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Research Article

The effect of (3-aminopropyl) triethoxysilane and curcumin coating on the physico-chemistry of Fe_3O_4 particles as theragnostics of oral cancer: a descriptive study

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ABSTRACT

Introduction: Superparamagnetic iron oxide nanoparticles (SPION) have been used in MRI and have the capability to conjugate with different ligands. One of the active ingredients of interest in biomedical applications is curcumin (Cur), which has been shown to have anti-inflammatory effects, wound healing properties, and anti-cancer activity. However, such conjugation may need to be facilitated by polymers, such as 3-aminopropyltrimethoxysilane (AMPTS). This study aims to describe the effect of coating materials (AMPTS and Cur) on the physicochemistry of Fe_3O_4 particles as a diagnostic of oral cancer. **Methods:** this research employed descriptive research. Modified SPIONs were synthesized by a simple coprecipitation method. In a molar ratio of 2:1, Fe^{3+} and Fe^{2+} were dissolved and mixed in 40 ml of water. The mixture was then heated at 40 °C until a brown precipitate was formed. AMPTS and Curcumin (20 mg/ml DMSO) were added for modifications. Fe_3O_4 particles were characterized using Fourier transform infrared spectroscopy (FTIR) to analyze the conjugation yield. Modified SPIONs were analyzed using dynamic light scattering (DLS) to examine their size distribution. Furthermore, the zeta potential of the particles was examined. **Results:** DLS showed size increases after modification of SPION with different materials. In addition, there were slight changes in zeta potential. However, FTIR showed no differences in peaks, indicating that no conjugation was successful. **Conclusion:** Although FTIR showed no differences in peaks, DLS and zeta potential showed changes with different coatings, which may indicate conjugation. However, further analyses must be carried out to quantify the conjugation yield.

KEYWORDS: AMPTS, curcumin, iron oxide, oral cancer, coprecipitation

Pengaruh bahan pelapis AMPTS dan kurkumin terhadap fisikokimia partikel Fe_3O_4 sebagai teragnostik kanker mulut: studi deskriptif

ABSTRAK

Pendahuluan: *Superparamagnetic iron oxide nanoparticle (SPION) telah digunakan dalam MRI dan memiliki kemampuan untuk dikonjugasi dengan berbagai ligan. Salah satu bahan aktif yang menjadi sorotan di bidang biomedis adalah curcumin yang memiliki sifat anti-inflamasi, penyembuhan luka, dan aktivitas anti-kanker. Akan tetapi, konjugasi tersebut biasanya memerlukan fasilitator berupa polimer, seperti* 3-aminopropyltrimethoxysilane (AMPTS). *Penelitian ini bertujuan untuk mendeskripsikan pengaruh bahan pelapis 3-aminopropyltriethoxysilane (AMPTS) dan kurkumin (Cur) terhadap fisikokimia partikel Fe_3O_4 sebagai teragnostik kanker mulut.* ***Metode:*** *Jenis penelitian ini adalah penelitian deskriptif. SPION disintesis dan dimodifikasi dengan bahan-bahan pelapis tersebut melalui metode kopresipitasi sederhana. Fe^{3+} dan Fe^{2+} dengan rasio molar 2:1 dilarutkan dan dicampurkan dalam 40 ml air. Campuran dipanaskan pada 40°C hingga presipitat cokelat terbentuk. AMPTS dan Curcumin (20 mg/ml DMSO) ditambahkan dalam larutan untuk modifikasi permukaan. Untuk menganalisis hasil konjugasi, sampel dikarakterisasi dengan fourier transform infrared spectroscopy (FTIR). Berikutnya, sampel dianalisis dengan dynamic light scattering (DLS) untuk melihat distribusi ukuran. Kemudian, potensial zeta sampel diukur.* ***Hasil:*** *DLS menunjukkan peningkatan ukuran setelah modifikasi SPION dengan bahan pelapis. Selain itu, ada perubahan dalam potensial zeta. Akan tetapi, analisis FTIR tidak menunjukkan perbedaan puncak serapan yang mengindikasikan ketiadaan konjugasi.* ***Simpulan:*** *Meskipun FTIR tidak menunjukkan perbedaan puncak serapan setiap sampel, DLS dan potensial zeta menunjukkan perubahan nilai untuk setiap sampel dengan coating berbeda, yang boleh jadi mengindikasikan konjugasi. Akan tetapi, analisis lebih lanjut perlu dilakukan untuk mengkuantifikasi konjugasi.*

KATA KUNCI: *AMPTS, kurkumin, besi oksida, kanker mulut, kopresipitasi*

INTRODUCTION

Oral cancer is a significant global health problem, with an assumption of 275.000 cases and 128.000 deaths each year.[1] Of the cases of oral cancer, more than 90% are *oral squamous cell carcinoma* (OSCC).[2] Conventional cancer therapy, which involves surgery, radiation therapy, and chemotherapy, has various drawbacks, including the lack of specificity, the resistance of cancer cell to treatment, and the toxicity related to the use of medications.[3] In order to overcome these limitations, a new approach has been developed using the Fe_3O_4 particles, known as magnetic nanoparticles.This new innovation aims to selectively target cancer cells without affecting the surrounding normal cells.[4] There are three different ways that Fe_3O_4 particles can be used for cancer treatment: specific antibody conjugation to selectively bind the tumor receptor and obstruct growth; hyperthermia therapy targeting tumor using Fe_3O_4 particles; and holding anticancer medicine.[5] In addition, Fe_3O_4 particles can also be used as contrast agents in magnetic resonance imaging (MRI).[6]

