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Synthesis and characterization of Pullulan Acetate coated magnetic nanoparticle for Hyperthermic therapy

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Abstract

Magnetic nanoparticles have attracted increasing attention due to their potential applications in many industrial fields, even extending their use in biomedical applications like in drug delivery system, Implants, biosensors, lab on a chip, image enhancement, hyperthermia therapy and so on.For better experimental applications magnetic nanoparticles are encapsulated. The coating material used in this study is pullulan. Pullulan is a non toxic, non immunogenic, plasma expanders which are coated with the magnetic nanoparticles. Acetylation of pullulan is carried out using Motozato's method and along with this magnetic gel was prepared . Pullulan acetate coated magnetic nanoparticles was the outcome of pullulan acetate and magnetic nanoparticles by solvent diffusion method. The sample was characterized using XRD (X-ray Diffraction), FTIR (Fourier Transform Infrared Spectroscopy), SEM (Scanning Electron Microscopy), VSM (Vibrating Sample Magnetometry) and DLS (Dynamic Light Scattering). Biocompatibility studies show the percentage of cell viability with different concentration of samples. The material was checked for the increase in temperature by varying magnetic field and field intensity.

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Keywords: Nanoparticles; pullulan; biomedical applications; Hyperthermia.

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1. Introduction

Nanotechnology is currently the focus of intense development in the field of nanomedicine. Recent advances in nanotechnology have led towards the development of multifunctional nano particle probes for molecular and cellular imaging, nanoparticle drugs for targeted therapy, and integrated nanocarriers for early cancer detection and screening [J. Kreuter et.al (1995); M. Lewin et.al (2000); Y. Anzai et.al. (1994)]. By altering the surface properties of the magnetic nanoparticles it could be used in various biomedical applications such as drug delivery system [A. S. Lubbe et.al (1996)], hyperthermia [D. C. F. Chan et.al (1993); J. H. Park et.al (2005); D. H. Kim et.al.(2006)], immunoassay [T. Matsunaga et.al. (1999)] and for contrast enhancement in magnetic resonance imaging (MRI) [T. Islam et.al (2009); B. R. Jarrett et.al (2007); S. J. Cho et.al (2006); J.Y.Park et.al (2008)]. Concerning magnetic nanoparticles, the main advantage in their employment in Nanomedicine is that they can be visualized in Magnetic Resonance Imaging (MRI) techniques, acting therefore as magnetic contrast agents (CA). The ability of self heating when irradiated by electromagnetic field in the range of kHz–MHz is expected to provide a great deal of technical advantages, accelerating a new form of therapeutic hyperthermia cancer treatment, the so called Magnetic Fluid Hyperthermia (MFH) [R.E. Rosenweig et.al.(2002)]. Magnetic nanoparticles have hydrophobic surfaces and large surface area to volume ratio, in vivo use of nano-magnetic particles tends to result in agglomeration and very rapid clearance from the circulation. In addition, a common problem of uncoated magnetic nanoparticles is their chemical instability with respect to oxidation in air [M. Chastellain et.al. (2004)]. Surface modification or coating of iron oxide nanoparticle with biocompatible polymer is often dispensable for in vitro biomedical applications in addition to prevent oxidation of the particles.

