

SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO	
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DEP: 2020 DIRECCION DE NUEVAS CREACIONES	EVE: 379 FASE NACIONAL INCLUIDO CAPITULO II
TRA: 11 PCT-PATENTE DE INVENCION CAPITULOS I Y II	FOLIOS: 9
ACT: 430 REQUERSOLICITA	

DIRECCIÓN DE NUEVAS CREACIONES

2020
Bogotá, D.C.,

Abogado(a)/Señor(a)
LUZ CLEMENCIA DE PÁEZ

Asunto: Radicación: 14-26546- -7
 Trámite: 11
 Evento: 379
 Actuación: 430
 Oficio: 7352
 Folios: 9

Patente de Invención	X			Patente de Modelo de Utilidad	
Vía Nacional					
Vía PCT (Fase Nacional)	X	Capítulo I		Capítulo II	X
No. Solicitud Internacional	PCT/EP2012/003381				
Fecha de Presentación Internacional	07/08/2012				

Como resultado del examen de fondo realizado, con fundamento en el Artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se comunica:

1. Documentos estudiados

Descripción:	Radicado N° 14-026546-00000-0000	07/Febrero/2014
Reivindicaciones:	Radicado N° 14-026546-00000-0000	07/Febrero/2014
	Radicado N° 14-026546-00006-0000	11/Noviembre/2014
Oposición:	Radicado N° 14-026546-00004-0000	LafrancoI 06/Agosto/2014
Respuesta de la solicitante:	Radicado N° 14-026546-00006-0000	11/Noviembre/2014
Prioridad:	US 61/522,217	10/Agosto/2011

2. Análisis de la Prioridad

Se acepta la reivindicación de prioridad porque ésta solicitud fue presentada dentro del plazo establecido (Art. 9 D 486) y su contenido técnico corresponde con el de la prioridad reivindicada.

3. Objeto de la invención

De acuerdo con lo establecido en el numeral 1.2.2.2 del capítulo primero del Título 10 de la Circular Única para efectos del examen de patentabilidad, se tendrá en cuenta la tasa

pagada por la solicitante al momento de comenzar el examen para las reivindicaciones adicionales; esto es que cuando la solicitud contenga más de diez (10) reivindicaciones y no se haya pagado la tasa complementaria, sólo se estudiarán las cubiertas por la tasa pagada. En el presente caso se estudiarán las reivindicaciones 1 a 29 del capítulo reivindicatorio (Radicado N°. 14-026546-00006-0000).

Título de la solicitud: “AGLOMERACIÓN DE NANOPARTICULAS RECUBIERTAS CON ALCOXISILANO MAGNÉTICO”.

El problema planteado en esta solicitud consiste en la necesidad de proporcionar opciones de tratamiento eficaces y rentables para tumores sólidos.

Para resolver este problema técnico la solicitud en estudio proporciona nanopartículas magnéticas biocompatibles mejoradas para el tratamiento de tumores.

El objeto de la presente solicitud se relaciona con la Clasificación Internacional de Patentes (CIP): H01F 1/00.

4. Excepción a la Patentabilidad (Art 20 D 486 literal (d))

El objeto de las reivindicaciones 24, 28 y 29 no es patentable de acuerdo con el Art citado, toda vez que reclaman métodos de tratamiento. Esto es evidente pues dichas reivindicaciones se caracterizan por la inyección de una suspensión a un tumor, o por su aplicación en el tratamiento de una enfermedad mencionando además condiciones de exposición del paciente tratado.

5. Reivindicaciones relativas a un uso (Art 14 D 486)

Si bien las reivindicaciones 28 y 29 se encabezan como suspensiones, hacen referencia a usos; se le recuerda a la solicitante que los usos no son patentables de acuerdo con el Art 14, puesto que no hacen referencia a las categorías de producto o procedimiento.

6. De la solicitud de patente

Reivindicaciones (Art 30 D 486, Art 22 PCT)

1. La reivindicación 1 no es clara porque:

- No se presentan las características técnicas esenciales de las etapas comprendidas en el método que se reclama, la definición de estas características es necesaria para realizar la comparación con el estado de la técnica. Se sugiere incluir características tales como: identidad de los reactivos, es decir de los llamados “*metal magnético*” y “*alcoxisilano magnético*”, así como el tiempo, temperatura y composición del medio en el que se lleva a cabo la incubación.
- El término “*nanopartículas*” es muy general, por lo que no permite la comparación con el estado de la técnica. Se sugiere definir, mediante un rango concreto el tamaño de dichas partículas.
- El término “*esencialmente en ausencia*” es impreciso, por lo que no limita de manera adecuada la materia reclamada. Se sugiere reemplazarlo por un rango concreto de concentraciones.

2. La reivindicación 2 no es clara porque no define de manera concreta las sales de hierro utilizadas, se sugiere definir su composición.
3. La reivindicación 4 no es clara porque no define las sales o los complejos de hierro utilizados, se sugiere definir su composición. Por otra parte, tampoco definen las condiciones en las que se dan las llamadas etapas de “*precipitación*” y “*descomposición térmica*”, se sugiere definir dichas características para realizar de manera adecuada la comparación con el estado de la técnica.
4. La reivindicación 5 no es clara porque la expresión “*son tratadas con H₂O₂*” no define las características técnicas esenciales de la etapa reclamada. Se sugiere indicar la concentración de H₂O₂, la temperatura y los medios de reacción en los cuales se da el tratamiento mencionado.
5. Las reivindicaciones 9, 10 y 12 no son claras porque las expresiones “*son desintegradas*” y “*desintegrar*” no definen las características técnicas esenciales de la etapa reclamada. Se sugiere indicar el mecanismo usado para la etapa de desintegración y el tamaño de las partículas obtenidas en dicha etapa.
6. La reivindicación 11 no es clara porque la expresión “*eliminar nanopartículas recubiertas de forma incompleta*” no define las características técnicas esenciales de la etapa reclamada. Se sugiere indicar el mecanismo usado para la eliminación de las nanopartículas, así como los criterios utilizados para realizar dicha selección.
7. Las reivindicaciones 13 y 21 no son claras porque la expresión “*por lo menos aproximadamente*” es impreciso y no permite delimitar de manera adecuada la materia reclamada. Se sugiere sustituirlo por un rango concreto de valores.
8. Las reivindicaciones 14 y 25 no son claras porque definen un producto en términos del proceso por el cual este fue producido, el cual no es una característica técnica esencial que lo defina de manera adecuada. Se sugiere la inclusión de características técnicas esenciales correspondientes a los productos reclamados o la eliminación de estas reivindicaciones.
9. La reivindicación 16 no es clara porque el término “*esencialmente en ausencia*” es impreciso, por lo que no limita de manera adecuada la materia reclamada. Se sugiere reemplazarlo por un rango concreto de concentraciones.
10. La reivindicación 18 no es clara porque no define características técnicas adicionales a las ya mencionadas, por lo cual se considera redundante y se sugiere su eliminación.
11. La reivindicación 20 no es clara porque no se define la composición de la solución amortiguadora, se sugiere a la solicitante definir el rango de pH y concentraciones, así como la identidad química de los componentes que actúan como agentes amortiguadores que la conforman.
12. Las reivindicaciones 22-24 no es clara porque caracteriza la composición reclamada en referencia a resultados a alcanzar, como la viscosidad de la suspensión a una velocidad de cizallamiento determinada o la tasa de absorción específica (SAR), las cuales no son características técnicas esenciales. Se sugiere incluir las características esenciales, estructurales o funcionales, que contribuyen a la obtención de ese efecto.
13. La reivindicación 26 no es clara porque caracteriza al dispositivo medico reclamado únicamente en función de la sustancia contenida, la cual no es una característica estructural del dispositivo. Se sugiere definir el sistema en función de sus características esenciales estructurales y/o funcionales.

14. Las reivindicaciones 26 y 27 no están sustentadas por la descripción porque en ella no se presenta evidencia del desarrollo de dispositivos o medicamentos de acuerdo a lo solicitado. Se sugiere eliminar dichas reivindicaciones.

Se sugiere hacer las aclaraciones necesarias.

7. Análisis de las Oposiciones

Argumentaciones de Lafrancol

La opositora manifiesta que *"(...) composiciones y combinaciones farmacéuticas que comprenden aglomerados de nanopartículas de metal recubiertas con alcoxisilano magnético, en donde los aglomerados tienen un tamaño promedio de 30-450 nm, preferiblemente de 50 a 350nm y especialmente de 70 a 300nm como es determinado por dispersión de luz, son prácticas y eventos totalmente conocidos en la industria farmacéutica"*, por lo que manifiesta que carecen de los requisitos de novedad y nivel inventivo.

Asimismo la opositora señala que algunas de las reivindicaciones reclaman métodos de tratamiento y considera que no cumplen con el requisito de aplicación industrial, adicionalmente afirma que la solicitud pretende reivindicar combinaciones, las cuales no se consideran invenciones porque *"la mezcla (combinaciones) de productos (moléculas) conocidas y su variación, bien sea en uso, forma, dimensión o materia, no se consideran invenciones por cuanto no son susceptibles de aplicación industrial, nivel inventivo y novedad, luego no pueden acceder a beneficios patentarios"*.

Por último la opositora manifiesta que la solicitud carece de los requisitos de Novedad y Nivel inventivo, fundamentales para otorgársele el privilegio de patente de invención, si se tiene cuenta la materia revelada en documentos como:

- FENG B ET AL: "Synthesis of Fe₃O₄/APTES/PEG diacid functionalized magnetic nanoparticles for MR imaging". COLLOIDS AND SURFACES. A, PHYSICACHEMICAL AND ENGINEERING ASPECTS, ELSEVIER, AMSTERDAM, NL, vol. 328, no. 1-3, 1 October 2008 (2008-10-01).
- SASMITA MOHAPATRA ET AL: "A simple synthesis of amine-derivatised superparamagnetic iron oxide nanoparticles for bioapplications", JOURNAL OF MATERIALS SCIENCE, KLUWER ACADEMIC PUBLISHERS, BO, vol. 42, no. 17, 24 May 2007 (2007-05-24).
- ROQUE A C A ET AL: "Biocompatible and bioactive gum Arabic coated iron oxide magnetic nanoparticles", JOURNAL OF BIOTECHNOLOGY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 144, no. 4, 1 December 2009 (2009-12-01).
- DEL CAMPO ARANZAZU SEN T ET AL: "Multifunctional magnetite and silica-magnetite nanoparticles: Synthesis, surface activation and applications in life sciences", JOURNAL OF MAGNETISM AND MAGNETIC MATERIALS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 293, no. 1, 1 May 2005 (2005-05-01)

Respuestas de la Solicitante

Con respecto a la oposición presentada por la sociedad Lafrancol S.A la solicitante responde presentando un nuevo capítulo reivindicatorio conformado por 16

reivindicaciones e indica que la modificación no implica una ampliación de la materia inicialmente divulgada.

En respuesta a los argumentos de la opositora según los cuales la materia reclamada carece de Novedad y Nivel Inventivo a la luz de las enseñanzas de los documentos citados, la solicitante afirma que la opositora no presenta un análisis que permita determinar por qué dichos documentos afectarían la novedad y el nivel inventivo de la presente solicitud, puesto que no se existe una evaluación o análisis de las características técnicas de la invención, o como estas podrían derivarse de manera obvia a partir de los documentos señalados.

Por otra parte, frente a los documentos citados por la opositora, la solicitante presenta una serie de argumentos referidos a las diferencias entre las características técnicas de la solicitud y aquellas divulgadas en dichos documentos, como se muestra a continuación:

1. Frente a *FENG et al*, 2008: La solicitante afirma que la opositora hizo caso omiso de la característica técnica relacionada con el potencial Z de las nanopartículas de metal reclamadas, y menciona que este documento divulga partículas con un diámetro promedio diferente al de las partículas reclamadas, por lo que asegura que la materia reclamada es novedosa frente a lo enseñado en este documento. Por otra parte, la solicitante indica que el problema técnico planteado por *Fena et al*, es la necesidad de proporcionar un método para producir partículas para uso como agente de contraste MRI para administración intravenosa, por lo que asegura que la persona versada en la materia no se vería motivada a usar las enseñanzas allí divulgadas para derivar de forma obvia u evidente la presente invención, dándole así nivel inventivo.
2. Frente a *Aranzazu del Campo et al*, 2005: La solicitante manifiesta que las partículas divulgadas en este documento presentan diferencias de tamaño y potencial Z, mientras que asegura que este documento se orienta a proporcionar superficies adecuadas como superficiesceptoras para el acople con oligonucleótidos.
3. Frente a *Roque et al*, 2005: La solicitante manifiesta que las partículas divulgadas en este documento presentan diferencias de tamaño y potencial Z, puesto que en este documento este presenta un valor negativo, por lo que una persona versada en la materia no derivaría de manera obvia o evidente las características técnicas esenciales de la presente invención.
4. Frente a *Mohapatra et al*, 2007: La solicitante manifiesta que las partículas divulgadas en este documento se diferencian de las reclamadas por el potencial Z y señala que el mismo se refiere a aminosilanos como un recubrimiento para la unión de bio-moléculas, recalando que en este documento la aglomeración es vista como un problema, de modo que concluye que la persona versada en la materia no se vería motivada a incluir aglomerados de nanopartículas a partir de las enseñanzas proporcionadas por este documento.

Respuesta del Examinador Técnico

En primer lugar, sobre el nuevo capítulo reivindicatorio presentado junto con los argumentos de respuesta a la oposición, esta Oficina acepta las modificaciones realizadas puesto que no se considera una ampliación de la materia inicialmente divulgada.

En segundo lugar, con respecto al argumento de la opositora según el cual la actual solicitud reclama métodos de tratamiento, esta Oficina encuentra que dicha afirmación es

acertada para las reivindicaciones 24, 28 y 29, tal como se explicó en el numeral 4, por lo que se considera que dicho argumento esta fundado, y se sugiere a la solicitante que haga la corrección necesaria.

Con respecto a los documentos citados en el escrito de oposición esta oficina considera que, contrario a lo afirmado por la solicitante, los documento de Feng et al y Mohapatra et al., divulgan métodos para la preparación de nanopartículas de magnetita recubiertas con trietoxisilano, por lo que anticipan algunas de las características técnicas y se consideran relevantes para el estudio del nivel inventivo de la actual solicitud.

