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Radiolanthanide-loaded Agglomerated Fe$_3$O$_4$ Nanoparticles for Possible Use in Treatment of Arthritis: Formulation, Characterization and Evaluation in Rats

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Abstract

This investigation reports the preparation of agglomerated Fe$_3$O$_4$ nanoparticles and evaluation of its utility as a viable carrier in the preparation radiolanthanides as potential therapeutic agents for treatment of arthritis. The material was synthesized by chemical route and characterized by XRD, FT-IR, SEM, EDX and TEM analysis. Surface of agglomerated particle possessed ion pair (-O-:Na$^+$) after dispersing particles in NaHCO$_3$ solution at pH = 7 which is conducive for radiolanthanide ($^\ast$Ln = $^{90}$Y, $^{153}$Sm, $^{166}$Ho, $^{169}$Er, $^{177}$Lu) loading by replacement of Na$^+$ ions with tripositive radiolanthanides ion. Radiolanthanide-loaded particulates exhibited excellent in vitro stability up to ~3 half-lives of respective lanthanide radionuclides when stored in normal saline at 37 °C. Radiochemical purities of the loaded particulates were found to retain to the extent of >70% after 48 h of storage when challenged by strong chelator DTPA present in concentration as high as 5 mM, indicating fairly strong chemical association of lanthanides with agglomerated Fe$_3$O$_4$ nanoparticles. Biodistribution studies of $^{90}$Y and $^{166}$Ho-loaded particulates carried out after intra-articular injection into one of the knee joints of a normal Wistar rat revealed near-complete retention of the radioactive preparations (> 98% of the administered radioactivity) within the joint cavity even after 72 h post injection. This was further confirmed by sequential whole-body bioluminescence imaging. These experimental results are indicative of the potential use of radiolanthanide-loaded agglomerated Fe$_3$O$_4$ nanoparticles for treatment of arthritis.

Key Words: Radiation synovectomy (RSV), iron oxide (Fe$_3$O$_4$) nanoparticles, agglomeration, radiolanthanides.
Introduction

Arthritis is one of the most common forms of slowly progressive, systemic, autoimmune inflammatory disorders of synovial joints which affect ~2% of adult population worldwide [1-4]. This progressive degenerative disease is characterized by uncontrolled proliferation of tissue in the inner layer of the synovial membrane leading to chronic joint inflammation [1,3,4]. This in turn leads to restricted joint mobility and partial disability and thereby severely affects the quality of life of the affected population. If synovial inflammation is left untreated, it can even lead to loss of cartilage, erosion and weakening of bones. The treatment strategy involves ablation of diseased synovial membranes followed by the regeneration of disease-free synovium which eventually results in the reduction of inflammation in the joints, alleviation of the pain, improvement in mobility and preservation of joint functions [1]. Ablation of the synovium can be accomplished through a number of surgical and non-surgical procedures including laser surgery, use of chemicals such as osmic acid rifampicin, and use of inoizing radiation by intra-articular administration of β− emitting radionuclides in suitable chemical formulation. Among all these treatment modalities in practice, intra-articular administration of β− emitting radionuclides in the form of colloidal formulation or radiolabeled particulate (preferably of 1-10 µm size range) into the articular cavity, referred as ‘radiation synovectomy (RSV)’ or ‘radiosynoviorthesis’, has emerged as one of the most effective option having satisfactory clinical efficacy and minimum side-effects [2-9].

While a myriad of factors contribute to the utility of RSV, selection of an appropriate β− emitting radionuclide of optimum tissue penetration range along with desirable radioactive decay characteristic is a key determinant that underpins its success. The β− radiation energy should be sufficient to penetrate and ablate proliferating layer of the inflamed synovium with minimum
radiation induced damaged to the underlying articular cartilage or adjacent bone underneath [5,10-12]. The radionuclide should have a half-life adequate to deliver cytotoxic radiation dose to the synovium and at the same time should be substantially less than the retention time of the radiolabeled formulation in the joint to be treated. Among the various radionuclides used for RSV, the lanthanide radionuclides such as, $^{90}$Y (yttrium is considered as a pseudo-lanthanide), $^{153}$Sm, $^{165}$Dy, $^{166}$Ho, $^{169}$Er, $^{175}$Yb and $^{177}$Lu (Table 1) have dominated the field significantly and have been profusely explored for the development of a broad panoply of radiolabeled particles/colloids/macroaggregates [2,11-24]. This is principally due the availability of a wide variety of therapeutically useful $\beta^-$ emitting radionuclides among lanthanide elements having a wide range of energy (0.34-2.28 MeV). As the thickness of the proliferating membrane varies in different joints, treatment of diseased synovium in joints of disparate size requires radionuclide of different $\beta^-$ particles energies. In this premise, the scope of using a single carrier platform loaded with different lanthanide radionuclides resulting in a series of radiolanthanide-loaded particulates of same material would be an interesting proposition. This is expected to pave the way for the treatment of arthritis of human joints of different sizes from finger joint than for a knee, for example, by selecting particulate loaded with the suitable lathanide radionuclide. In pursuit of such a strategy, the possibility of using $^{90}$Y, $^{153}$Sm, $^{166}$Ho, $^{169}$Er and $^{177}$Lu seemed attractive as they offer the prospect of designing radiolabeled particles of wide spectrum of $\beta^-$ particle energies.

In the quest of an innovative and more effective carrier platform to deliver cytotoxic dose of radiolanthanes to the diseased synovium, our attention was turned toward use of nanomaterials as they provided unprecedented opportunities to design and synthesis of novel materials which preferentially target proliferating cells in biological systems [25,26]. While the usefulness of iron
oxide nanoparticles as the platform to deliver imaging probes including radionuclides and therapeutic drugs [27-32] and in hyperthermia therapy [27,33-35] have been copiously exploited, potential utility of this substrate in the preparation of RSV agents has not yet been explored. Based on these successful propositions of iron oxide nanoparticles in medical use and given the excellent biocompatibility of Fe$_3$O$_4$, it is envisaged to use agglomerated Fe$_3$O$_4$ nanoparticles (Agl-MNP) of 1-10 µm size range as the carrier platform for developing potential radiotherapeutic agents for treatment of arthritis. The ability of magnetic Fe$_3$O$_4$ nanoparticles to absorb different elements has been utilized in recent studies on intrinsic or chelator free formulation of different radiolabeled Fe$_3$O$_4$ nanoparticles as probes for positron emission topography (PET) imaging [32,36].

