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JST

Journal homepage: http://penerbit.uthm.edu.my/ojs/index.php/jst
ISSN : 2229-8460 e-ISSN : 2600-7924

Journal of Science and Technology

Characterization of Amino-Functionalized Ferromagnetic Nanoparticles with Glutaraldehyde Cross-linking

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DOI: https://doi.org/10.30880/jst.2021.13.01.005
Received 31 March 2021; Accepted 12 May 2021; Available online 25 May 2021

Abstract: Characterization of amino-functionalised magnetic nanoparticles (FMNs) modified surfaces with glutaraldehyde cross linker, which enables the attachment to a specific antibody against Salmonella was examined using FTIR spectroscopy. The magnetism property of the particles before and after attachment to the target cells was studied. Synchrotron FTIR spectroscopy technique was employed to investigate the chemistry of the crosslinking reaction to amino-functionalized ferromagnetic nanoparticles. Results indicated that the bonding pattern imine bond (C=N) and amide bond (-CONH-) were detected. The finding indicated the attachment of aldehyde group (CHO) to amino region of the particle and the other free end to link to the antibodies against *Salmonella*. The bonding allowed the antibodies to bear reactive sites to catch the target *Salmonella* in food samples. The FMNs demonstrated hysteresis characteristics that could be changed due to cell attachment that caused reduction of loop coercive force (H). Results showed evidence of glutaraldehyde crosslinking that could be also used as quality control for immobilization of antibodies to the particles. Magnetism properties (the coercive force H) and FTIR characteristics could be further used for signal of attachment of cells to the FMNs as well.

Keywords: Magnetic nanoparticles, FTIR spectroscopy, glutaraldehyde, magnetism

1. Introduction

Over the past few decades, magnetic iron oxide nanoparticles have gained much attention. There are applications in the various fields like protein immobilization, bioseparation, biomedicine, biotechnology, environmental treatment, food analysis, and material science [1-4]. Biomedicine applications include controlled drug delivery system (DDS),

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magnetic resonance imaging, magnetic fluid hyperthermia (MHF), macromolecules and bacterial detection, cancer therapy and so on [2,5]. The benefits of their properties are super-paramagnetism, high surface area, large surface-to-volume ratio, easy for separation under external magnetic fields and the ability of being easily chemical modified for biocompatibility, and dispersibility [1, 3, 6].

Magnetic nanoparticles have been used for target bacterial capture to reduce the total time of analysis and improve the sensitivity of detection of pathogenic microorganisms. Usually, the magnetic nanoparticles surface functionality will be modified to facilitate immobilization of specific molecules onto the nanoparticles. Attachment of specific ligands and proper surface modification are critical [1, 2]. There are several methods for effective surface modification of magnetic nanoparticles. Physical immobilization, covalent conjugation and biological-mediated specific interaction are frequently used. These methods have the advantages and disadvantage [1, 6].

Physical immobilization is simple and mild, this method generally involves weak interaction such as electrostatic interactions, hydrogen bonds, van der Waals forces, and hydrophobic interaction, and the binding stability of adsorbed species is highly affected by environmental conditions. The biomolecules tend to break away from the support, and lead to the loss of activity [6]. Biologically Mediated Specific Interaction has offered a novel route to solve the problem of selectivity, which could be achieved by the formation of bonds between the active groups on the supports and specific residues on the proteins. The proper modification of the support surfaces and protein alteration, such attachments could be strategically realized under mild conditions. The approach has considerably reduced the risk of protein degradation. Though this method has contributed greatly to the popularity for protein immobilization, the protein of interest must first be labelled with biotin if site-selective attachment is desired [6]. The covalent conjugation method for attachment of antibody, via its functional groups, to chemically engineered substrates, has resulted in further improvement in antibody [7].

In this case, we selected the covalent conjugation method for modification of magnetic nanoparticle, which could be carefully regulated with specific functional groups to bind to biomolecules such as antibodies for specific target [1, 6]. Glutaraldehyde (GA) is one of the popular coupling reagents for covalent cross-linking the modified magnetic nanoparticles and biomolecules, DNA and antibodies, because their functional group include aldehyde (-CHO) group can react between functional groups (e.g. – amino (-NH_2) group) on the surface of magnetic nanoparticles and amino (-NH_2) groups on the biomolecules [1, 6, 7, 8]. Moreover, GA is widely used in various applications such as histochemistry, microscopy, cytochemistry, leather tanning industry, enzyme technology, chemical sterilization, and biochemical and pharmaceutical sciences [9].

GA had great success as a result of its commercial availability and low cost. In addition, GA reacted rapidly to amine group at neutral pH in a more efficient way compared to other aldehydes in generating thermally and chemically stable crosslinks [9]. We hypothesize that aldehyde (-CHO) groups of the GA react with amino (-NH_2) group abundantly distributed over antibodies surface. The attachment of aldehyde group to terminal amino groups of antibodies near the recognition place and the active sites in the fragment antigen binding (Fab) domain of antibody could lead to totally blocking the active sites causing antibodies to lose binding ability and reduce efficiency to the analysis [10]. The binding of GA as cross linker was not well understood.