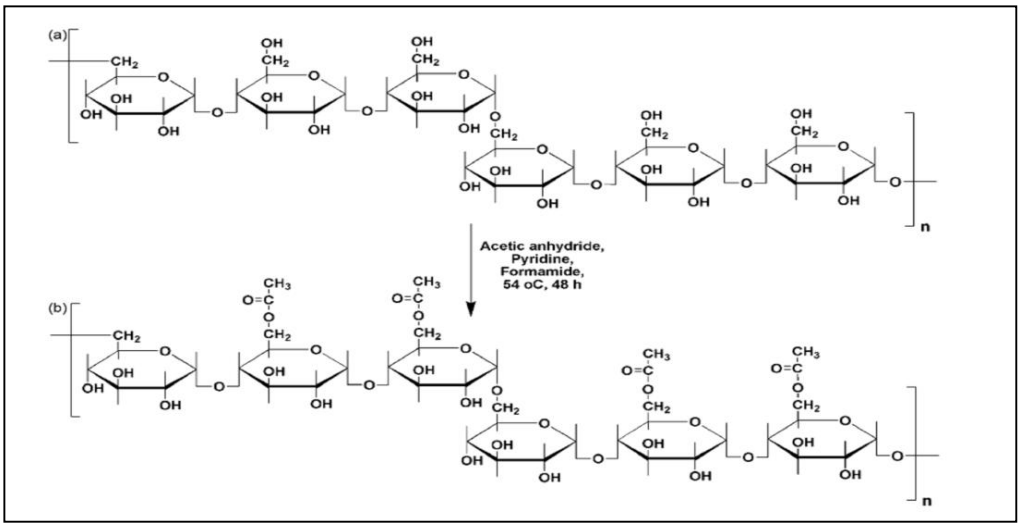

Fig 1: Chemical structure of pullulan and its acetylated form

Pullulan is a nonionic polysaccharide and is blood compatible, biodegradable, non-toxic, nonimmunogenic, non-mutagenic and noncarcinogenic [U.S Congress Publications, (1993)]. The properties of the PA-coated magnetic nanoparticles (PAMNs), such as their morphology, particle size and distribution, composition and magnetic properties were investigated by X-ray Diffraction (XRD), transmission electron microscopy (TEM), dynamic light scattering (DLS), thermogravimetric analysis (TGA), and vibrating sample magnetometer (VSM). The presence of the coating of PA on the magnetic nanoparticles was confirmed by Fourier transform infrared spectroscopy (FTIR). Cell viability of white blood cells at different concentration ranges of the sample were also studied using biocompatibility test. Based on the characterization results, the PAMN could be used in Hyperthermia for cancer therapy.

2. **Experimental method**

2.1 .Acetylation of pullulan – Motozato's method

Acetyl moieties were chemically introduced to pullulan to induce amphiphilic properties. Pullulan (2g) was suspended in 20mL of formamide and dissolved via vigorous stirring at 50 ◦C. Pyridine (12 ml) and acetic anhydride (30 ml) were added and the mixture was stirred for 48 h at 54 ◦C. Pullulan acetate (PA) was obtained via precipitation against 400mL of water [K. Na et.al. (2002)].

2.2. *Magnetic nanoparticle synthesis*

11.60 g $FeCl_3.6H_2O$ and 4.30 g $FeCl_2.4H_2O$ were dissolved in 400 ml deionized water under nitrogen gas with vigorous stirring at 90 ºC. 15 ml of 25 weight% NH_4OH was added to the solution. Then, 9ml oleic acid was added dropwise into the suspension. After several minutes, the upper solution became colorless and the black magnetic gel precipitated and was isolated by magnetic decantation. The bare magnetite was prepared by the same recipe except for the omission of oleic acid. To increase the magnetization, the magnetic gel prepared could be thoroughly washed with ethanol or acetone to remove excess oleic acid [Xianqiao Liu et.al. (2006)].

Fig 2:Synthesis of pullulan Acetate coated magnetic nanoparticle

2.3 Preparation of PAMNs – solvent diffusion method

50 mg of wet magnetite gel and 100 mg of PA were dissolved in separate 5 ml portions of dimethyl formamide (DMF). The two portions were mixed by vortex [Fig 2]. The resulting solution was then added to an aqueous solution of 90 ml of poly (vinyl alcohol) (PVA) (0.5%, *w/w*) by means of a syringe positioned with the needle directly in the medium under moderate mechanical stirring. The magnetic particles were isolated by ultracentrifugation (Hitachi, CF 16RX) at 15 000 RPM for 20 min at 4 °C and washed three times with deionized water. The free PA was removed by magnetic separation of the magnetic particles which were subsequently freeze-dried for characterization [Fuping Gao et.al. (2010)].

