Cobalt Nanoparticles for Biomedical Applications: Facile Synthesis, Physiochemical Characterization, Cytotoxicity Behavior and Biocompatibility

S. M. Ansari¹, R. D. Bhor², K.R. Pai², D.Sen³, S. Mazumder³, Kartik Ghosh⁴, Y. D.

Kolekar^{1,*}, and C. V. Ramana^{5,#}

¹Department of Physics, Savitribai Phule Pune University, Pune-411007, Maharashtra, India

²Department of Zoology, Savitribai Phule Pune University, Pune-411007, Maharashtra, India

³Solid State Physics Division, Bhabha Atomic Research Centre, Mumbai-400 085, India

⁴Department of Physics, Astronomy and Materials Science, Missouri State University,

Springfield, Missouri 65897, USA

⁵Department of Mechanical Engineering, University of Texas at El Paso,

El Paso, Texas 79968, USA

*Corresponding Author(s):

rvchintalapalle@utep.edu (C.V. Ramana)

ydkolekar@gmail.com (Y.D. Kolekar)

C.V. Ramana: Tel: +1-9157478690-2678; Fax: +1-9157475019

Y.D. Kolekar: Tel: +91-20-2569-2678; Fax: +91-20-2569-1684

ABSTRACT

Cobalt (Co) nanoparticles (NPs) were produced by a simple, one step hydrothermal method with the capping of oleic acid. Intrinsic structural, physiochemical and magnetic properties of Co NPs were investigated and demonstrated their applicability in biomedicine. X-ray diffraction, Raman spectroscopy and infrared (IR) spectroscopic studies confirm the single phase Co NPs with a high structural quality. The IR data revealed the capping of oleic acid via monodentate interaction. Small angle scattering studies suggest the existence of sticky hard sphere type of interaction among the Co NPs because of magnetic interaction which is further evidenced by electron microscopy imaging analyses. The Co NPs exhibit a ferromagnetic character over a wide range of temperature (20-300 K). The temperature dependence of magnetic parameters namely, saturation magnetization, remanent magnetization, coercivity and reduced remanent magnetization were determined and correlated with structure of Co NPs. The Cytotoxicity studies demonstrate that these Co NPs exhibit the mild anti-proliferative character against the cancer cells (cisplatin resistant ovarian cancer (A2780/CP70)) and safe nature towards the normal cells. Haemolytic behaviour of human red blood cells (RBC) revealed (<5%) haemolysis signifying the compatibility of Co NPs with human RBC which is an essential feature in vivo biomedical applications without creating any harmful effects in the human blood stream.

Keywords: Cobalt nanoparticles; hydrothermal process; biomedical applications; cytotoxicity; haemolytic activity

1. Introduction

Nanoparticles (NP) based materials and/or materials with tailored nano-architectures have been the subject of intense research by the scientific research community and industry as they manifest fascinating and many useful properties, which are often not attainable by the bulk materials. Due to exceptional properties at the reduced dimensions, nanomaterials can be exploited for a variety of industrial and biomedical applications [1-3]. Specifically, the metal nanoparticles are interesting because they offers efficient means to manipulate or modify their optical, mechanical, magnetic, chemical and electronic properties, which can be readily utilized in a wide variety of technological applications [2,3]. Among the metallic NPs, Co is one of the most promising materials and are of great interest over a long time for researchers from a wide range of fields for different applicability. Typically, Co NPs possess excellent magnetic, electrical and catalytic properties, which are of scientific and technological interest in various fields, including recording media, magnetic sensors, magnetic memories, magnetic fluids, magnetic composites and catalysis etc [1-4]. Maximum saturation magnetization and large anisotropy field of Co NPs makes them suitable for high-density information storage and permanent magnet applications [5]. Recently, Co NPs were also found to be attractive for electromagnetic (EM) wave absorption applications such as development of wireless communications and high frequency circuit devices [6]. Most importantly, the Co NPs also have an intrinsic advantage in biomedical related fields, e.g., drug delivery and magnetic resonance imaging [1, 7], where all these applications demand high quality and purity to avoid alteration in their magnetization or response stability [8].

Functionality and activity of magnetic NPs depend on the microstructure, size and morphology. From microstructure point of view, Co has been studied extensively due to fact that it exhibits variable crystal structures; the hexagonal close-packed (HCP), the face centered cubic (FCC) and the ϵ -phases [9]. HCP is the stable phase at room temperature,

whereas FCC is stable at temperatures above 450°C [10]. Moreover, the excellent catalytic properties have been reported for different morphologies of Co; therefore, attempts have been made to obtain different morphologies and shapes such as nanosheets, spheres, snowflakes or cauli-flowerlike particles, leaf-shaped flakes, and microchains [11]. A wide variety of synthetic procedures, such as solvothermal, solvent-thermal route, evaporation of metal atoms, microemulsion in an inert atmosphere, sonification, reduction of Co salt, reduction in inverse micelles, electrochemical generation in the presence of surfactants, were adopted in the past to produce Co with varying morphology and size distribution [11-13]. In the present work, Co NPs were synthesized employing a facile hydrothermal route due to its relative merits such as low cost, single step process, convenience, and an environmental friendly approach based on an aqueous medium and easier to conduct. As the ultimate goal is processing Co NPs without needing higher processing temperatures while maintaining their high structural quality and biocompatibility, attention is directed towards evaluating the biocompatibility of Co NPs. Note that, for magnetic NPs, biocompatibility is the key for their integration into health science and biomedicine. Typically, the magnetic NPs are coated with surfactants and polymers to enhance biocompatibility, provide functionality, and prevent agglomeration [14]. A variety of ligands have been used to control particle growth, stabilize the metal dispersions, and limit their surface oxidation. It is known that the metal particles experience the strong van der Waals interaction which combined with the magnetic dipole interactions and to **stabilize such systems are very challenging** [14]. Therefore, in the present work, oleic acid (OA) has been used as a capping agent during synthesis because it provides the steric stabilization of the NPs against the van der Waals interactions and also magnetic attractive interactions and thereby prevents their agglomeration. Moreover, this approach limits the growth of the NPs and prevents the Ostwald ripening process since its surface layer acts as a barrier to mass transfer [15]. Therefore, by controlling the stability,

size distribution and shape, the surface activity and performance in the targeted applications can be influenced. However, in the literature, Co NPs are mainly investigated for their magnetic and catalytic applications with different morphologies **while relatively less efforts exist toward biomedical applications.** For instance, Colognato et. al. [16] have studied the comparative genotoxicity of Co NPs and ions on human peripheral leukocytes in vitro. Their results demonstrated that the nanosized Co can be internalized by human leukocytes and can interact with DNA leading to the observed genotoxic effects, which are, however, modulated both by donor's characteristics and/or by Co^{2+} release. Studies performed by Sabbioni et. al. demonstrate that both the chemical and the physical properties of Co particles contribute to cytotoxicity and morphological transformation [17]. Sabbioni et. al. and colleagues have studied the dissolution, interaction with medium components, bio-availability in culture medium, uptake and intracellular distribution of radiolabelled Co forms (Co NP, Co microparticles and Co^{2+} ions) in Balb/3T3 mouse fibroblasts [18]. Elena Papis et. al. have evaluated the cellular uptake of Co oxide NPs [19]. An interesting investigation was made by Gornati et. al [20] on zero-valent on cell cultures. They compared Co NPs cytotoxicity with other metals namely, Ni and Fe and their cytotoxicity; gene expression experiments were performed on SKOV-3 and U87 cells [20]. The outcome of their experiments is the fact that, in both cell lines, Co NPs and Ni NPs were definitely more toxic than Fe NPs [20]. Recently, Bossi et. al. [21] have considered the possibility of a direct crossing of the plasma membrane. It is clear from this brief review that, along with the expanding applications of magnetic nanoparticles (MNPs), the potential toxic effects of MNPs have been of wide concern [22, 23]. In fact, multiple results show that the MNPs can significantly reduce the cell viability of human macrophage, epithelial cell lines [24], human mesothelioma [25], and inhibit the normal formation of PC12 neuronal cell morphology [26]. At

higher concentrations, DMSA-coated MNPs decrease mitochondrial activity of human fibroblasts [27].

The primary objective of this work is, thus, to synthesize the Co NPs at lower temperatures along with their physiochemical characterization. The impetus is to perform a thorough investigation to evaluate the cytotoxicity of Co NPs against the cancer and normal cells and the primary goal is to demonstrate the potential application of such as-synthesized Co NPs for biomedical and **biomedicine** area, especially for cancer research. Therefore, in this work, detailed cell proliferation inhibition rate (CPIR) of the Co NPs was studied using cancer cell (cisplatin resistant ovarian cancer (A2780/CP70)) as well as on normal human peripheral blood mononuclear cells (PBMC) by modified MTT assay. As it is well known that **the MTT assay procedure, which is a non-radioactive, colorimetric assay, has been utilized extensively for in vitro toxicity evaluation due to the fact that the method allows rapid assessment of cell viability, cell survival, cell growth and provides excellent reproducibility [28].** In addition, biocompatibility of the Co NPs with human red blood cells (RBC) is also evaluated using Haemolytic assay. Typically, cancer is a group of diseases characterized by uncontrolled growth and spreading of abnormal cells, which lead to cause of death. A key characteristic by all cancer cells is escape from their natural growth mechanisms resulting in uncontrolled proliferation. Present treatments for cancer include surgery, radiation, drugs, herbal drugs and other therapies [29]. It has been reported that the majority of cases, especially for advanced stage cancer, are still treated with conventional chemotherapy. Since the problem of drug resistance and its toxicity is a major obstacle in chemotherapeutic treatment [30], there is a compelling need for the development of new therapeutic drugs which will be more efficient and will synergize with the existing ones. Therefore, results on Co NPs, as presented and discussed in this paper, are expected to significantly contribute to the further advancement in this field.

2. Materials and Methods

2.1.Synthesis of Co Nanoparticles

To synthesize pure Co NPs by hydrothermal method we have used the analytical reagent (AR) grade chemicals, viz, cobalt chloride hexahydrate, (CoCl₂.6H₂O, 99%) as the cobalt precursor, potassium hydroxide (KOH) and hydrazine monohydrate (NH₂-NH₂.H₂O, 85%) as the precipitant and reducing agent, respectively, whereas oleic acid (OA) (C₁₈H₃₄O₂, 90%) is used as the capping agent. In a typical synthesis procedure, 0.35 M CoCl₂.6H₂O was added into deionized (DI) water and stirred for 10 minutes. Simultaneously, KOH (1M) solution was added drop wise followed by constant stirring for at least 15 mins. Then the hydrazine monohydrate 1 M solution was added drop wise and 0.05 M OA also added with constant stirring for at least 20 mins so that the total solution volume becomes as 2/3 of the total volume of autoclave (250 mL). The above prepared chemical mixture was stirred then vigorously for 2 hr until the complete dissolution of solid reagents was observed. After that, the solution mixture was poured into a Teflon-lined autoclave and was placed in an electric oven where the reaction temperature was maintained at 160 °C for about 24 hr. After 24 hr, the autoclave was allowed to cool to room temperature naturally. The chemical reaction for the synthesis of Co NPs can be expressed as [31]:

The resulting Co NPs were separated from the liquid phase by centrifuge the final solution several times with D.I water and ethanol at 10000 rpm for 20 mins. The liquid phase was discarded and the resulting black precipitates were dried in an oven at 100 °C overnight and finally, as-prepared Co NPs were used for further characterization without subsequent sintering.