A non-destructive Fourier transform infrared (FTIR) technique is rapid, sensitive and versatile [12]. This technique is a measurement of wavelength and intensity of the absorption of IR radiation by a sample [13]. It is useful and applied for biological such as detection, discrimination, and classification of bacteria [10,14], protein structural studies [13], diagnosis of breast cancer [15], investigation of biological tissues [16] and studies of crosslinking processes for moulding sand [17]. The aim of this study was to prove binding and suitability of GA as a cross linker with antibodies by using FTIR technique. This was to prove the chemistry behind the interaction between GA, amino functionalized FMN particles (amino-FMNs) surfaces and at the same time facilitate attachment of antibodies against *Salmonella* on the other end of GA as indicated in Fig. 1.

2. Materials and Method

2.1 Reagents

Iron (III) chloride hexahydrate, ethylene glycol, sodium acetate and ethanol (99.9%) were purchased from QREC (New Zealand). Sodium hydroxide was purchased from Ajax Finechem (Australia). Ethylenediamine was purchased from Merck (Sweden). GA was purchased from Fisher Chemical (U.S.A.). Deionized water was obtained from the purification system.

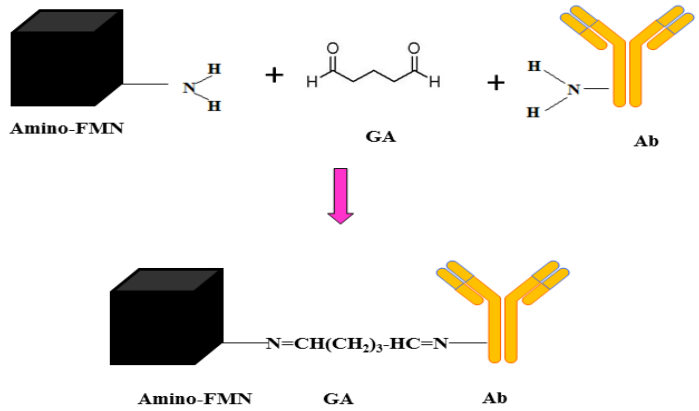

Fig. 1 - The schematic illustration of the functionalization process of amino-FMNs with glutaraldehyde (GA) crosslinking and antibody (Ab) attachment

2.2 Apparatus

The autoclave Sanyo Model MLS-3020 (Japan) was used for preparation of magnetic nanoparticles. Fourier transform infrared (FTIR) was employed to investigate functional groups and bonding on the surface of magnetic nanoparticles (Vertex 70, Bruker).

2.3 Preparation of Amino Functionalized Ferromagnetic Nanoparticles (amino-FMNs)

The synthesis of amino-FMNs was prepared by polyol technique as described by Songworavit et al. [5] with some modification. In the synthesis, amino-FMNs were synthesized using ethylene glycol as solvent and reducing agent. Amino-FMNs were prepared to have amino group on the surface of particles in one step of synthesis with ethylenediamine as an amino group source. Firstly, 2g of $FeCl_3.6H_2O$ was dissolved in 40 ml ethylene glycol and mixed until the solution was cleared yellow. Then 6 g of CH_3COONa, 1.68 g of NaOH and 20 ml of ethylenediamine was added in the flask and stirred for 30 min. After the mix, the solution was further heated in a laboratory autoclave at 121 °C, 105 KPa, 2 h per cycle, for 3 cycles. Between the cycles, the mixed solutions were shaken for 5 min. When the reaction was completed, amino-FMNs were separated by magnetic force and washed five times with deionized water and five times with 95% ethanol to remove the solvents. Ultrasonication was used to facilitate washing. The amino-FMNs were oven dried at 50 °C for 24 h., milled with mortar and pestle then kept in amber bottles until use.

2.4 Characteristic Magnetic Nanoparticles

Characterization of nanoparticles followed that described previously in [5]. FMNs were dispersed in distilled water for 15 min with ultrasonicator and the top of the particle suspension was dropped on copper grid. It was air dried for 20 min before the morphology and size of the nanoparticles were determined by images from scanning electron microscope (SEM: FEI Quanta 450). The samples were analysed by using SEM-EDS. Structure of nanoparticles was characterized by X-ray Diffractometer (XRD; Bruker AXS Model D8 Discover, Germany) with target: Cu, 40kV, 40mA, angle 20-80 degree, increment 0.02 degrees/step, scan speed 0.3 s/step. The magnetic properties were characterized by vibrating sample magnetometer (VSM) developed by the Department of Physics, Kasetsart University, Thailand.

2.5 Synchrotron FTIR Spectroscopy

Measurements were performed with the Synchrotron IR spectroscopy facility, at the Synchrotron Light Research Institute (Public Organization), Thailand. The Bruker Hyperion 2000 microscope (Bruker Optics Inc., Ettlingen, Germany), equipped with a nitrogen cooled MCT (HgCdTe) detector with a 15×IR objective coupled to a Bruker Vertex 70 spectrometer, was used for FTIR data acquisition. The FTIR spectra were obtained in transmission mode. Spectra were collected from 64 scans, 20µm ×20µm aperture size at a resolution of 4 cm^{-1} over a measurement range of 4,000-800 cm^{-1}. Spectral acquisition and instrument control were performed using OPUS 6.5 software (Bruker Optics Ltd, Ettlingen, Germany).