A su vez, esta oficina considera que si bien los documentos de Aranzazu del Campo et al. y Roque et al., enseñan métodos para la preparación de nanopartículas magnéticas, no se encuentran relacionados con el campo técnico de la actual solicitud, por lo que no se consideran relevantes para el análisis de nivel inventivo de la actual solicitud.

En conclusión los documento de Feng et al. y Mohapatra et al., citados por la sociedad Lafrancol S.A. en el escrito de oposición, anticipan algunas de las características esenciales de la actual solicitud, y por lo tanto se consideran relevantes para la evaluación de la novedad y el nivel inventivo de la materia ahora reclamada y en consecuencia la oposición presentada es considerada como fundada.

8. Determinación del Estado de la Técnica

El estado de la técnica se determinó con base en la fecha de presentación de la solicitud de prioridad: 10 de agosto de 2011 y se encontraron los siguientes documentos que anticipan el objeto de esta solicitud:

Ítem	Documento	Título	Fecha de Publicación
D1	B. Feng et al. Colloids and Surfaces A: Physicochem. Eng. Aspects 328 (2008) 52–59	Synthesis of Fe ₃ O ₄ /APTES/PEG diacid functionalized magnetic nanoparticles for MR imaging	19/Junio/2008
D2	Mohapatra S. et al. J Mater Sci. (2007) 42:7566–7574	A simple synthesis of amine-derivatised superparamagnetic iron oxide nanoparticles for bioapplications	24/Mayo/2007

El documento D1¹ divulga el uso de nanopartículas magnéticas recubiertas con PEG para imágenes por resonancia magnética nuclear (MRI) (Pág. 52, Resumen).

El documento D2² divulga dispersiones coloidales acuosas de aglomerados de nanopartículas de magnetita recubiertas con 3-aminopropiltrietoxisilano de 45.8nm (Pág. 7566, Col.1, Resumen).

9. Examen de Patentabilidad (Art 14 D486)

Novedad (Art 16 D486)

La reivindicación 15 consiste en: *“Una composición que comprende aglomerados de nanopartículas de metal magnéticas recubiertas con alcoxisilano, caracterizada porque los aglomerados tienen un tamaño promedio de 30 a 450nm, (...)”* (Radicado N°. 14-026546-00006-00000).

Comparación de las características técnicas esenciales, con las del estado de la técnica:

1<http://www.sciencedirect.com/science/article/pii/S0927775708004184>

2<http://link.springer.com/article/10.1007/s10853-007-1597-7#page-1>

Características esenciales	D2
Composición que comprende aglomerados de nanopartículas de metal magnéticas recubiertas con alcoxisilano	Dispersión acuosa coloidal de aglomerados de nanopartículas de magnetita recubiertas con 3-aminopropiltriethoxisilano
Aglomerados con 30 a 450nm de tamaño	El tamaño de partícula de los aglomerados es de 45.8nm (Pág. 4566, Resumen).

Composiciones con las características esenciales de la reivindicación 1 habían sido divulgadas previamente en el documento D2, el cual da a conocer dispersiones acuosas de aglomerados de nanopartículas de magnetita recubiertas con 3-aminopropiltriethoxisilano, los cuales presentan un diámetro de 45.8nm, mientras que las partículas presentan un tamaño promedio entre 7-12 nm (Pág. 4566, Col. 1, resumen)..

En consecuencia, la reivindicación 15 no es nueva o carece de novedad, según lo divulgado en el documento D2.

Las reivindicaciones dependientes 16 – 23, 26 y 27 no mencionan características esenciales que hagan nuevas las composiciones de la reivindicación 15, por lo cual se considera que la materia allí reclamada no es nueva.

Nivel inventivo (Art 18 D486)

La reivindicación 1 consiste en: *“Un método para producir una suspensión de aglomerados de nanopartículas de metal recubiertas con alcoxisilano magnético, en donde una suspensión acuosa de nanopartículas de metal magnético se incuba con alcoxisilano (...)”* (Radicado N°. 14-026546-00006-00000).

Comparación de las características técnicas esenciales, con las del estado de la técnica:

Características esenciales	D1
Método para preparar una suspensión de aglomerados de nanopartículas de metal recubiertas con alcoxisilano magnético, en el que	Reacción de silanización para recubrir partículas MNP (Pág. 53, numeral 2.4)
Una suspensión acuosa de nanopartículas de metal magnético	A 100mL de un fluido magnético que comprende nanopartículas de Fe ₃ O ₄
Se incuba con triethoxisilano en ausencia de un solvente orgánico	Se adiciona una solución de polímero de silano producido por la hidrólisis de 3-aminopropiltriethoxisilano (Pág. 53, numeral 2.3)
Donde 0.3-0.6x10 ⁻³ mol de triethoxisilano son agregados por 0.9mol de metal	No se menciona

El documento D1 se considera el estado de la técnica cercano a la solicitud definida en la reivindicación 1 porque divulga métodos de preparación de partículas magnéticas para imágenes por resonancia magnética. Las partículas obtenidas por el método divulgado en el documento D1 presentan un tamaño de 30.4nm (Pág. 55, tabla 1).

La diferencia entre el método solicitado en la reivindicación 1 y el descrito en el documento D1 es que el método reclamado define la proporción molar entre el

alcoxisilano y el metal.

El efecto técnico logrado por la definición de la proporción molar entre el alcoxisilano y el metal, es la obtención de nanopartículas magnéticas recubiertas con alcoxisilano que presentan una proporción específica de alcoxisilano-metal.

Por lo tanto, el problema técnico objetivo que pretende resolver esta invención se puede formular como la necesidad de proporcionar métodos para la preparación de nanopartículas magnéticas recubiertas con alcoxisilano que presenten una proporción específica de alcoxisilano-metal.

Sin embargo, la definición de proporciones es un proceso rutinario, conocido por la persona del oficio normalmente versada en la materia, por lo que no se considera como una actividad inventiva.

En consecuencia, la persona del oficio normalmente versada en la materia, consideraría el cambio en las proporciones de alcoxisilano-metal, llegando así al objeto de la reivindicación 1 por lo que la materia allí reclamada se considera obvia.

Puesto que las reivindicaciones dependientes 2–14 no mencionan características inventivas adicionales tampoco se pueden considerar inventivas.

Conclusión:

Las reivindicaciones 1– 14 cumplen el requisito de novedad (Art. 16), pero no cumplen con el requisito de nivel inventivo (Art. 18).

10. Conclusión

Los requisitos de Patentabilidad de la presente solicitud están afectados así:

Ítem	Documento	Reivindicaciones afectadas	Requisito que afecta
D1	B. Feng et al. Colloids and Surfaces A: Physicochem. Eng. Aspects 328 (2008) 52–59	1-14	Nivel inventivo
D2	Mohapatra S. et al. J Mater Sci. (2007) 42:7566–7574	15-23, 26 y 27	Novedad

En cumplimiento del artículo 45 de la Decisión 486 de la Comisión de la Comunidad Andina, se le indica que debe dar respuesta dentro del término de sesenta (60) días contados a partir del día siguiente de la fecha de notificación de la presente comunicación. En caso de no dar respuesta al presente requerimiento, o si a pesar de la respuesta subsistieran los impedimentos para la concesión, se denegará la patente.

Se invita al solicitante se sirva indicar, si así lo considera, su renuncia al término faltante de sesenta (60) días para dar respuesta a este requerimiento y presentarla por escrito.

Finalmente, se le recuerda al solicitante que si como respuesta a este requerimiento llegara a modificar el capítulo reivindicatorio y éste contenga más de diez (10) reivindicaciones, no pagará la tasa de modificación voluntaria pero sí la correspondiente al pago por reivindicación adicional.

Notifíquese el presente oficio conforme a lo dispuesto en el numeral 5.2, literal e), del capítulo quinto del título primero de la Circular Única No. 10 de 2001.



JOSÉ LUIS SALAZAR LÓPEZ
Director de Nuevas Creaciones (c)

El anterior requerimiento fue notificado por fijación en lista, de fecha 2015-07-07

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A simple synthesis of amine-derivatised superparamagnetic iron oxide nanoparticles for bioapplications

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Abstract Adsorption of 3-aminopropyltriethoxysilane (APTS) on magnetite nanoparticles during its formation has been investigated to optimise the preparation of stable aqueous dispersion of amine derivatised magnetite nanoparticles. APTS adsorbs chemically on the surface of magnetite particle modifying its surface which is evident from thermal and C, H, N analysis. The variation of particle size has been observed with change of APTS concentration. X-ray diffractogram shows the formation of pure inverse spinel phase magnetite with average crystallite size 7 nm when equimolar (Fe_3O_4 : APTS = 1:1) quantity of APTS was used during its synthesis. The presence of free surface $-\text{NH}_2$ groups and Fe–O–Si bonds was observed by FTIR. Raman spectrum further confirms the presence of surface $-\text{NH}_2$ groups. Transmission electron microscopy shows formation of particles of average size between 7 nm and 12 nm. The effective hydrodynamic diameter of the APTS coated particle agglomerates is 45.8 nm in stable aqueous colloidal dispersion, which is evident from photon correlation spectroscopy. VSM measurements at room temperature of both silanised and

unsilanised magnetite shows their superparamagnetic nature with saturation magnetisation 41 e.m.u/g and 56 e.m.u/g, respectively. Avidin has been immobilised on the surface through glutaraldehyde, which demonstrates the possibility of the synthesised material to be used in protein immobilisation to form bioactive magnetic particles.

Introduction

Study of bio-functionalised superparamagnetic iron oxide nanoparticles (SPION) is emerging as an interesting multidisciplinary area of research because of their potential applications in biology and medicine such as enzyme and protein immobilisation, magnetic resonance imaging (MRI), RNA and DNA purification, cell separation, targeted drug delivery and hyperthermia treatment [1–6]. The most important factor which influences the applicability of these magnetic particles is the successful attachment of biomolecules to the solid magnetic particle supports. As various biomolecules exhibit great diversities, a versatile approach of surface modification of the solid support is always required, which carefully balances the intermolecular forces between the molecules to be grafted and the outer layer of the solid support. In this context, surface modification with organosilanes is an interesting approach as these are compatible with many material used for biological purpose [7]. Organosilanes are bifunctional molecules having general formula $\text{X}-(\text{CH}_2)_n-\text{SiR}_n(\text{OR}')_{3-n}$ where X = functional group and $-\text{Si}(\text{OR})_n$ are anchoring groups by means of which they can be easily attached to the inorganic surfaces by forming strong Si–O–M bond (M is the metal from inorganic substrate) [8]. The most

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common functional target for immobilizing protein molecules is the amine group, which is present on the vast majority of proteins due to the abundance of lysine side chain ϵ -amines and N-terminal α -amines. Though alkoxy-silanes with various X (functionalities) are available, amino group is the most frequently used for biological purpose. The process of surface modification of iron oxides by silanisation is complex and depends upon a number of parameters like solvent type, temperature, and time as well as on the catalyst and type of concentration of organosilane used. The available literatures and patents describe silanisation of iron oxide after its synthesis [9–11]. But practically these naked magnetic particles heavily interact to agglomerate and form 200–400 nm clusters due to high dipole–dipole interaction [12]. Also the effects of various parameters like temperature, pH and silane concentration on surface functionalisation have been hardly considered. The optimisation of all these parameters is very much important to produce well-dispersed functionalised magnetic particles for subsequent tagging with biomolecules.

In this paper, amine derivatised magnetite nanoparticles have been synthesised in a single step by co-precipitation of $\text{Fe}^{2+}/\text{Fe}^{3+}$ in presence of water-soluble 3-aminopropyltriethoxysilane (APTS). The quantity of surface adsorbed APTS during the formation of magnetite particle was determined by C, N and thermal analysis. The effect of APTS concentration of particle size and stability of the colloid was studied. The surface functional groups, structural purity, microstructure and magnetic property were studied by Fourier transform infrared spectroscopy (FTIR), Raman spectroscopy, X-ray diffraction (XRD) analysis, transmission electron microscopy (TEM), dynamic light scattering (DLS) and magnetisation by vibration sample magnetometry (VSM) respectively.

Materials and methods

Chemicals

All chemicals and reagents used were analytical grade and used without further purification. FeCl_3 and $\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$ were obtained from Merck (Germany). 3-aminopropyltriethoxysilane was taken from Aldrich (USA). Millipore water was obtained from Milli-Q system (Millipore, Bedford, MA). Tetramethylrhodamine isothiocyanate (TRITC)-Avidin was taken from Sigma–Aldrich (USA).

Synthesis of APTS coated magnetite particles

Magnetite was synthesised according to the procedure developed by Massart [13, 14] with a little modification. In a 100 mL three necked flask equipped with a mechanical

stirrer were taken 0.324 g of FeCl_3 and 0.278 g of $\text{FeSO}_4 \cdot 6\text{H}_2\text{O}$ in 40 mL of absolutely deoxygenated millipore water under argon. To the stirred reaction mixture variable amount of APTS (molar ratio of APTS: magnetite remains 1:4, 1:2, 1:1, 3:2, 2:1) was added over a period of 15 min during which a black precipitate formed, which spontaneously dissolved to give a clear solution. Black precipitate again reappeared by subsequent injection of 25% NH_3 solution. The reaction mixture was further stirred for 30 min. Then the particles were separated and washed five times with millipore using a magnetic concentrator.

Protein immobilisation study and determination of protein coupling efficiency

For activation of magnetic particles 1 mL of the particle suspension (50 mg/mL) was transferred to a 25 mL conical tube, washed with 0.01 M pyridine (pH 6.0) (4×5 mL) and incubated with 2 mL of 5% glutaraldehyde for 3 h at room temperature with vigorous shaking followed by washing with 0.01 M pyridine (2×5 mL). A total of 10 mg of protein (0.5 mg of Avidin-TRITC conjugate + 9.5 mg BSA) was taken in 1 mL 0.01 M pyridine. A total of 75 μL of this protein solution was added to 925 μL 0.01 M pyridine and kept as pre-coupling solution. Glutaraldehyde activated magnetic beads were incubated with about 75 μL protein solution for 16 h. The supernatant was removed magnetically, and saved as post-coupling solution. Then beads were washed with (3×100 μL) wash buffer (0.01 M Tris, 0.1% NaN_3 , 0.1% w/v BSA, 0.1 M NaCl, 0.001 M EDTA, pH 7.4) magnetically and the supernatant added to the post coupling solution. The post coupling solution was made up to 1 mL by adding 0.01 M pyridine and saved for coupling efficiency determination. To the beads 5 mL 1 M glycine (quenching solution) was added and kept for 30 min. Avidin coupled magnetic beads were washed three times with washing buffer (0.01 M Tris, 0.1% NaN_3 , 0.1% w/v BSA, 0.1 M NaCl, 0.001 M EDTA, pH 7.4). After washing particles were observed on glass slide under Leica Epi Fluorescent Microscope (DMR-MPS60). The same analysis was done with particles precipitated without using any silane.