2.4 In vitro cell experiments

2.4.1 Proliferation test

Blood samples from healthy volunteers were collected by venepuncture and transferred into 15 ml heparin coated test tubes. It was diluted in 1:1 ratio with PBS, layered onto Ficoll-Histopaque 1077 at a volume ratio of 3:1 and centrifuged at 1,000 x g for 30 min. During the centrifugation the PBMCs moved from the plasma and were suspended in the density gradient, isolating them from erythrocytes and granulocytes. The PBMCs layer was removed and then washed twice with PBS. The supernatant was then removed and the cells were resuspended in RPMI 1640 medium supplemented with 1 mM L-glutamine, 100 Units/ml penicillin and 0.1 mg/ml streptomycin, 10% inactivated FCS, and adjusted to pH 7.2 by the addition of 15 mM HEPES. Cell viability was determined by the trypan-blue dye exclusion method. The PBMC cell density used in the cytotoxicity study was 1 x 10^5 cells/ well of the 96-well tissue culture plate. Dose-response 50- 1000µg/ml between the percentage of cell viability and concentrations of the extracts were constructed.

2.4.2 In vitro cytotoxicity/ viability studies

The compound was dissolved in 10% Dimethyl Sulfoxide (DMSO) to give a final concentration of DMSO not

more than 0.5% and did not affect cell survival. The viability of cells was assessed by MTT assay (Mosmann et.al, 1993) using hepG2 cell lines. The cells were plated separately in 96 well plates at a concentration of 1×10^4 cells/well. Then add 200 µl of complete DMEM medium. After 24 hours of incubation in co2 incubator, cells were washed twice with 100 µl of serum-free medium and starved for an hour at 37°C. After starvation, cells were treated with different concentrations of test compound (10-500µg/ml) for 24 h. At the end of the treatment period the medium was aspirated and serum free medium containing MTT (0.5 mg/ml) was added and incubated for 4 h at 37ºC in a CO_2 incubator.

The MTT containing medium was then discarded and the cells were washed with PBS (200 µl). The crystals were then dissolved by adding 100 µl of DMSO and this was mixed properly by pipettting up and down. Spectrophotometrical absorbance of the purple blue formazan dye was measured in a microplate reader at 570 nm (Biorad 680). Cytotoxicity was determined using Graph pad prism5 software.

2.4.3 Hyperthermia study

Magnetic nanoparticles are heated with induction to selectively heat tumor cells. The Sample was tested for change in the temperature by varying magnetic field and power. An increase in the temperature need to be checked for hyperthermia applications.

3. Results and discussion

3.1. Experimental results

Firstly prepared magnetic Fe_3O_4 nanoparticles was modified with oleic acid, and the amphiphilic polymer PA was then coated onto the surfaces of the magnetic nanoparticles using the solvent diffusion method as shown in Fig 3. The parent nanoparticles were synthesized by co-precipitation of ferrous and ferric ions in aqueous solution by addition of ammonium hydroxide to obtain well-dispersed iron oxide colloidal solutions.

Fig 3: Pullulan Coated Magnetic Nanoparticle

3.2 .Characterization results

XRD data confirm the crystalline nature of the sample is shown in Fig 4 (a). The FTIR spectra of PAMN confirms the introduction of acetate groups in PA, which is indicated by C=O stretch at 1600 cm^{-1}, CH_3 deformation at 1425 cm^{-1}, O-C=O bend at 472 cm^{-1}. The maximum absorption peak is found to be at 3425 cm^{-1} which is plotted in Fig 4 (b). The particle size distribution was found to be wide for PAMN which was confirmed using DLS as given in Fig 4 (c).