2.6 Surface Modification of Amino-FMNs with GA

The objective of modifying the surface of amino-FMNs was to immobilize antibodies on the particle surface. In this study, GA was used as the crosslinking reagent to use aldehyde group to the particles and to amine groups of antibodies with amine linkage [7]. The reaction improved the immobilization capacity of antibodies on surface of the particles [10]. For the reaction, aliquots of 0.1 g amino-FMNs were washed and re-suspended in 50 ml of phosphate buffered saline (PBS, pH 7.4) to a concentration of 2 mg/ml. Then, 5 ml of GA (2.5% v/v) was added and gently stirred at room temperature for 2 h. The surface modified amino-FMNs were separated by the external magnetic field. The particles were washed three times with PBS (pH 7.4) to remove any unreacted chemicals and re-suspended in PBS before the next step.

2.7 Preparation of Antibodies Conjugated Amino-FMNs

This step was to attach antibodies to the other end of GA already attached on the particle surface. Before the antibodies conjugation the antiserum against *Salmonella* was tested by slide agglutination [18] to confirm activity of antibodies against *Salmonella*. The test was prepared by making two adjacent suspensions of the test organism in drops of saline on a slide. Then, a drop of the antiserum was added to one suspension and mixed. Agglutination of the suspension indicates reaction of antibodies and *Salmonella* cells. Positive result was agglutination of the suspension, and negative result was indicated when the suspension remains turbid. The coupling of the antibodies onto the modified amino-FMNs was carried out using 500 µl of antibodies (0.1 mg/ml) to mix with 500 µl of the particles (2 mg/ml). The mixture was incubated at room temperature for 24 h and washed three times with PBS (pH 7.4). Finally, the antibodies –amino-FMNs complex was re-suspended in 1 ml of PBS and stored at 4 °C before use. The antibody concentration on the particles was quantified using a Nano Drop spectrophotometer (NanoVue Plus ™, UK) [19].

2.8 Sample Preparation for Synchrotron FTIR Spectroscopy Examination

An aliquot of 5 µl sample was dropped onto barium fluoride windows, spread very thinly from the droplet in a linear fashion on the slide surface using pasture pipette, and then vacuum-dried for at least two days in a desiccator prior to analysis.

3. Results

3.1 SEM Micrograph of Amino-FMNs, After Modification with GA, and Amino-FMNs Conjugated with Antibodies

SEM micrograph of the amino-FMNs is shown in Fig. 2. The synthesized amino-FMNs have rather uniform cubic shapes. After modification, it was found that the sizes of the particles are larger after each modification step. After conjugation with antibodies, the particles appeared to be even larger in size (Fig. 2(a), 2(b) and 2(c)). The image of the nanoparticles indicated the cubic shape with an average size of about 50 nm.

3.2 Magnetic Properties

Magnetization curves of amino-FMNs at room temperature indicated that the FMNs were ferromagnetism but nearly complete superparamagnetism with a saturation magnetization (Ms) of about 48 emu/g, remanence (Mr) of 1.7 emu/g and coercivity (Hc) of 23.5 Oe (Fig. 3). The amino FMNs were well dispersed in water and also can be separated from the solution by attraction of a magnet.

Fig. 3 showed magnetization curve of the particles with hysteresis characteristic of the FMNs particles. The coercive force is the force that acts on the particles in order to produce their magnetization (M). As more force is introduced, the magnetization would reach the saturated point. Upon lower coercive force, the magnetization should decrease. However, due to its hysteresis property, the magnetization does not decrease at the same path resulting in the remanence (Mr). In this case, to achieve the zero magnetization, the opposite (negative) value of coercive force must be applied. In the same way, as the opposite coercive force increase, the opposite magnetization would also reach. All of these show the hysteresis property of the particles. By using this property tracking the changes in coercive force, remanence and saturated magnetization, the attachment of particles to other materials such as bacterial cells could be monitored or measured.

The hysteresis of pure particles has coercive force, a measurement of the reverse field needed to drive the magnetization to zero after being saturated, of about 23.5 Oe. The value is represented by the arrow in Fig. 3. Changes of the coercive force could be used to demonstrate the attachment of this particle to other materials such as bacterial cells.