In the present study, we have adopted similar strategy in synthesizing radiolanthanide loaded Agl-MNP. The agglomerated nanoparticles can have pores as well as -OH groups over surface which lead to generation of surface negative charge when these particles are dispersed in neutral to alkaline pH. This would be useful for loading many metal cations on Agl-MNP including lanthanide ions. Working towards this, we describe herein synthesis, radiochemical and physicochemical characterization of Agl-MNPs loaded with lanthanide radionuclides namely $^{90}$Y, $^{166}$Ho, $^{153}$Sm, $^{177}$Lu and $^{169}$Er for their possible utilization in treatment of arthritis of different joints of human body. Biological properties of $^{90}$Y- and $^{166}$Ho-loaded particulates were evaluated after intra-articular administration into the knee joint of Wistar rats. An optimized formulation strategy of radiolanthanide-loaded Agl-MNPs possessing adequate in vitro and in vivo stability has been the propitious outcome. To our knowledge, this is the first example where the unique physical and chemical properties associated with a nanomaterial-based carrier platform have been exploited in the preparation of radiotherapeutic agents for RSV.
EXPERIMENTAL

Materials and equipments

Radionuclides used in the present study were produced by radiative neutron capture (n,γ) route in research reactor. Spectroscopic grades (> 99.99% pure) of yttrium oxide (Y₂O₃) and holmium oxide (Ho₂O₃) used as the targets for the production of ⁹⁰Y and ¹⁶⁶Ho, respectively, were procured from American Potash, USA. These are naturally mononuclidic in ⁸⁹Y and ¹⁶⁵Ho. For the production of ¹⁵³Sm, ¹⁶⁹Er and ¹⁷⁷Lu, isotopically enriched Sm₂O₃ (99.8% in ¹⁵²Sm), Er₂O₃ (98.6% in ¹⁶⁸Er) and Lu₂O₃ (82% in ¹⁷⁶Lu) (all > 99.99% pure) were used as the target materials. These were all procured from Trace Science International, Canada. No-carrier-added (NCA) ⁹⁰Y was obtained from a ⁹⁰Sr/⁹⁰Y generator system based on electrochemical separation technique developed in-house [37]. Ferric chloride (FeCl₃.6H₂O), ferrous sulphate (FeSO₄.7H₂O), suprapure HCl and de-ionized water (resistivity higher than 18.2 MΩ.cm) were procured from Merck, Darmstadt, Germany. All other chemicals used in the experiments were of AR grade and procured from Aldrich Chemical Company, USA.

The radiochemical processing of irradiated targets were performed in 100 mm lead shielded glove box with remote handling provisions maintained under aseptic condition. Radioactivity assay of all the radionuclides except ⁹⁰Y was carried out by high resolution gamma ray spectrometry using an HPGe detector (EGG Ortec/Canberra detector) coupled to a 4000 multichannel analyzer (MCA) system. Energy and efficiency calibration of the HPGe-MCA system was carried out using ¹⁵²Eu and ¹³³Ba reference source obtained from Amersham, USA. Assay of gamma emitting radionuclidic impurities present in the radionuclides produced, if any, were also carried out using the same system. The radioactivity of ⁹⁰Y was measured using a liquid scintillation counter (Tri-Carb 3100TR Liquid Scintillation analyzer, Perkin Elmer, USA),
calibrated for the assay of $^{90}$Y. In case of $^{90}$Y, assay of Sr radionuclides present was carried out by extraction paper chromatography (EPC) technique [38]. Measurements of $^{90}$Y and $^{89/90}$Sr activities during EPC studies were carried out using liquid scintillation counter. All other radioactivity measurements were carried out by using a well type NaI(Tl) scintillation counter (Mucha, Raytest, Germany).

Xylazine hydrochloride and ketamine hydrochloride used for anaesthetizing the animals during animal were procured from local suppliers. Bioluminescence images of the animals after administration of the radiolabeled preparation were recorded using Photon Imager (Biospace Lab, France). All the animal experiments were conducted in strict compliance of the relevant national laws relating to the conduct of animal experiments in India.

**Synthesis of agglomerated Fe$_3$O$_4$ nanoparticles (Agl-MNP)**

Aqueous solutions of ferric chloride (0.1 M, 20 mL) and ferrous sulphate (0.1 M, 10 mL) were mixed with stirring. The resultant solution was made alkaline by addition of 20 mL of 0.2 M NaOH. The black precipitate evolved was separated using a magnet, washed five times with water and dried after stirring for 1 hour. The precipitate was further dried using acetone. Subsequently, 1 g of Fe$_3$O$_4$ obtained was treated with 10 mL of 0.1 M NaHCO$_3$. This process leads to the formation of highly agglomerated nanoparticles of Fe$_3$O$_4$, which gradually settles in aqueous medium.

**Physicochemical characterization**

The prepared samples of Agl-MNP were characterized using X-ray diffractometry (using Rigaku Miniflex 600 X-ray diffractometer) and crystallite size (t) was calculated using the Scherrer equation $t = (0.9 \lambda)/(B \cos \theta)$, where $\lambda$ is the wavelength of Cu Kα, B the half width at maximum intensity and $\theta$ the Bragg’s angle. The surface functionalization of Fe$_3$O$_4$ particle was
characterized by Fourier Transform Infrared (FT-IR) Spectrometer (Bomem FTIR). TEM images of the particles were recorded using Transmission electron microscope (2000 FX, JEOL, Japan). The X-ray photoelectron spectroscopy (XPS) spectra were recorded in a UHV chamber (base pressure $< 2 \times 10^{-8}$ mbar) using a VG CLAM-2 analyzer with a nonmonochromatic twin Mg X-ray ($h\nu \sim 1253.6$ eV) source. All binding energies were referenced to the C1s peak at 284.6 eV.