2.2.Characterizations of Co Nanoparticles

2.2.1. Crystallography, Morphology and Compositional Studies

The crystalline phase of Co NPs was studied with D8-Advanced Bruker diffractometer equipped with Cu K α radiation ($\lambda = 0.154$ nm) along with 40 kV acceleration voltage and 40 mA tube current. The X-ray diffraction (XRD) data were collected at room temperature over a 2θ scattering range 20-80° with a step of 0.05°. Scanning electron microscopy (SEM) (JEOL JSM 6360) along with energy dispersive spectroscopy (EDS) was used to study the surface morphology and compositional analyses of as-synthesized powder.

2.2.2. X-ray and Neutron Scattering Experiments

The small angle X-ray scattering (SAXS) and small angle neutron scattering (SANS) experiments were carried out to obtain the structural information at two different length scale ranges. The scattered intensities $I(q)$ were recorded as a function of scattering vector transfer ($q = \frac{4\pi \sin \theta}{\lambda}$), where 2θ being the scattering angle and λ is the X-ray wavelength ($\lambda = 0.154$ nm) and sample to detector distance was kept about 1070 mm. The higher q regime (i.e. 0.1-2.5 nm $^{-1}$) have been investigated by performing the small-angle X-ray scattering (SAXS) using a laboratory based point collimation small angle scattering instrument. The intensities were corrected for sample absorption. Lower q region (0.003-0.173 nm $^{-1}$) was probed by medium resolution small-angle neutron scattering (SANS) experiments. A double crystal based medium resolution SANS (MSANS) facility at the Guide Tube Laboratory of Dhruva Reactor at Trombay, India was used for this purpose [32, 33]. The instrument consists of a non-dispersive (1, -1) setting of (111) reflections from a pair of single crystals with specimen between them. The scattered intensities were recorded as a function of wave vector transfer similar to SAXS. In the present experiments, we have used the incident neutron wavelength of 0.312 nm. The specimens under SANS investigation were placed on a sample holder with a circular slit of 1.5 cm diameter. The measured SANS profiles have been corrected for

background and resolution effects [34]. SAXS and SANS data were normalized at the common q region of the convenience of model fitting for such hierarchical structures. This may be possible because, the functionality of the scattering data at common q region matches fairly well.

2.3. Stability Study (Dynamic Light Scattering and Zeta Potential Measurements)

The stability of OA coated Co NPs in DI water and DMEM was further determined by measuring the zeta potential of the NPs using dynamic light scattering (DLS) (Brookhaven NanoBrook 90plus particles size analyzer). DLS was also used to measure the hydrodynamic diameter of Co NPs in DI water and DMEM.

2.4. Chemical Bonding and Magnetic Properties

Fourier transform infrared spectra (FTIR) were recorded with the FTIR spectrometer (JASCO Model-6100) in the range of 400-4000 cm^{-1} . Raman studies were performed on an InVia Micro RAMAN (Renishaw) spectrophotometer with 532 nm laser excitation wavelength. The scattered light was analyzed by using the charge coupled device (CCD) detector. Magnetic properties were measured using vibrating sample magnetometer (VSM) (Lake Shore 7000), with a maximum applied field of ± 10 KOe. The hysteresis loops were collected at different temperatures as 300, 200, 150, 100, 50 and 20 K respectively.

2.5. Cytotoxicity Studies

2.5.1. Cell Culture and Reagents

Cisplatin resistant ovarian cancer (A2780/CP70) cells were obtained from National Centre for Cell Science (NCCS), Pune, India. Dulbecco's modified essential medium (DMEM), Trypsin 0.25% were purchased from Invitrogen. Foetal Bovine Serum (FBS-10%), and the Phosphate buffer saline (PBS), Penicillin 100 Units/mL, Streptomycin 100 $\mu\text{g/mL}$, Sodium Lauryl Sulphate (SLS), ethanol, dimethyl sulfoxide (DMSO), MTT dye (3-(4,5-

dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), were obtained from Sigma Aldrich and Ficoll-paque from GE Healthcare. In vitro cell viability of as-synthesized Co NPs, (A2780/CP70) cells were cultured in regular growth medium consisting of DMEM supplemented with 10% heat-inactivated FBS, penicillin and streptomycin as an antibiotic solution. All the cells were maintained in humidified atmosphere with 5% CO₂ and 95% air at 37 °C for viability assay. Upon 80 to 90% confluency, the cells were subculture for every 4-5 days. To passage the cells, 0.25% of trypsin was added to detach cells.

2.5.2. Cell Proliferation Inhibition by MTT Assay

It is well-known that the MTT assay is a colorimetric process to measure the mitochondrial and metabolic activities of enzymes. It reduces the yellow tetrazolium dye MTT, to insoluble formazan crystals, giving a purple color. The amount of formazan formed is proportional to the number of living cells. In the present case, modified MTT assay was used to find out how the NPs get affected by cellular metabolic activity of the cell [35]. A2780/CP70 Cisplatin resistant ovarian cancer cells were seeded at 1×10^5 cells/mL density and incubated for 24 hr at 37 °C. After completing the incubation period, cells were treated with different concentration (10, 50, 100, 150, 200, 250, 300 µg/mL) of Co NPs for further 24 hr. Similar procedure was adapted for setting the control treatment (without Co NPs) [36]. After 24 hr of treatment with Co NPs, 20 µL/well MTT was added to each well (5 mg/mL) at 37 °C. **The Formazan crystals formed after the action of mitochondrial enzyme does not solubilize and forms crystals which may give unreliable results. Therefore, the MTT assay modified slightly by centrifuged the 96 well plates for 5 mins and then removed 100 µl from the treated and untreated wells.** And the microtiter plate was incubated for 4 hr. After 4 hr of incubation, the insoluble formazan formed inside the cells was dissolved with the addition of DMSO (100 µL/well) to each well. **The 96 well plates were shaken for**

10 sec in the multimode reader. Earlier we did not use this step of centrifugations and shaking. In the present study, we have employed this modified MTT assay [37, 38].

The absorbance was then recorded at 550 nm in a microplate reader (BMG LABTECH Fluostar OPTIMA spectrophotometer). Cell survival was expressed as the absorption percentage of treated cells compared to that of control cells (not incubated with Co NPs). The cell viability (%) was calculated by using the following relation [39]:

$$\text{Cellular viability} = \frac{\text{O.D. of treated cells}}{\text{O.D. of untreated cells}} \times 100 \quad (2)$$

Where O.D. implies the optical density, both the mean value and the standard deviation were obtained from at least three independent experiments and finally the data was represented graphically as mean. Microscopic observation and photographs of treated and untreated A2780/CP70 cells were performed using evos XL core digital bright field microscope.

2.5.3. Isolation of Peripheral Blood Mononuclear Cells (PBMC)

For PBMC isolation, blood was drawn from healthy volunteers and human PBMC were separated from heparinised, by Ficoll-paque (GE Healthcare) gradient centrifugation method [40]. Ficoll solution (15 mL) was gently added to the diluted blood and the mixture was centrifuged at 800 rpm for 25 min at room temperature [41]. After the centrifugation, PBMC were collected which were found at the interface and again washed several times with PBS (pH~7.2) by centrifugation. Finally, PBMCs were counted and re-suspended in a complete DMEM medium, supplemented with FBS (10%), L-glutamine (2 mM), penicillin (100 Units/mL), and streptomycin (100 µg/mL), and were incubated at 37 °C in 5% CO₂, 95 % air humidified atmosphere [42]. The protocol of the study was approved by the Ethics Committee of the Savitribai Phule Pune University (formerly University of Pune) (Ethics-UoP/2012/19). Moreover, a written informed consent was obtained from each of the healthy donor.

2.5.4. Cytotoxicity Assay of PBMC

PBMC (2×10^4 cells/mL) were seeded in the microtiter plates and kept for adherence at 37 °C in a 5% CO₂ atmosphere for 24 hr. After incubation, cells were treated with Co NPs for further 24 hr with the indicated concentrations added to the individual wells, in triplicate, except in the control well. Only medium with PBMC was used as positive control. Cell survival was determined by the modified MTT method as discussed earlier.

The protocols for cytotoxicity assessments on A2780/CP70 cisplatin resistant human ovarian cancer cells and on PBMCs are same except for slight differences. The A2780/CP70 cisplatin resistant human ovarian cancer cells are adherent cell line and they are of human epithelial cells in origin which are adherent in nature. Whereas, normal human peripheral blood mononuclear cells (PBMCs) are freshly isolated cells from peripheral blood. PBMCs are suspension cells which are the mixture of adherent (monocytes) and non-adherent mononuclear cells (T, α , β cells and natural killer cells). Thus, the protocols for seeding and incubation are different for A2780/CP70 cells and on PBMCs. However, from addition of MTT and termination of MTT assay is same in both the cases. In case of Cytotoxicity assay of PBMC, after 24 hr of treatments with our pristine Co NPs, we have added MTT solution (5 mg/ml) to each well. And sample were further incubated for 3-4 hr and then centrifuged at 1500 rpm for 10 mints. Supernatant of 100 μ l was removed and followed by addition of DMSO (100 μ l). So, using these we don't lose of PBMC. Finally, the absorbance was measured at 550 nm.

2.5.5. Haemolysis Assay

The lysis and distrupction of erythrocytes in response to NPs can be a measure of both the membrane disruption of the cell and extreme cellular toxicity (i.e., necrosis) and is especially important to the NPs that are intended to be directly introduced into the bloodstream. Percent haemolysis of human red blood cells (RBC) treated with Co NPs was

assessed by haemolysis assay as per US FDA guidelines [43] and performed by using human erythrocytes. RBC obtained from heparinised pooled and whole blood of healthy volunteers was washed several times with sterile isotonic PBS solution (pH~7.2). Consequently, 1% RBC suspension was prepared in 96 well plate and the subsequent erythrocyte suspension was incubated at 37 °C for 1 hr with as stated different concentrations of Co NPs, positive control (1% Triton X-100) and vehicle control (PBS) set as a negative control [44]. Following the incubation, the NPs were centrifuged at 400rpm for 10 min and the absorbance of the supernatant was recorded at 550 nm using spectrophotometer. The haemolysis (%) was calculated as:

$$Haemolysis(\%) = \left[\frac{(O.D. \text{ sample} - O.D. \text{ PBS})}{(O.D. \text{ Triton} - O.D. \text{ PBS})} \times 100 \right] \quad (3)$$

Percent haemolysis was determined following the comparison with 100% lysed erythrocytes [45].

2.5.6. Statistical Analysis

Each assay was performed in triplicate with three independent experiments. The data reported as percent cytotoxicity of cancer cells, PBMC viability and hemolysis were expressed as mean $\pm$ standard deviation. Significance of the difference was determined by Student's t-test method with p value < 0.05 which was considered to be significant.

2.5.7. Microscopic Study of Treated and Untreated Cells with Co Nanoparticles

Human cisplatin resistant ovarian cancer (A2780/CP70) cells (5×10^5 /mL) were seeded in Petri dishes with complete culture medium incubated in CO₂ incubator. After 24 hr of incubation or the confluency up to 70-100 % , they were treated with Co NPs concentrations 5X (10-300 μ g/mL). Cells only with complete medium were considered as

control. After 24 hr of incubation, we have observed morphology of treated and untreated cells under bright field microscope.

3. Results and Discussion

3.1. Structure, Phase Analysis and Chemical Bonding

3.1.1. X-ray Diffraction (XRD)

The XRD pattern of Co NPs is shown in Fig.1. The diffraction peaks observed at 2θ values of 41.55° , 44.47° , 47.41° , 62.52° and 75.87° can be assigned to the (100), (002), (101), (102) and (110) planes of HCP-Co (JCPDS 05-0727) and the lattice constants were estimated to be $a=2.51\text{\AA}$ and $c=4.07\text{\AA}$, respectively. Additionally, the weakly intense peak observed at 51.26° was found and could be assigned to the (200) plane of FCC-Co (JCPDS file:15-0806). Importantly, the ratios of peak intensities were well agreed those predicted for HCP Co. After the detailed analysis of intensity of individual peaks, it is observed that the HCP and FCC phases of metallic Co present in the final products with minor presence of FCC phase. Both the HCP and FCC phases of Co are close-packed structures, and the only difference between them is the stacking sequence of the atomic planes along the cubic (111) direction. It has been reported that the combination of FCC and HCP phases in the same sample is introduced by the lower activation energy required for the formation of stacking faults [46]. It has also been reported that at higher alkali concentrations, Co preferred to crystallize into HCP structure rather than the FCC structure [11]. Furthermore, it is well known that under atmospheric conditions, Co could be easily oxidized to cobalt oxide (CoO) or cobalt hydroxide but in the present case those were not detected by using XRD analysis (see Fig.1).