Characterisation

The APTS concentration per gram of the dried particles was approximately estimated from C, H, and N analysis, using a Series II, Model 2400, Perkin Elmer, USA C, H, N analyzer.

The X-ray diffraction analysis was performed on the Phillips PW 1840 X-ray diffractometer after drying the samples prepared with different concentrations of APTS in vacuum. The lattice parameters were calculated by least

squares method and the crystallite size of the nano-crystalline samples obtained by X-ray line broadening analysis using Scherer's formula.

FTIR spectra of the prepared samples were done in KBr medium in the range $400\text{--}4,000\text{ cm}^{-1}$ with a model Thermo Nicolet Nexux FTIR (model 870).

Laser Raman Micro spectrometry was done with Renishaw Raman System 1000B coupled with a Leica DMLM microscope, using an argon laser with laser power 8 mW at the sample.

Thermal analysis (TG/DTG, DTA) was done with a thermal analyzer (Pyris Diamond TG/DTA) with a heating rate $8\text{ }^{\circ}\text{C}$ per minute with in temperature range $50\text{--}850\text{ }^{\circ}\text{C}$.

TEM micrographs were obtained on a Phillips CM 200 transmission electron microscope with an acceleration voltage of 200 kV. The nanoparticles were thoroughly dispersed in water by ultra-sonication and placing a drop of solution on the carbon coated copper grid.

DLS analysis was done by taking one drop of aqueous suspension of as prepared nanoparticles in 10 mL millipore water by Brookhaven 90 plus particle size analyzer. The laser light of wavelength ($\lambda = 660\text{ nm}$) was scattered with an angle $\theta = 90^{\circ}$ at $27\text{ }^{\circ}\text{C}$ placing the dispersion in a polystyrene cuvette.

Room temperature magnetisation data of the freeze-dried samples were taken with magnetometer Lake Shore, Model-7410, USA.

Mössbauer measurements were carried out in the standard transmission mode by a PC based set-up working in the constant acceleration mode. ^{57}Co embedded in Rh matrix was used as the source. The Mössbauer data were deconvoluted using least squares fitting program.

Results and discussion

Two main steps involved in synthesis of nanocrystals by bottom-up method are (1) nucleation, (2) growth of nanocrystals. It is well known that the size of the nucleus is controlled by the solubility of the precipitate and super saturation of the reaction system.

The Fe_3O_4 magnetic nanocrystals are prepared by injecting NH_4OH to the mixture of Fe^{3+} and Fe^{2+} . A large amount of OH^- is added in order to increase the super saturation of the system. In fact, the ionic product of Fe^{2+} or Fe^{3+} and OH^- becomes much greater than solubility product, confirming the super saturation. The surface Fe atoms in magnetite are co-ordinatively unsaturated. As they carry unoccupied atomic orbitals, in aqueous systems they therefore co-ordinate with hydroxyl ions or water molecules which share their lone electron pair with Fe. Upon adsorption, the water molecules usually dissociate, resulting a surface covered by hydroxyl groups [15]. The silanol

groups gradually formed by stirring APTS in acidic $\text{Fe}^{2+}/\text{Fe}^{3+}$ solution are transformed to siloxane (Si-O-Si) bonds via condensation reaction at low pH [16]. Stirring the solution at high pH (9–10) for a long time, a coating of siloxane is formed through surface condensation on iron oxide surface [16].

The hydrodynamic diameter (HD) of the synthesised iron oxide particles prepared with increasing the concentration of APTS has been measured using DLS method. The intensity–intensity time correlation function has been accepted when the measured and calculated baselines are in good agreement. With increasing the amount of APTS the mean hydrodynamic diameter (MHD) calculated taking five consecutive effective HDs decreases, but after a certain concentration it again starts increasing (Fig. 1a). Aqueous naked Fe_3O_4 particles have tendency to agglomerate and form clusters as observed in case of AS-0 due to magnetic dipole interaction. But the use of APTS prevents the magnetic interaction and hence the particle-aggregate size decreases. But after a certain concentration of APTS again agglomeration is observed which can be explained on the basis of DLVO theory based on the consideration of balance between the molecular forces of attraction and ionic forces of repulsion that act between the particles. Coating of APTS on Fe_3O_4 reduces the possibility of adsorption of potential-determining ions (Fe^{2+} , Fe^{3+} , OH^-) on the surface. As a result the surface potential (φ_0) decreases. As the charge diminishes the electric force of repulsion between the particles weakens, particles stick together as they approach each other. At a sufficiently low $\varphi_{0\text{ crit}}$ at which the energy barrier disappears and the thickness of $1/x$ (or h) of diffuse layers which surround the particles is expressed by the equation

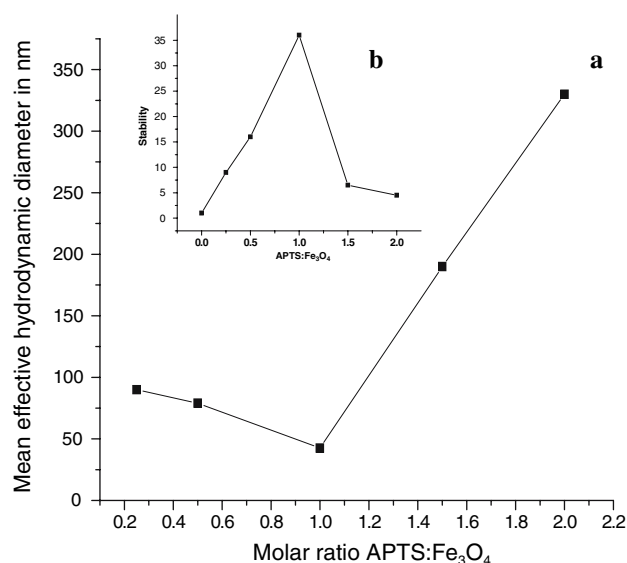


Fig. 1 Variation of MHD with of APTS conc

$$\varphi_{0,\text{critical}} = \sqrt{c \frac{A}{\varepsilon \cdot \frac{1}{x}}} = \sqrt{c \frac{Ax}{\varepsilon}}$$

where $c = \text{const.}$; $A = \text{attraction constant}$; $\varepsilon = \text{dielectric constant of the medium}$.

The relationship between $\varphi_{0 \text{ critical}}$ and x , which follows from the above equation has been empirically found by H. Eilers and J. Korff in 1936. According to them and the given equation

$$\Phi_0^2 h = C \frac{A}{\varepsilon} < > B$$

$< B$ coagulation occurs; $> B$ system is stable; B is a certain critical quantity and can be determined experimentally.

As more amount of APTS is adsorbed on Fe_3O_4 surface there is drop in Φ_0 potential and $\Phi_0^2 h < B$. Hence coagulation occurs. The effect of aminosilane concentration on the final product has been quantified in terms of its organic content and the density of surface $-\text{NH}_2$ groups (Table 1). It is clear that using equimolar amount of APTS as Fe_3O_4 (AS-3) the particle of minimum diameter has been obtained.

The X-ray diffraction patterns of the samples AS-0 and AS-3 are shown in Fig. 2. It is clear that there is no appreciable shift in peak position due to use of aminosilane. The lattice parameters calculated from least square method for both AS-0 and AS-3 are found to be 8.37 Å and 8.39 Å, respectively. The reported lattice parameter for inverse spinel magnetite is 8.37 Å. The d -values match well with that of inverse spinel magnetite (JCPDS card no 82-1533). The appearance of broad peaks indicates that both the samples are nano-crystalline in nature. The average mean crystallite sizes of all samples calculated taking into account the line broadening of each peak are given in Table 1.

The bright field TEM micrographs along with particle size distribution of AS-0 and AS-3 are presented in Fig. 3. AS-0 shows slight agglomeration, which may be due to the magnetic interaction of naked particles. It shows broad distribution of particles between 15 nm and 40 nm with a

mean average particle size 25 ± 1 nm. AS-3 shows well dispersion of spherical particles and a significantly narrow particle size distribution between 7 nm and 12 nm. The mean average particle size is 9 ± 0.5 nm. It clearly indicates silanes/siloxanes prevent the growth of Fe_3O_4 nanoparticles by forming a thin coating on its surface. SAED pattern of AS-3 shows usual polycrystalline nature of the particles. The d -spacings calculated from electron diffraction accord with XRD. Figure 3c shows high resolution TEM of a single particle of AS-3 showing lattice imaging of [400] plane ($d = 2.08$ Å).

In order to verify the exact phase of the magnetite present Raman spectra was recorded with 10% laser power for 30 s integration time. Figure 4a presents the Raman spectrum of AS-3 between 200 cm^{-1} to $1,000 \text{ cm}^{-1}$. Though metal oxides are poor Raman scatterers, still Raman peaks of Fe_3O_4 and $\gamma\text{-Fe}_2\text{O}_3$ are sufficiently different to distinguish [17]. Less intense but distinct peaks at 684, 530 and 320 cm^{-1} correspond to magnetite core [18] and also it is further evidenced by Mössbauer study. The room temperature Mössbauer spectrum of the sample shows a doublet (Fig. 4b), which is typical of a sample undergoing superparamagnetic relaxation. The doublet was fitted with

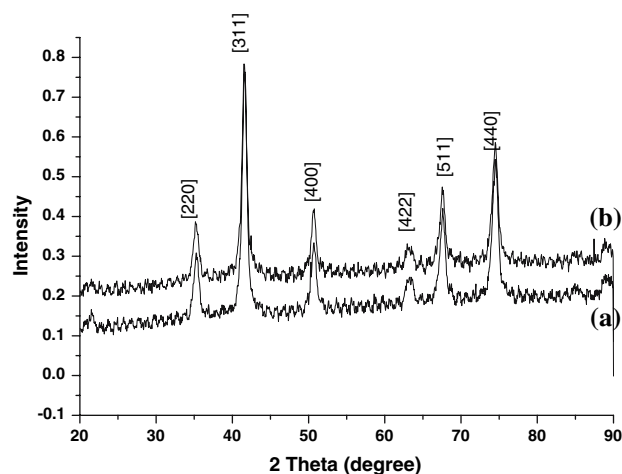


Fig. 2 X-ray diffractogram of (a) AS-0 (b) AS-3

Table 1 Physical properties of the samples prepared with varying Fe_3O_4 : APTS ratio

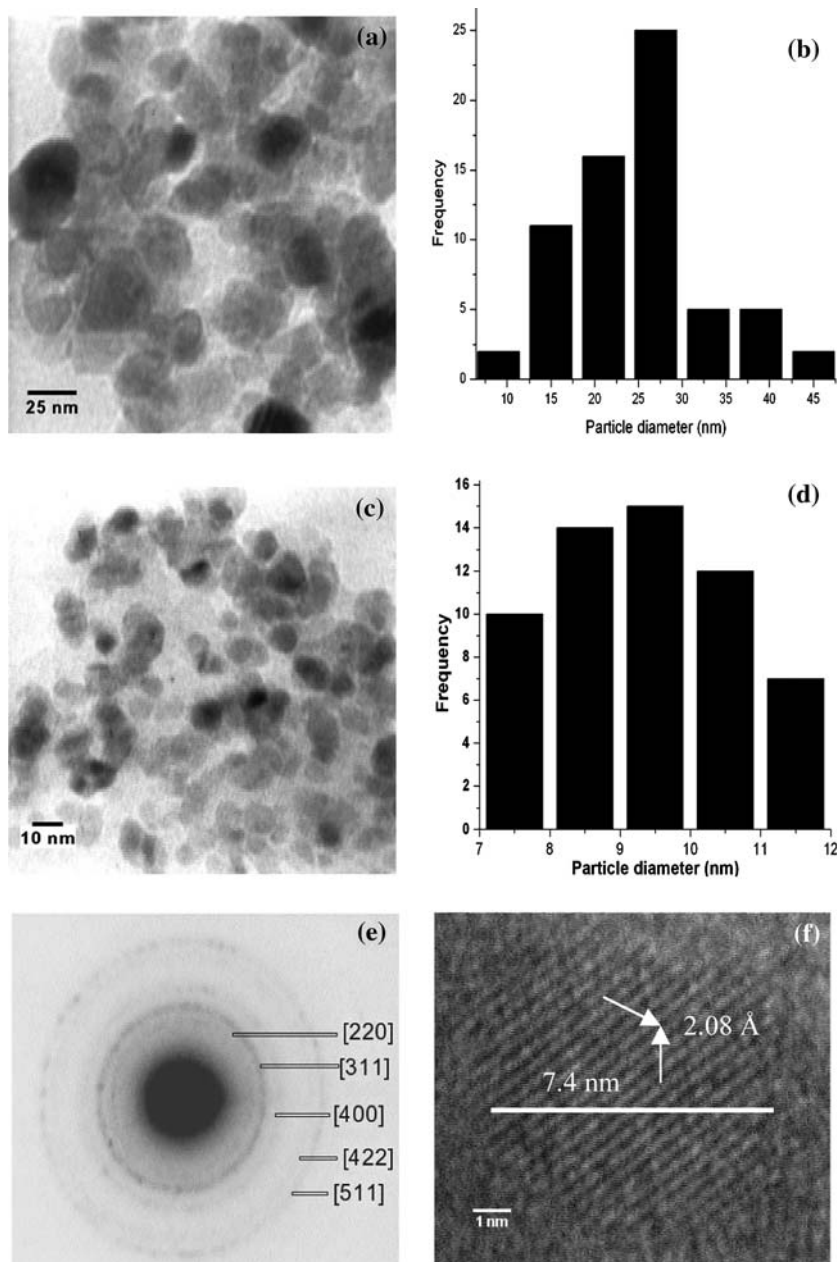
Sample ID	APTS: Fe_3O_4	Hydrodynamic diameter (nm)	C, H, N (%)	Crystallite size (nm)	Surface $-\text{NH}_2$ groups (nmol/mg)	Stability (h)
AS-0	0.0	206	—	11.0	0	1.0
AS-1	0.25	90	1.46, 0.31, 0.465	10.9	10.5	9.0
AS-2	0.50	79	3.21, 0.928, 0.88	10.0	18.3	16
AS-3	1.0	45.8	4.45, 1.32, 1.50	7.0	32.4	36
AS-4	1.5	190	6.72, 1.93, 1.74	7.0	15.4	6.0
AS-5	2.0	330	8.72, 2.74, 2.62	6.5	11.2	4.5

a single symmetric doublet. The parameters obtained are given in Table 2. The isomer shift matches well with that reported by Hartridge et al. [19] thereby suggesting that the sample is nano-crystalline magnetite. The value of the quadruple splitting is slightly larger which may be due to the extremely small size of the particles in the sample. When the temperature of the sample is lowered down to 20 K, a clear sextet (Fig. 4c) is obtained which confirms the presence of superparamagnetism in the sample at room temperature. A single sextet was fitted to the spectrum, which is essentially two closely overlapping sextets arising from the A and the B sites of the spinel lattice. The isomer

shift and the hyperfine field obtained matches well with that of magnetite. As mentioned, the slightly higher value of quadruple splitting is ascribed to the extremely small size of the sample.