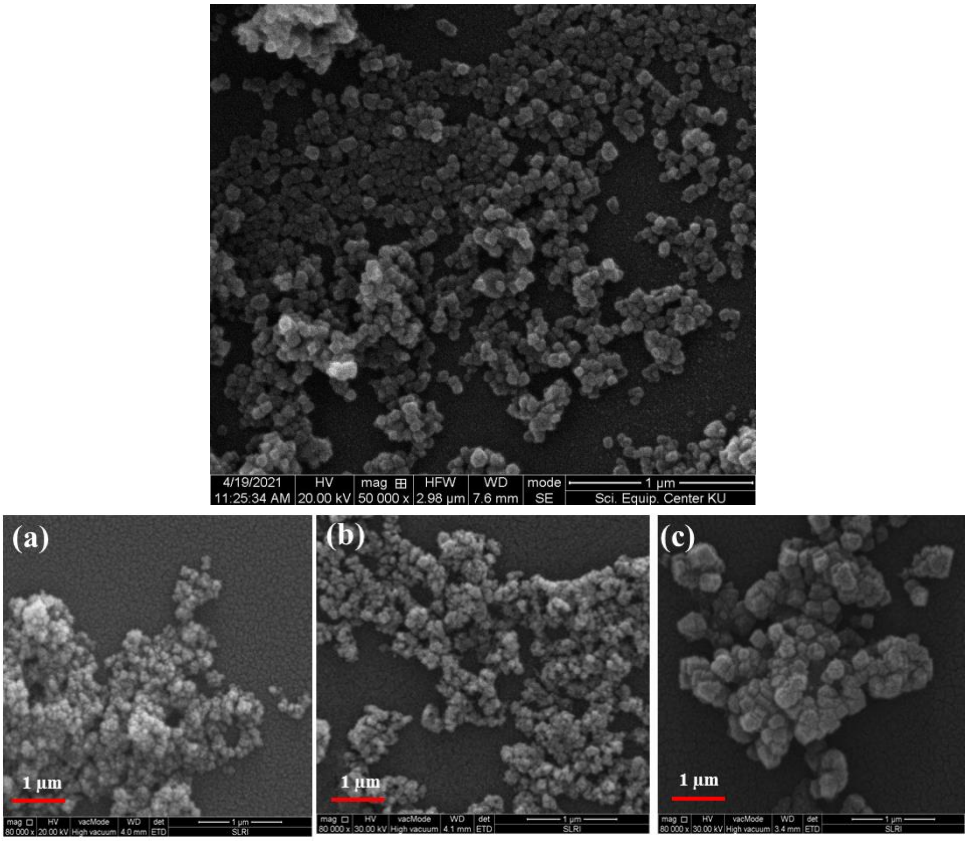

Fig. 2 - SEM image of amino-FMNs particles under 20kV, 50,000x magnification. A bar represents 1 µm in length. SEM images a, b and c represent FMNs alone, after crosslink with glutaraldehyde (GA), and conjugate with antibodies (Ab), respectively

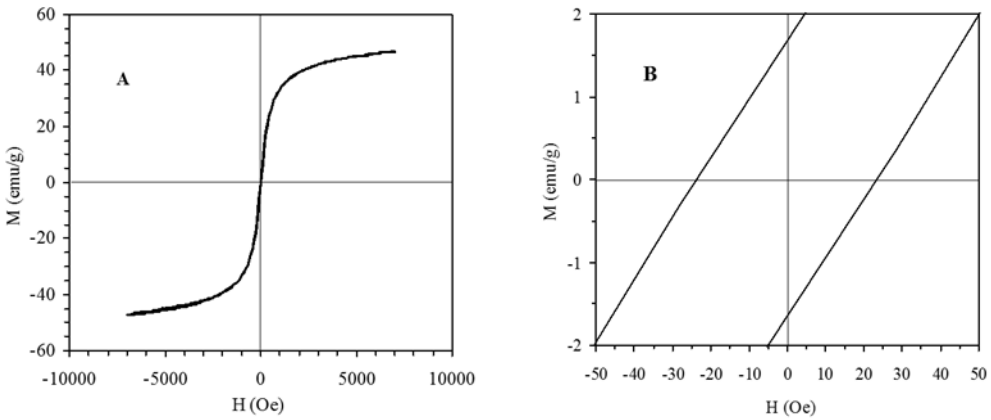

Fig. 3 - Magnetization curves of (A) amino-FMNs and (B) hysteresis loop of magnetization curve. The coercive force is the force that brings the magnetization to zero, in this case, demonstrated by the arrow

3.3 FTIR Surface Characterisation of Amino-FMNs

Surface characterization of amino-FMNs was performed by a Synchrotron FTIR spectroscopy. The FTIR spectra of amino-FMNs and GA alone were shown in Fig. 4. A peak at 1672cm^{-1} corresponds to N-H scissoring vibration of NH_2 in aliphatic primary amine [20]. Results indicated the existence of amino group on particle surface. It also indicated that the synthesis of amino-FMNs was successful.