3.1.2. Raman Spectroscopy

Raman spectroscopy is highly sensitive to the structural chemistry in terms of phase transitions, lattice distortion, charge-lattice, spin-lattice couplings, local cation distribution

and magnetic ordering. Therefore, the presence of oxide or hydroxide forms of Co NPs is examined by using this powerful technique. Figure 2 shows the Raman spectra of as-synthesized Co NPs recorded at room temperature. The sample **exhibits a set of** weak peaks at 313 cm^{-1} and 802 cm^{-1} , which can be assigned to CoO (OH). And also peaks were observed at 473, (193, 516, 614, 448) and 680 cm^{-1} corresponding to the E_g , F_{2g} and A_{1g} Raman active modes of CoO [47,48]. It is clearly seen from the spectra that, only A_{1g} mode is more intense than the other peaks. Thus, the spectra demonstrate that the presence of oxidized Co in the form of CoO. Note that, the laser power used in Raman characterization was extremely less (0.1 mW) and the exposure time was very short (i.e. 10 sec); therefore laser induced phase transformation can be neglected. In the present study there are no vibrations originating from the metallic Co nanocrystallites and it is well agreed with the studies reported by Nurlaela et.al. [49].

Interestingly, the above results suggest that the Co NPs might be covered with a very thin layer of CoO, which may exists in the form of oxide on the surface of the Co core [9]. Nitrogen gas produced during the redox reaction could protect the precipitated Co in solution against being oxidized [50]. However, after the reaction, unwanted surface oxidation of the NPs creates thin layer of CoO during sample handling. The difference in the result of XRD and Raman spectroscopic techniques (Raman) can be understood by the fact that the CoO content present is minor (<5%). It is very hard to detect such minor content using diffraction analyses; therefore, presence of CoO was not seen in XRD. The average crystallite sizes was estimated using Scherrer's formula [51] which is based on the X-ray diffraction line broadening and is as follows: $D = \frac{K\lambda}{\beta \cos(\theta)}$ where λ is the wavelength of the x-rays ($1.5406\text{ \AA}$), K is shape factor, here ($K= 0.9$) assuming spherical crystallite shapes, β is the pure diffraction line broadening (in radians) can be easily found by measuring the full width at half maximum (FWHM) of the (hkl) reflection and θ is the Bragg's angle. From the

broadening of the diffraction peaks the average crystallite size (D) of the Co particle was estimated to be $\sim 27.43 \pm 0.15$ nm. The FWHM was narrower, suggesting the larger grain size. Moreover, in present case the (101) diffraction shows higher intensity than other peaks, which indicates that the (101) planes tend to be preferentially oriented.

3.1.3. Infrared Spectroscopy

To determine the capping of OA and better understanding of adsorption mechanism of the OA on the surface of Co NPs, FTIR measurements were performed on the pure OA and the Co NPs capped with OA. A typical FTIR spectrum, for the pure OA is shown in Fig. 3a. Peak identification was carried out in accordance with the literature [52-54]. The FTIR spectrum of pure OA shows more intense vibration bands at 2924cm^{-1} and 2854 cm^{-1} and they are attributed to the CH_2 asymmetric and CH_2 symmetric stretch, respectively. In addition, the intense peak at observed 1709 cm^{-1} was derived from the existence of $\text{C}=\text{O}$ stretch and the band at 1285 cm^{-1} indicate the presence of $\text{C}-\text{O}$ stretch, respectively. The $\text{O}-\text{H}$ in-plane and out-of-plane bands observed at 1457 and 939 cm^{-1} , respectively [52].

The FTIR spectrum for Co NPs capped with OA is shown in Fig. 3b. It shows that a broad peak at 3408 cm^{-1} corresponding to the stretching vibrations of the hydroxide groups due to the moisture present in the sample and is assigned to $\text{O}-\text{H}$ bond. It has been well documented in the literature that, the absence of $\text{C}=\text{O}$ stretch bond of the carboxyl group (OA) and the presence of two characteristics bands of asymmetric ($\nu_{\text{as}}(\text{COO}^-)$) and symmetric ($\nu_{\text{s}}(\text{COO}^-)$) stretch, confirm the capping of OA [37]. Here, it is important to note that the $\text{C}=\text{O}$ stretch band of the carboxyl group present at 1709cm^{-1} in the IR spectrum of pure OA (Fig.3a). This band is absent in the spectrum for Co NPs capped with OA (Fig.3b). However, instead of the band at 1709 cm^{-1} , the two new bands at 1625 and 1387 cm^{-1} , respectively, were observed which are characteristic of the asymmetric $\nu_{\text{as}}(\text{COO}^-)$ and the symmetric $\nu_{\text{s}}(\text{COO}^-)$

stretching vibrational modes. This demonstrates that the OA was chemisorbed as a carboxylate onto the NPs and the formation of deprotonated carboxylate means that there is no free liquid OA present in the sample. The absence of (-COOH) characteristic peak suggests that the non-existence of physically absorbed OA in the samples and the formation of OA mono layer on the surface of Co NPs.

The type of interaction present between the carboxylate head and metal atoms on the surface of particles was investigated by the wave-number separation (Δ) between the asymmetric (-COO^-) and symmetric (-COO^-) stretching [53]. These interactions were categorized into four types: monodentate, bridging bidentate, chelating bidentate and ionic interaction. In monodentate interaction, one metal ion is binding with one carboxylic oxygen atom and if both the oxygen atoms are involved in the binding process, they form complexes with either two different metal atoms or a single one. These interactions are referred to as bridged bidentate interaction and chelating bidentate interaction, respectively (Fig.4a) [53]. Experimentally, the actual binding interaction may be distinguished by IR spectroscopy due to the characteristically shifted carbonyl band [54]. The largest Δ ($\sim 200\text{-}320\text{cm}^{-1}$) corresponds to the monodentate interaction and the smallest Δ ($<110\text{ cm}^{-1}$) corresponds the chelating bidentate. The medium range ($\sim 140\text{-}190\text{ cm}^{-1}$) of wave-number separation ascribed to the bridging bidentate interaction. In the present study, the $\Delta \sim 238\text{ cm}^{-1}$ was ascribed to the monodentate interaction where the single oxygen atom forms covalent bond with the metal atoms on the surface of particles. Furthermore, we have attempted to visualize the possible mechanism showing that how the OA interacts with Co NPs and is shown in Fig. 4b.

3.2. Small Angle Scattering Analysis

It is known that density fluctuation in the mesoscopic length scale gives rise to scattering of X-ray or thermal neutrons at small angle. In the present case, the observed scattering contribution at small angle, originates from the density fluctuation due to the

presence of sub-micrometric structure. The SANS and SAXS are well established non-destructive characterization tools [55, 56]. Thus, the comprehensive structural information in the two different length scales has been obtained via SAXS and SANS analyses. Small angle scattering methods provide the statistically averaged information about the structure and with the correlation from the measured angular variation of the scattering intensity. The combined SANS and SAXS profiles are presented in Fig. 5, where the data is represented in double logarithmic scale, for Co NPs. The SAS profile has been fitted with a mathematical model. The total scattering intensity can be written as:

$$I_{total}(q) = I_{subunit}(q) + I_{aggllo}(q) \quad (4a)$$

Where $I_{subunit}(q)$ represents the scattering contribution from nanostructured subunits and can be written as:

$$I_{subunit}(q) = C_1 \int_0^{\infty} [P_{subunit}(q, R) \cdot D_{subunit}(R) \cdot V_{subunit}^2(R) \cdot S_{subunit}(q, R)] dR \quad (4b)$$

And second term in eq. (4a) represents the second contribution to our mathematical model i.e. $I_{aggllo}(q)$ scattering contribution due to overall agglomerated particles.

$$I_{aggllo}(q) = C_2 \int_0^{\infty} [P_{aggllo}(q, R) \cdot D_{aggllo}(R) \cdot V_{aggllo}^2(R) \cdot S_{aggllo}(q, R)] dR \quad (4c)$$

Scattering of NPs is considered as that from polydisperse spherical particles with lognormal size distribution under local monodispersed approximation [57, 58]. In this approach, it is assumed that a particle of a certain size is always surrounded by particles of same size. In the eq. (4b) and (4c), ‘ C_1 and C_2 ’ represent the respective scale factors, independent of q and denotes the product of number density of NPs and contrast factor (i.e., the square of the scattering length density difference between the particles and the matrix). Here $V(R)$ represents the volume of a particle of radius ‘ R ’; the parameters, $P_{subunit}(q, R)$ and

$P_{\text{aggllo}}(q, R)$ are the form factor of subunit and agglomerated particles. Form factor depends on the shape and size of the particles. $S_{\text{subunit}}(q, R)$ and $S_{\text{aggllo}}(q, R)$ represents the inter-particles structure factor of subunit and agglomerated particles. For the spherical particles, the form factor $P(q, R)$ with radius 'R' can be solved and can be written analytically as follows [59]:

$$P(q, R) = \left[\frac{3 \sin(qR) - qR \cos(qR)}{(qR)^3} \right]^2 \quad (5)$$

On the other hand, $S(q, R)$ depends on the nature of the interaction between the particles and gives the information about the spatial arrangement of the particles. Sticky hard sphere [58, 60] type of interaction has been considered that explains the present data reasonably well. Furthermore, the SEM micrographs also support this fact by elucidating that basic particles significantly stick to each other because of magnetic interaction. The sticky hard-sphere potential can be described by combination of hard sphere repulsion potential with an extra attractive square-well at the tail. The interaction potential for a sticky hard sphere system can be expressed as:

$$\begin{aligned} U(r) &= \infty \dots \dots \dots \text{for } (r \leq \sigma) \\ U(r) &= -U_o \dots \dots \dots \text{for } (\sigma < r < \sigma + \Delta) \\ U(r) &= 0 \dots \dots \dots \text{for } (r > \sigma + \Delta) \end{aligned} \quad (6)$$

Where r is the centre to centre separation, Δ and σ - are the width of the attractive square well pair potential and the hard core diameter, respectively. The ration $[\Delta/(\Delta + \sigma)]$ is defined as the perturbation parameter. The stickiness parameter τ , which is a measure of strength of the attractive force, is defined as:

$$\tau^{-1} = 4 \left[\exp \left(\frac{-U_o}{K_B T} \right) - 1 \right] \left[\left(1 + \frac{\Delta}{\sigma} \right)^3 - 1 \right] \quad (7)$$

