Research Paper

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Formulation and Characterization of Silanised Magnetic Nanoparticles Conjugated with Folic Acid: A Nanocarrier System for Anticancer Drug Delivery

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KEYWORDS : 3-aminopropyl triethoxy silane (ϒ-APTES), Silanisation, Folic acid(FA), Fourier transforms infra red spectroscopy (FTIR).

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ABSTRACT

Nanotechnology has emerged as one of the very promising fields of biomedical research in the past few decades. Drug delivery is one of the areas where this technology has made remarkable progress. The article presents the formulation and characterization of the Magnetic nanoparticle (MNP) mediated targeted anticancer drug delivery system. Modifying the MNP nanocarrier with organic silane 3-aminopropyl triethoxy silane makes it more effective as it turns more biocompatible and the surface amino groups provide way to further functionalization. Conjugation with folic acid (FA) increases specific targeted drug delivery towards folate receptor bearing cancer cells to improve anticancer effectiveness by increasing the tissue's local concentration of drugs.

INTRODUCTION

Major challenge to successful cancer chemotherapy is achieving specific drug accumulation at the tumor sites. Also most of the available anticancer agents cannot distinguish between cancerous and healthy cells, leading to a systemic toxicity and undesired side effects (Langer, 1998).Current attempts to solve such hurdles in cancer therapy are focusing on the use of targeted magnetic nanoparticles (Mahmoudi et al.,2010).

Surface modifications of NPs facilitate their use in biomedical applications, especially the "'targeted drug delivery in cancer therapy''. To control the surface properties of SPIONs, they are coated with a biocompatible polymer during or after the synthesis process in order to prevent the formation of large aggregates, changes from the original structure and biodegradation when exposed to the biological system. In addition, polymer coating can also allow binding of drugs by covalent attachment, adsorption or entrapment on the particles (Tartaj and Serna, 2003). Surface modification of the MNPs with 3-aminopropyl triethoxy silane (ϒ-APTES) (silanisation) creates a self assembled monolayer (SAM). Silanisation renders the MNPs biocompatible, prevents them from oxidation in suspension, isolate them from each other, avoid the formation of magnetic aggregates, and serves as a support for chemical bonding (i.e.,can form covalent bonds through their surface amino groups) or physical adsorption of biomolecules on the surface of the particles. The main reason behind choosing the silane is its well known surface chemistry.

An effective approach to improving the targeting capability and drug release efficiency is to conjugate nanoparticles with chemical or biological reagents including antibodies or low molecular weight targeting agents that have strong affinity for target cells and high efficiency for nanoparticle internalization. Identification of specific targeting agents or drugs that can be effectively released from nanoparticles inside target cells remains a challenge and is the central focus of the current studies in the field. Folic acid (FA) is a good choice as targeting ligand as the folate receptors are overexpressed on the cell membranes of many cancer cells including ovarian, endometrial, colorectal, breast, lung, renal cell carcinomas, brain metastases derived from epithelial cancers and neuroendocrine carcinomas.(Sudimack and Lee,2000.,Gabizon et al.,1999)

MATERIALS AND METHODS

1-MATERIALS

EDC {(3-dimethylaminopropyl)-N^1-Ethyl carbodimide hydrochloride} and NHS (N-hydroxysuccinamide were purchased from Sisco research Lab. Pvt. Ltd., Mumbai. 3-Aminopropyl triethoxysilane (ϒ-APTES)(Polysciences Inc.), Folic Acid (Fluka, Sigma Aldrich, Belgium) and ultrapure water (Millipore Milli-Q purification system-Bedford, U.S.A) were also used in the experiment.

2-METHODS

2-1(a). Surface modification of the SPIONs by Silanization

For functionalization of the MNP surface with amino groups, 20mL of ϒ-APTES was added to solid MNPs (20mg), and the reaction mixture was stirred at reflux temperature (403K) on a magnetic stirrer with hot plate. After 20 h at reflux, the mixture was cooled and then washed three times with ultrapure water and ethanol and dried at 353 K in an oven (Moritake et al.,2007).

2-1(b). Conjugation of the targeting ligand (Folic acid) with the Silanised SPIONs

Folic acid conjugates (FNPs) were prepared by carbodiimide coupling chemistry. The silanised SPIONs were added to the mixture of 1 ml 10 mM folic acid solution in dimethylsulfoxide (DMSO), 1.5 ml 15 mM NHS and 1.5 ml 75 mM EDC solution in water, using triethylamine as a catalyst. The pH was adjusted to 9.0. After incubation at 37°C for 4 h, the suspension was centrifuged and the precipitate was washed with de-ionized water, and vacuum dried overnight (Zhang et al., 2001).

2-2.Characterization:

Fourier transform infra red spectroscopy (FTIR) measurement was taken to investigate on the chemical interaction in the MNP formulations. A small amount of the MNPs (20 mg) were milled with KBr (180mg), and a mixture of them was pressed into a pellet with a pressure 150 kg/cm^2. The pellet was subjected for analysis using FTIR spectrometer (NICOLET 6700, FT-IR, Thermo Scientific) with a resolution of 2 cm^{-1} in the range of 500-2500 cm^{-1}.