The FTIR spectra of AS-3, AS-0 and APTS have been given in Fig. 5.1 and the corresponding band assignments are given in Table 3. In the FTIR spectrum of AS-3 absorption at $3,460\text{ cm}^{-1}$ and $1,625\text{ cm}^{-1}$ is due to O–H stretching and bending vibrations of physically adsorbed H_2O and surface –OH groups. Bands at $2,930\text{ cm}^{-1}$ and $2,853\text{ cm}^{-1}$ corresponds to – CH_2 – group of aminopropyl group attached to Si which are absent in AS-0. Bands at

Fig. 3 (a) Transmission electron micrograph of AS-0 (b) Particle size distribution (c) Transmission electron micrograph of AS-3 (d) Particle size distribution of AS-3 (e) SAED pattern of AS-3 (f) HRTEM of a single particle of AS-3 showing the lattice imaging of [400] plane



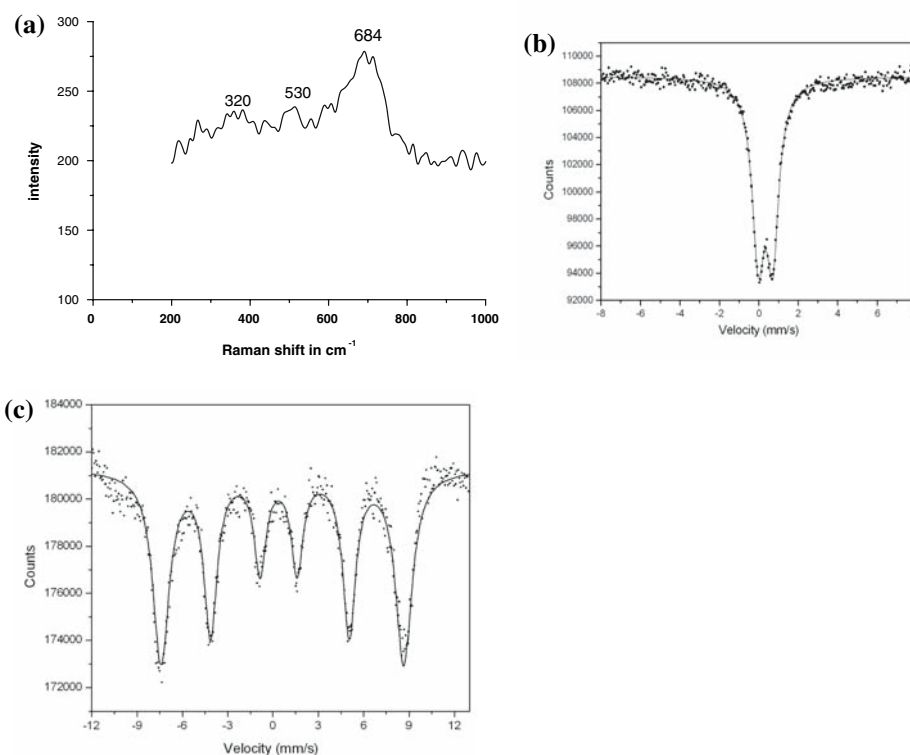
1,123 cm^{-1} and 1,023 cm^{-1} contributed by C–N and Si–O bonds are not observed in the uncoated sample AS-0. For bulk Fe_3O_4 samples the vibration for Fe–O bond appears at 570 cm^{-1} and 375 cm^{-1} . But in this case there is blue shift to 670 cm^{-1} and 570 cm^{-1} due to size reduction [20]. Contributions from $-\text{NH}_2$ group were probably overlapped with that of vibration bands associated with water. To verify the presence of surface amino groups Raman spectrum was recorded at very low laser power (0.008 mW at sample for 300 s integration time). The Raman spectrum shows two distinct peaks at 3,220 cm^{-1} and 3,490 cm^{-1} (Fig. 5b), which may be attributed to $-\text{NH}_2$ and $-\text{OH}$ peaks, respectively [21]. Thermal analysis (TG) further supports the presence of aminopropyl chain in the sample. Weight loss in APTS coated sample is in three stages (Fig. 6) which includes a small weight loss between 150 °C to 200 °C due to physically adsorbed water. Weight loss in between 300 °C and 400 °C is due to the loss of surface hydroxyl groups, which is present in both coated and uncoated samples (AS-0 and AS-3). Between 750 °C and 800 °C there is a small weight loss in AS-3, which is absent in uncoated sample AS-0, corresponds to the loss of organic moiety. This result shows that the APTS, which was used during precipitation of magnetite, forms a coating and is stable up to 750 °C. The density of the surface amine groups was measured using 4-nitrobenzaldehyde [22] and has been given in Table 1.

The magnetisation curves (Fig. 7) of both uncoated magnetite (AS-0) and silane coated magnetite (AS-3) show no hysteresis opening and are completely reversible at room temperature. Coercivity and remanence were not observed in both the samples, which indicate superparamagnetism. From M vs. $1/H$ plot the saturation magnetisation σ_s for uncoated magnetite is 56 e.m.u/g while that of coated particle is 41 e.m.u/g. Saturation magnetisation of bulk magnetite is 92 e.m.u/g. The decrease in saturation magnetisation of as prepared naked magnetite particles in comparison to bulk magnetite may be attributed to the decrease in particle size. It is reported in the literature that saturation magnetisation of ferrite nanoparticles decreases with decrease in particle size [12]. In addition, this decrease in saturation magnetisation may be due to different chemical composition on the surface like oxidation of Fe_3O_4 to Fe_2O_3 , surface effect such as nonlinearity of spins of magnetically inactive layer with the magnetic field. Also the discrepancy could be explained by the variation in synthetic methods employed, which can generate particles

Table 2 The Mössbauer parameters obtained for AS-3

Sample temp (K)	Isomer shift (mm/s)	Quadruple splitting (mm/s)	Hyperfine field (T)
300	0.33	0.73	–
20	0.48	0.26	498

Fig. 4 (a) Raman spectrum of AS-3 at 0.08 mW laser power (b) Mössbauer spectrum of AS-3 at room temperature (c) Mössbauer spectrum of the same at 20 K



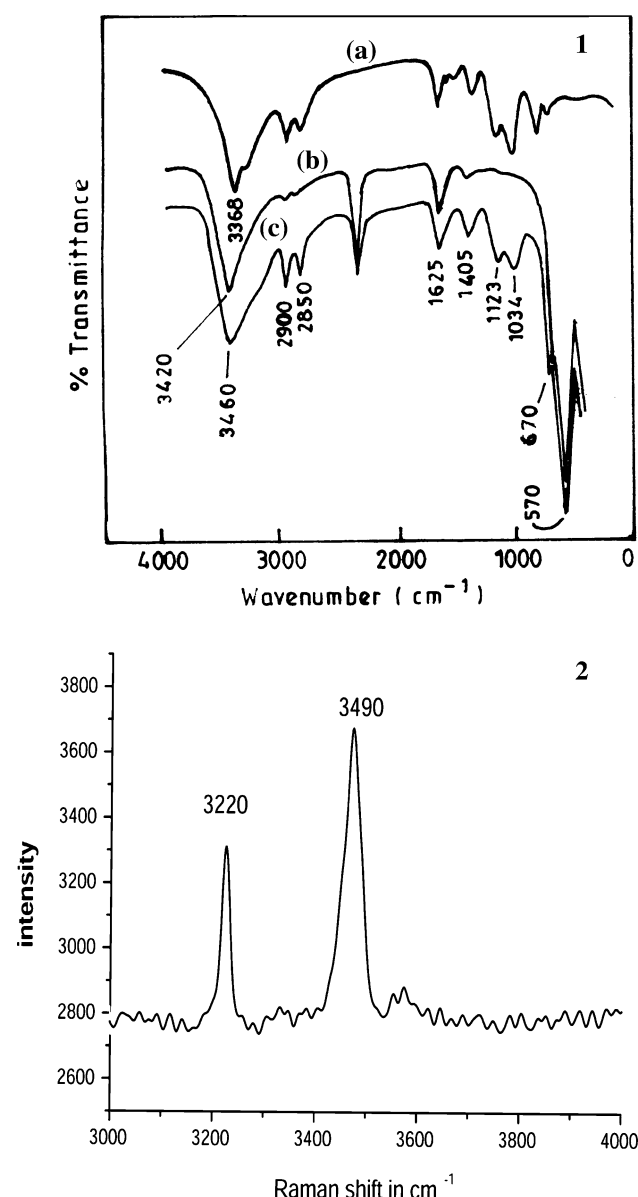


Fig. 5 (1) FTIR spectra of (a) APTS (b) AS-0 (c) AS-3 (2) Raman spectrum of AS-3 at 0.08 mW laser power

of different size. However, the saturation magnetisation value obtained here is consistent with the range of values reported in literature [23]. The saturation magnetisation of APTS coated magnetite particles (AS-0) is 41 e.m.u/g. This decrease in saturation magnetisation than naked

Table 3 Assignments of absorption bands in FTIR spectra

APTS (cm ⁻¹)	Fe ₃ O ₄ coated with APTS (AS-3) (cm ⁻¹)	Uncoated Fe ₃ O ₄ (cm ⁻¹)	Assignments
3368	—	—	ν^* NH...H
—	3420	3460	ν^* HO...H
—	—	—	ν^* O...H
2942	2900	—	ν^* ass CH...H
2874	2850	—	of -CH ₂
1625	1625	1625	ν^* bend O...H
1405	1405	—	δ^* C-OH
1123	1123	—	ν^* C...N
1070	1030	—	ν^* Si-O-Si
—	578	575	Fe-O-Fe
—	—	—	ν^* Fe-O

ν^* , Stretching vibration; ν^* bend, Bending vibration; δ^* , Deformation vibration; ν^* ass, Asymmetric stretching

as-prepared magnetite particles is an indication of formation of diamagnetic APTS coating contributes as a non-magnetic mass to the total sample volume, which is also supported by thermal analysis. Additionally a small decrease may be due to the detachment from dipole coupling due to well dispersion of APTS modified Fe₃O₄ particles. From slope of the magnetisation curve near $H = 0$, the magnetic particle diameter for both samples were calculated using the equation [24],

$$D_{\text{mag}} = \left[\frac{18kT (dM/dH)_0}{\Pi \rho M_s^2} \right]^{1/3}$$

where k is the Boltzmann constant, T is the absolute temperature, $[dM/dH]_0$ is the initial slope of the magnetisation curve near origin. ρ is the density of the Fe₃O₄ ($=5.24 \text{ g/cm}^3$) [25]. The particle sizes calculated for uncoated (AS-0) and coated (AS-3) samples are 19 nm and 6.5 nm, respectively.

TRITC conjugated avidin was coupled with magnetic nanoparticles through glutaraldehyde. The quantitative adsorption of proteins on amine functionalised magnetite particles was determined in terms of its coupling efficiency. Coupling efficiency was obtained by measuring OD at 280 nm for both pre-coupling and post-coupling solutions and using the formula $D = \text{Dilution factor}$ ($=13.3$).

$$\frac{[(OD_{280} \text{ Pre - Coupling Solution} \times D) - (OD_{280} \text{ Post - Coupling Solution} \times D)] \times 100}{OD_{280} \text{ Pre - Coupling Solution} \times D}$$

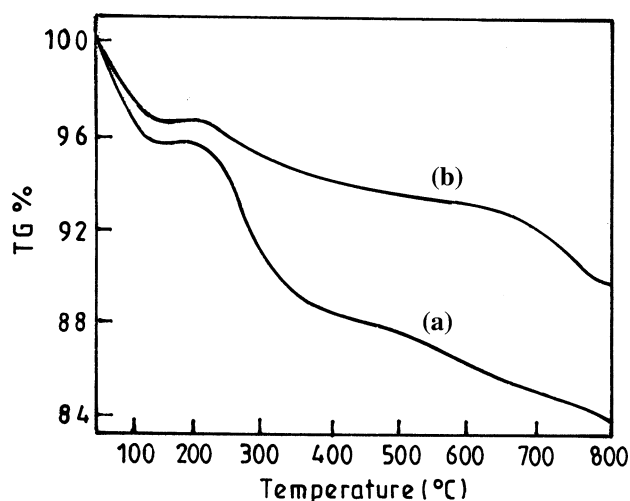


Fig. 6 TG curve of (a) AS-0 (b) AS-3

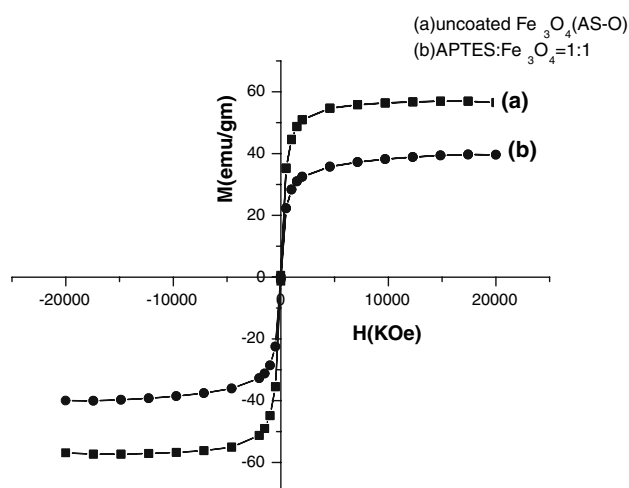


Fig. 7 Magnetisation curve of (a) AS-0 (b) AS-3

In this case coupling efficiency for TRITC conjugated avidin was found to be 73.5%. We have used TRITC conjugated avidin to visualise the conjugation under the fluorescent microscope (Fig. 8). Same approach can be taken to immobilise any other protein of interest like enzymes, antibodies etc. depending on the need. We have not found any binding with the uncoated magnetic nanoparticle. We have also immobilised a glycoprotein through glutaraldehyde with very high efficiency (82%) to these magnetic particles.