Where K_B is the Boltzmann constant and T is the temperature. If the stickiness parameter is very high ($\tau \gg 1$), the sticky hard sphere interaction resembles the hard sphere interaction and

smaller the value of τ , implies more is the stickiness. Here, fitting the model intensity profile to the experimental data, gives τ value as 1.5 and 10 for subunit and agglomerated structure, respectively. Note that the τ value is greater than unity and sticking is more for the agglomerated structure compared to the subunit structure. As the structure factor depends on volume fraction, it allows estimating the local volume fractions, f_{subunit} and f_{agglo} , of subunit and agglomerated particles, respectively. In the present case, these values were found to be 0.55 and 0.41, while fitting the model intensity profile. Volume fraction of subunit was observed to be larger than the agglomerate value. Thus, overall these observations imply that the presence of nearly hard sphere interaction among the NPs. $D(R)$ represents the size distribution of the basic constituents, i.e. $D(R) dR$ represents the probability of finding a particle with radius R to $R + dR$. Here, the normalized log-normal distribution is assumed and is expressed as:

$$D(R, \mu, \sigma) = \frac{1}{\sqrt{2\pi\sigma^2 R^2}} \exp\left(-\frac{[\ln(R/\mu)]^2}{2\sigma^2}\right) \quad (8)$$

where μ and σ are the two controlling parameters representing median and polydispersity index, respectively and are related to the mean radius of the particle as follows ;

$$\left[R_{av} = \mu \exp\left(\frac{\sigma^2}{2}\right) \right] \quad (9)$$

The value of σ observed to be varies between 0 and 1. From fitting the model with combined data, polydispersity index (σ) and median radius (μ) were found to be is found to be $\sim(0.44$ and $22.89 \text{ nm})$ and $(0.56$ and $192 \text{ nm})$ for subunit and agglomerate structure, respectively. Using the fitted parameter σ and μ , the average size of subunit and agglomerated particles were estimated to be $\sim 50 \text{ nm}$ and $\sim 448 \text{ nm}$, respectively. The nature of profile at low q regime is determined by the nature of structure factor. For an attractive potential, the intensity at low q increases because of the formation of aggregated structure of the subunits which can

be also clearly seen by SEM micrographs (see Fig.6). Since, the applied scattering tools are believed to give the statistically averaged information about the system, unlike SEM which provides local and the selective information based on the region of interest. In this context, it will be more reliable to believe on median radius of agglomerate given by scattering experiments. The fit of the model to the data is shown in Fig. 5. It is found that the model is indeed capable of explaining the scattering data in the whole accessible q range and the values of the fitting parameters are tabulated in Table 1.

3.3. Morphology and Chemical Composition

The morphologies of as-synthesized products were examined by SEM micrographs. The low as well as high magnification SEM images of Co NPs are shown in Fig. 6. A low magnification image (Fig.6a,b) show the agglomeration of particles and it seems to be flower like structure. Whereas the high magnification images (Fig.6c,d) shows that most of the particles are spherical in shape. Moreover, the close examination of Fig. 6d demonstrates that these spherical shape particles were made up of small subunits. Among the individual MNPs, magnetic attraction is present which reinforces the subunit particles, and creates more regular flower like assemblies. Therefore, agglomeration of subunit occurs and forms the larger secondary particles or agglomerated structure. The separate individual particle could be seen more clearly and is shown in Fig.6e. Energy dispersive X-ray spectroscopy (EDS) of the Co is shown in Fig.7, which demonstrates that the as-prepared sample is the pure Co. Only a very small amount of oxygen was detected which results possibly from the slight oxidation of the surface and presence of carbon was expected as OA which was added as capping agent.

3.4. Chemical Reaction and Growth Mechanism

A possible mechanism for the formation of Co NPs may be explained as follows. Under the alkaline conditions, well grown Co flower can be obtained using hydrazine monohydrate as a reducing agent. When the potassium hydroxide is added to the aqueous

metal salt solutions, metal-hydroxide complexes can be formed. Upon the addition of hydrazine monohydrate solution to the metal-hydroxide complex, it is decomposed to form the metal NPs. It is well known that the hydrazine monohydrate (HyH) exhibits strong reduction ability under the alkaline condition. Interestingly, the formation of Co NPs takes place through the reduction of Co^+ ions species and simultaneous oxidation of hydrazinium ions from hydrazine monohydrate. Actually, in aqueous $\text{NH}_2\text{-NH}_2\cdot\text{H}_2\text{O}$ solution, the chemically active free ion of HyH is hydrazinium (N_2H_5^+) [1]

The oxidation of hydrazinium ion with standard electrode potential of -0.23 V results into electron generation:

The electrons generated due to the oxidation of hydrazinium ions reduce the Co^+ ions, and Co NPs are formed in the chemical solution. The overall reaction for the formation of Co NPs is as follows:

In the initial step, the Co^{2+} cations were rapidly reduced by the reductive hydrazine monohydrate to corresponding metal nuclei in chemical solution. These magnetic Co nuclei will generate a magnetic field that induces magnetic force of attraction for newly reduced atoms and it may initiate the growth of nuclei. To avoid the further growth or agglomeration of nuclei, OA is used as a capping agent and in addition, nitrogen gas produced during the redox reaction could protect the precipitated Co in solution against the being oxidized. Both the factors (i.e. the OA and nitrogen gas) are important to control and prevent the further growth of nuclei. It is reported that the crystal morphology and size are dependent on the

competition between nucleation and growth that can be adjusted through the controlling reaction rate.

3.5. Dynamic Light Scattering and Zeta Potential Measurements

The particle size distribution of Co NPs suspension in solvent (DI water and DMEM) has been performed using the log-normal fitting using the parameters i.e., hydrodynamic radius with polydispersity obtained from dynamic light scattering (DLS) measurements. The results obtained are presented in Fig 8. It has been observed that the NPs exhibit the smaller hydrodynamic radius in DMEM ($R_{H-medium} = 297.95 \pm 0.21 \text{ nm}$) compared to the hydrodynamic radius in DI water ($R_{H-water} = 382.50 \pm 0.26 \text{ nm}$) as solvent. This may be due to the reason that DMEM suppresses the double layer of ions around the particles, enhancing the diffusion speed and resulting in smaller hydrodynamic diameter [61]. It is also observed that the NPs size obtained from DLS measurements are larger than the SAS, Fe-SEM and XRD results and this may be due to the hydrodynamic diameter of particles and their surrounding solvent layers. Furthermore, it is known that the zeta potential is a very important parameter for NPs or colloidal system because the zeta potential value can give an indication of the potential stability of the colloidal system. If all the particles in suspension have a large negative or positive zeta potential, then they will tend to repel each other and there is no tendency to flocculate. However, if the particles have lower zeta potential values then there is no force to prevent the particles coming together and flocculating. The zeta potential value is useful for understanding and predicting interactions between particles in suspension and therefore, in present study, this concept has been used to study the stability of NPs in DI water and DMEM [62].

The zeta potential value in this case has been calculated from the electrophoretic mobility based on the Henry equation,

$$U_E = \frac{2.\varepsilon_r.\xi.f(ka)}{3\eta} \quad (13)$$

where ξ is the zeta potential, U_E is the measured electrophoretic velocity, η is the viscosity, ε_r is the dielectric constant and $f(ka)$ is Henry's function (adopted value: 1.5, referring to the Smoluchowski approximation). The Z-average values obtained by DLS measurements were calculated (using the Brookhaven Instruments 90Plus software) as the average of ten measurements of the same suspension, each one obtained registering ten scans. The ξ values were calculated as the average of ten measurements of the same suspension. In the present study, zeta potentials values are -15.544 mV and -15.573 mV in DI water and DMEM solvent, respectively. In general, the NPs with negative zeta potential are stable due to the electrostatic repulsion among the NPs. It is well known that the NPs suspension said to be stable if a zeta potential value exists in the +30 mV to -30 mV range [63]. Therefore, from the observed zeta potential values, we conclude that the OA coated Co NPs synthesized by low temperature hydrothermal route are stable.

3.6. Magnetic Properties

Magnetizations (M) versus the applied magnetic field (H) measurements, i.e. M-(H) hysteresis loops obtained at different temperatures are presented in Fig. 8. The magnetic parameters namely, saturation (maximum) magnetization (M_S), remanent magnetization (M_r), reduced remanent magnetization (M_r/M_S), and coercive field (H_C) extracted from the measured hysteresis loops and are given in Table.2. Co NPs demonstrate the ferromagnetic behavior at all temperatures (20- 300K)(see Fig.8). It can be noted from the M-(H) plots that the maximum magnetization of Co is ~99.51emu/g at 300 K and is lower than that of bulk Co (168 emu/g) [60,64]. The reduction in magnetization for Co NPs may be due to the presence of organic capping molecules (OA), antiferromagnetic (AFM) CoO on the surface and spin disorder. This may be one of the possibilities because the presence of AFM CoO and capping

of OA on the surface of NPs have been proved by the Raman and FTIR analyses. As a result, it may create magnetically dead layer on the surface of NPs and thickness of the dead layer was determined [65] by using following eqⁿ:

$$M_s = M_s(bulk) \left(1 - \frac{6t}{D} \right) \quad (14)$$

where t is the thickness of the dead layer, M_s (168 emu/g) and D is the particle size. Here, D value is taken as the average radius of subunits (~ 50 nm) and accordingly the thickness of dead layer is estimated to be 3.4 nm at room temperature. There are possibilities have been proposed to understand the presence of nonmagnetic or disordered layer such as the canting of the surface spins, a high anisotropy layer, or the cation site disorder of the magnetic ions in the surface layer [66]. A possible existence of the dead layer (3.4 nm), motivated us to check the presence of exchange bias (EB) effect or the horizontal shift (H_E) in M-(H) loops measured at different temperature in the range 20 -300 K. Generally, it is believed that the exchange bias effect is derived from the interfacial exchange coupling between FM and AFM layers and is always characterized by a shift in hysteresis loop (H_E) along the field direction and an enhancement of the coercivity [67]. Therefore, the quantity H_E is calculated based on $H_E = (H_{C1} + H_{C2})/2$, where H_{C1} and H_{C2} denote the coercive fields at the descending and the ascending branches of the M-(H) hysteresis loop, respectively [68]. In the present case H_E value found to be ~ 30.17 Oe at 20 K which is twice of its room temperature value (Fig. 9a). The larger H_E values are observed at temperatures (< 300 K) which could also be due to the pronounced growth of magnetic anisotropy of the core-shell in NPs. This anisotropy growth increases the core-shell (spin) interaction which increases the H_E values for temperature, less than 300 K [69]. Moreover, with increase in temperature from 50 K to 200 K, the magnitude of H_E increases and exhibits a maximum value of $H_E = 53.33$ Oe at 200 K. Typically, the atomic coordination number of the surface spins is different from that of the core and this

variation of atomic coordination number causes perturbations in the crystal field which can destabilizes the ferrimagnetic order at the surface [70]. Furthermore, the alignment of spin can have a multiplicity of forms with several different ground states and as a result, the surface layer acts like “spin glass ” behavior [52-70]. Thus, in present study, we expect that the core-shell interaction at the interface gives rise the phenomenon of exchange bias effect and this observed exchange bias effect can support the fact that the surface spins have spin-glass-like behavior and surrounds the ferromagnetic core material [71].

The coercivity (H_C) value ~ 128.41 Oe observed at 300 K, is much larger than that for bulk Co (10 Oe) [9, 64]. It is known that the coercivity of any material referred to measure of the magnetic field strength required to change the direction of magnetization which depends on several factors like magnetic anisotropy, defects, strain, size, doping, nature of the surface, and also the interparticle interaction [72]. The total magnetic anisotropy is a combination of magnetocrystalline anisotropy and surface anisotropy, expressed as [73]:

$$K = K_A + \left(\frac{6}{d}\right)K_s \quad (15)$$

Where K is the total magnetic anisotropy, K_A is the magnetocrystalline anisotropy of material, d is the diameter of particle, and K_s is the surface anisotropy that depends upon the surface properties and surface coating. In the present case, as the surface of Co is coated with OA therefore the surface anisotropy may be developed through the OA molecules. Moreover, as stated earlier that the as-synthesized NPs demonstrate the ferromagnetic behavior over a wide range of temperature with average subunit size ~ 50 nm therefore the enhancement in H_C is ascribed to the ferromagnetic particles with smaller size [74]. It is also reported that the magnetic anisotropy of the core and the surface anisotropy mainly contribute to the coercivity without considering the shape anisotropy of spherical nanomaterials [75]. Moreover, as the smaller size particles exhibit large surface area, which induces the high surface anisotropy due to spin disorder, therefore an increased magnetic energy was required to change the

magnetization direction of dipoles resulting in the coercivity enhancement. The increase in coercivity of Co can be attributed to the presence of nano-regime, anisotropic shape, and stacking faults [76, 77].