**Production and quality control of radionuclides**

A measured quantity (few mg) of natural $\text{Y}_2\text{O}_3$ (100% $^{89}\text{Y}$), $\text{Ho}_2\text{O}_3$(100% $^{165}\text{Ho}$), enriched $\text{Sm}_2\text{O}_3$ (99.8% in $^{152}\text{Sm}$) and $\text{Er}_2\text{O}_3$ (98.6% in $^{168}\text{Er}$) targets were weighed separately into clean quartz ampoules which were subsequently flame sealed. The sealed ampoule was put inside a standard aluminum container, sealed and irradiated in DHRUVA research reactor at Bhabha Atomic Research Centre, India, at a thermal neutron flux of $\sim 1 \times 10^{14}$ neutrons per cm$^2$ per second (n.cm$^{-2}$s$^{-1}$). The durations of irradiation were 7 d (for $\text{Ho}_2\text{O}_3$ and $\text{Sm}_2\text{O}_3$), 14 d (for $\text{Y}_2\text{O}_3$) and 28 d for $\text{Er}_2\text{O}_3$. In case of $\text{Lu}_2\text{O}_3$, a stock solution of lutetium target (85.5 % in $^{176}\text{Lu}$) was prepared by dissolving enriched $\text{Lu}_2\text{O}_3$ powder in 0.01 M suprapure HCl to obtain 2 mg/mL Lu concentration. Measured aliquot of this solution (typically 0.1 mL) was dispensed in quartz ampoule and carefully evaporated to dryness. The ampoule was subsequently flame sealed, encapsulated in Al container and irradiated in DHRUVA reactor at a thermal neutron flux of $\sim 1 \times 10^{14}$ n.cm$^{-2}$.s$^{-1}$ for a duration of 21 d. Following neutron irradiation, the targets were retrieved from the quartz ampoules and dissolved in 0.1 M suprapure HCl (0.01 M suprapure HCl, in case of Lu target) by heating under reflux for a period of 15 min in a sterile round bottom flask. The resultant solution was evaporated to near dryness, cooled and reconstituted in 5 mL of deionized water. NCA $^{90}\text{Y}$ was obtained from an $^{90}\text{Sr}/^{90}\text{Y}$ generator system developed in-house [37].
is obtained as $^{90}\text{YCl}_3$ solution in 0.1 N HCl from this generator following the procedure reported earlier [37], which was subsequently used.

The total activity of all the radionuclides produced was individually assayed using gamma ray spectrometry using HPGe detector coupled to a 4K-MCA system, except that of $^{90}\text{Y}$. Energy and efficiency calibration of the detector was carried out using standard $^{152}\text{Eu}$ and $^{133}\text{Ba}$ sources of the same geometry as the test samples. Prominent photo peak/s of the radionuclides (Table 1) were used for measurements of the radioactivity content. The activity of $^{90}\text{Y}$ produced was determined using a pre-calibrated liquid scintillation counter. For the determination of radionuclidic purity, the trace level of co-produced gamma emitting radionuclide impurities were assayed by recording the gamma ray spectra of the sample aliquot from the batch processed, initially having high radioactive concentration, after complete decay ($8 \cdot 10^{12}$ T$_{1/2}$) of principle radionuclide in all the cases. In the case of $^{90}\text{Y}$ while gamma ray spectrometry was used to assay any gamma emitting radionuclidic impurity, propable impurities emitting only $\beta^-$ ($^{89}\text{Sr}$ for reactor produced $^{90}\text{Y}$ and $^{90}\text{Sr}$ in case of NCA $^{90}\text{Y}$) was assayed by extraction paper chromatography (EPC) technique [38]. The procedure is based on the selective retention of $^{90}\text{Y}$ by bis(2-ethyl hexyl)phosphonic acid. In brief, 10 µL of the reagent was impregnated at a distance of 2 cm from one end of a Whatman 3 mm chromatography paper (12×1 cm) upon which 5 µL of $^{90}\text{Y}$ solution (37 MBq/mL) was applied. The paper was dried and developed in 0.9% saline. Subsequently, the activity in each 1 cm segment of the paper was counted using a liquid scintillation counter.

Under these conditions, any $^{89/90}\text{Sr}$ impurity, if present, migrates to the solvent front ($R_f = 0.9-1.0$), while $^{90}\text{Y}$ remains at the point of application ($R_f = 0$). The counts obtained at the solvent front were then compared with the total spotted activity to determine the $^{89/90}\text{Sr}$ content in the $^{90}\text{Y}$ sample.
Optimization of protocol for formulation of radiolanthanide-loaded Agl-MNPs

Loading of agglomerated Fe₃O₄ magnetic nanoparticles (Agl-MNP) with ⁹⁰Y (reactor produced and NCA), ¹⁵³Sm, ¹⁶⁶Ho, ¹⁶⁹Er and ¹⁷⁷Lu was achieved by mixing the radioactivity in the form of *LnCl₃ (Ln = Y, Sm, Ho, Er and Lu) solution (~185 MBq for ⁹⁰Y, ¹⁵³Sm, ¹⁶⁶Ho and ¹⁷⁷Lu; ~37 MBq for ¹⁶⁹Er) with a suspension of particles in 0.1 M NaHCO₃ solution such that pH of the reaction medium was ~8 after addition of *LnCl₃ solution. The mixture was kept under constant stirring at room temperature. Subsequently, the suspended particulates were allowed to settle at the bottom of the reaction tube and the supernatant was carefully separated. The *Ln-loaded particles thus obtained were subjected to a further washing using 1 mL of sterile 0.9% saline to ensure the removal of unlabeled [*Ln⁺³] activity, if any. Finally, the radiolanthanide-loaded particulates were suspended in sterile 0.9% and autoclaved.

Several experiments were carried out by varying the reaction parameters such as, concentration of particles, pH of the reaction mixture and mixing time etc in order to obtain the optimized protocol for maximum yield of radiolabeled particles. In a reaction volume of 1 mL, the amount of particles was varied between 1 mg to 10 mg and the radiolabeling yield was determined in each case. The effect of variation of pH on radiolabeling yield at room temperature was studied by adjusting the pH of the reaction mixture to different from 2 to 10 using either 1 M HCl or 1 M NaOH solutions. The mixing time required to obtain maximum labeling yield was optimized by carrying out reactions for different time periods (0, 10, 20, 30, 60 and 120 min) at room temperature and determining the yield in each case.