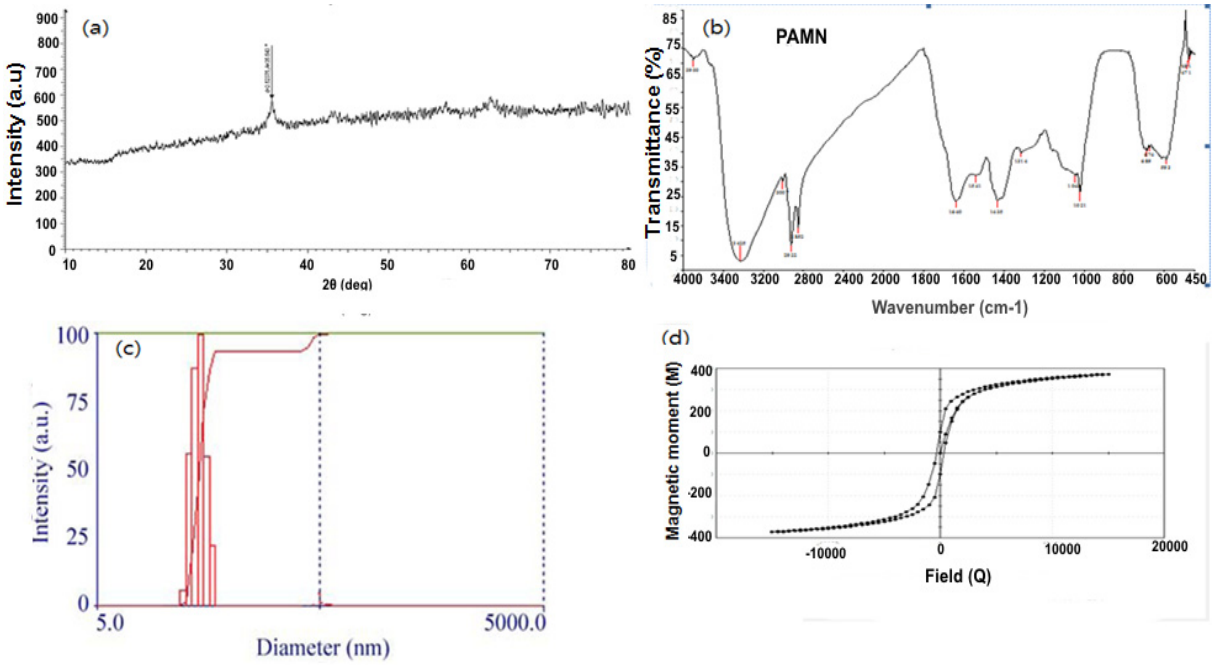

Fig 4: (a) XRD data, (b) FTIR spectrum analysis of the sample, (c) Particle size distribution (d) VSM analysis

Ferromagnetic solids are those in which the permanent magnetic moments are already aligned due to bonding forces. By using VSM analysis the magnetic property was checked by a varying magnetic field and magnetic moment The susceptibility is found to be very large and positive in the range of 10^2 -10^5. The sample is found to have strong magnetic lines of force [Fig.4 (d)]. The SEM analysis is mainly based on the kinetic energy of the compounds which results in secondary electrons, backscattered electrons, diffracted backscattered electrons, photons and heat. The SEM image is shown in Fig 5 (a). The SEM with EDAX provides the weight percentage and atomic percentage of elements in the sample as shown in Fig 5 (b).

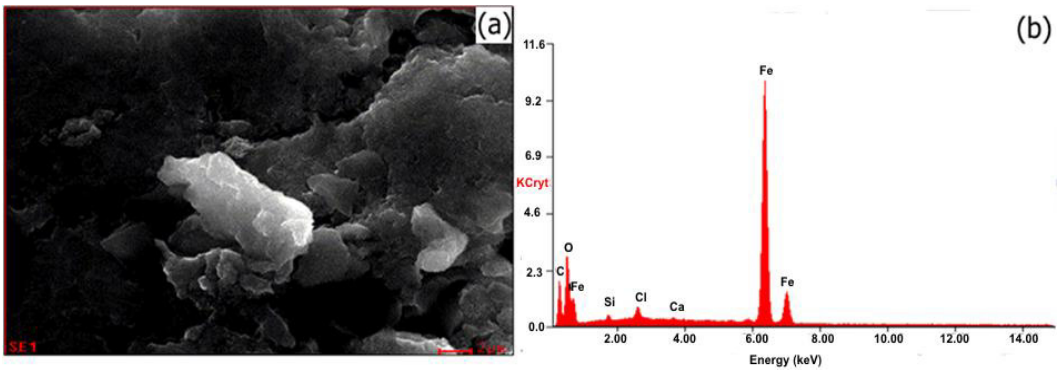

Fig 5: (a) Magnified SEM image of the sample (b) Graph showing the elemental analysis(EDAX) of the sample

H. Invitro Cell viability studies

The MTT assay for cell viability evaluation has been described as a suitable method for detection of biomaterial toxicity [T.Mosmann et.al.]. The WBCs were treated with different concentration of the magnetic nanoparticle 25µg to 250µg and respective absorbance value was noted after 24 hours and 72 hours. Based on these observations a graph of percentage of cell viability versus concentration of magnetic nanoparticles was plotted which is shown in Fig 6.