3-RESULTS AND DISCUSSION

3-1. Investigation on the chemical integrity of MNPs and its various conjugates.

Fourier Transform Infra-red (FT-IR) spectral analysis disclosed the surface chemistry of the native MNPs and the surface functionalised MNPs. The un-modified iron oxide (Fe_3O_4) nanoparticles has shown a broad band between 750 and 1300 cm^{-1}, which is indicative of the O-H bond bending in and out of plane. For nanoparticles modified with 3-aminopropyl triethoxy silane, newly formed peaks in the frquency range of 1350-1470 depicts the bending vibrations on CH_3 deformation.Peaks corresponding to 750-900cm^{-1} and the frequency variations at 1550-1650cm^{-1} confirmed the bending vibrations of NH_2 group (Fig.1a). Absorption bands associated with C=O

bond strtching are very strong because a large change in the dipole takes place in that mode. Such a change occurred on folic acid conjugation is due to the presence of its C=O groups involved in the formation of amide bond with the NH_2 groups present on silanated MNP surface (Fig.1b).

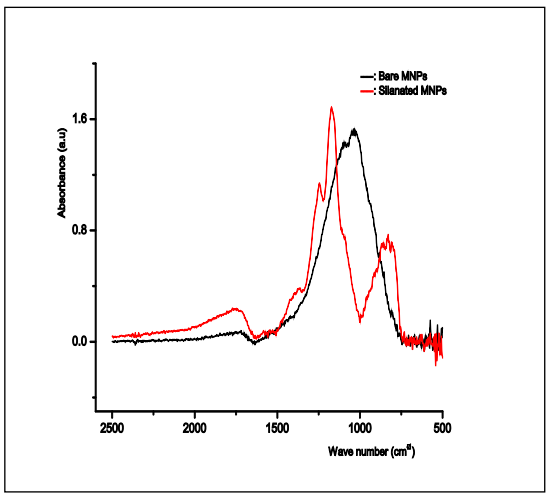

Fig.1a: FTIR spectra of pure and silanated MNPs where the latter shows specific peaks due to the functionalization of amino groups on its surface .

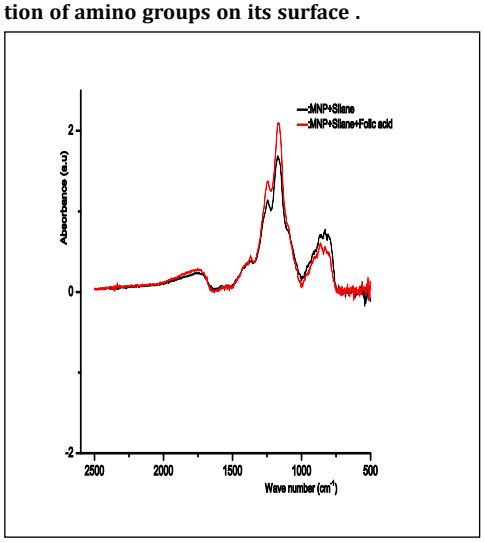

Fig.1b: FTIR spectra of silanated MNPs on folic acid (FA) functionalization where peaks depicts the presence of amide bond between carboxyl group of FA and amino groups on silanated MNPs.

Folic acid (targeting ligand) and the Methotrexate (Anticancer drug) were immobolized on the silanised MNP surface through the reaction between amino and carboxyl roups in presence of EDC (1-ethyl-3-(3-(dimethylamino)-propyl) carbodimide) and NHS (N-hydroxysuccinimide). Carbodiimides are unlike other conjugation reactions in that no spacer exists between the molecules being coupled (Kohler et al., 2005).

REFERENCE

1. Gabizon A, Horowitz, A T, Goren D, Tzemach D, Mandel- baum-Shavit F, Qazen & M M, Zalipsky S (1999) Bioconjugate Chem., 10: 289-298. | 2. Kohler N, Sun C, Wang J & Zhang M (2005) Langmuir, 21: 8858-8864. | 3. Langer,R. Drug delivery and targeting, (1998)Nature, 392 (6679) 5–10.s, 518 (15): 4281–4289./ | 4. Mahmoudi M, Simchi A, Imani M, Stroeve P & Sohrabi A (2010) Thin Solid Films, 518 (15): 4281–4289. | 5. Moritake S, Taira S, Ichiyanagi Y, Morone N, Song S Y, Hatanaka Y, Yuasa S & Setou M (2007) J Nanosci Nanotechnol.,7:937-944. | 6. Sudimack J & Lee R J (2000) Adv Drug Delivery Rev., 41: 147-162. | 7. Tartaj,P. and Serna, C.J. (2003). Synthesis of monodisperse superparamagnetic Fe/silica nanospherical composites, Journal of the American Chemical Society, 125 (51) :15754–15755. | 8. Zhang Y, Kohler N & Zhang M (2001) Biomaterials, 23: 1553-1561. |