Interestingly, the nature of the M-(H) loop of as-synthesized NPs didn't change significantly with respect to temperature. A smooth S-shaped unsaturated hysteresis loop is obtained (Fig.9). The non-saturation of the hysteresis loop observed at all temperatures for NP may be due to the glass-like freezing of surface spins at low temperature [78] and low applied field (± 10 KOe).

The maximum saturation magnetization (M_S) versus temperature plot for Co NPs is shown in Fig.10 (b-I). Saturation magnetization is mainly determined by magnon and their orientation in the system. It is expected that the temperature dependent magnetization for NPs may differs from that of bulk materials, since the spatial confinement reduces the number of degrees of freedom generating an energy gap in the corresponding spin wave spectrum [79]. To analyze the temperature dependence of the M_S for Co NPs; the M_S versus T plot can be divided into three regions: region I ($T < 50$ K), region II (T in the range 50 K - 200 K) and region III ($T > 200$ K) (Fig. 10 (b-I)). It can be evident that M_S exhibits a slight increase with reducing temperature from 300 K, reaching a maximum value of 103.19 emu/g at 200 K (region III). However, in region II, there is a decrease in M_S observed with decrease in temperature from 200 K to 50 K, and attains the lower value $M_S = 94.284$ emu/g at 50 K. Moreover, the M_S obtained at 50 K and 100 K is lower as compared to the room temperature value due to the inability to rotate the moments at low temperature. According to Stoner-Wohlfarth model, the inability to saturate the moments is attributed to the larger ratio of the effective energy barrier (anisotropy energy-Zeeman energy) to the thermal energy ($K_B T$) [80]. When the temperature is further reduced, M_S increases again and achieving the maximum value of 100.76 emu/g at 20 K (region I). Furthermore, from the temperature

dependence plots (Fig.10 (b-I and b-II)) it is seen that for $T < 50$ K magnitudes of both i.e., M_s and M_r are higher than the value measured at 50 K which can be considered as a result of the decrease in thermal energy (region I) [81]. It is known, in general that the applied field offers the energy to make the moments ordered, while the thermal energy tries to disturb such order. With decrease in temperature, the effect of thermal energy becomes weaker, thus making it easier for the magnetic moments to flip along the direction of applied magnetic field. To clarify the temperature dependence of M_s and M_r , above 50 K, the effective anisotropy constant (K_E) at different temperatures is determined and for this we have fitted the high magnetic field part of $M(H)$ data to ‘law of approach’ (LA) to saturation. For a polycrystalline magnetic material, the ‘law of approach’ (LA) to saturation equation can be written as [58]:

$$M(H) = M_s \left(1 - \frac{a}{H} - \frac{b}{H^2} - \dots \right) + \chi_p H \dots \dots (a) \quad (16)$$

where $M(H)$ is the magnetization at the applied field H , and M_s is the saturation magnetization. The term χ_p is the forced magnetization coefficient that describes the linear increase in spontaneous magnetization at higher magnetic fields. This factor is applicable at high field and high temperature. However, our data is recorded at ± 10 KOe field with temperature range 20 to 300 K (low temperature). Hence, we have neglected this term during fitting. The term a/H attributed to the strain field around dislocation in the material and is neglected in the present study. The term b/H^2 is the magnetic anisotropy and the coefficient b is related to K_E by:

$$b = \frac{4K_E^2}{15.M_s^2} = \frac{4 \left(K_{sh}^2 + K_{st}^2 + K_{sf}^2 + \frac{2}{7} K_1^2 \right)}{15.M_s^2} \quad (17)$$

where K_{sh} , K_{st} , K_{sf} and K_1 are the shape anisotropy, stress anisotropy, surface anisotropy and magnetocrystalline anisotropy, respectively. The parameter, b and M_s as obtained through the fitting are used to determine the K_E using equation (16) at different temperatures. For

instance, at 300 K, $K_E = 5.935 \times 10^5 \text{ erg/cm}^3$, which is less than that of bulk FCC Co ($K = 2.7 \times 10^6 \text{ erg/cm}^3$) [82] and bulk HCP Co ($K = 5.2 \times 10^6 \text{ erg/cm}^3$) [83]. Variation of K_E with temperature (Fig.10 (c-I)) shows a slight increase with reducing temperature, reaching a maximum value of $6.870 \times 10^5 \text{ erg/cm}^3$ at 150 K. However, a decrease ($K_E = 6.459 \times 10^5 \text{ erg/cm}^3$) in K_E is observed at 50 K (region II) which can be attributed to the weakening of spin-orbit interactions, and when the temperature is reduced further K_E increases again, achieving a maximum value of $6.942 \times 10^5 \text{ erg/cm}^3$ at 20 K. And above 200 K, the values of K_E observed to be decreased because it mainly results from the magnetocrystalline anisotropy.

Furthermore, for an ensemble of NPs, it is understood that the magnetic properties are greatly influenced by interparticle interactions, including magnetic dipole–dipole or exchange coupling between the surface atoms of the adjacent particles. The nature and strength of interparticle interactions depend on the arrangement of NPs. As the surface coating of MNPs alters the interparticle magnetic interaction therefore; we have studied the temperature dependence of dipolar interaction. The strength of interparticle dipolar interactions present in the samples is estimated by the maximum dipolar field (H_{dip}) between the nearest neighbor particles by using the equation as: $H_{\text{dip}} = 2\mu/d^3$, where μ is the particle moment ($\mu = M_s \times V_m$) with M_s is maximum saturation magnetization, V_m is the magnetic grain volume and d is the distance between the centers of two particles [68]. Variation of the H_{dip} against temperature is plotted in Fig. 10 (c-II) and it also exhibits maxima and minimum values as 108.01 Oe and 98.68 Oe at 200 K and 50 K, respectively. It is observed that maximum saturation magnetization and smaller size of subunit particles plays an important role to alter the dipolar interparticle interaction among the particles. Therefore, the temperature dependent behavior of M_s and M_r can be ascribed to the change of intrinsic effective magnetic anisotropy and dipolar interaction. In conclusion, all of the parameters are strongly correlated with each other.

As the coercivity (H_C) is a very important parameter deciding the particular application of material in magneto-recording. We have studied the H_C as a function of temperature and are shown in Fig. 11(a). It can be clearly seen that, the H_C decreases monotonously with increasing temperature following the typical superparamagnetic nature of uniaxial single domain particles [58]. This behavior can be understood by considering the effect of thermal fluctuations of the blocked moments across the anisotropy barriers. For the single-domain non-interacting NPs the temperature dependence of H_C can be described by Kneller's law given by:

$$H_c = H_{c_0} \left[1 - \left(\frac{T}{T_B} \right)^{\frac{1}{2}} \right] \quad (18)$$

where H_{C0} is the coercivity at 0 K and T_B is the superparamagnetic blocking temperature. The data of H_C , in the temperature range 20-300 K, indicates the deviation with $T^{1/2}$ as shown in inset of Fig. 11(a). According to this model, neglecting the interparticle interactions, it is expected that when the temperature is above T_B , the intrinsic anisotropy energy (E_A) will be overcome by the heat energy, and the H_C will become zero Oe. However, the value of $H_C \sim 128.41$ Oe is still observed event at 300 K which shows the deviation from Kneller's law and the departure from Kneller's law is attributed to the interparticle interaction [84]. Moreover, it is predicted that the interparticle interaction among the subunit particles causes the deviation from the normal Kneller's law for as-prepared products.

It is known that the reduced remanent magnetization have major implications for technological applications [85]. Remanent magnetization (M_r) arises from the blocked particles whose moments cannot undergo reversal after removing the saturating magnetic field. In general, the normalized remanent magnetization is given by [86]:

$$\frac{M_r}{M_s} = \int_{\Delta E_{crit}}^{\infty} f(\Delta E) d\Delta E \quad (19)$$

where M_r is the remanent magnetization, M_s is the saturation magnetization, E is the energy barrier, and ΔE_{crit} is the critical energy barrier above which the thermal energy can induce switching of the magnetic moments. Since ΔE_{crit} is directly proportional to the temperature, temperature dependence of (M_r/M_s) is displayed in Fig. 11(b). The ratio (M_r/M_s) obtained from the hysteresis curve shows the strong deviation from unity at all temperatures (20 to 300 K) and the (M_r/M_s) observed to be increases with decrease in temperature, (Fig.11 (b)) similar to H_C .

3.7. Cytotoxicity against Cancer and Normal (PBMC) Cells

Cytotoxicity study of any given NPs is critical to validate their possibility for integration into biomedical applications. The objectives of these experiments were, therefore, to investigate the cellular viability response of as-synthesized Co NPs against the cancer cell and also on normal cell, via modified MTT assay. In present study, A2780/CP70, Cisplatin resistant ovarian cancer cells were preferentially selected for Co NPS modified Cyotoxicity analysis as a model cell line of cisplatin resistant ovarian cancer, a common cancer in women. A2780/CP70 cells were induced by Co NPs to provide insights to ovarian cancer therapeutics. Ovarian cancer is the leading cause of death from gynaecologic cancer in women worldwide. According to the National Cancer Institute, (USA), ovarian cancer has the highest mortality rate among all the reproductive cancers in women. Advanced stage diagnosis and chemo/radio-resistance is a major obstacle in treating advanced ovarian cancer. The most commonly employed chemotherapeutic drug for ovarian cancer treatment is cisplatin. As with most chemotherapeutic drugs, many patients eventually become resistant to cisplatin and therefore, diminishing its effect. The efficacy of current treatments may be improved by increasing the sensitivity of cancer cells to chemo/radiation therapies [87].

The cytotoxicity data of as-synthesized and sonicated NPs against A2780/CP70 is shown in Fig.12(a). It was found that, the proliferation of cisplatin resistant ovarian cancer cells reduced in a dose dependent manner. Significantly, cell viability of NPs was observed above 86% for all the different Co NPs concentrations. In this work, at higher concentration of NPs (300 $\mu\text{g/mL}$), viability was found to be 87.36 % and the lowest viability for the present case was observed ~ 87 % (at 250 $\mu\text{g/mL}$). The cytotoxicity against the cisplatin resistant ovarian cancer cells was observed to be 5–13 % (viability : 87 - 95%) for 10-300 $\mu\text{g/mL}$ concentration of Co NPs which is poor. However, in clinical settings, 30% of response of patient to anti-cancer drugs is taken as a good therapeutic index and moreover, *in vivo* conditions there is an immune-based recovery as immune cells similar to macrophages and natural killer (NK) cells are rendered activated to anticancer therapeutics drugs [88,89]. Therefore, we can state that there is a novelty and the results of this work are significant. Fig. 12(a) suggest that as-synthesized Co NPs are mildly cytotoxic against human cisplatin resistant ovarian cancer cells (A2780/CP70).