**Determination of yield and radiochemical purity**

The loading yields of radiolanthanides on Agl-MNP were determined in the following way. An aliquot (typically, 20 µL) was withdrawn from the supernatant solution of reaction...
mixture after the precipitation of Agl-MNP and the radioactivity was measured. Same aliquot was withdrawn from ‘blank’ (solution having the identical composition of *Ln-Agl-MNP reaction mixture without particles) and the activity was measured. Percentage radiochemical yield was determined from the activity data using the following formula:

\[
\text{Percent Radiolabeling yield} = \left(100 - \frac{R}{B}\right)\%
\]

where, B and R are the background-corrected *Ln activities associated with the aliquots withdrawn from the blank and supernatant solution of the reaction mixture, respectively. The radiochemical purity of the radiolabeled preparation was determined using the same technique subsequent to the removal of unlabeled *Ln activity by washing of loaded Agl-MNP using normal saline.

**In vitro stability studies**

*In vitro* stability of radio-lanthanide loaded Agl-MNPs was studied in normal saline. For this, loaded particles were suspended in 1 mL of normal saline. The suspensions were stored at 37 °C up to 3 half-lives of the individual radionuclide. The radiochemical purities of the suspended *Ln-Agl-MNPs were determined at the end of different time intervals by following the technique described previously. The *in vitro* stability of the radiolabeled particulates was also ascertained by DTPA challenge (DTPA = Diethylene Triamine Pentacaetic Acid). For this, the radiolabeled formulation was suspended in 1 mL of 5 mM aqueous solution of DTPA (pH ~6.5) and the mixture was stored at room temperature. The percentage radiochemical purity *Ln-Agl-MNPs in presence of strong chelator DTPA was determined at regular time intervals upto 48 h of storage following the same technique as described above.
Synthesis of cold Y/Ho-Agl-MNPs

Agl-MNP (50 mg) was suspended in 4 mL of 0.1 M NaHCO$_3$ solution. To this suspension, 1 mL of YCl$_3$/HoCl$_3$ solution in 0.01 M suprapure HCl containing 50 mg of yttrium/holmium was added and it was mixed thoroughly at room temperature for 24 h using magnetic stirrer. The pH of the mixture was found to be ~7-8. Subsequently, the mixture was centrifuged and supernatant was removed carefully. The precipitated Y/Ho-loaded particles were washed thrice with de-ionized water and dried under IR lamp. Subsequently, these particulates were analysed by SEM and EDX techniques.

SEM and EDX analyses

The surface morphology and particle size distribution of Y/Ho-loaded Agl-MNPs were examined using SEM (AIS2100 SERON Technologies, South Korea). For SEM analysis powder samples were spread over mirror polished Si substrate using acetone, dried and tapped to remove any loose particle prior to loading into SEM chamber. All the micrographs were recorded at the same magnification so as to compare the size and shape of samples. Elemental analyses were carried out using the same samples by EDX technique.

In vivo studies in animal model

The pre-clinical biological evaluation of $^{90}$Y- and $^{166}$Ho-loaded Agl-MNPs was studied by carrying out bio-distribution and radio-luminescence imaging studies in normal Wistar rats. Radio-luminescence imaging (also known as Cerenkov luminescence imaging) technique is based on the emission of visible light during the passage of particulate radiation from the decay of certain radionuclides through the condensed phase (that of biological tissue in the present case) [39]. For biodistribution studies, loaded particles (~2 MBq) suspended in 100 µL of normal saline was injected intra-articularly into one of the knee joints of each animal. For intra-arterial
administration, the rats were first anaesthetized using a combination of xylazine hydrochloride and ketamine hydrochloride. Subsequently, the left knee area of the animals was clipped and prepared aseptically. The knee joint was approached craniolaterally in between lig. collaterale laterale genus and m. gastrocnemius lateralis, below condulis lateralis osis femoris and the radiolabeled particulates were administered. $^{90}$Y-loaded Agl-MNP was synthesized using $^{90}$Y produced by (n,$\gamma$) route. Normal saline (100 $\mu$L) was injected into the other joint (control). The animals administered with $^{90}$Y-Agl-MNP were sacrificed by CO$_2$ asphyxiation at the end of 3, 24 and 72 h post-injection (p.i.). On the other hand, animals administered with $^{166}$Ho- MNPs were sacrificed at 3, 24 and 48 h p.i. (p. i. = post injection) time points. Four rats were used for each time point. Major organs and tissues were excised, washed with saline (except blood), dried, weighed and the activity associated with each of them was measured in a flat-type NaI(Tl) scintillation counter. Distribution of the activity in different organs was calculated as percentage of injected activity (dose) (%ID) per organ and percentage of injected activity per gram of the organ (%ID/g) from these data. Activity accumulated per gram of femur was considered for obtaining the total skeletal uptake assuming skeletal weight to be 10% of the total body weight [21,22]. The total uptake in blood, skeleton and muscle were calculated by considering that the respective tissue constitute 7%, 10% and 40% of the total body weight [21,22]. The percentage of activity excreted is indirectly ascertained by subtracting the activity accounted in all the organs from the total injected activity. For radio-luminescence imaging, $^{90}$Y- and $^{166}$Ho-Agl-MNP preparations (~ 10 MBq) suspended in 100 $\mu$L of normal saline were injected into the joints. Prior to the acquisition of images, the animals were anesthetized using a combination of xylazine hydrochloride and ketamine hydrochloride. Sequential whole-body radio-luminescence
images were acquired in Photon Imager at the same time points p.i. when the bio-distribution studies were carried out.

All animal experiments were performed in compliance with the relevant laws and institutional guidelines of the Bhabha Atomic Research Centre, and also state that the institutional Animal Ethics Committee has approved the experiments.

Results

The primary objective of this investigation aims at developing a synthetic route for the large scale preparation of agglomerated Fe$_3$O$_4$ nanoparticles, structural characterization of the material, and evaluation of its usefulness as a viable carrier platform for the preparation of radiolanthanide-loaded particles for use in RSV. With a view to accomplish the desired goal, a systematic approach was pursued.

Synthesis and characterization of agglomerated Fe$_3$O$_4$ nanoparticles

The schematic diagram for formation of agglomerated Fe$_3$O$_4$ nanoparticles is shown in Fig. 1. The adopted synthetic protocol led to the formation of agglomerated nanoparticles with size (1-10 µm agglomerated size) optimal for retention within the joint cavity without extra-articular leakage to other healthy organs. Moreover, the protocol enables cost-effective preparation of agglomerated Fe$_3$O$_4$ nanoparticles in large quantities with high reproducibility with only minor batch-to-batch variations in yield.