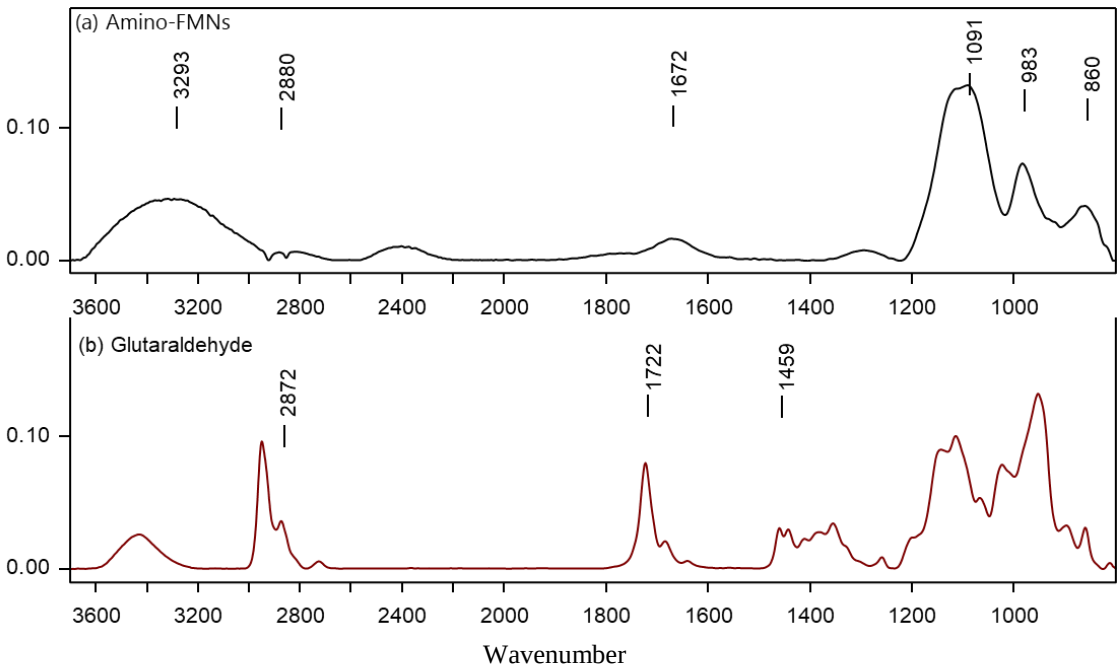

Fig. 4 - FTIR spectra of amino-FMNs (a) glutaraldehyde; (b) alone. A peak of amino-FMNs (a) at 1672cm^{-1} indicates NH scissoring vibration of the amino group on particle surface. Glutaraldehyde (GA); (b) has specific peaks at wave numbers 1722 and 1459

3.4 Amino-FMNs with Glutaraldehyde Cross-linking and Antibody Conjugation

The FTIR spectra of amino-FMNs-GA linking were shown in Fig. 5. A new imine (C=N) peak appeared at about 1653 cm^{-1}. The imine bond (C=N) can be ascribed to the interaction between aldehyde (-CHO) group of GA and amino (-NH_2) group on surface of amino-FMNs. Moreover, the unique peak at 1366 cm^{-1}, assigned as C-H bending vibration of CH_2 in aliphatic compounds, was observed. Results indicated successful surface modification of amino-FMNs with GA.

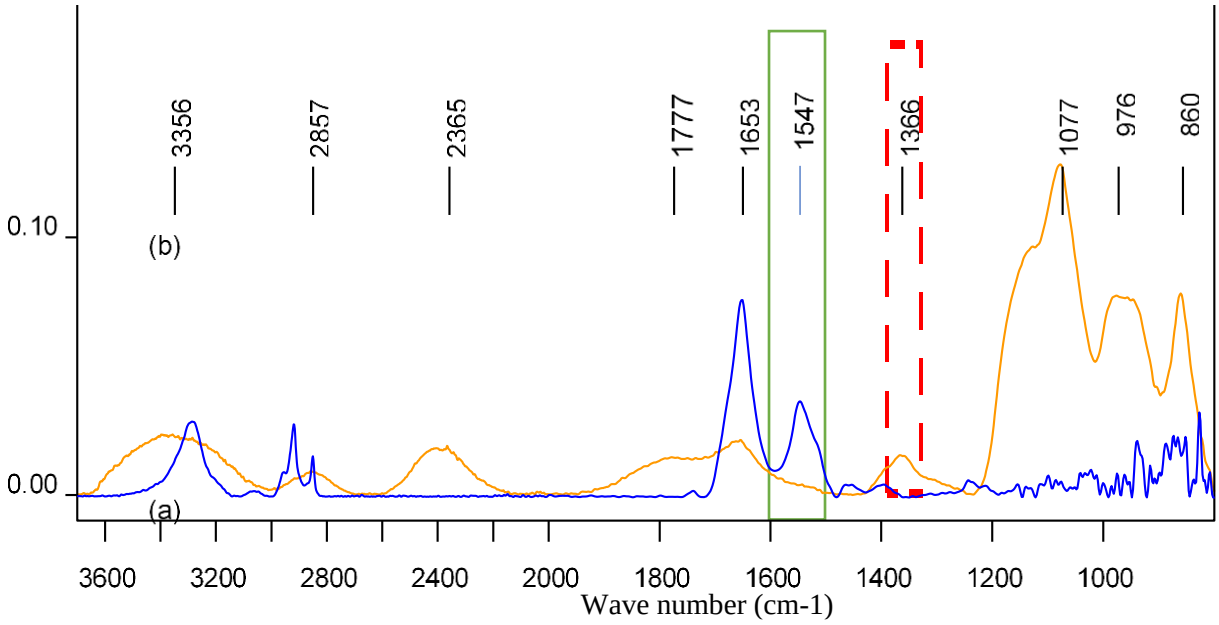

Fig. 5 - FTIR superimposed spectra of reaction steps of amino-FMNs cross linked with glutaraldehyde, (a) FMNs+GA and attached with antibodies: FMNs+GA+Ab (b)