In continuation with the cytotoxicity of NPs, the effect of Co NPs on the proliferation on normal PBMC *in vitro* was shown in Fig. 12(b). Interestingly, PBMC exhibits higher cellular viability (above 81%) over a wide range of NPs concentration (10-300 $\mu\text{g/mL}$). Cytotoxicity against PBMC observed to be between 14.89–18.61 % (viability: 81.39- 85.11 %) from 10-300 $\mu\text{g/mL}$ concentration of Co NPs. It is well known that when magnetic NPs exposed to the healthy cells, presence of NPs on the cell surface affect the plasma membrane over a period of time and causes lysis of the cells hence causes cell death as compared to control. Nevertheless, the cytotoxicity data of MNPs is difficult to compare since the toxic effects of MNPs are influenced by many parameters such as size distribution, surface coating, magnetic properties, etc. [27]. It has been

reported that the surface coating modifies the surface properties of NPs and as can be inferred from the literature survey, the OA plays an important role in the control of the size, shape, and agglomeration of the magnetic nanoparticles [15]. In addition, studies of Limaye et al. reported that the bonding nature of the oleic acid was determined to have a critical effect on the magnetic properties [68]. However, in the present case as mentioned earlier, maximum saturation magnetization (M_s at 300 K) was found to be lower than the bulk Co with size of individual particles around ~ 50 nm. It is important to mention here that OA is used as capping agent during the synthesis not after the synthesis. The IR data revealed the capping of oleic acid via monodentate interaction on the surface of particles. Yes, it could be possible that capping agent (OA) might be protective for cells or in other words capped NPs less toxic than the bare the NPs. It has been reported by several research groups, such as Malvindi et al. [90], that NPs surface passivation reduces the oxidative stress and alteration of iron homeostasis and, consequently, the overall toxicity, despite bare and passivated NPs show similar cell internalization efficiency. Malvindi et al. [90] found that the higher toxicity of bare NPs is due to their stronger in-situ degradation, with larger intracellular release of iron ions, as compared to surface passivated NPs. Their results indicate that surface engineering of MNPs plays a key role in improving particles stability in biological environments reducing both cytotoxic and genotoxic effects. Additionally, studies performed by Shukla et al. [91] also indicate that the chitosan oligosaccharide coating on iron oxide nanoparticles results in the decrease in cellular damage and moderate ROS production, thereby, significantly decreasing the cytotoxic impact of bare iron oxide nanoparticles.

From the careful cytotoxicity analyses of Co NPs against the cancer cell and normal cell, we suggest that Co NPs within concentrations range 10-300 μ g/mL can be used for biomedical applications as it shows the viability more than 81% against the

normal cell (PBMC) and cancer cell (A2780/CP70) respectively. Therefore, the present data suggest that, as-synthesized NPs are mildly antiproliferative against cisplatin resistant human cancer cells. Moreover, it is evident from Fig. 12(b) that the NPs did not show adverse effect on PBMC as compared to its control. As a consequence, we suggest that these NPs can be tested as the candidates for anticancer agents either alone or with existing drugs. It can also be used for treatment as a vector to deliver a drug in the affected area of body [92].

3.8 Haemolytic Activity

For the in vivo applications of the magnetic NPs, excellent blood compatibility is desirable, such as very low hemolytic effect. In the present work, hemolytic assays were performed to evaluate the blood compatibility of as-synthesized Co NPs at different concentrations (10-300 $\mu\text{g/mL}$). Figure 12(c) depicts the percent hemolysis profile in the control as well as in the presence of NPs. Percent haemolysis are compared with the control. The absorbance values obtained to the different concentrations of NPs showed no significant differences when compared to the control. No visible hemolytic effect can be observed visually in the experiment over a broad concentration range of 10-300 $\mu\text{g/mL}$. Even maximum hemolytic activity was observed for the present experiment $\sim 4.25\%$ only. Thus, it can be concluded that the Co NPs have negligible hemolytic activity means, Co NPs are compatible with human RBC, which is a essential feature for future in vivo biomedical applications such as drugs, in drug delivery, intravenous drug delivery applications without creating any harmful effects in the blood stream [92].

3.9. Microscopic Observations on Cell Morphology

Co NPs treated cancer cells showed marked morphological changes (Fig. 13) when compared to their untreated counterparts (control). Briefly, Fig.13 describes the effect of pristine Co on cells after incubation of 24 hr where Fig. 13 (a) shows the

A2780/CP70 Cisplatin-resistant human ovarian cancer cells when treated with medium alone (Control) at 40X magnification and A2780/CP70 Cisplatin-resistant human ovarian cancer cells treated with cobalt nanoparticles at 10 $\mu\text{g/mL}$ (b), 50 $\mu\text{g/mL}$ (c), 100 $\mu\text{g/mL}$ (d), 150 $\mu\text{g/mL}$ (e), and 250 $\mu\text{g/mL}$ at 40X magnification. At lower Co concentration i.e. at 10 $\mu\text{g/mL}$ and 50 $\mu\text{g/mL}$ change is not remarkable (Fig.13 (b-c)). Basically, Cisplatin- resistant human ovarian cancer cells (A2780/CP70) are epithelial cells in origin which have lost the characteristics of contact inhibition exhibited by normal cells. Contact inhibition is a process of arresting cell growth when cells come in contact with each other. As a result, normal cells stop proliferating when they form a monolayer in a culture dish. Contact inhibition is a powerful anticancer mechanism that is lost in cancer cells [93]. Our pristine Co NPs appear to have disrupted the contact between the cells which can be clearly observed from Fig. 13d. Figure 13d shows loss of contact among Cisplatin- resistant human ovarian cancer cells (A2780/CP70) when treated with pristine Co NPs at 100 $\mu\text{g/mL}$ (at 40 X magnifications). Treatment of NPs causing detachment of cancer cells, contact inhibition with cytoplasmic shrinking, no intact cell organelles and cell dispersion with membrane blebbing pointing towards apoptosis as compared with control where the size of cells are large with intact cell organelles and looks healthy with no granulation which was seen in treated cells.

4. Conclusions

We have presented an approach, which is facile, one step hydrothermal without needing higher processing temperatures, for the synthesis of Co particles for biomedical and health science applications. The results demonstrate that the inexpensive process could potentially be applicable for many applications. The median radius of subunit and agglomerated structure was found to be ~ 22 nm and 192 nm, respectively. X-ray diffraction analysis revealed that both HCP as well as FCC phases of metallic cobalt coexist in the final

products due to low activation energy of formation for the stacking faults. Raman analysis gives the signature of existence of poorly Raman active mode of CoO which couldn't detect using XRD. **The observed zeta potential values and DLS measurements demonstrates that the Co NPs (coated with OA) synthesized by low temperature hydrothermal route are stable.** The horizontal shift (H_E) observed in M-(H) loops were less (< 54 Oe) at all temperatures (20 to 300 K). The magnetic exchange between the surface spins (spin glass like) and the core FM spins is responsible for the horizontal loop shifting. The enhancement of coercivity may have resulted from the small size, anisotropic shape, horizontal loop shifting and stacking faults. The toxicological activity of the as-synthesized NPs against the cisplatin resistant human ovarian cancer cells and normal human peripheral blood mononuclear cells (PBMC) suggest that the NPs were mildly anti-proliferative against cancer cells and safe towards normal cells. Assessment of toxicity toward human RBC revealed $< 5\%$ haemolysis up to the concentration $250 \mu\text{g/mL}$, suggesting the safe nature of the NPs on human cells. Thus, these Co NPs may fulfil the emerging need for cheaper drugs or drug-delivery vehicles with no side effects to the patients and biocompatible with human RBC.

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REFERENCES

- 1) N.T.K. Thanh, L.A.W. Green, Functionalisation of nanoparticles for biomedical applications, *Nano. Today*. 119 (2015) 503-516.
- 2) D. L. Feldheim, A. Colby, J. Foss, *Metal Nanoparticles: Synthesis, Characterization, and Applications*, Marcel Dekker Inc, New York, 2002.
- 3) J. K. Lim, S. A. Majetich, Composite magnetic-plasmonic nanoparticles for biomedicine, *Nano. Today*. 8 (2013) 98-113.
- 4) V. F. Puentes, K. M. Krishnan, A. P. Alivisatos, Colloidal nanocrystal shape and size control: the case of cobalt, *Science*. 291 (2001) 2115–2117.
- 5) F. Dumestre, B. Chaudret, C. Amiens, M. C. Fromen, M. J. Casanove, P. Renaud, P. Zurcher, Shape control of chemo-dynamically stable cobalt nanorods through organometallic chemistry, *Angew. Chem.* 114 (2002) 4462-4465.
- 6) H. Cuizhu, Q. Song, W. Xinzheng, L. Jiurong, L. Liqiang, L. Wei, I. Masahiro, K. Machida, Facile synthesis of hollow porous cobalt spheres and their enhanced electromagnetic properties, *J. Mater. Chem.* 22 (2012) 22160–22166.
- 7) Q. A. Pankhurst, N. T. K. Thanh, S. K. Jones, J. Dobson, Progress in applications of magnetic nanoparticles in biomedicine, *J. Phys. D: Appl. Phys.* 42 (2009) 224001-224015.
- 8) M. G. Montiel, P. S. Jacinto, J. A. I. D. Gongora, E. Reguera, G. R. Gattorno, Synthesis and thermal behavior of metallic cobalt micro and nanostructures, *Nano. Micro. Lett.* 3 (2011) 12-19.
- 9) Y. J. Zhang, Q. Yao, Y. Zhang, T. Y. Cui, D. Li, W. Liu, W. Lawrence, Z. D. Zhang, Solvothermal synthesis of magnetic chains self-assembled by flowerlike cobalt sub-microspheres, *Cryst. Growth. Des.* 8 (2008), 3206-3212.

- 10) V. F. Puentes, K. M. Krishnan, A. P. Alivisatos, Synthesis, self-assembly, and magnetic behavior of a two-dimensional superlattice of single-crystal ϵ -Co nanoparticles, *Appl. Phys. Lett.* 78 (2001) 2187–2189.
- 11) Y. Dong, H. Zhao, Y. Liu, X. Wu, J. Pei, Shape-controlled synthesis of cobalt particles by a surfactant-free solvothermal method and their catalytic application to the thermal decomposition of ammonium perchlorate, *Cryst. Eng. Comm.* 17 (2015) 9062-9069.
- 12) H. Cuizhu, Q. Song, X. Wang, L. Jiurong, L. Liqiang, W. Liu, I. Masahiro, K. Machid, Facile synthesis of hollow porous cobalt spheres and their enhanced electromagnetic properties, *J. Mater. Chem.* 22 (2012) 22160-22166.
- 13) M. Respaud, J. M. Broto, H. Rakoto, A. R. Fert, Surface effects on the magnetic properties of ultrafine cobalt particles, *Phys. Rev. B.* 57 (1998) 2925-2935.
- 14) S. Shouheng, C. B. Murray, Synthesis of monodisperse cobalt nanocrystals and their assembly into magnetic superlattices, *J. Appl. Phys.* 85 (1999) 4325-4330
- 15) S. Jovanović, M. Spreitzer, M. Tramšek, Z. Trontelj, D. Suvorov, Effect of oleic acid concentration on the physicochemical properties of cobalt ferrite nanoparticles, *J. Phys. Chem. C.* 118 (2014) 13844-13856.
- 16) **R. Colognato, A. Bonelli, J. Ponti, M. Farina, E. Bergamaschi, E. Sabbioni, L. Migliore, Comparative genotoxicity of cobalt nanoparticles and ions on human peripheral leukocytes in vitro, *Mutagenesis.* 23(5) (2008) 377–382.**
- 17) **E. Sabbioni, S. Fortaner, M. Farina, R. D. Torchio, I. Olivato, C. Petrarca, G. Bernardini, R. M. Costantini, S. Perconti, L. D. Giampaolo, R. Gornati, M. D. Gioacchino, Cytotoxicity and morphological transforming potential of cobalt nanoparticles, microparticles and ions in Balb/3T3 mouse fibroblasts: an in vitro model, *Nanotoxicology.* (2013) 1–10.**