X-ray diffraction (XRD) pattern shows cubic structure of Fe$_3$O$_4$ with lattice parameter $a = 8.38$ Å (Fig. 2(i)), which is matching with the reported value [40]. Fourier transform infrared (FTIR) spectra of Fe$_3$O$_4$ particles before and after treatment with NaHCO$_3$ suggest that both have Fe–O characteristic peak at 600 cm$^{-1}$ (Fig. 2(ii)) [41]. Additionally, surface –OH groups on Fe$_3$O$_4$ particles were identified by characteristic peaks at 3400 (stretching) and 1650 cm$^{-1}$
(bending) [40,41]. However, after treatment of NaHCO$_3$ solution, the IR spectrum does not show the peak at 3400 cm$^{-1}$. This observation is probably attributed to the formation of O$^-$:Na$^+$ ion-pair on the surface of the particles. This will lead to negative surface charge on the particles and expected separation of the particles. However, magnetic dipole-dipole interaction might have dominated over electrostatic separation force resulting in the agglomeration of smaller crystallites. The peak at 1640 cm$^{-1}$ will be related to the carbonate residue from NaHCO$_3$ solution. TEM image of Fe$_3$O$_4$ particles shows the highly agglomerated particles (Fig. 2(iii)) and its selected area electron diffraction (SAED) pattern (Fig. 2(iv)) suggests the highly crystalline phase with the cubic structure.

Since XRD pattern of Fe$_3$O$_4$ is similar to $\gamma$-Fe$_2$O$_3$, it is difficult to say whether compound is Fe$_3$O$_4$ or $\gamma$-Fe$_2$O$_3$. In order to identify compound, we have carried out XPS experiments of sample. Figure 3(a) and (b) show the XPS spectra of Fe 2p and O 1s. The position of Fe 2p$_{3/2}$ peak is found to be at 709 eV. There is a very weak satellite peak at ~ 719 eV. In the literature, Fe 2p$_{3/2}$ peak positions in Fe$_3$O$_4$ and $\gamma$-Fe$_2$O$_3$ were 709 and 711 eV, respectively and also $\gamma$-Fe$_2$O$_3$ has an extra peak (known as satellite) at 719 eV [42]. It is concluded that our prepared compound is Fe$_3$O$_4$. The position of O 1s peak is found at 530 eV, which is similar to the reported one [42]. However, there is asymmetric nature in peak at higher binding energy side. This is suggested that oxygen stoichiometry is not maintained. This is due to inhomogeneity of oxygen at core as well as surface of nanoparticles.

**Production and quality control of the radionuclides**

The specific activity and radionuclidic purity of the radionuclides produced in the reactor at 6 h after the end of irradiation (EOI) are shown in Table 2. Apart from the reactor-produced radionuclides, NCA $^{90}$Y was separated from $^{90}$Sr/$^{90}$Y following electrochemical separation.
technique as described in the experimental section. It is evident from the Table 2 that all the radionuclides under consideration were produced with very high radionuclidic purity of >99.9% and hence suitable for clinical utilization. Depending upon the cross section of radiative neutron capture of the corresponding target nuclide, the specific activities of the radionuclides vary widely from $^{169}$Er ($0.4 \pm 0.05$ GBq/mg) to $^{177}$Lu ($930.8 \pm 25.6$ GBq/mg).

**Formulation of radiolanthanide-loaded agglomerated Fe$_3$O$_4$ nanoparticles**

With an aim to arrive at the optimum conditions for formulation of radiolanthanide Agl-MNPs with maximum yield, a detailed study on the influence of various experimental parameters on the radiolabeling yield were carried out. All the optimization studies were carried out using ~185 MBq activity of $^{90}$Y, $^{153}$Sm, $^{166}$Ho and $^{177}$Lu; and ~37 MBq activity of $^{169}$Er, which could be considered as the clinically relevant doses for treatment of arthritis for various joints [10].

The effect of variation of the concentration of Agl-MNP on the yield of each of the radiolanthanide loaded Agl-MNP is shown in Fig. 4. In all the cases reactions were carried out at pH ~7 for 30 min at room temperature. It was found that while NCA $^{90}$Y-loaded Agl-MNP could be prepared in high yield of $96.4 \pm 0.8\%$ using 2 mg particles in 1 mL reaction volume, for other radionuclides including (n,γ) produced $^{90}$Y, the minimum amount of particles required for obtaining >95% yield was 5 mg. Consequently, 5 mg/mL was considered as the optimum particulate concentration for formulation of clinically relevant doses of radiolanthanide loaded particulates. Variation of pH of the reaction mixture between 2 to 10 showed that the pH did not have any significant effect on the yield within the range of 5-10. Below pH 5, the yields were found to be poor. The optimum pH for formulation of radiolanthanide loaded particulates was therefore considered to be ~7-8, which is advantageous as it is close to the physiological pH. Further, it was found that when the reaction was carried out in 0.1 M NaHCO$_3$ medium, the pH was automatically adjusted within 7-8 when $^{*}$MCl$_3$ (M = Y, Sm, Ho, Er and Lu) solution was
added to the suspension of Fe$_3$O$_4$ particles. When loading yields were determined at different time points during the reaction, it was observed that the yield gradually increased with reaction time and reached maximum when the reactants were mixed for 30 min of incubation at room temperature.

Ion pair formation (-O$:Na^+$) helps in loading of Ln$^{+3}$ ions over the particle. Ln$^{+3}$ ion exchanges 3 Na$^+$ ions to form -O$: Ln^{+3}$ and then finally, hydroxylation takes place to form-O$^-$:RE-OH. The loading of RE ions over surface of Fe$_3$O$_3$ is schematically shown in Fig. 1.

**In vitro stability studies**

Radiolanthanide-loaded Agl-MNPs showed excellent *in vitro* stability upto a study period of ~3 half-lives of respective lanthanide radionuclides when stored in normal saline at 37 °C. The radiochemical purities of all the preparations were found to retain to the extent of >98% during the entire study period. In the DTPA challenge study, it was observed that the radiochemical purities of all the preparations under investigation gradually degraded in 5 mM DTPA solution as shown in Fig. 5. This gradual degradation is most likely due to leaching of lanthanide ions from the loaded particles and formation of Ln-DTPA complex. However, it is pertinent to note that the radiochemical purities of the loaded particulates were found to retain to the extent of >70% even after 48 h of storage, even when they were challenged by strong chelator DTPA present in concentration as high as 5 mM. This indicates fairly strong chemical association of lanthanides with particles, an essential prerequisite in designing radiotherapeutic agents for radiation synovectomy.