After antibodies attachment onto the surface of particle cross-linked by GA (Fig. 5b), a new strong absorbance at 1652 cm^{-1} the characteristic of amide I with C=O stretching appeared. The peak at 1547 cm^{-1} characteristic of amide II

band with C-N stretching and N-H bending was found. It was evident that peak at 3299 cm^{-1} N-H stretching in amide of antibodies was still intact after GA crosslinking. The peak shifted slightly to 3286 cm^{-1} after GA attachment to antibodies. The proteins served as antigen binding site of the antibodies. This is also characteristic to the absorption spectrum of protein, thus confirming the tethering of the antibodies. The antibodies remained active after the attachment. The peak at 2041 cm^{-1}, N=N=N asymmetric stretching, of antimicrobial azide in the antibody. It was absent after being multiple washed with PBS.

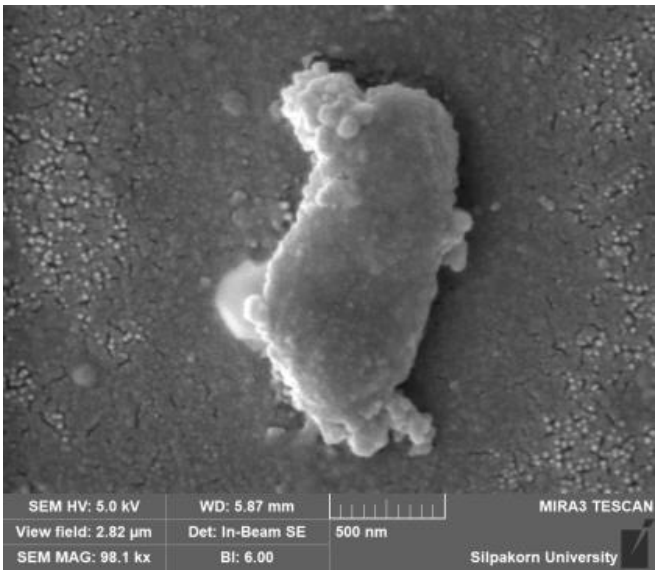

Fig. 6 - Scanning electron micrograph, 98.1kx magnification, of *Salmonella* cell attached with FMN-GA-Ab particles. A bar represents 500 nm

The attachment of bacterial cells with the FMNs particles was achieved as intended. This is to verify that the FMN with GA cross linking to antibody (Ab) could successfully function as antibody conjugated FMN. It was evident as shown in Fig. 6. The FMNs particles of 50 nm in size were attached on the surface of the target cells and made visible under a 5.0KV SEM imaging at 98.1kx magnification. The target cell was well surrounded by the antibody-conjugated FMNs particles.

4. Discussion

4.1 Surface Characterization of Amino-FMNs

Preparation of amino-FMNs employed polyol technique using ethylenediamine as source of amino group ($-NH_2$). Ethylenediamine ($NH_2CH_2CH_2NH_2$) contains two amino group ($-NH_2$) functional groups and CH_2. Thus, Synchrotron FTIR spectra of amino-functionalized FMN showed vibrational modes ascribed to the amino group at 1673 cm^{-1} and 858 cm^{-1}, while the characteristic absorption of CH_2 were observed at 2881 cm^{-1}, 2810 cm^{-1}, and 983 cm^{1} [21-22]. The C-N stretching at 1090 cm^{-1} was the core of chemical structure of ethylenediamine [20]. These results were clearly evident that the magnetic nanoparticles were successfully functionalized with amino groups from ethylenediamine.

4.2 Magnetic Properties

Magnetization curves of amino-FMNs at room temperature indicated that the FMNs were ferromagnetism but nearly complete superparamagnetism with a saturation magnetization (Ms) of about 48 emu/g, remanence (Mr) of 1.7 emu/g and coercivity (Hc) of 23.5 Oe (Fig. 3). The amino-FMNs were well dispersed in water and also can be separated from the solution by attraction of a magnet. Magnetite particles would exhibit complete superparamagnetic properties when their size below 25 nm which is a critical size of magnetite [23]. At this size, each particle has only one magnetic domain, and when a magnetization curve is plotted, the curve would show intersection at the zero point and has no remanence and coercivity. Although, the amino-FMNs produced in this work was not super-paramagnetism because their size was larger than the critical size and their ferromagnetic properties were very low. They were sufficient to be used in any biological applications because they could be well dispersed in an aqueous solution and separated by a magnet as well. The results were similar to those reported in [24] and [5]. The FMNs particles were also reported of having ferromagnetism with saturation magnetization (Ms) of 48 emu/g, remanence (Mr) of 1.7 emu/g, and coercivity (He) of 23.5 [5]. The changes of coercive forces could be used to report attachment of this particle to other materials such as bacterial cells.

