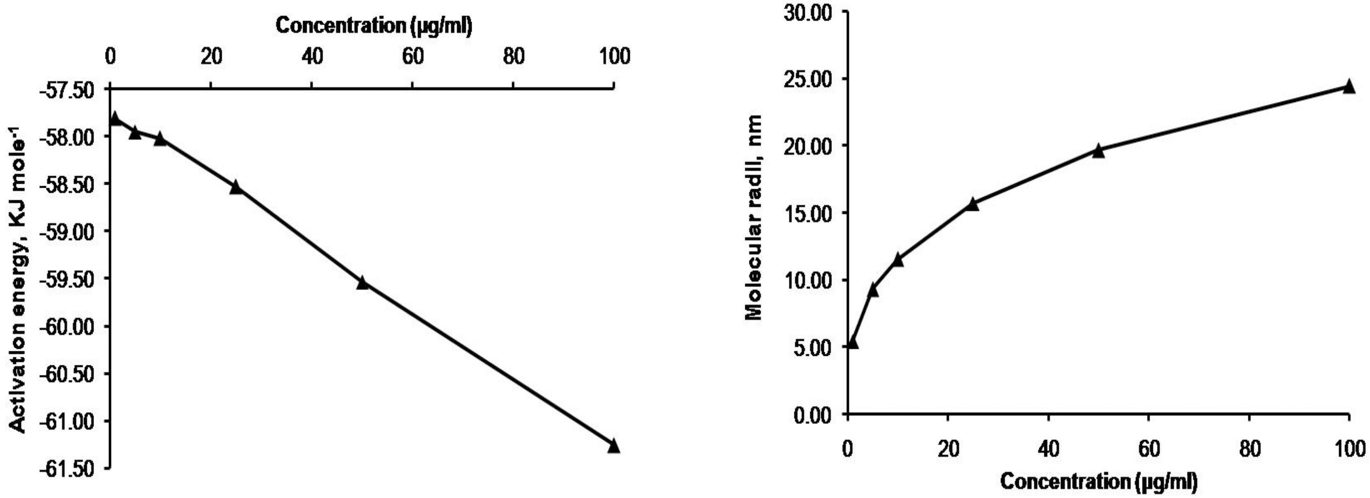

Figure 4.5: Density, Viscosity, Surface tension, Friccohesity, Activation energy and Molecular radii (A-F) of TiO_2 NPs in egg albumen

Table 4.7: Limiting friccohesity (σ^0, s.m.$^{-1}$), and its 1st slope (S_σ, s.m.$^{-1}$ kg^2 mol^2), Limiting activation energy ($\Delta\mu_2^{*0}$, kJ mol^{-1}), and its 1st slope ($S\Delta\mu_2^*$, kJ L/mM^2), Limiting molecular radii (r^0, nm), and its 1st slope (S_r, nm) of TiO_2 NPs

System	Friccohesity		Activation energy		Molecular radii	
	σ^0	S_σ	$\Delta\mu_2{*}^0$	$S\Delta_{\mu 2}*$	r^0	S_r
TiO_2 NPs + Water	0.009582	0.000001	-57.87	0.09	20.45	0.42
TiO_2 NPs + Saline	0.01064	0.000005	-58.18	0.05	8.95	0.18
TiO_2 NPs + Albumen	0.002093	0.000002	-57.73	0.04	8.81	0.17

DISCUSSION

Nanomaterials in various biological systems are extensively studied to obtain clarity on their behavior in physiological environment. Proteins form a layer around the nanomaterials to form a "protein corona" (Lynch & Dawson, 2008) by interacting through their structural constituents with respect to the environment. Nanomaterials based on their size, shape and surface chemistry, interact with protein in unique ways and undergo changes in their "biological identity" (Walkey & Chan, 2012). Nanomaterials with definite friccohesity attain certain levels of Boltzmann distribution energy so as to achieve enhanced activity to perform localized movement. This results in an alteration in size, aggregation indices and interfacial properties in combination with fellow molecules. Hence, physiological changes in egg albumen following interaction with Fe_2O_3 NPs was studied to unravel its impact on chicken embryo by assessing physicochemical parameters such as density, viscosity, surface tension, friccohesity, activation energy and molecular radii. The data obtained reflects the internal activities of peptide bonds and state of Lennard Jone potential responsible for causing spontaneity and strengthening or weakening of the interactive forces.

The density of albumen was 3.4% and 4.1% higher than that of saline and water respectively which is due to complex composition of albumen, also, saline and water constitute of sodium salt and hydrogen and hydroxyl ions with strong ionic-peptide forces. Viscosity is a fluid dynamics driven parameter which reflects entangling or interacting activities of albumen with saline and water. Higher intramolecular forces account for an increase in viscosity and the same was observed in water, saline and albumen. Stronger interactions of Fe_2O_3 NPs with albumen resulted in an increase in viscosity of albumen. Hence, tendency of Fe_2O_3 NPs to form more structured complex by binding strongly with albumen was observed herein. These results are in agreement with previously assessed RPMI-1640- TiO_2 NP interaction in our lab (Patel *et al.*, 2016).