**SEM and EDX analyses of Ho-loaded Fe$_3$O$_4$ particles**

A typical scanning electron micrograph of holmium labelled Agl-MNP is shown in Fig. 6. The SEM result reveals that the particles have porous architecture throughout the matrix. The
porous surface has an advantage because it can facilitate the diffusion of lanthanide for adsorption on the internal surface of the particulates. The micrograph also revealed that the size of holmium loaded particles were ranged from 1-10 µm.

To verify the elemental composition and ensure the incorporation of Ho into the matrix, an EDX profile of holmium loaded particles have also been recorded as shown in Fig. 7. The EDX spectrum of Ho-Fe₃O₄ particles shows the presence of Fe, O and Ho confirming the incorporation of holmium into the particles. The chemical composition of Ho-Fe₃O₄ surface were determined from the intensity of the peak pertaining to different elements and the quantification of results is given in Table 3.

**In vivo studies in animal model**

The results of the bio-distribution studies carried out in normal Wistar rats after local-regional administration of ⁹⁰Y- and ¹⁶⁶Ho-loaded Agl-MNPs into one of the knee joint cavities of the animals are summarized in Tables 4 and 5, respectively. The results showed retention of > 98% of the injected activity within the joint cavity even after 72 h p.i. for ⁹⁰Y- and 48 h for ¹⁶⁶Ho-loaded particles upto which studies were carried out. Activity detected in blood and other major organ/tissue was insignificant. The percentage of administered activity estimated to be excreted from the animals were also very low. These results indicated that there was almost no leakage of injected activity from the joint cavity of the animals.

The Sequential whole-body radio-luminescence images of the Wistar rat acquired 30 min, 3 h, 24 h, and 72 h post-injection of ⁹⁰Y-loaded particles into one of the knee joints are shown in Fig. 8(a) to (d), respectively. Similarly, Fig. 9(a) to (d) show the images of animals administered with ¹⁶⁶Ho-loaded particles at 30 min, 3 h, 24 h, and 48 h post-injection. It is evident from these figures that almost all the injected activity remained localized in the synovium till the time post-administration upto which studies were carried out (> T₁/₂ of ⁹⁰Y and
\( \sim 2 \, T_{1/2} \) of \(^{166}\text{Ho}\). No activity could be detected in any other organs/tissue thereby confirming that practically no leakage of instilled particles had occurred. This observation has been corroborated with the results of the bio-distribution studies.

**Discussion**

The role of RSV as a safe, minimally invasive and cost effective therapeutic option in the management of different kinds of arthritis needs hardly to be reiterated [43]. The progress in this radiotherapeutic modality has been largely due to availability of more potent radioactive agents. The biological mechanism of RSV essentially involves the absorption of radioactive particulates by superficial cells of the synovium followed by phagocytosis by the macrophages of the inflamed synovium. Beta radiation results coagulation necrosis, fibrosis and sclerosis of the proliferating synovial tissue and leads to destruction of diseased pannus and inflamed synovium [3,5,8,9]. This prevent the secretion of fluid and accumulation of inflammation causing cellular compounds and the joint surfaces become fibrosed, offering rapid and sustain pain relief. The effective cytotoxic radiation dose required to be delivered to the joints is determined principally by the size of the joint along with other factors such as synovial thickness, synovial structures (smooth, villous-fine/rough edematous), condition of the joint fluid (watery or gelatinous) and inflammatory activity of synovium.

The exciting perspective of radiolanthanides in RSV is primarily attributed to their ideally suited nuclear decay properties, favorable production logistics and availability of biocompatible materials where lanthanides can be irreversibly loaded. *Table 1* gives the list of such radionuclides with their decay properties and the routes of their production. The selection of a radiolanthanides for a specified application is primarily dictated by the physical half live, the mean tissue penetration range of the emitted \( \beta^+ \) particles and the size of the joint to be treated.
The penetration depth of the β− radiation energy should correspond to the thickness of the synovium in the joint to be treated as inadequate penetration will give an inferior therapeutic effect, and excessive penetration would lead to radiation dose to healthy tissue. These factors have been the motivation to use $^{90}$Y, $^{166}$Ho, $^{153}$Sm, $^{177}$Lu and $^{169}$Er for this investigation.

The inherent success of RSV also reside on the selection an effective particulate carrier for the radionuclide. The particle used in RSV should be biocompatible, non-toxic, preferably biodegradable and have surface sites that allow absorption or binding of the radionuclide. The binding between the radionuclide and the particle should be essentially irreversible through the course of radiotherapy in order to prevent the leakage of radioactive material from the joint and to avoid whole-body radiation and its systemic effects. Our interest on the use of iron oxide nanoparticles spawned from its inimitable physicochemical characteristics including low toxicity, chemical inertness, biocompatibility, and possibility of surface functionalization for absorption/binding of metal ions [27,30]. Iron oxide nanoparticles are clinically approved metal oxide nanoparticles and have been shown to be associated with low toxicity in the human body [44]. Recent studies reported with supermagnetic iron oxide nanoparticles showed that the administered nanoparticles were gradually degraded form the organs and biotransformed into poorly magnetic iron species, which were believed to be stored into ferritin proteins over a period of three months [45, 46].

Owing to large surface-to-volume ratio, iron oxide nanoparticles possess high surface energies and thus tend to aggregate so as to minimize the surface energies. The agglomeration tendency offer the scope for preparing agglomerated $\text{Fe}_3\text{O}_4$ nanoparticles of 1-10 µm size range. While a number of strategies for radiolabeling of $\text{Fe}_3\text{O}_4$ nanoparticle have been reported, chelator free radiolabeling by noncovalent physisorption appeared to be enticing and the least intricate
route [34,38]. This strategy has been successfully followed in the present work. Fe$_3$O$_4$ nanoparticle exhibits intrinsic surface reactivity and in aqueous solution coordinated with water to bear surface hydroxyl groups [47]. These reactive -OH groups on subsequent dissociation generate ion pair (-O-:Na$^+$) when these particles are dispersed in NaHCO$_3$ solution at pH = 7. The ion pair (-O-:Na$^+$) formation on the nanostructured Fe$_3$O$_4$ scaffolds provides a versatile binding site and made it a viable strategy to attach or bind positively charged radiolanthanide by replacing Na$^+$ ions. Such a strategy constitutes an advantageous attribute in the endeavor of preparing radiolanthanide conjugates based on a single carrier platform.