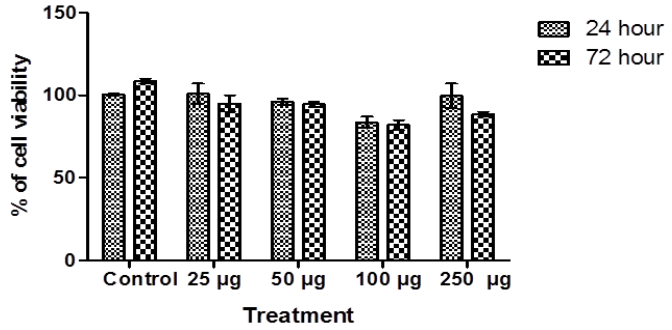

Fig 6: cell viability profile , the Graphical representation of biocompatibility study.

I. Hyperthermic studies

The induction heating system has the facility to vary the frequency from 150-400kHz and power from 1-10kW. Using this feasibility frequency and power was varied and the corresponding temperature was noted down in Table 1. The initial temperature of the sample was 32 ºC. Based on this observation, the graph has been plotted considering frequency versus concentration of the sample and power versus concentration of the sample which is given in Fig 7 (a) and 7 (b). It is found that the sample has the capacity to increase the temperature based on variation in frequency and power. Hence this material can be used for hyperthermia therapy for heating cancer cells.

Table 1: Hyperthermic studies–with the variation of temperature

Frequency (kHz)	**150**	**200**	**250**	**300**	**350**	**400**
Power (kW)	2	3.5	5	6.5	8.5	10
Temp(°C)	48	52	58	64	70	78

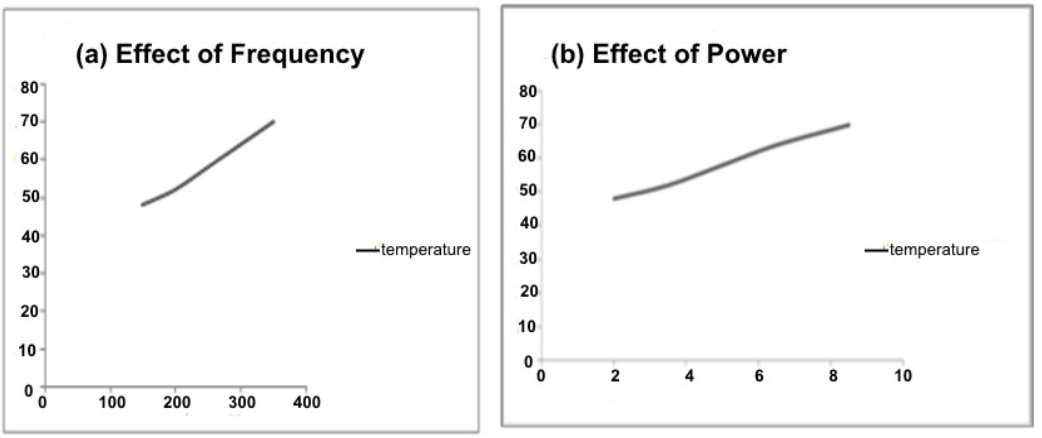

Fig 7: Hyperthermic studies (a) Effect of frequency in changing the temperature (b) Effect of power in changing the temperature

4. Conclusions

PAMNs was prepared using the solvent diffusion method and then the sample was characterized using various analyses such as XRD, SEM, VSM, DLS, FTIR. Biological studies such as Cell viability studies were also being studied. The sample prepared was tested whether it has the ability to change the temperature by varying the field intensity using precision induction system. Based on this results hyperthermia can also be tested with the cell lines and also in tumor induced animal models.

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