4.3 FTIR Characterization of Amino-FMNs with Glutaraldehyde Crosslinking

In this experiment, hypothesis was that the glutaraldehyde (GA) is used as crosslinker to bind amine of amino-FMNs on one end and another end with amine group of biomolecules such as antibodies [7]. GA was an organic compound that contains a linear of 5 carbon and dialdehyde groups (-CHO) on both ends with chemical formula of $C_5H_8O_2$ or $OHC(CH_2)_3CHO$ or $HCO(CH_2)_3CHO$ [9]. The functional groups of GA are 2 aldehyde groups (-CHO) and alkyl group (CH_2) [25]. The absorption characteristics of GA are shown in Fig. 4(b). The carbonyl groups of aldehydes (-CHO) absorb energy at 1722 cm^{-1}, while C-H stretching of aldehyde (-CHO) absorbs energy at 2872 and 2726 cm^{-1}. The strong band observed at 2948 cm^{-1} corresponds to C-H symmetric stretching in aliphatic compounds. The vibrational modes ascribed to the alkyl group (CH_2) such as 1460 (CH_2 scissors), 1355 (CH_2 wagging) and 952 (C-H out of plane bending). Furthermore, the peak appeared at 1114 cm^{-1} was assigned to C-C stretching [21,25]. The FTIR spectra characteristics of glutaraldehyde were conducted to compare before and after surface modification of magnetic nanoparticle with glutaraldehyde. Aldehyde group at one end of the glutaraldehyde reacts with amine of the surface of FMN and forms amide bond. A new peak appearing at about 1653 cm^{-1} was due to imine bond (-C=N-) [21,25-27]. Moreover, the peak at 2858 cm^{-1} refers to C-H symmetric stretching of CH_2 in aliphatic compound and 1366 cm^{-1} is aldehydic C-H bending vibration [21-22].

5. Conclusion

This Synchrotron FTIR spectroscopy study showed the chemistry changes during reaction stage of glutaraldehyde as cross linker on the surface of magnetic nanoparticles. Results showed that there were changes of amino ($-NH_2$) group of amino-FMNs after reacted with aldehyde (-CHO) group of glutaraldehyde resulting in imines (C=N) formation. It is evident that glutaraldehyde can serve as cross linker between the particles and biomolecules. Though, FTIR technique cannot identify location of aldehyde (-CHO) group reaction with amino ($-NH_2$) group in the Fab region of the antibody, we reported different unique signals of glutaraldehyde reaction stage. The first stage in preparation of magnetic nanoparticle showed a specific peak at 1672 cm^{-1}. The second stage of surface modification with glutaraldehyde, had a specific peak at 1653 cm^{-1}. The data obtained from Synchrotron FTIR spectroscopy showed unique signal changes during reaction stages. Unique peaks could be used for quality control and identify presence of target biomolecules such as bacteria or virus. The findings confirmed glutaraldehyde cross linking that could lead to further attachment of target antibodies.

Coercive force of the particles hysteresis characteristics could be of benefit to indicate their attachment to other materials such as bacterial cells. The results from this study could benefit further immobilization of antibodies to FMNs and the design of non-destructive testing for detection of foodborne pathogens.

Acknowledgement

The research was facilitated and supported by The Office of Disease Prevention and Control 4, Saraburi, Department of Disease Control, Ministry of Public health, BL4.1 IR-spectroscopy Synchrotron- Light Research Institute (Public Organization), and S & A Reagent Co. Ltd.

Funding Statement

Financial supports from The Agriculture Research and Development Agency (ARDA), and Thailand Research Fund (TRF) Thailand.

References

[1] Xu, J. K., Zhang, F., Sun, J., Sheng, J., Wang, F., & Sun, M. (2014). Bio and nanomaterials based on Fe_3O_4: review, Molecules, 19, 21506–21528

[2] Niemirowicz, K., Markiewicz, K. H., Wilczewska, A. Z., & Car, H. (2012). Magnetic nanoparticles as new diagnostic tools in medicine, Advances in Medical Sciences, 57, 196–207

[3] Ni, H., Sun, X., Li, Y., & Li, C. (2015). Solvothermal self-assembly of magnetic Fe_3O_4 nanochains by ethylenediamine functionalized nanoparticles for chromium (VI) removal, Journal of Materials Science, 50, 4270–4279

[4] Sobczak-Kupiec, A., Venkatesan, J., AlAnezi, A. A., Walczyk, D., Farooqi, A., Malina, D., Hosseini, S., & Tyliszczak, B. (2016). Magnetic nanomaterials and sensors for biological detection: Review Article, Nanomedicine: Nanotechnology, Biology, and Medicine, 12, 2459–2473

[5] Songvorawit, N., Tuitemwong, K., & Tuitemwong, P. (2011). Single step synthesis of amino-functionalized magnetic nanoparticles with polyol technique at low temperature, International Scholarly Research Network, 2011, 483129

[6] Xu, J., Sun, J., Wang, Y., Sheng, J., Wang, F., & Sun, M. (2014). Application of iron magnetic nanoparticles in protein immobilization, Molecules, 19, 11465–11486