- 18) E. Sabbioni, S. Fortaner, M. Farina, R. D. Torchio, C. Petrarca, G. Bernardini, R. M. Costantini, S. Perconti, L. D. Giampaolo, R. Gornati, M. D. Gioacchino, Interaction with culture medium components, cellular uptake and intracellular distribution of cobalt nanoparticles, microparticles and ions in Balb/3T3 mouse fibroblasts, *Nanotoxicology*, 8(1) (2014) 88–99.
- 19) E. Papisa, F. Rossi , M. Raspantib, I. D. Donnec, G. Colomboc, A. Milzanic, G. Bernardini, R. Gornati, Engineered cobalt oxide nanoparticles readily enter cells, *Toxicology Lett.*, 189 (2009) 253–259.
- 20) R. Gornati, E. Pedretti, F. Rossi, F. Cappellini, M. Zanella, I. Olivato, E. Sabbioni, G. Bernardini, Zerovalent Fe, Co and Ni nanoparticle toxicity evaluated on SKOV-3 and U87 cell lines, *J. Appl. Toxicol.* 36 (2016) 385-393.
- 21) E. Bossi, D. Zanella, R. Gornati, G. Bernardini, Cobalt oxide nanoparticles can enter inside the cells by crossing plasma membranes, *Sci. Rep.* 6 (2016) 22254.
- 22) R. F. Service, *Nanotoxicology. Nanotechnology grows up. Science.* 304 (2004)1732-1734.
- 23) X. Wu, Y. Tan, H. Mao, M. Zhang, Toxic effects of iron oxide nanoparticles on human umbilical vein endothelial cells. *Int. J. Nanomedicine.* 5 (2010) 385-399.
- 24) K. Soto, K. M. Garza, L. E. Murr, Cytotoxic effects of aggregated nanomaterials. *Acta Biomater.* 3 (2007) 351-358.
- 25) T. J. Brunner, P. Wick, P. Manser, P. Spohn, R. N. Grass, L. K. Limbach, A. Bruinink , W. J. Stark, In vitro cytotoxicity of oxide nanoparticles: comparison to asbestos, silica, and the effect of particle solubility. *Environ Sci. Technol.* 40 (2006) 4374-4381.

- 26) T. R. Pisanic, J. D. Blackwell, V. I. Shubayev, R. R. Finones, S. Jin, Nanotoxicity of iron oxide nanoparticle internalization in growing neurons. *Biomaterials*. 28 (2007) 2572-2581.
- 27) M. Auffan , L. Decome, J. Rose, T. Orsiere, D. Meo, V. Briois, C. Chaneac, L. Olivi, J. L. Berge-Lefranc, A. Botta, M. R. Wiesner, J. Y. Bottero, In vitro interactions between DMSA-coated maghemite nanoparticles and human fibroblasts: a physicochemical and cyto-genotoxic study. *Environ. Sci. Technol.* 40 (2006) 4367-4373.
- 28) M. Mahmoudi, A. Simchi, A. S. Milani, P. Stroeve, Cell toxicity of superparamagnetic iron oxide nanoparticles, *J. Colloid. Interface. Sci.* 336 (2009) 510-518.
- 29) W. Xu, J. Liu, C. Li, H. Wu, Y. Liu, Kaempferol-7-O-b-D-glucoside (KG) isolated from *Smilax china* L. rhizome induces G2/M phase arrest and apoptosis on HeLa cells in a p53-independent manner. *Cancer Lett.* 264 (2008) 229–240.
- 30) H. O. Smith, M. F. Tiffany, C. R. Qualls, C. R. Key, The rising incidence of adenocarcinoma relative to squamous cell carcinoma of the uterine cervix in the United States – a 24-year population-based study. *Gynecol. Oncol.* 78 (2000) 97–105.
- 31) L. Zhang, L. Tianmin, J. Wang, W. Liangmin, Z. Yang , Y. Zhang, Template-free synthesis of one-dimensional cobalt nanostructures by hydrazine reduction route., *Nanoscale. Res. Lett.* 6 (2011) 58.
- 32) S. Mazumder, D. Sen, T. Saravanan, P. R. Vijayaraghavan, A medium resolution double crystal based small-angle neutron scattering instrument at Trombay, *Curr. Sci.* 81 (2001) 257-262.

- 33) S. Mazumder, D. Sen, T. Saravanan, P. R. Vijayaraghavan, Performance and Calibration of the Newly Installed Medium Resolution Double Crystal Based Small-angle Neutron Scattering Instrument at Trombay, *J. Neutron. Res.* 9 (2001) 39-57.
- 34) J. A. Lake, An iterative method of slit –correcting small angle X-ray data, *Acta. Cryst.* 23 (1967) 191-194.
- 35) T. Mosmann, Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods.* 65 (1983) 55-63.
- 36) M. Ramovatar, K. K. Kesari, M. Rani, R. Paulraj, Effects of hydroxyapatite nanoparticles on proliferation and apoptosis of human breast cancer cells (MCF-7). *J. Nanopart. Res.* 14 (2012) 712–722.
- 37) M. Oka, S. Maeda, N. Koga, K. Kato, T. Sait, A modified colorimetric MTT assay adapted for application to proliferation and cytotoxicity assays, *Biosci. Biotech. Biochem.* 56 (9) (1992) 472-473.
- 38) H. Tada, O. Shiho, K. Kuroshima, M. Koyama, K. Tsukamoto, An improved colorimetric assay for interleukin2, *J. Immunological Methods*, 93 (1986) 157-165.**
- 39) Wang G.-H., Zhao Y.-Z., Tan J., Zhu S.-H., Zhou K.-C. Arginine functionalized hydroxyapatite nanoparticles and its bioactivity for gene delivery. *Transactions of Nonferrous metals Society of China.* 25 (2015) 490-496.
- 40) A. Boyum, Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g, *Scand. J. Clin. Lab. Invest. Suppl.* 97 (1968) 77–89.

- 41) R. W. Y. Sun, R. Chen, N. P. Y. Chung, C. M. Ho, C. L. Steve, C. M. Che, Silver nanoparticles fabricated in Hepes buffer exhibit cytoprotective activities toward HIV-1 infected cells. *Royal. Soc. Chem.* 1 (2005) 1-11.
- 42) I. M. M. Paino, V. S. Marangonia, R. C. S. Oliveirab, L. M. G. Antunes, V. Zucolotto, Cytotoxicity and genotoxicity of gold nanoparticles in human hepatocellular carcinoma and peripheral blood mononuclear cells. *Toxicol. Lett.* 215 (2012) 119-125.
- 43) FDA, Guidance for Industry - Nonclinical studies for the safety evaluation of pharmaceutical excipients. Rockville, MD, 2005.
- 44) L. Y. Shen, H. L. Christy, Impacts of mesoporous silica nanoparticle size, pore ordering, and pore integrity on hemolytic activity. *J. Am. Chem. Soc.* 132 (2010) 4834–4842.
- 45) J. Laloy, V. Minet, L. Alpan, F. Mullier, S. Beken, O. Toussaint, S. Lucas, J. M. Dogné, Impact of silver nanoparticles on haemolysis, platelet function and coagulation. *Nano. Biomedicine.* 1 (2014) 4.
- 46) D. P. Dinega, M. G. Bawendi, A solution-phase chemical approach to a new crystal structure of cobalt, *Angew. Chem., Int. Ed.*, 38 (1999) 1788-1791.
- 47) Q. Guan, J. L. Cheng, X. D. Li, B. Wang, L. Huang, F. D. Nie, W. Ni, Low temperature vacuum synthesis of triangular CoO nanocrystal/graphene nanosheets composites with enhanced lithium storage capacity, *Sci. Rep.* 5 (2015) 10017.
- 48) S. H. Johnson, C. L. Johnson, S. J. May, S. Hirsch, M. W. Cole, J. E. Spanier, Co@CoO@Au core-multi-shell nanocrystals, *J. Mater. Chem.*, 20 (2010) 439-443
- 49) E. Nurlaela, S. O. Chikh, I. Llorens, J. L. Hazemann, K. Takanabe, Establishing efficient cobalt-based catalytic sites for oxygen evolution on a Ta₃N₅ photocatalyst, *Chem. Mater.* 27 (2015) 5685-5694.

- 50) Z. T. Liu, L. Xin , Z. W. Liu, J. Lu, Synthesis and catalytic behaviors of cobalt nanocrystals with special morphologies, *Powder Technol.* 189 (2009) 514-519.
- 51) B. D. Cullity, *Elements of X-Ray Diffraction*, Addison-Wesley: London, UK, 1978.
- 52) W. Nianqiang, F. Lei, S. Ming, M. Aslam, K. C. Wong, V. P. Dravid, Interaction of fatty acid monolayers with cobalt nanoparticles, *Nano. Lett.* 4(2004) 383-386.
- 53) A. Weddemann, I. Ennen, A. Regtmeier, C. Albon, A. Wolff, K. Eckstädt, N. Mill, M. K. H. Peter, J. Mattay, C. Plattner, N. Sewald, A. Hütten, Review and outlook: from single nanoparticles to self-assembled monolayers and granular GMR sensors, *J. Nanotechnol.* 1 (2010) 75-93.
- 54) K. Nakamoto, *Infrared Spectra of Inorganic and Coordination Compounds*; Wiley: New York, 1963.
- 55) O. Glatter, O. Kratky, *Small Angle X-ray Scattering*, Academic Press, New York, NY, 1982
- 56) D. Sen, J. Bahadur, Avik Das, S. Mazumder, J.S. Melo, H. Frielinghaus, R. Loidl, E. Coli, Imprinted nano-structured silica micro-granules by spray drying: Optimization of calcination temperature, *Colloids and Surfaces B: Biointerfaces*, 127 (2015) 164-171.
- 57) J. S. Pedersen, Determination of Size Distribution from small angle scattering data for systems with effective hard sphere interactions, *J. Appl. Crystallogr.* 27 (1994) 595-608.
- 58) A. Das, D. Sen, S. Mazumder, A. K. Ghosh, C. B. Basak, K. Dasgupta, Formation of nano-structured core-shell microgranules by evaporation induced assembly, *RSC Adv.* 5 (2015) 85052-85060.
- 59) A. Guinier, G. Fournet, B. C. Walker, L. K. Yudowith, *Small Angle Scattering of X-Rays*, Wiley, New York 1955

- 60) S. V. G. Menon, C. Manohar, K. S. Rao, A new interpretation of the sticky hard sphere model, *J. Chem. Phys.* 95 (1991) 9186-9190.
- 61) L. V. Stebounova, E. Guio , V. H. Grassian, **Silver nanoparticles in simulated biological media: a study of aggregation, sedimentation, and dissolution**, *J. Nanopart. Res.* 13 (2011) 233–244.
- 62) S. Singh, A. Bharti, V. K. Meena, **Structural, thermal, zeta potential and electrical properties of disaccharide reduced silver nanoparticles**, *J. Materials Sci: Materials in Electronics.* 25(9) (2014)747–3752.
- 63) N. Chandrasekar, K.M.M. Kumar, K. S. Balasubramnian, K. Karunamurthy, R. Varadharajan, **Facile synthesis of iron oxide, iron-cobalt and zero valent iron nanoparticles and evaluation of their antimicrobial activity, free radicle scavenging activity and antioxidant assay**, *J. Nanomaterials. Biostructures.* 8(2) (2013) 765-775.
- 64) B. Q. Xie, Y. T. Qian, S. Y. Zhang, S. Q. Fu, W. C. Yu, A hydrothermal reduction route to single-crystalline hexagonal cobalt nanowires, *Eur. J. Inorg. Chem.* (2006) 2454-2459.
- 65) S. Ayyappan, S. Mahadevan, P. Chandramohan, M. P. Srinivasan, John Philip, Baldev Raj, Influence of Co^{2+} ion concentration on the size, magnetic properties, and purity of CoFe_2O_4 spinel ferrite nanoparticles, *J. Phys. Chem. C.* 114 (2010) 6334-6341.
- 66) R. Wu, J. Z. Wei, X. L. Peng, J. B. Fu, S. Q. Liu, Y. Zhang, Y. H. Xia, C. S. Wang, Y. C. Yang, J. B. Yang, The asymmetric magnetization reversal in exchange biased granular Co/CoO films, *Appl. Phys. Lett.* 104(2014) 182403, 1-4.
- 67) W. B. Rui, Y. Hu, A. Du, B. You, M. W. Xiao, W. Zhang, S. M. Zhou, J. Du, Cooling field and temperature dependent exchange bias in spin glass/ferromagnet bilayers, *Sci. Rep.* 5 (2015) 13640, 1-10.