Conclusion

Superparamagnetic amine derivatised magnetite nanoparticles have been prepared in a single step at room temperature by simple co-precipitation method. The

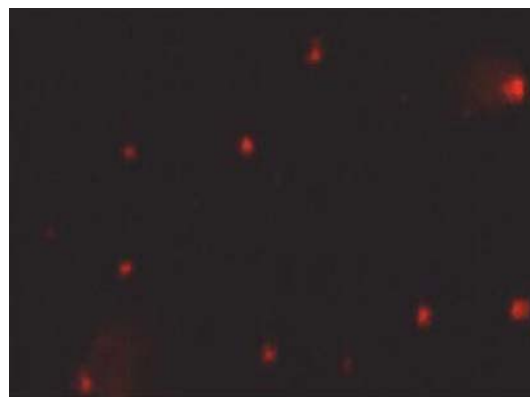


Fig. 8 Fluorescent micrograph of TRITC conjugated Avidin coated magnetite nanoparticles

aminosilane concentration influences particle size. Particles of mean average size 9 ± 0.5 nm with high density of surface free $-NH_2$ have been obtained when molar ratio of Fe_3O_4 :aminosilane was maintained as 1:1 in the reaction. Particle diameters calculated from different observations are reasonably in good agreement. Particles are highly hydrophilic and form stable aqueous suspension. Proteins, drugs and other bio-molecules can be attached through the surface amino group.

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Synthesis of Fe₃O₄/APTES/PEG diacid functionalized magnetic nanoparticles for MR imaging

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ABSTRACT

The magnetite (Fe₃O₄) nanoparticles (MNPs) coated with PEG diacid via covalent bonds were prepared for magnetic resonance imaging (MRI). The surface of MNPs was first coated with (3-aminopropyl) triethoxysilane (APTES) by a silanization reaction and then linked with PEG diacid via the reaction between –NH₂ and –COOH to form well-dispersed surface functionalized biocompatible MNPs. The obtained nanoparticles were characterized by X-ray powder diffraction (XRD), transmission electron microscopy (TEM), scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FT-IR), vibrating sample magnetometer (VSM) and thermogravimetry (TG). The MNPs had an average size of 20 nm and exhibited superparamagnetism and high saturation magnetization at room temperature. In addition, PEG-6000 diacid coated Fe₃O₄ nanoparticles were used to perform the MRI experiments on the living rabbits with VX2 malignant tumor, the results showed that these nanoparticles appear to be a promising vehicle for MR imaging.

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1. Introduction

In the last decade, nanoscaled magnetic particles have attracted much attention since magnetic nanoparticles have shown great potential applications in the field of in vitro and in vivo biomedicine [1], including cellular therapy in cell labeling, separation and purification [2], target-drug delivery [3–6], and hyperthermia treatment of cancers [7,8], as well as MRI (magnetic resonance imaging) [9–11].

MRI is a noninvasive technique routinely used in clinics for diagnostic imaging. It has become very important diagnostic modalities in hospitals [12,13]. The conventional, gadolinium compounds [14] have been used as T₁ contrast agents that cause positive contrast enhancement and provide a bright state in the image where the compounds are accumulated [15]. However, with the increasing use of paramagnetic gadolinium chelates for MRI, shortcomings of gadolinium chelates have been found, such as fast elimination

in tissue; non-specific distribution in vivo and limited effect in improving MR imaging.

Superparamagnetic Fe₃O₄ magnetic nanoparticles are suitable for MRI contrast since the MNPs can enhance the alterations of proton relaxation in the tissue microenvironment [16,17], and thus provide better MR imaging and longer life times in the bloodstream than Gd-chelate compounds [18,19]. However, relatively high toxicity of magnetic nanoparticles restricts the use of these materials to human beings [20]. Therefore, many approaches have been focused on the encapsulation of magnetic nanoparticles with biocompatible materials, such as dextran, chitosan, gelatin, poly(ethylene glycol) (PEG), PLGA, poly(D, L-lactide) (PLA), and poly(glycolide) (PGA), etc. because these materials have biocompatible and biodegradable properties as well as low toxicity [21,22]. Among these, PEG is a typical non-toxic, non-immunogenic, non-antigenic, and protein-resistant polymer [23], which is widely used in the pharmaceutical field, has attracted much attention. It is water soluble, inert in biological systems and does not influence cell viability. The PEG coating could enhance the compatibility between nanoparticles and aqueous medium, prevent particle surface from oxidation, reduce toxicity, and facilitate storage or transport, etc. In some research groups, magnetite/PEG composites were prepared by a

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one-step method and PEG was linked with MNPs through Van-der Waals force and hydrogen bond [24]. However, these bonds are not strong enough to prevent the aggregation among nanoparticles, which will further influence the properties of magnetic nanoparticles.

In the present study, we carried out chemical synthesis and characterization for PEG-functionalized magnetite (Fe_3O_4) nanoparticles. The preparation consists first magnetite particles synthesis by co-precipitation reaction of ferrous and ferric salts with NH_4OH , then the surface modification of these particles through the grafting of aminopropylsilane groups $(-\text{O})_3\text{Si}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{NH}_2$ and the coupling of PEG diacid via formation of covalent bonds which are bound to the particle surface. The morphology, structure and composition of PEG-functionalized magnetite nanoparticles were examined by IR, TEM, VSM, XRD and TG. Furthermore, the PEG-functionalized Fe_3O_4 MNPs were dispersed in water to form magnetic fluids (MFs). The New Zealand white rabbits were intravenously injected with MFs 20 mg/kg body weight. In vivo effect of magnetic targeting on the extent and selectivity of nanoparticle accumulation in tumors of rabbits harboring VX2 malignant tumor was quantified with MRI. Image analysis revealed that the as-prepared Fe_3O_4 MNPs were potential as MRI contrast agents to detect tumor.

2. Experimental

2.1. Materials

All chemicals used are analytical grade without further purification. Iron (III) chloride hexahydrate ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), iron (II) sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), aqueous ammonia (25 wt%), hydrazine hydrate ($\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$, 50 wt%), PEG-2000 ($\text{Mw} \approx 2000$), PEG-6000 ($\text{Mw} \approx 6000$), PEG-20000 ($\text{Mw} \approx 20000$), chlorhydric acid, Potassium permanganate (KMnO_4) were all purchased from the SCRC (Sinopharm Chemical Reagent Co., Ltd.). 3-Aminopropyltriethoxysilane (APTES) was a coupling agent. In all experiments, deionized water was used.

2.2. Poly(ethylene glycol) diacid preparation

Polyethylene glycol diacid ($\text{HOOC}-\text{PEG}-\text{COOH}$) was prepared by oxidizing poly(ethylene glycol) in the presence of KMnO_4 as oxidizer. In typical synthesis, the PEG (0.015 mol, molecular weight = 2000, 6000, 20000, respectively) and excess amount of KMnO_4 were dissolved in water (150 ml) by stirring. After reaction for 12 h at room temperature, the resulting MnO_2 precipitate was filtered out, and a colorless transparent solution was obtained. Hydrochloric acid was added to the solution until its pH decreased to about 2.0. Excess KMnO_4 , hydrochloric acid and hydrazine were removed by dialysis for 24 h using a membrane bag with a 1000 cut-off molecular weight. The solution was evaporated in a rotary evaporator at 70°C . The solid product was further dried in a vacuum oven at 100°C to removal all water.

2.3. Organosilane hydrolyze

10 ml of (3-aminopropyl) triethoxysilane (APTES) is placed into an aqueous solution of an acid ($\text{pH} = 4$) that acts as a catalyst. As shown in Fig. 1, the APTES is hydrolyzed, then a condensation reaction takes place to form a silane polymer [25]. In the hydrolysis reaction (Fig. 1), alkoxide groups $-\text{OC}_2\text{H}_5$ are replaced by hydroxyl groups (OH) to form reactive silanol groups, which condense with other silanol groups to produce siloxane bonds ($\text{Si}-\text{O}-\text{Si}$). Alcohol ($\text{C}_2\text{H}_5\text{OH}$) and water are produced as by-products.

2.4. Preparation of MNPs and coating procedure by silanization reaction

Synthesis of the Fe_3O_4 MNPs was based on our previous study [26,27]. In typical synthesis, 5 g of iron (III) chloride hexahydrate dissolved in 30 ml of deionized water was put into a three-necked flask equipped with a mechanical stirrer, and the 0.5 ml of hydrazine hydrate was added into the flask. After the mixture was stirred and mixed completely, 2 g of sulfate heptahydrate was added. After 15 min, sulfate heptahydrate was dissolved completely, and some dosage of ammonia solution was quickly dropped into the mixture with vigorous stirring under argon protection, followed by slowly dropping additional ammonia solution until the pH of the solution reached 10. Soon afterwards the solution was stirred for an additional 3 h in argon atmosphere at the temperature of 60°C . The slurry was washed repeatedly with distilled water. Then particles were magnetically separated from the supernatant and redispersed in aqueous solution at least three times, until obtaining pH 7. After that, the solution of silane polymer was added to 100 ml of the MFs in a 250 ml three-necked round-bottom flask. Under argon protection, the mixture was stirred for 5 h at 60°C . After cooling at room temperature, the product was washed with deionized water and ethanol for several times respectively, and then dried in vacuum at room temperature. By the above treatment, the surface of nanoparticles was modified with amino-silane. The resulting solid product was washed with distilled water and dried in the vacuum for 24 h.

2.5. PEG diacid coated MNPs

To synthesize PEG diacid-functionalized nanoparticles, the amino-silane coated magnetite particles were mixed with the PEG diacid solution (molecular weight = 2000, 6000, 20000, respectively) in the ratio of 1:2 and stirred overnight at 60°C under Ar protection. Then, the water-based MFs were prepared. A low ratio was preferred to avoid encapsulation of nanoparticles with a thicker layer of PEG diacid, based on electron microscopic examination of polymer-coated nanoparticles [28]. The as-prepared MFs were typically dialyzed against pure water for 2 days to remove all species with molecular weights less than 1000. The resulting solid particles were obtained by dried MFs in vacuum for further measurements.

2.6. Characterization

2.6.1. XRD

The bare and composite Fe_3O_4 MNPs were characterized respectively by X-ray powder diffraction (XRD) (D/Max-IIIC, Japan) using $\text{Cu K}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$). Distances between peaks were compared to the JCDPS 5-0664 of International Center for Diffraction Data to determine crystalline structures.

2.6.2. IR

Adsorption of functional groups on the surface of Fe_3O_4 MNPs was examined by A Nicolet Avatar 360 Fourier transform infrared spectroscopy (FT-IR). Fourier transform infrared (FT-IR) spectra were obtained using with KBr method.

2.6.3. TEM

The bare Fe_3O_4 , APTES/ Fe_3O_4 and PEG diacid/APTES/ Fe_3O_4 MNPs were observed by transmission electron microscopy (TEM, Hitachi H-600-II) with an acceleration voltage of 200 kV. The TEM

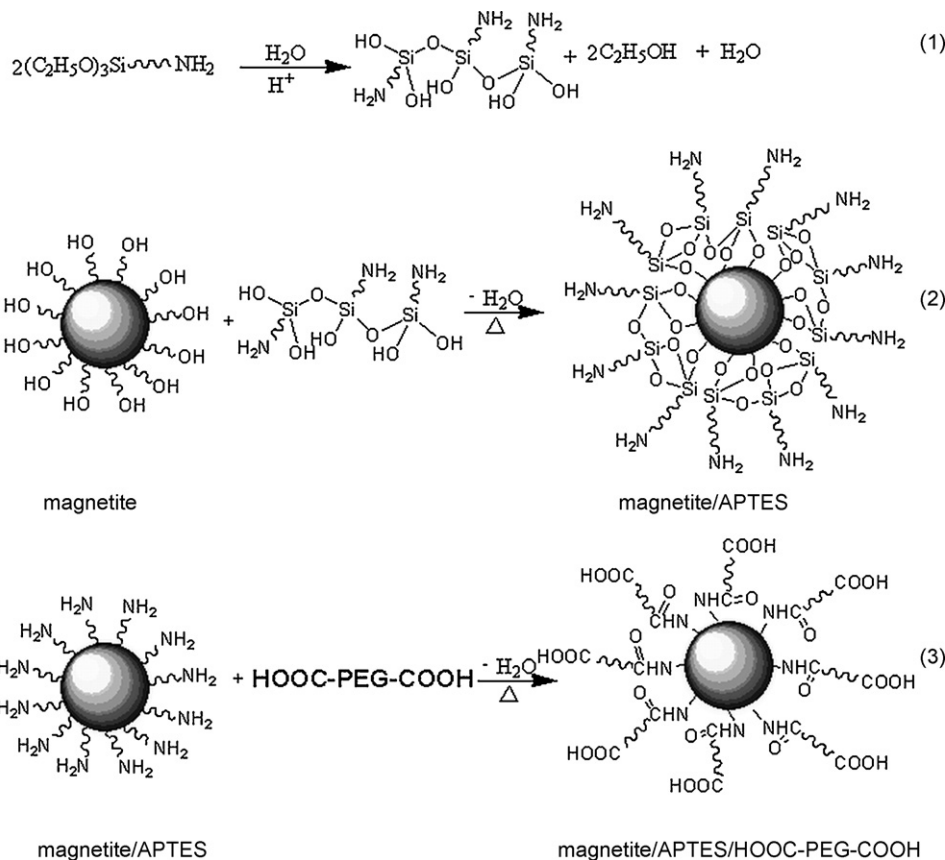


Fig. 1. Synthesis route for the PEG diacid coated magnetite nanoparticles: (1) simplified reaction of hydrolysis and condensation with production of silane polymer; (2) scheme of the simplified silanization reaction of APTES on the magnetite surface; (3) simplified reaction of HOOC-PEG-COOH on the magnetite surface after treated by APTES.

samples for analysis were prepared by dropping the dilute suspensions of powders onto the carbon-coated copper grids and let the solvent evaporate.