[7] Welch, N.G., Scoble, J. A., Muir, B. W., & Pigram, P. J. (2017). Orientation and characterization of immobilized antibodies for improved immunoassays: Review, Biointerphases, 12, 1–13
[8] Shen, M., Rusling, J. F., & Dixit, C. K. (2016). Site-selective orientated immobilization of antibodies and conjugares for immunodiagnostics development, Methods, 116, 95–111
[9] He, J., Huang, M., Wang, D., Zhang, Z., & Li, G. (2014). Magnetic separation techniques in sample preparation for biological analysis: A review, Journal of Pharmaceutical and Biomedical Analysis, 101, 84–101
[10] Migneault, I., Dartiguenave, C., Bertrand, M. J., & Waldron, K. C. (2004). Glutaraldehyde: behavior in aqueous solution, reaction with protein, and application to enzyme crosslinking: Review, BioTechniques, 37, 790–802
[11] Wang, Z., Yue. T, Yuan. Y, Cai, R., Niu, C., & Guo, C. (2013). Preparation of immunomagnetic nanoparticles for the separation and enrichment of Alicyclobacillus spp. in apple juice, Food Research International, 54, 302–310
[12] Davis, R. & Mauer, L. J. (2010). "Fourier transformed infrared (FT-IR) spectroscopy: A rapid tool for detection and analysis of foodborne pathogenic bacteria, in Méndez-Vilas,A.(Ed.), Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, 2, 1582 – 1594
[13] Buensanteai, N., Thumanu, K., Sompong, M., Athinuwat, D., & Prathuangwong, S. (2012). The FTIR spectroscopy investigation of the cellular components of cassava after sensitization with plant grow promoting rhizobacteria, Bacillus subtilis CaSUT007, African Journal of Microbiology Research, 6, 603–610
[14] Kong, J., & Yu, S. (2007). Fourier transformed infrared spectroscopic analysis of protein secondary structures, Acta Biochimica et Biophysica Sinica, 39, 549–559
[15] Gupta, A. D., & Sivakumaran, K. (2016). Individual and combined toxic effect of nickel and chromium on biochemical constituents in E. coli using FTIR spectroscopy and Principle component analysis, Ecotoxicology and Environmental Safety, 130, 289–294
[16] Thumanu, K., Sangrajrang, S., Khuhaprema, T., Kalalak, A., Tanthanuch, W., Pongpaichan, S., & Heraud, P. (2014). Diagnosis of liver cancer from blood sera using FTIR microscopy: a preliminary study, Journal of Biophotonics, 1–10
[17] Talari, A. C. S., Martinez, M. A. G., Movasaghi, Z., Rehman, S., & Rehman, I. U. (2017) Advances in Fourier transform infrared (FTIR) spectroscopy of biological tissues, Applied Spectroscopy Reviews, 52, 456–506
[18] Grabowska, B., Sitarz, M., Olejnik, E., & Kaczmarska, K. (2015). FT-IR and FT-Raman studies of cross-linking process with Ca^{2+} ions, glutaraldehyde and microwave radiation for polymer composition of poly (acrylic acid)/ sodium salts of carboxymethyl starch – Part I, Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 135, 529–535
[19] Yin, M., Yang, B., Wu, Y., Wang, L., Wu, H., Zhang, T., & Tuohetaribayi, G. (2016). Prevalence and characterization of Salmonella enterica serovar in retail meats in market place in Uighur, Xinjiang, China, Food Control, 64, 165–172
[20] Najafi, R., Mukherjee, S., Hudson, Jr. J., Sharma, A., & Banerjee, P. (2014). Development of a rapid capture-cum- detection method for Escherichia coli O157 from apple juice comprising nano-immunomagnetic separation in tandem with surface enhanced Raman scattering, International Journal of Food Microbiology, 189, 89–97
[21] Phalang, K., & Chaichanakun, S. (2014). Organic Chemistry (7th ed). Chulalongkorn: Chulalongkorn University Press
[22] Shurvell, H. F. (2002). Spectra-Structure Correlation in the Mid-and Far-Infrared, In Handbook of Vibration Spectroscopy Volume 3, (pp.1791– 1793). Chichester: John Wiley & Sons Ltd
[23] Liu, X., Kaminski, M. D., Guan, Y., Chen, H., Liu, H., & Rosengart, A. J. (2006). Preparation and characterization of hydrophobic superparamagnetic magnetite gel, Journal Magnetic and Magnetic Materials, 306, 248–253
[24] Tuitemwong, P., Songvorawit, N., & Tuitemwong, K. (2012). Facile and sensitive epifluorescent silica nanoparticles for the rapid screening of EHEC, Journal of Nanomaterials, 2013, 706354
[25] Silverstein, R. M., Webster, F. X., & Kiemle, D. J. (2005). Spectrometric identification of organic compounds (7th ed). John Wiley & Sons
[26] Fryhle, C. B., Snyder, S. A., & Solomons, T. W. G. (2018). Organic Chemistry (pp.86–103). John Wiley & Sons
[27] Rani, M., Agarwal, A., & Negi, Y. S. (2011). Characterization and biodegradation studies for interpenetrating polymeric network (INP) of chitosan-amino acid beads, Journal of Biomaterials and Nanobiotechnology, 2, 71–84