Intramolecular forces comprising of solute-solute and solvent-solvent cohesive forces collectively contribute to the surface tension of liquids. Interestingly, larger involvement of medium or solvent with albumen or salts weakens the cohesive forces of the medium which supports distribution of the albumen at a cost of surface tension or the energy. An increment in surface tension following an increase in concentration of Fe_2O_3 NPs + albumen may have contributed to formation of protein corona (Lynch & Dawson, 2008) and also the possible formation of hydration sheath type structure due to active water-water cohesive forces coupled with higher surface tension. Higher dose of Fe_2O_3 NPs (200µg/ml) had recorded a significant increment in surface tension of Fe_2O_3 NPs + saline immobilizing the interacting molecules with stronger binding forces. At this dose, a spherical shaped corona with a smaller surface area and a stronger attractive force may have been formed that could cross *in ovo* barriers. This is hypothesized because 200µg/ml dose of Fe_2O_3 NPs accounted for 100% mortality of chick embryos and therefore needs further scrutiny.

Friccohesity is an assessment that deals with the dual force theory which is a product of frictional and cohesive forces within similar and dissimilar molecules (Patel *et al.*, 2016). Fundamentally, higher friccohesity initiates and improves coordination among the molecules present in a liquid mixture. A decrement in friccohesity of Fe_2O_3 NPs +

albumen indicates a poor frictional force and weak Fe_2O_3 NPs -albumen interactions. Also, the same were found to be weaker in saline as compared to water. Weakening of cohesive force between the molecules with lower product of intramolecular forces contributes to increased friccohesity of Fe_2O_3 NPs + saline that is in contrast to TiO_2 NP + RPMI-1640 reported earlier (Patel *et al.*, 2016).

The Fe_2O_3 NPs molecules gain energy from the solvent system to interact with the egg albumen that further impacts their extent of interaction. This energy is referred as the activation energy that also is an indicator of an increase in proportion of collision/chemical reaction between solute and solvent (Patel *et al.*, 2016). Higher activation energy recorded at 200µg/ml in Fe_2O_3 NPs + saline and Fe_2O_3 NPs + albumen also explains the 100% mortality of chicken embryos observed at the said dose. A dose dependent increase of Fe_2O_3 NPs and variations in its interaction with egg albumen causes alterations in molecular radii. Molecular radii are an important parameter because an increase in effective radii positively impacts pattern of aggregation and subsequent dispersion of biomolecules. Observations of molecular radii of Fe_2O_3 NPs + saline were similar to albumen at all the lower doses (10-100 µg/ml) but showed an increment at 200µg/ml. The same may have caused an alteration in size aggregation indices and interfacial properties accounting for a 100% mortality of chicken embryos.

Dose dependent increase in density of albumen was observed following addition of TiO_2 NPs, but relatively lowest density observed at 10 µg/ml hints at effective dispersion of TiO_2 NPs in egg albumen. Previous studies in our lab had shown that TiO_2 NPs interact with protein components of RPMI-1640 medium (Patel *et al.*, 2016) and results in 0.84%

intermolecular interaction than TiO_2 NP + water. Higher intermolecular forces and cohesion are the key factors that determine viscosity and surface tension of liquids. In our study, a dose dependent decrement in viscosity and a reciprocal increment in surface tension are in support of our claim that higher intermolecular forces are as a result of higher concentration of TiO_2 NPs. A dose dependent decrement in friccohesity suggests weaker inter conversion between cohesive and frictional forces implying towards a stronger nanoparticles-egg albumen interaction. Relatively higher indices of activation energy recorded in TiO_2 NPs + albumen (10 µg/ml) is suggestive of more quantum of interaction between TiO_2 NPs and egg albumen. A dose dependent increment in molecular radii resulting due to TiO_2 NPs-egg albumen interactions implies towards formation of nanoparticle agglomerates at higher doses. It can be concluded that metal complexes such as TiO_2 or Fe_2O_3 interact with biomolecules and undergo subtle physicochemical alterations that have been reported herein. These results support our hypothesis that nanometal oxides interact with biomolecules, undergo physicochemical change and can manifest toxicity. This study is the first that assesses the physicochemical interaction of Fe_2O_3 and TiO_2 NPs with egg albumen and the findings inspired us to conduct a detailed study on developmental toxicity using chicken embryo model that is showcased in chapters 4 and 5.

SUMMARY

Nanoparticles have an ability to interact with biological matter resulting in a change in its physicochemical properties. Hence, in this study we aim to assess the changes in physicochemical parameters such as density, viscosity, surface tension, friccohesity, activation energy and molecular radii of nanoparticles (Fe_2O_3 and TiO_2 NPs) after dispersion in saline, water and egg albumen. Alterations in limiting density, viscosity, friccohesity and molecular radii of Fe_2O_3 NPs+ egg albumen implied towards its binding with albumen resulting in 100% mortality at 200 µg/ml dose possibly due to Fe^{2+} ions overload. Changes in the physicochemical indices indicated stronger intermolecular forces of attraction between TiO_2 NPs and egg albumen. Also, increment in molecular radii of TiO_2 NPs-egg albumen implies towards formation of nanoparticle agglomerates at higher doses due to which they do not impact the chicken embryo and cause toxicity at the lower doses only.