2.6.4. Size distribution

The size distribution of coated Fe_3O_4 MNPs in water was obtained using Malvern HPPS5001 laser particle-size analyzer.

2.6.5. TG

PerkinElmer TGA-7 was employed to perform the thermogravimetric analysis (TGA). Dried sample (1–5 mg) was placed in the TGA furnace and the measurements were carried out under nitrogen with a heating rate of $15^\circ\text{C}/\text{min}$ from 50°C to 600°C .

2.6.6. VSM

The magnetic properties of Fe_3O_4 MNPs were measured on a BHV-55 vibrating sample magnetometer (VSM).

2.6.7. MRI

MRI measurements were carried out on a Philips MR spectrometer with magnetic field intensity of 1.5 T. (Philips Medical Systems, Inc.). A volume coil was used for both transmission of radio frequency pulses and signal reception. The parameters for in vivo experiments were $\text{FOV}=160\text{ mm}\times 160\text{ mm}$, matrix size = 256×256 , slice thickness = 0.6 mm , $\text{TE}=15.66\text{ ms}$, $\text{TR}=660\text{ ms}$, and $\text{NA}=2$ for T_2^* -weighted MR images.

3. Results and discussion

3.1. Oxidation percent of PEG

The percent of PEG diacid was determined using NaOH titration method. By calculation, it was found that the oxidation percent of PEG was 100%, which was also proved by Fourier-transform infrared (FTIR) spectroscopy. Compared with FTIR spectroscopy of PEG (Fig. 2a), the adsorption peaks at around 1736 and 3249 cm^{-1} were observed in Fig. 2b after oxidation process [29]. The characteristic

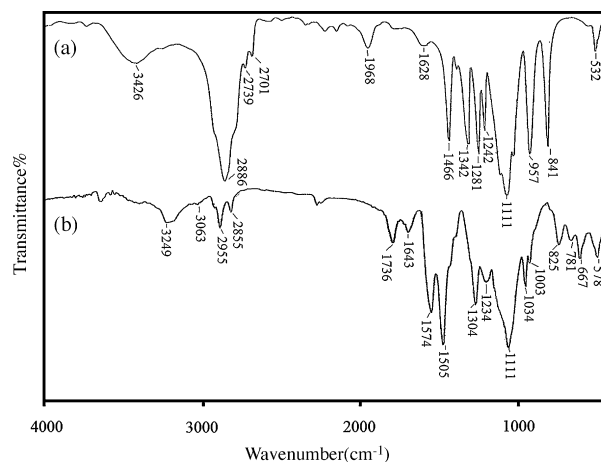


Fig. 2. FTIR spectra of (a) PEG-6000; (b) PEG-6000 diacid.

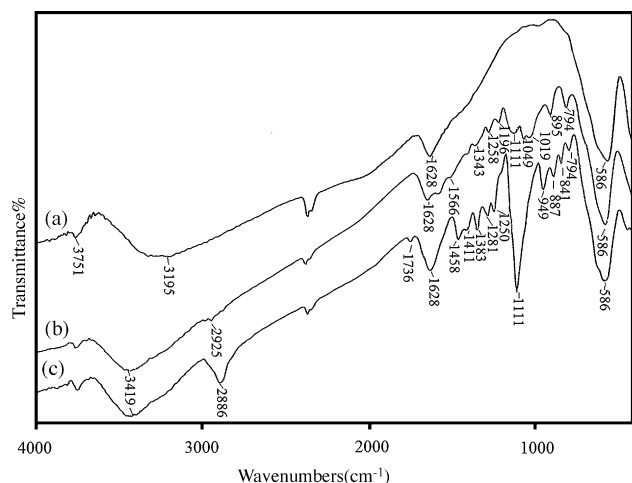


Fig. 3. FTIR spectra of (a) bare Fe_3O_4 MNPs; (b) Fe_3O_4 MNPs treated by APTES; (c) Fe_3O_4 MNPs coated by PEG diacid.

absorption peaks of 1736 and 3249 cm^{-1} were corresponding to C=O and COOH bonds, respectively, which confirmed formation of carboxylic group in the oxidation of PEG.

3.2. IR

The surface chemical structure of Fe_3O_4 MNPs was characterized by Fourier-transform infrared (FTIR) spectroscopy. Fig. 3b shows the APTES-coated Fe_3O_4 MNPs. The adsorption peaks at around 563 or 580 cm^{-1} were the characteristic absorption of Fe–O bond which confirmed the presence of magnetite nanoparticles [30,31]. The covalent anchoring of aminopropylsilane groups is mainly due to their self-polycondensation leading to a highly cross-linked polysiloxane film entrapping each magnetite nanoparticle. But the presence of Fe–O–Si bonds cannot be seen in the FTIR spectrum because it appears at around 584 cm^{-1} and therefore overlaps with the Fe–O vibration of magnetite nanoparticles [32–33]. So, the adsorption of silane polymer onto the surface of magnetite particles was confirmed by bands at 1111, 1049 and 1018 cm^{-1} which correspond to the Si–O–H and Si–O–Si groups. The absorption bands at 895 and 794 cm^{-1} are due to the stretching of Si–O–H and vibrations of OH on the surface of magnetite. Furthermore, the two broad bands at 3419 and 1628 cm^{-1} can be ascribed to the N–H stretching vibration and NH_2 stretching [34,35]. All the figures indicate that the coupling agent APTES had been introduced onto the Fe_3O_4 MNPs surface.

Fig. 3c shows the PEG diacid coated Fe_3O_4 MNPs. The adsorption peaks at 1736.5 cm^{-1} were the characteristic absorption of C=O stretching, which indicated that the formation of carboxyl group on the surface of iron oxide nanoparticles. Moreover, the characteristic bands of amorphous PEG at 2886.4 cm^{-1} (C–H asymmetric stretching) and 1111.4 cm^{-1} (C–O–C stretching) further support the attachment of PEG diacid onto the Fe_3O_4 MNPs surface.

3.3. X-ray diffraction

The X-ray diffraction patterns of pure magnetite, amino-silane treated magnetite and PEG diacid coated magnetite samples were

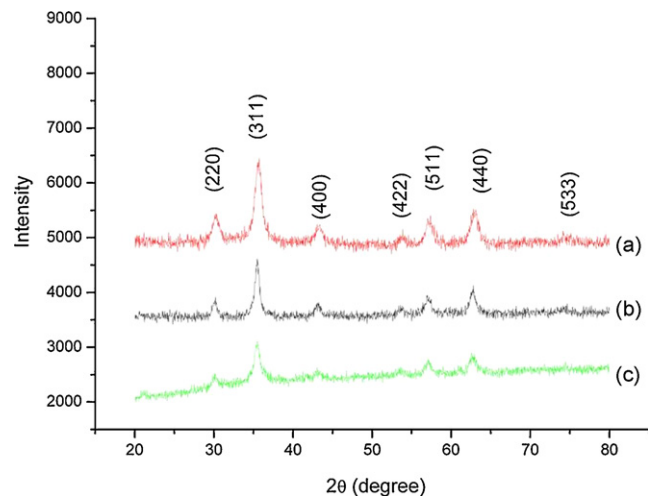


Fig. 4. XRD patterns of (a) Fe_3O_4 MNPs; (b) Fe_3O_4 MNPs treated by APTES; (c) Fe_3O_4 MNPs coated by PEG diacid.

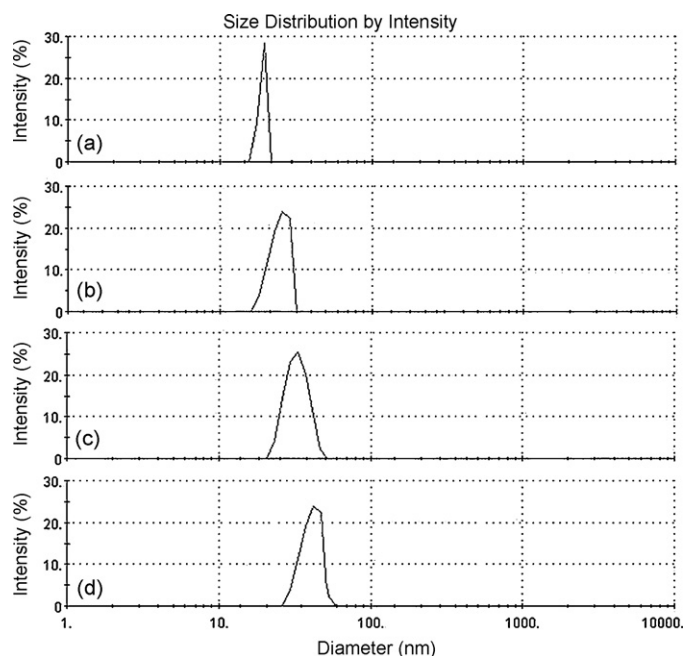


Fig. 5. Size distribution of magnetite/APTES and magnetite/PEG nanocomposites obtained by using particle-size analyzer (a) Fe_3O_4 nanoparticles treated by APTES; (b) Fe_3O_4 nanoparticles coated by PEG diacid 2000; (c) Fe_3O_4 nanoparticles coated by PEG diacid 6000; (d) Fe_3O_4 nanoparticles coated by PEG diacid 20,000.

shown in Fig. 4a–c, respectively. Fig. 4a is the spectrum of Fe_3O_4 MNPs prepared by co-precipitation. The peaks agree with the standard Fe_3O_4 (cubic phase) XRD spectrum. The crystallographic parameters calculated using software provided by Panalytical company (Holland) are: $a=b=c=8.3740\text{ Å}$, $\alpha=\beta=\gamma=90.0000^\circ$, they are indexed to the cubic spinel phase of Fe_3O_4 . The average crystallite size D was calculated using the Debye–Scherrer formula $D=K\lambda/(\beta\cos\theta)$, where K is Sherrer constant, λ the X-

Table 1

Particles size of magnetite/APTES and magnetite/PEG nanocomposites obtained by using laser particle-size analyzer

Sample	Fe_3O_4 /APTES	Fe_3O_4 /PEG 2000	Fe_3O_4 /PEG 6000	Fe_3O_4 /PEG 20,000
$d(0.99)_{\text{nm}}$	30.4	40.3	49.8	60.2

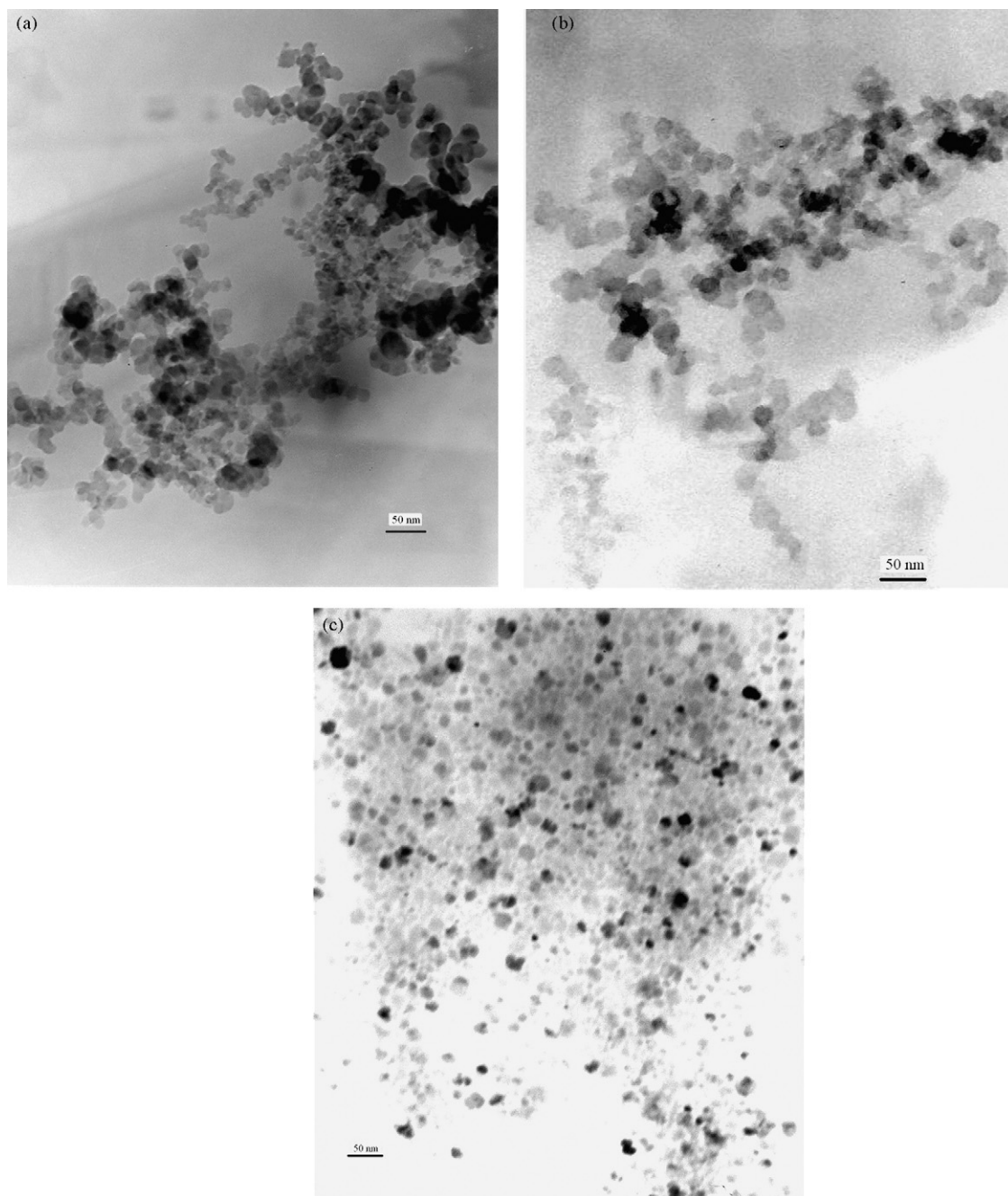


Fig. 6. TEM of (a) Fe_3O_4 MNPs; (b) Fe_3O_4 MNPs treated by APTES; (c) Fe_3O_4 MNPs coated by PEG diacid.

ray wavelength, β the peak width of half-maximum, and θ is the Bragg diffraction angle. The crystallite size thus obtained from this equation was found to be about 19 nm, which is basically in accordance with the transmission electron micrographs.