In last few decades, a large number of radiolabeled agents have been extensively studied in animal models and some in human patients for their use in RSV. Among these, a few agents namely, $^{90}$Y-silicate/citrate colloid, $^{186}$Re-sulfide colloid and $^{169}$Er-citrate colloid have been approved for use in large, medium and small joints, respectively. All these approved agents are basically radiocolloids. However, one of the drawbacks associated with these agents are lack of control of particle size during their syntheses. This sometime may lead to the leakage of the radioactivity from the diseased joints in case very small (nm dimension) radioactive particles are instilled. Any such leakage would result in accumulation of radioactivity in non-target organs such as liver, lung, spleen, etc. Contrary to this, in case of the radiolanthnide loaded Agl-MNPs proposed in the present study the radionuclide is loaded onto the preformed particles of appropriate size. In vitro stability studies and in vivo biodistribution as well imaging studies in Wistar rats carried out using $^{90}$Y- and $^{166}$Ho-loaded particles clearly demonstrated that the synthesized radiolanthanide-loaded preparations are highly stable and there is almost no dislodging of radiolanthanides form the loaded particles in vivo. Based on these encouraging
features together with the biocompatible nature of iron oxide nanoparticles, the developed formulations are presumed to have excellent therapeutic efficacy with minimal side effects.

Conclusions

The objective of development of a synthetic protocol based on simple co-precipitation method that led to the formation of agglomerated Fe$_3$O$_4$ nanoparticles, with size distribution (1-10 $\mu$m) optimal for use in RSV, has been successfully achieved. The surface chemistry of Fe$_3$O$_4$ nanoparticles have been utilized to achieve irreversible binding of radiolanthanides (*Ln$^{3+}$) ions with the particle. Following the optimized procedure, it was possible to prepared radiolanthanide loaded particulates with $>95\%$ yields and $>99\%$ radiochemical purities. All the radioactive preparations exhibited very good in vitro stability. In vivo studies in animal model confirmed the near complete retention ($>98\%$ of administered radioactivity) of $^{90}$Y- and $^{166}$Ho-loaded particles into the knee joint cavities of normal Wistar rats even after 72 h post injection. These results amply indicate the potential utility of these agents for treatment in arthritic patients. The important details available from this investigation would be of considerable value and would stimulate researchers to pursue development of potent radioactive particle based on nanoparticle platform for use in RSV.

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Atomic Research Centre for their keen interest and providing necessary supports during this study. The authors also gratefully acknowledge all the staff members of Isotope Production and Applications Division of Bhabha Atomic Research Centre associated with the production of the radionuclides used in the present investigation. KSS thanks UGC, Delhi for providing JRF-NET fellowship.

**Disclosure Statement**

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**References**


Table 1. Lanthanide radionuclides proposed for radiation synovectomy [2,16,19,20,23,24].

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Half-life</th>
<th>$E_{\beta\text{(max)}}$ (MeV)</th>
<th>Max. tissue penetration (mm)</th>
<th>$E_{\gamma}$ (keV) (% ab)</th>
<th>Production route</th>
<th>$\sigma_{\text{th}}$ (barns)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{90}$Y</td>
<td>64.1 h</td>
<td>2.28</td>
<td>11.3</td>
<td>-</td>
<td>$^{89}$Y(n,γ)</td>
<td>1.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$^{89}$Y/ $^{90}$Y Gen.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{166}$Ho</td>
<td>26.9 h</td>
<td>1.85</td>
<td>8.7</td>
<td>80.6 (6.4)</td>
<td>$^{165}$Ho(n,γ)</td>
<td>61.2</td>
</tr>
<tr>
<td>$^{165}$Dy</td>
<td>2.35 h</td>
<td>1.29</td>
<td>5.6</td>
<td>94.7 (3.6)</td>
<td>$^{164}$Dy(n,γ)</td>
<td>1040</td>
</tr>
<tr>
<td>$^{153}$Sm</td>
<td>46.9 h</td>
<td>0.81</td>
<td>3.5</td>
<td>103.0 (28.0)</td>
<td>$^{152}$Sm(n,γ)</td>
<td>206</td>
</tr>
<tr>
<td>$^{177}$Lu</td>
<td>6.65 d</td>
<td>0.497</td>
<td>2.0</td>
<td>113.0 (6.4), 208.0 (11.0)</td>
<td>$^{176}$Lu(n,γ)</td>
<td>2090</td>
</tr>
<tr>
<td>$^{176}$Yb</td>
<td>4.18 d</td>
<td>0.48</td>
<td>2.0</td>
<td>282.0 (3.1), 396.0 (6.5)</td>
<td>$^{174}$Yb(n,γ)</td>
<td>69</td>
</tr>
<tr>
<td>$^{169}$Er</td>
<td>9.39 d</td>
<td>0.34</td>
<td>1.0</td>
<td>110.9 (0.0014)</td>
<td>$^{168}$Er(n,γ)</td>
<td>1.95</td>
</tr>
</tbody>
</table>
Table 2. Specific activity and radionuclidic purities of radionuclides produced for loading on Agl-MNPs at 6 h post EOI.