- 68) K. Maaz, M. Usman, S. Karim, A. Mumtaz, S. K. Hasanain, M. F. Bertino, Magnetic response of core-shell cobalt ferrite nanoparticles at low temperature, *J. Appl. Phys.* 105 (2009) 113917, 1-5.
- 69) R.H. Kodama, A.E. Berkowitz, Surface spin disorder in NiFe_2O_4 nanoparticles, *Phys. Rev. Lett.* 77 (1996) 394-397.
- 70) R. Topkaya, O. Akman, S. Kazan, B. Aktas, Z. Durmus, A. Baykal, Surface spin disorder and spin-glass-like behavior in manganese-substituted cobalt ferrite nanoparticles, *J. Nanopart. Res.* 14 (2012) 1156.
- 71) M. V. Limaye, S. B. Singh, S. K. Date, D. Kothari, V. R. Reddy, A. Gupta, V. Sathe, R. J. Choudhary, S. K. Kulkarni, High coercivity of oleic acid capped CoFe_2O_4 nanoparticles at room temperature, *J. Phys. Chem. B.* 113 (2009) 9070-9076.
- 72) C. R. Vestal, Z. J. Zhang, Synthesis and magnetic characterization of Mn and Co spinel ferrite-silica nanoparticles with tunable magnetic core, *Nano Lett.* 3 (2003) 1739-1743.
- 73) V. F. Puentes, D. Zanchet, C. K. Erdonmez, A. P. Alivisatos, Synthesis of hcp-Co nanodisks, *J. Am. Chem. Soc.* 124 (2002) 12874-12880.
- 74) C. R. Vestal, Z. J. Zhang, Effects of surface coordination chemistry on the magnetic properties of MnFe_2O_4 spinel ferrite nanoparticles, *J. Am. Chem. Soc.* 125 (2003) 9828-9833.
- 75) S. E. Shirsath, M. L. Mane, Y. Yasukawa, X. Liu, A. Morisako, Self-ignited high temperature synthesis and enhanced super-exchange interactions of $\text{Ho}^{3+}\text{-Mn}^{2+}\text{-Fe}^{3+}\text{-O}^{2-}$ ferromagnetic nanoparticles, *Phys. Chem. Chem. Phys.* 16 (2014) 2347-2357.
- 76) S. L. Wen, Y. Liu, X. C. Zhao, J. W. Cheng, H. Li, Synthesis, dual-nonlinear magnetic resonance and microwave absorption properties of nanosheet hierarchical cobalt particles, *Phys. Chem. Chem. Phys.* 16 (2014) 18333-18340.

- 77) R. H. Kodama, Magnetic nanoparticles, *J. Magn. Magn. Mater.* 200 (1999) 359-372.
- 78) B. K. Chatterjee, K. Bhattacharjee, A. Dey, C. K. Ghosh, K. K. Chattopadhyay, Influence of spherical assembly of copper ferrite nanoparticles on magnetic properties: orientation of magnetic easy axis, *Dalton Trans.* 43 (2014) 7930-7944.
- 79) A. Mumtaz, K. Maaz, B. Janjua, S. K. Hasanain, M. F. Bertino, Exchange bias and vertical shift in CoFe_2O_4 nanoparticles, *J. Magn. Magn. Mater.* 313 (2007) 313, 266-272.
- 80) D. H. Chen and Y. Y. Chen, Synthesis of barium ferrite ultrafine particles by coprecipitation in the presence of polyacrylic acid, *J. Colloid Interface Sci.* 235 (2001) 9-14.
- 81) J. P. Chen, C. M. Sorensen, K. J. Klabunde, G. C. Hadjipanayis, Magnetic properties of nanophase cobalt particles synthesized in inversed micelles, *J. Appl. Phys.* 76 (1994) 6316-6318.
- 82) J. Schallibaum, F. H. D. Torre, W. R. Caseri and J. F. Loffler, Large-scale synthesis of defined cobalt nanoparticles and magnetic metal-polymer composites, *Nanoscale* 1(2009)374-381.
- 83) S. T. Xu, Y. Q. Ma, G. H. Zheng, Z. X. Dai, Simultaneous effects of surface spins: rarely large coercivity, high remanence magnetization and jumps in the hysteresis loops observed in CoFe_2O_4 nanoparticles, *Nanoscale* 7 (2015) 6520-6526.
- 84) Y. Zhang, Y. Liu, C. Fei, Z. Yang, Z. Lu, R. Xiong, D. Yin, J. Shi, The temperature dependence of magnetic properties for cobalt ferrite nanoparticles by the hydrothermal method, *J. Appl. Phys.* 108 (2010) 084312, 1-6.
- 85) Y. Song, H. Modrow, L. L. Henry, C. K. Saw, E. E. Doomes, V. Palshin, J. Hormes, S. Challa, S. R. Kumar, Microfluidic synthesis of cobalt nanoparticles, *Chem. Mater.* 18 (2006) 2817-2827.

- 86) X. M. Lin, C. M. Sorensen, K. J. Klabunde, G. C. Hadjipanayis, Temperature dependence of morphology and magnetic properties of cobalt nanoparticles prepared by an inverse micelle technique, *Langmuir* 14 (1998) 7140-7146.
- 87) S. Kumar, A. Kumar, P. P. Shah, S. N. Rai, S. K. Panguluri, S. S. Kakar, **Micro RNA signature of cis-platin resistant vs. cisplatin sensitive ovarian cancer cell lines**, *J. Ovarian Res.* 4(17) (2011) 1-11.
- 88) A. S. Narang, D. S. Desai, **Anticancer Drug Development Unique Aspects of Pharmaceutical Development**, Y. Lu, R.I. Mahato, *Pharmaceutical Perspectives of Cancer Therapeutics*, (eds.), 2009, XVI, 694, illus 31-19
- 89) K. Pai, A. Shrivastava, R. Kuma, S. Khetarpal, B. Sarmah, P. Gupta, A. Sodhi, **Activation of P388D1 macrophage cell line by chemo therapeutic drugs**, *Life Sci.* 60(15)(1997) 1239-1248.
- 90) M. A. Malvindi, V. D. Matteis, A. Galeone, V. Brunetti, G. C. Anyfantis, A. Athanassiou, R. Cingolani, P. P. Pompa, **Toxicity assessment of silica coated iron oxide nanoparticles and biocompatibility improvement by surface engineering**, *PLoS ONE.* 9(1) (2014), e85835, 1-11
- 91) S. Shukla, A. Jadaun, V. Arora, R. K. Sinha, N. Biyani, V.K. Jain, **In vitro toxicity assessment of chitosan oligosaccharide coated iron oxide nanoparticles**, *Toxicol. Reports.* 2 (2015) 27–39.
- 92) A. Seluanov, C. Hine, J. Azpurua, M. Feigenson, M. Bozzella, Z. Mao, K. C. Catania, V. Gorbunova **Hypersensitivity to contact inhibition provides a clue to cancer resistance of naked mole-rat**. *Proc. Natl. Acad. Sci. U S A.* 17,106(46) (2009)19352-19357
- 93) S. M. Ansari, R. D. Bhor, K. R. Pai, S. Mazumder, D. Sen, Y. D. Kolekar, C. V. Ramana, **Size and chemistry controlled cobalt-ferrite nanoparticles and their anti-proliferative effect against the MCF-7 breast cancer cells**, *ACS Biomater. Sci. Eng.* 2 (2016), 2139-2152.

Figures

Fig.1. X-ray diffraction pattern of Co nanoparticles

Fig.2. Raman spectrum of Co nanoparticles recorded at room temperature

Fig.3. FTIR spectra of (a) pure OA (b) OA capped Co nanoparticles.

Fig. 4(a). OA interaction with Co nanoparticles

Fig. 4(b). Possible mechanism showing OA interacts with Co nanoparticles

Fig.5. SAXS and SANS profiles in double logarithmic scale (solid line represent the fitted curve) and inset at top right and bottom left, are the lognormal size distributions of subunit and agglomerated particles obtained from SAXS and SANS data.

Fig.6. SEM micrographs of Co nanoparticles

Fig.7. EDAX spectra of Co nanoparticles

Fig.8. Size distribution of Co nanoparticles in (a) DI water and (b) DMEM, fitted under log normal distribution using the parameters obtained via Dynamic Light Scattering (DLS)

Fig.9. M-H loops of Co nanoparticles obtained at (a) 20 K (b) 50 K (c) 100 K (d) 150 K (e) 200 K and (f) 300 K (inset at top left show the magnified M-(H) loop)

Fig.10. Temperature dependence of values of (a) Hysteresis loop shift (H_E), (b-I) Maximum saturation magnetization (M_S), (b-II) Retentivity (M_r), (c-I) effective anisotropy constant (K_E) and (c-II) maximum dipolar field (H_{dip}) of the co nanoparticles

Fig.11. Variation of (a) Coercivity (H_C) (inset at bottom left described deviation from Kneller's law) and (b) reduced remanent magnetization (M_r/M_s), of the Co nanoparticles as a function of temperature

Fig.12. Percent cell viability against (a) A2780/CP70 cells (Cisplatin-resistant human ovarian cancer) , (b) human peripheral blood mononuclear cells (PBMC) after incubation for 24 hr at different cobalt nanoparticles concentrations, as measured by modified MTT assay, and (c) Percentage of hemolysis of RBCs treated by cobalt nanoparticles (where Triton-X : Positive control).

Fig.13. A2780/CP70 Cisplatin-resistant human ovarian cancer cells treated with complete medium alone (control) at 40X magnification (a) and A2780/CP70 Cisplatin-resistant human ovarian cancer cells treated with cobalt nanoparticles at 10 $\mu\text{g/mL}$ (b), 50 $\mu\text{g/mL}$ (c), 100 $\mu\text{g/mL}$ (d), 150 $\mu\text{g/mL}$ (e), and 250 $\mu\text{g/mL}$ at 40X magnification.

Tables

Table1. Summary of parameter obtained from small angle scattering analysis

Co Sample	Scattering parameters			
	Polydispersity index (σ)	Stickiness Parameter (τ)	Median radius (μ) (nm)	Volume Fraction
Subunit	0.44	1.5	22.89	0.55
Agglomerate	0.56	10	192	0.41

Table2. Summary of magnetic parameter, M_S - saturation (maximum) magnetization, M_r - remanent magnetization, (M_r/M_S)- reduced remanent magnetization, H_{dip} - maximum dipolar field, H_C -coercivity, H_E -hysteresis loop shift, K_{eff} - effective anisotropy constant.

Temperature (K)	$(M_S)(\text{emu/g})$		$(M_r)(\text{emu/g})$	(M_r/M_S)	$H_{dip}(\text{Oe})$	$H_C(\text{Oe})$	$H_E(\text{Oe})$	$K_E(\times 10^5)(\text{erg/cm}^3)$
	Experimental	Fitted						
20	100.76	111.17	6.24	0.062	105	199	30	6.94
50	94.28	104.44	5.70	0.060	99	197	45	6.46
100	99.04	104.46	5.84	0.059	104	188	49	6.77
150	102.13	111.63	5.69	0.055	107	175	50	6.87
200	103.19	112.44	5.38	0.052	108	162	53	6.87
300	99.51	102.42	4.23	0.043	104	128	15	5.94