Same characteristic peaks can also be found in Fig. 4b and c, which illustrated that the characteristic peaks did not change but only the peak intensity and width after coated with amino-silane and PEG diacid, showing the crystalline structure of the modified nanoparticles was not varied. In addition, it indicated that the coating process was not sufficient to cause growth and therefore affect the physical properties of the magnetite particles [36]. No peaks of impurities were detected.

3.4. Size distribution of nanoparticles/aggregates in MFs

In order to study the effect of PEG length on the size distribution of nanoparticles/aggregates in MFs, a series of PEG diacid with molecular weight (M) of 2000, 6000 and 20,000, respectively, were coupled to the surface of the nanoparticles, as shown in Fig. 5. From the figure, we found that the size of nanocomposites increased gradually with the increasing molecular weight of PEG (Table 1). This phenomenon illuminated the longer chains of PEG are more apt to entangle with magnetite nanoparticles, which thickens the coating layer. By comparing with the TEM image, the size of nanocomposites obtained by dynamic light scattering was much larger. This can be explained that Fe_3O_4 MNPs do not exist as individual particles and some primary particles aggregated due

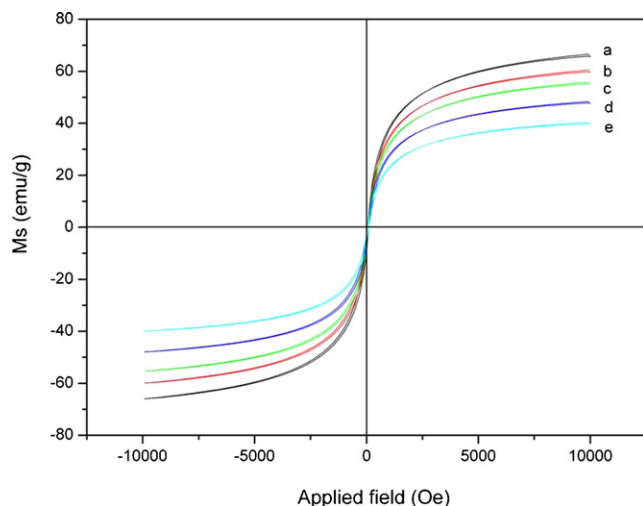


Fig. 7. Magnetization curves obtained by VSM at room temperature of (a) Fe_3O_4 MNPs; (b) Fe_3O_4 MNPs treated by APTES; (c) Fe_3O_4 MNPs coated by PEG diacid 2000; (d) Fe_3O_4 MNPs coated by PEG diacid 6000; (e) Fe_3O_4 MNPs coated by PEG diacid 20,000.

to the magnetism of nanoparticles and the entanglement of long chains of PEG.

3.5. Transmission electron microscopy

The particles size and morphology of magnetite particles, amino-silane treated magnetite and PEG diacid coated magnetite particles were investigated by TEM, as shown in Fig. 6a–c. Particles with an approximate spherical shape and an average diameter of 20 nm were observed. This result was in accordance with that of X-ray diffraction in Section 3.3. Due to large specific surface area and high surface energy, some bare nanoparticles (Fig. 6a) were aggregated. While after being coated with PEG diacid, the nanoparticles were well dispersed, as shown in Fig. 6c. Therefore, PEG diacid coating reduced the aggregation and improved the particle dispersion.

3.6. Magnetic properties of Fe_3O_4 MNPs

The magnetic properties of Fe_3O_4 MNPs were characterized by vibrating sample magnetometer (VSM). Fig. 7 shows the typ-

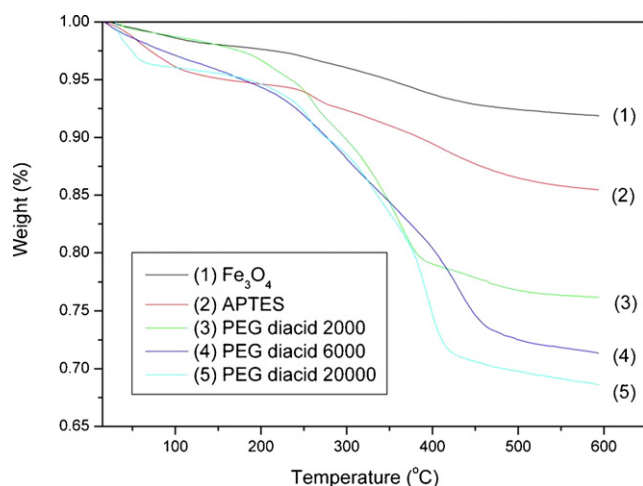


Fig. 8. Weight loss curves of (1) Fe_3O_4 MNPs; (2) Fe_3O_4 MNPs treated by APTES; (3) Fe_3O_4 MNPs coated by PEG diacid 2000; (4) Fe_3O_4 MNPs coated by PEG diacid 6000; (5) Fe_3O_4 MNPs coated by PEG diacid 20,000.

Table 2

MNPs content and weight ratio of iron to PEG

Sample	Magnetic content (%)	Weight ratio of iron to PEG
PEG 2000	76.18	1:0.27
PEG 6000	71.34	1:0.85
PEG 20,000	68.59	1:0.99

ical room temperature magnetization curves of bare Fe_3O_4 MNPs (Fig. 7a), APTES-coated Fe_3O_4 MNPs (Fig. 7b), and PEG diacid coated Fe_3O_4 MNPs with different molecular weight (M) 2000 (Fig. 7c), 6000 (Fig. 7d) and 20,000 (Fig. 7e), respectively. As shown in this figure, the saturation magnetization (M_s) of samples b–e is 59.5, 55.0, 47.6 and 39.7 emu/g, respectively. All the samples show a typical superparamagnetic behavior. However, the saturation magnetization of the nanoparticles is 65.6 emu/g (Fig. 7a), which is significantly smaller than that of bulk magnetite. The reduction of measured saturation magnetization is due to rather small size of the Fe_3O_4 MNPs and the added mass of the monolayer which was nonmagnetic on them [37]. In addition, it was found that with the increase of the molecular weight of PEG diacid, the saturation magnetization became lower. This phenomenon may be explained that the high molecular weight of PEG diacid thickens the coating layer, which lowers the content of Fe_3O_4 . However, we could not exclude the oxidation of magnetite nanoparticles during the process of reaction, which leads to the formation of some non-magnetic iron oxide.

3.7. Thermogravimetric analysis

The magnetite contents of amino-silane treated magnetite and PEG diacid coated magnetite were measured through the TGA runs in the condition of nitrogen atmosphere at the heating rate of $15^\circ\text{C}/\text{min}$. Fig. 8 illustrates the TGA curves, depicting the variations of the residual masses of the samples with temperature. The organic materials and magnetite of the samples are completely burned to generate gas products and converted into iron oxides at the elevated temperature, respectively. The magnetite amounts of the samples can be estimated from the residual mass percentages (see Table 2). From Fig. 8 (2), thermogravimetric chart of amino-silane Fe_3O_4 nanoparticles pointed that the magnetite contents is about 85.46%. As shown in this Fig. 8(3–5), the first weight loss stage (below 130°C) can be ascribed to the evaporation of water molecules in the polymer matrix, while the other stage beginning at about 220°C was due to the decomposition of PEG diacid. The PEG diacid coated magnetite nanoparticles with higher molecular weight of PEG would have the smaller percentage of the residual mass.

3.8. MRI

To examine the prepared Fe_3O_4 MNPs potential as MRI contrast agent to detect malignant tumor, the PEG-6000 coated Fe_3O_4 MNPs were chosen to perform the MRI experiments on a living rabbit. In these studies, the rabbit was a gift from The First Affiliated Hospital of Soochow University. A piece of VX2 malignant tumor was inoculated subcutaneously in rabbit at the lateral side of the back.

The rabbit was intravenously injected with 4 ml MFs (20 mg/kg body weight). T_2^* -weighted MR images acquired before and at different times after intravenous injection are shown in Fig. 9(a–e). In comparison with Fig. 9a (before injection), 30 min after the injection, the signals of the tumor (indicated by closed red dotted lines) decrease. The signal continued to decrease for another 2 h, this indicates that the Fe_3O_4 MNPs were accumulated in malignant tumor tissues. The reason for this phenomenon can be explained that in the comparison with benign malignant, one of the bio-

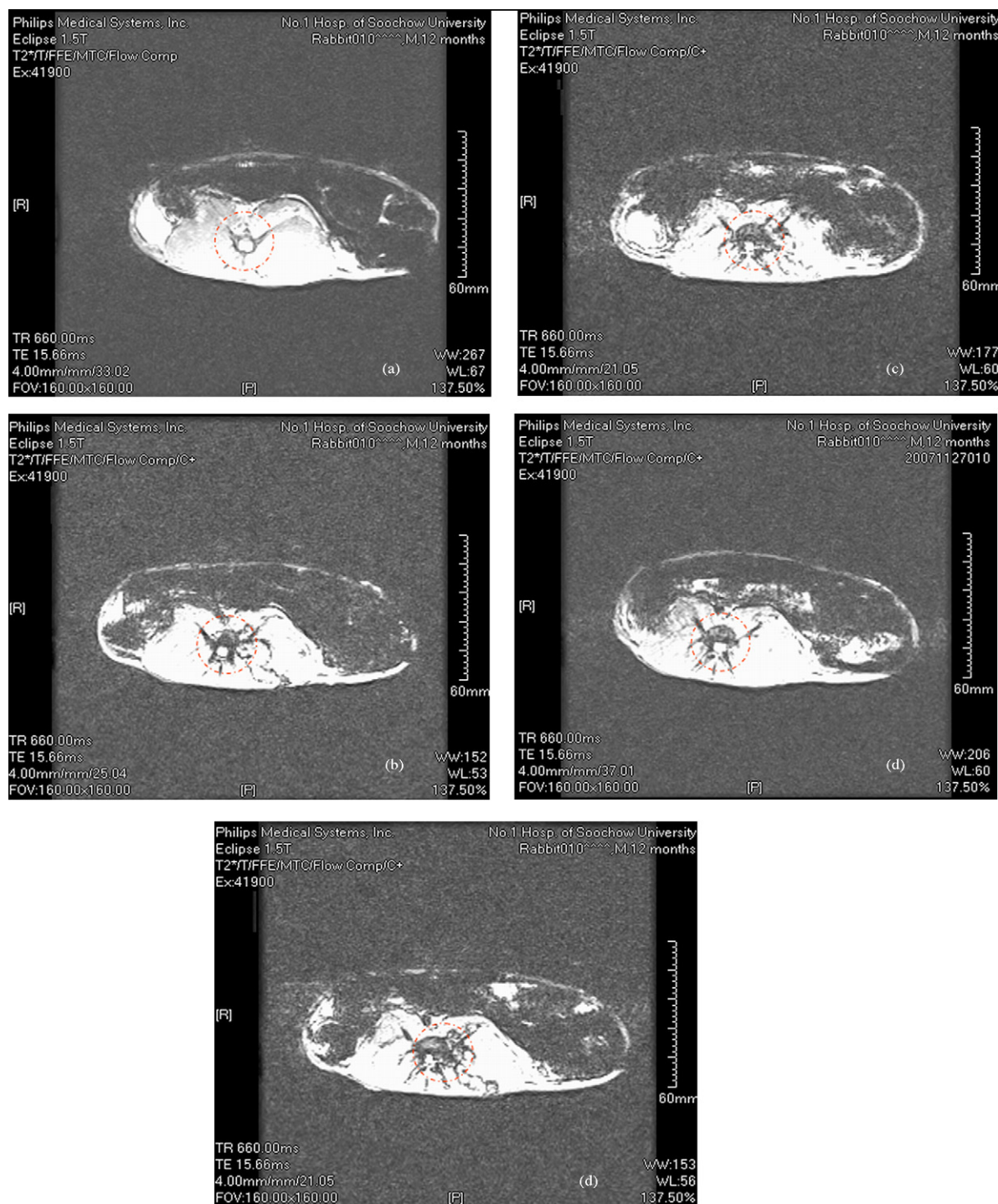


Fig. 9. T₂-weighted MR images acquired before (a) and at different times after the injection of Fe_3O_4 MNs (b: 30 min, c: 60 min, d: 90 min, e: 120 min).

logical characteristics of malignant tumor is angiogenesis on the effect of angiogenic growth factors and existed abundance blood supply in malignant tumor. The pathogenesis, development and metastasis of malignant tumor are closely related to angiogenesis in tumor, blood supply and microcirculation. The higher degree of malignancy, the higher degree of microvascular permeability is in the tumor. Thus, the Fe_3O_4 MNPs can easily enter into malignant tumor tissues through microvessel, and the MR signal of the tumor decreased. From that the malignant tumor can easily be identified. Also, the MRI experiments show that the Fe_3O_4 MNPs prepared

by the current synthetic approach possess good biocompatibility, which enables the Fe_3O_4 nanoparticles to circulate in the blood stream for a very long time.

4. Conclusions

In summary, by a two-step method through co-precipitation reaction of ferrous and ferric salts with NH_4OH , biocompatible magnetite nanoparticles were successfully synthesized by surface modification through the grafting of aminopropylsilane group and

the coupling of oxidized PEG via formation of covalent bond to form well-dispersed surface functionalized magnetite nanoparticles. Experimental results imply that PEG diacid is covalently modified on the surface of Fe_3O_4 particles via at least one of their carboxylic groups, leaving the second carboxylic group available for further chemical reaction. The molecular weight of PEG diacid has important effect on the size, morphology, coating efficiency, and magnetic property of magnetite nanoparticles. In vivo applications have demonstrated that the as-prepared Fe_3O_4 can potentially be used as effective MRI contrast agents for cancer diagnosis.