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Yield (GBq/mg)</th>
<th>% Radionuclidic purity</th>
<th>Impurities detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{90}$Y (reactor)</td>
<td>0.9 ± 0.06</td>
<td>99.93 ± 0.01</td>
<td>$^{89}$Sr, $^{91}$Y, $^{169}$Yb, $^{160}$Tb</td>
</tr>
<tr>
<td>$^{90}$Y (generator)</td>
<td>NCA</td>
<td>&gt;99.99</td>
<td></td>
</tr>
<tr>
<td>$^{166}$Ho</td>
<td>13.3 ± 0.4</td>
<td>99.99 ± 0.002</td>
<td>$^{166m}$Ho</td>
</tr>
<tr>
<td>$^{153}$Sm</td>
<td>44.4 ± 1.1</td>
<td>99.98 ± 0.005</td>
<td>$^{154}$Eu</td>
</tr>
<tr>
<td>$^{177}$Lu</td>
<td>931 ± 26</td>
<td>99.98 ± 0.005</td>
<td>$^{177m}$Lu</td>
</tr>
<tr>
<td>$^{169}$Er</td>
<td>0.4 ± 0.05</td>
<td>99.95 ± 0.01</td>
<td>$^{169}$Yb, $^{171}$Tm</td>
</tr>
</tbody>
</table>

# neutron irradiation at thermal flux of $1 \times 10^{14}$ n/cm$^2$.s for a duration of 7 d for Ho and Sm; 14 d for Y; 21 d for Lu and 28 d for Er

$n = 3$ for each radioniclide
Table 3. Chemical composition of the surface of Ho-loaded particles Fe$_3$O$_4$ as obtained from EDX analysis.

<table>
<thead>
<tr>
<th>Elements</th>
<th>Intensity Corrn.</th>
<th>Weight %</th>
<th>Atom %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxygen</td>
<td>1.116</td>
<td>30.8 ± 1.4</td>
<td>69.44</td>
</tr>
<tr>
<td>Iron</td>
<td>0.964</td>
<td>36.1 ± 1.0</td>
<td>23.34</td>
</tr>
<tr>
<td>Holmium</td>
<td>0.918</td>
<td>33.1 ± 1.2</td>
<td>7.23</td>
</tr>
</tbody>
</table>
Table 4. Biodistribution pattern of $^{90}$Y-loaded Fe$_3$O$_4$ particles administered in one of the knee joints of normal Wistar rats

<table>
<thead>
<tr>
<th>Organ</th>
<th>% Injected activity in organ/tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 h</td>
</tr>
<tr>
<td>Blood</td>
<td>0.01 (0.01)</td>
</tr>
<tr>
<td>Liver</td>
<td>0.23 (0.05)</td>
</tr>
<tr>
<td>GIT</td>
<td>0.07 (0.02)</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.02 (0.00)</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.00 (0.00)*</td>
</tr>
<tr>
<td>Heart</td>
<td>0.00 (0.00)*</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.05 (0.03)</td>
</tr>
<tr>
<td>Skeleton</td>
<td>0.11 (0.04)</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.04 (0.02)</td>
</tr>
<tr>
<td>Injected knee</td>
<td><strong>98.94 (0.28)</strong></td>
</tr>
<tr>
<td>Control knee</td>
<td>0.03 (0.01)</td>
</tr>
<tr>
<td>Excretion#</td>
<td>0.49 (0.15)</td>
</tr>
</tbody>
</table>

*Values in the parentheses represent standard deviation

At every time point 4 animals have been used

$^{90}$Y-Fe$_3$O$_4$ preparation was injected into the synovial cavity of the arthritis affected knee joints of each animal

*No detectable radioactivity above background

#Excretion has been calculated by subtracting the activity accounted in all the organs from the total activity injected
Table 5. Bio-distribution pattern of $^{166}\text{Ho}$-loaded $\text{Fe}_3\text{O}_4$ particles administered in one of the knee joints of normal Wistar rats

<table>
<thead>
<tr>
<th>Organ</th>
<th>%Injected activity in organ/tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 h</td>
</tr>
<tr>
<td>Blood</td>
<td>0.01 (0.01)</td>
</tr>
<tr>
<td>Liver</td>
<td>0.17 (0.06)</td>
</tr>
<tr>
<td>GIT</td>
<td>0.06 (0.02)</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.03 (0.01)</td>
</tr>
<tr>
<td>Stomach</td>
<td>0.00 (0.00)*</td>
</tr>
<tr>
<td>Heart</td>
<td>0.00 (0.00)*</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.06 (0.03)</td>
</tr>
<tr>
<td>Skeleton</td>
<td>0.09 (0.03)</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.00 (0.00)*</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.04 (0.03)</td>
</tr>
<tr>
<td><strong>Injected knee</strong></td>
<td><strong>99.01 (0.21)</strong></td>
</tr>
<tr>
<td>Control knee</td>
<td>0.02 (0.02)</td>
</tr>
<tr>
<td>Excretion</td>
<td>0.51 (0.12)</td>
</tr>
</tbody>
</table>

Values in the parentheses represent standard deviation

At every time point 4 animals have been used

$^{166}\text{Ho-Fe}_3\text{O}_4$ preparation was injected into the synovial cavity of the arthritis affected knee joints of each animal

*No detectable radioactivity above background

#Excretion has been calculated by subtracting the activity accounted in all the organs from the total activity injected
Fig. 1. Schematic diagram of the formation of agglomerated Fe$_3$O$_4$ particles and their sites where absorptions of radioalnthanide ions (indicated as ‘RE’) can take place.
Fig. 2 (i) XRD pattern of Fe$_3$O$_4$ particles. (ii) FTIR spectra of Fe$_3$O$_4$ and Fe$_3$O$_4$ after treatment with NaHCO$_3$ solution. (iii) TEM image of Fe$_3$O$_4$. (iv) SAED pattern of Fe$_3$O$_4$. 
Fig. 3 XPS spectra of Fe 2p and O 1s in Fe$_3$O$_4$ sample.
Fig. 4 The effect of variation of the concentration of Agl-MNP on the yield of radiolanthanide loaded particles.
Fig. 5 Variation of radiochemical purities of radiolanthanide loaded AgI-MNPs with time when stored in 5 mM aqueous solution of DTPA at room temperature
Fig. 6 A typical scanning electron micrograph of holmium loaded Agl-MNP
Fig. 7 EDX profile of holmium loaded Agl-MNP
Fig. 8. Sequential whole-body radio-luminiscence images of a normal Wistar rat acquired 30 min (a), 3 h (b), 24 h (c), and 72 h (d) post-injection of $^{90}$Y-loaded Agl-MNP into one of the knee joints.
Fig. 9. Sequential whole-body radio-luminiscence images of a normal Wistar rat acquired 30 min (a), 3 h (b), 24 h (c), and 48 h (d) post-injection of $^{166}$Ho-loaded AgI-MNP into one of the knee joints.