Acknowledgments

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INFORME DE BÚSQUEDA DE PATENTES PARA EL RADICADO 14-026546-00006-0000						
CRITERIOS PARA LA BÚSQUEDA						
Palabras clave utilizadas		Alkoxysilane magnetic nanoparticles				
CLASIFICACIONES INTERNACIONALES UTILIZADAS PARA LA BÚSQUEDA		H01F 1/00				
BASES DE DATOS CONSULTADAS.						
Google patents		Espacenet		Science Direct		Patbase
INFORMES DE EXÁMENES REALIZADOS EN OTRAS OFICINAS						
Oficina	Fecha del examen o de reporte de búsqueda de documentos	Anterioridades encontradas para el examen o reportados en el examen de búsqueda(relacionarlos todos)	Reiv. afectadas	RELEVANCIA (requisito que afecta)	FUERON TENIDOS EN CUENTA PARA EL EXAMEN EN LA OFICINA COLOMBIANA	
					SI	NO
EUROPEA	25/09/13	Synthesis of Fe ₃ O ₄ /APTES/PEG diacid functionalized magnetic nanoparticles for MR imaging	1-14	Nivel Inventivo	X	
		A simple synthesis of amine-derivatised superparamagnetic iron oxide nanoparticles for bioapplications	15-23, 26 y 27	Novedad	X	
		Biocompatible and bioactive gum Arabic coated iron oxide magnetic nanoparticles				X
		Multifunctional magnetite and silica-magnetite nanoparticles: Synthesis, surface activation and applications in life sciences				X
USPTO	08/06/15	US5695900				X
		US2004265233				X
		Yamamura et al., J. Magnetism Magnetic Materials, 2004, 279, 210-217				X
DOCUMENTOS ADICIONALES ENCONTRADOS POR EL EXAMINADOR DE ACUERDO A LOS CRITERIOS DE BÚSQUEDA.						
Synthesis, characterisation and application of silica-magnetite nanocomposites						
Progress in Solid State Chemistry 34 (2006) 237e247. Magnetic nanoparticle design for medical applications						
Langmuir, Vol. 23, No. 3, 2007. Silica- and Alkoxysilane-Coated Ultrasmall Superparamagnetic Iron Oxide Particles: A Promising Tool To Label Cells for Magnetic Resonance Imaging						
BÚSQUEDA DE SECUENCIAS						
Número de depósito / patente y N° de secuencia % de identidad						

SEQ ID NO (de la solicitud)	NCBI	The Lens	EMBL-EBI	DDJB	Genome Quest
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☐ Magnetic nanoparticles in MR imaging and drug delivery Review Article
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Journal of Magnetism and Magnetic Materials, Volume 277, Issues 1–2, June 2004, Pages 165-174
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Journal of Magnetism and Magnetic Materials, Volume 322, Issue 2, January 2010, Pages 179-185
Franja Šulek, Miha Drofenik, Maja Habulin, Željko Knez
AbstractPDF (361 K)

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Materials Science and Engineering: B, Volume 97, Issue 2, 25 January 2003, Pages 129-134
C. Calzer, C. Savli, M. Popovici
AbstractPDF (191 K)

☐ Cobalt-silica magnetic nanoparticles with functional surfaces Original Research Article
Journal of Magnetism and Magnetic Materials, Volume 293, Issue 1, May 2005, Pages 162-170
Michael L. Vadala, Michael A. Zalich, David B. Fuls, Tim G. St. Pierre, James P. Dailey, Judy S. Riffle
AbstractPDF (210 K)

☐ Asialoglycoprotein receptor-targeted superparamagnetic iron oxide nanoparticles Original Research Article
International Journal of Pharmaceutics, Volume 360, Issues 1–2, 6 August 2008, Pages 197-203
Guifang Huang, James Diakur, Zhenghe Xu, Leonard I. Wiebe
AbstractPDF (665 K)

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1) Family number: 30337175 (US6183658B)SimilarTranslateFull-textStatusCitations

Title: [EN] PROCESS FOR PREPARING AGGLOMERATE-FREE NANOSCALAR IRON OXIDE PARTICLES WITH A HYDROLYSIS RESISTANT COATING

Abstract:

Source: US6183658B [EN] A process for preparing an agglomerate-free suspension of nanosize iron-containing oxide particles coated in a stable manner comprises preparing an aqueous suspension of nanosize iron-containing oxide particles which are partially or completely in the form of agglomerates; adding a trialkoxysilane and a water-miscible polar organic solvent which has a boiling point at least 10 degrees centigrade higher than the boiling point of water; treating the resulting suspension with ultrasound until at least 70 percent of the particles present are in the range of plus or minus 20 percent of the average particle diameter; removing the water by distillation under the action of ultrasound; and separating the non-disintegrated agglomerates.

Classifications:

Classification Explorer

International (IPC 8-9): C01G49/00 C01G49/02 C09C1/24 C09C3/00 C09C3/12 C09D17/00 H01F1/34 (AdvancedInvention); C01G49/00 C09C3/12 C09D17/00 (AdvancedNon-invention); C01G49/00 C01G49/02 C09C1/22 C09C3/00 C09C3/12 C09D17/00 H01F1/12 (CoreInvention)

International (IPC 1-9): C01G49/00 C01G49/02 C09C1/24 C09C3/12 C09D17/00 H01F1/34

CPC: Y10T428/2995 B82Y30/00 C01G49/02 C01P2004/64 C01P2006/80 C01P2006/90 C09C1/24 C09C3/006

European: B82Y30/00 C01G49/02 C09C1/24 C09C3/00P M01P4/64 M01P6/80 M01P6/90

US: 106/456 106/460 252/62.52 252/62.54 252/62.56 427/220 427/600 427/601 428/405

Family:

Family Explorer

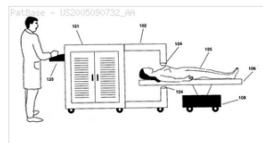
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CA2249609 AA	19980922	CA19972249609	19970409	
CA2249609 C	20060613	CA19972249609	19970409	
CN1083473 C	20020424	CN19971093415	19970409	
CN1214716 A	19990421	CN19971093415	19970409	

4) Family number: 31868729 (US2005090732A)

Similar • Translate • Full-text • Status • Citations

Title: [EN] Therapy via targeted delivery of nanoscale particles

Abstract: Source: US2005090732A [EN] Disclosed are compositions, systems and methods for treating a subject's body, body part, tissue, body fluid cells, pathogens, or other undesirable matter involving the administration of a targeted thermotherapy that comprises a bioprobe (energy susceptible materials that are attached to a target-specific ligand). Such targeted therapy methods can be combined with at least one other therapy technique. Other therapies include hyperthermia, direct antibody therapy, radiation, chemo- or pharmaceutical therapy, photodynamic therapy, surgical or interventional therapy, bone marrow or stem cell transplantation, and medical imaging, such as MRI, PET, SPECT, and bioimpedance. The disclosed therapies may be useful in the treatment of a variety of indications, including but not limited to, cancer of any type, such as bone marrow, lung, vascular, neuro, colon, ovarian, breast and prostate cancer, epitheloid sarcomas, AIDS, adverse angiogenesis, restenosis, amyloidosis, tuberculosis, cardiovascular plaque, vascular plaque, obesity, malaria, and illnesses due to viruses, such as HIV.



Classifications: [Classification Explorer](#)

International (IPC 8-9): A61B5/055 A61K41/00 A61K45/00 A61K51/00 A61N1/40 A61N2/00 A61N2/02 A61N5/06 A61N5/10 A61P25/28 A61P3/04 A61P31/06 A61P31/12 A61P31/18 A61P33/06 A61P35/00 A61P43/00 A61P9/00 G01T1/161 H01F1/00 (Advanced/Invention);

A61N5/04 A61N5/06 A61N5/10 (Advanced/Non-invention);

A61B5/055 A61K41/00 A61K45/00 A61K51/00 A61N1/40 A61N2/00 A61N5/06 A61N5/10 A61P25/00 A61P3/00 A61P31/00 A61P33/00 A61P35/00 A61P43/00 A61P9/00 G01T1/00

H01F1/00 (Core/Invention);

A61N5/02 A61N5/06 A61N5/10 (Core/Non-invention);

A61N (Subclass/Invention)

International (IPC 1-7): A61B5/055 A61N

CPC: A61N1/406 A61N2/002 A61N2/008 A61N5/04 A61N5/062 A61N5/10

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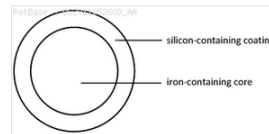
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9) Family number: 43503830 (US2011052609A)

Similar • Translate • Full-text • Status • Citations

Title: [EN] MAGNETIC TRANSDUCERS

Abstract: Source: US2011052609A [EN] Embodiments herein relate to the production of biocompatible magnetic nanoparticles with a high SAR-value which produce a large amount of heat when exposed to an alternating magnetic field. The produced heat can be used among others for therapeutic purposes, in particular for combating cancer.



Classifications: [Classification Explorer](#)

International (IPC 8-9): A61K31/7088 A61K33/26 A61K39/395 A61K41/00 A61K45/00 A61K45/06 A61K47/02 A61K48/00 A61K9/14

A61P19/02 A61P29/00 A61P31/04 A61P31/10 A61P31/12 A61P35/00 A61P43/00 A61P7/02 B01J19/10 B82B1/00 B82Y30/00 B82Y40/00 C01G

C01G49/00 C01G49/02 C01G49/06 C01G49/08 C09C1/24 (Advanced/Invention);

A61K47/02 A61P35/00 B01J19/10 C01G49/02 (Advanced/Non-invention);

A61K41/00 B01J19/10 C01G49/02 C09C1/22 (Core/Invention);

C01G (Subclass/Invention)

International (IPC 1-7): C01G49/02

CPC: A61K9/143 A61K33/26 A61K45/06 A61K41/0052 B82Y30/00 C01G49/02 C01G49/06 C01G49/08 C01P2004/42 C01P2004/64 C09C1/24

European: A61K41/00U B82Y30/00 C01G49/02 C01G49/06 C01G49/08 C09C1/24 M01P4/42 M01P4/64

US: 424/178.1 424/178.100P 424/490 424/646 424/646S 607/88 607/96

Family: [Family Explorer](#)

10) Family number: 43611323 (US2011223255A)

Similar • Translate • Full-text • Status • Citations

Title: [EN] IMPLANTABLE PRODUCTS COMPRISING NANOPARTICLES

Abstract: Source: US2011223255A [EN] The present disclosure relates to nanoparticle-containing implantable and preferably biodegradable medical products and their use for the thermotherapeutic after-treatment after surgical removal of tumors and cancerous ulcers.

Classifications: [Classification Explorer](#)

International (IPC 8-9): A61F13/00 A61F7/00 A61K A61K31/00 A61K31/33 A61K31/337 A61K31/395 A61K31/407 A61K31/704 A61K33/26 A61K38/16 A61K47/02 A61K47/30 A61K47/48 A61K9/00

A61K9/06 A61K9/14 A61K9/50 A61K9/51 A61K9/70 A61L15/42 A61L17/00 A61L31/00 A61L31/08 A61L31/14 A61P35/00 B82B1/00 B82Y5/00 (Advanced/Invention);

A61K9/06 A61K9/50 A61K9/70 A61L31/08 A61P41/00 A61P43/00 B82Y5/00 (Advanced/Non-invention);

A61F13/00 A61K31/00 A61K47/48 A61K9/00 A61K9/06 A61K9/50 A61K9/51 A61K9/70 A61L31/00 A61L31/08 (Core/Invention);

A61K (Subclass/Invention)

CPC: A61K9/0024 A61K9/70 A61K9/7015 A61K31/00 A61K41/0028 A61K41/0052 A61L15/42 A61L27/446 A61L27/54 A61L27/56 A61L2300/416 A61L2300/604 A61L2300/80

European: A61K31/00 A61K41/00M A61K41/00U A61K9/00M5D A61K9/70 A61K9/70D A61L15/42 A61L27/44R A61L27/54 A61L27/56 K61L300/416 K61L300/604 K61L300/80

US: 424/400 424/400S 424/489 424/489P 424/647 424/647S 514/183 514/183S 514/19.3 514/19.300S 514/34 514/34S 514/410 514/410S 514/449 514/449S 977/773 977/773S

Family: [Family Explorer](#)

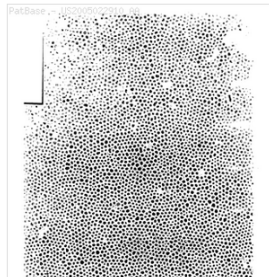
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AU2009214533 BB	20150129	AU20090214533	20090211	
CA2712832 AA	20090820	CA20092712832	20090211	
CN101938993 A	20110105	CN200980104353	20090211	

1) Family number: 31492456 (US2005022910A)

Similar • Translate • Full-text • Status • Citations

Title: [EN] MAGNETIC METAL PARTICLE AGGREGATE AND METHOD OF PRODUCING THE SAME

Abstract: Source: US2005022910A [EN] A method to produce dispersion of magnetic metal particle aggregates is provided. FePt **nanoparticles** are uniformly dispersed with the particles separated at controlled inter-particle spacing. The magnetic metal particle aggregate is composed of magnetic metal particles whose main components and the contents thereof are represented by the following general formula (1): $[TXM1-X]Y21-Y(1)$, where T is one or both of Fe and Co, M is one or both of Pt and Pd, Z is at least one member selected from the group composed of Ag, Cu, Bi, Sb, Pb and Sn, X represents 0.3 ~ 0.7, and Y represents 0.7 ~ 1.0, the balance being impurities unavoidably incorporated during production, wherein the proportion of face-centered tetragonal structure is in the range of 10 ~ 100 percent, the particles have an average diameter of 30 nm or less, and the particles are in a dispersed state spaced apart from one another.



Classifications: [Classification Explorer](#)

International (IPC 8-9): B22F1/00 B22F9/24 B32B5/16 B82Y25/00 C22C1/04 C22C19/07 C22C38/00 G11B5/70 G11B5/706 G11B5/714

H01F1/00 H01F1/06 H01F1/26 H01F1/44 H01F10/00 (Advanced/Invention);

B22F1/00 B22F1/02 C22C38/00 G11B5/70 H01F1/06 H01F10/00 (Advanced/Non-invention);

B22F1/00 B22F1/02 B22F9/16 B32B5/16 C22C1/04 C22C19/07 C22C38/00 G11B5/70 G11B5/706 H01F1/00 H01F1/032 H01F1/12 H01F1/44

H01F10/00 (Core/Invention);

H01F10/00 (Core/Non-invention)

International (IPC 1-7): B22F1/00 B22F1/02 B22F9/24 C22C19/07 C22C38/00 G11B5/70 G11B5/706 G11B5/714 H01F1/00 H01F1/06 H01F1/44

H01F10/00

CPC: H01F1/068 B22F1/0022 B22F9/24 B22F2998/00 B82Y25/00 B82Y30/00 C22C1/0491 H01F1/0054 H01F1/065 H01F10/007 Y10T428/12014