Various synthesis methods have been implemented to produce Fe_3O_4 particles in various sizes and surface loads or coating materials.[7] The most common method to synthesize Fe_3O_4 particles is coprecipitation[8], which is a very simple and efficient technique used in the synthesis of Fe_3O_4 particles.[9] However, it often results in large particles and broad size distribution.[10] The optimal particle size for Fe_3O_4 is 10-100 nm.[11] Particles exceeding 200 nm in diameter are usually cleaned by phagocytes in the spleen, while particles smaller than 10 nm are rapidly eliminated by kidney.[12]

To overcome the weakness, the Fe_3O_4 particles are covered by coating material to reduce the tendency of agglomeration, protect the surface oxidation, provide the surface for drug molecule conjugation and ligand targeting, increase blood circulation time, and minimize non-specific interaction.[13] Curcumin is a natural product that has anti-inflammatory and anti-cancer properties. As a phytochemical anticancer agent, it has a significant advantage as it is not poisonous for normal cells compared to synthetic chemical chemotherapy, yet curcumin shows low bioavailability due to its poor solvability and quick degradation in a basic environment.[14] The coating used in this study is AMPTS (3-aminopropyltrimethoxysilane), a silane that is able to prevent nanoparticle oxidation and provide complete protection for Fe_3O_4 particle structure.[15] AMPTS is proven to have the ability to to significantly limit the growth of Fe_3O_4 nanoparticles up to 6.5 nm through the hydrothermal method.[16] Furthermore, AMPTS provide silane for conjugation, which improves the loading of curcumin onto the surface of the SPION,[17] thus increases the bioavailability of curcumin and improves its efficacy.[18]

Altogether, this study presents an approach to improving the bioavailability of curcumin by conjugation with SPION and thus may improve both diagnostics by SPION and therapy by curcumin. AMPTS, Fe_3O_4 particle conjugation with curcumin gives general benefits: (i) facilitating the absorption of curcumin by cells; (ii) increasing the bioavailability of curcumin; (iii) increasing the cancer therapy index of curcumin (as a nutraceutical agent); and (iv) reducing nanoparticle opsonization of iron oxide.[19] This study aims to describe the effect of coating materials (AMPTS and Cur) on the physicochemistry of Fe_3O_4 particles as a diagnostic of oral cancer.

METHODS

This study employed descriptive study separation was accomplished through decanting, the most simple method for isolating solids from liquid. Materials such as iron (III) chloride hexahydrate ($FeCl_3.6H2O$), iron (II) chloride ($FeCl_2.4H2O$), 3-aminopropyltrimethoxysilane (AMPTS), dimethyl sulfoxide (DMSO), and curcumin powder was obtained from Sigma-Aldrich, while 28% of ammonium hydroxide (NH_4OH) were obtained from EMD Chemical Inc.

The synthesis of Fe_3O_4-AMPTS-Cur particles, curcumin-coated Fe_3O_4 particles, was made using the simple coprecipitation method. In brief, $FeCl_3.6H2O$ and $FeCl_2.4H_2O$ with a molar ratio of 2:1 were dissolved in 40 mL water. The solution was stirred using a hot *plate stirrer* for 15 minutes (40°C) until a brown-colored solution was formed. 100 µl AMPTS was dripped into the Fe(II) and Fe(III) mixtures. Besides that, the curcumin solution was made by dissolving 20 mg of curcumin in 1 ml of DMSO. Then 100 µl of the solution was dripped into the mixture of Fe(II), Fe(III), and AMPTS. Following the addition of 5 ml of ammonium hydroxide (28%) was done, the solution was heated until a black color appeared. The reaction was then continuously stirred at a temperature of 40°C for one hour. After cooling, black nanoparticles will settle on the bottom part, leaving the supernatant transparent on the top side.[19]

FTIR (*fourier transform infrared*) characterisation was measured to investigate the successful functionalization of Fe_3O_4 particles using AMPTS and curcumin.[20] Measurements of particle size distribution and zeta potential were conducted using the same equipment, HORIBA Scientific SZ-100.[21] The sample was prepared in water with a concentration of 100 µg/mL.[19] The wave peaks could not be observed resulting in unachievable curcumin conjugation. The red spectrum showed the FTIR spectrum of Fe_3O_4 coated with AMPTS and conjugated with curcumin. To determine the size distribution of the modified SPIONs, dynamic light scattering (DLS) was performed. Furthermore, an analysis of zeta potential of the particles was also conducted.

RESULTS

The FTIR spectrum of the three produced samples is illustrated in Figure 1. The Fe_3O_4 sample shows a peak at the wave number 595.8 cm^{-1}, which indicates the vibration of the Fe-O bond in Fe_3O_4.[22] the peak wave number of Fe_3O_4-Cur and Fe_3O_4-AMPTS-Cur shifted to 533.8 cm^{-1} and 602.4 cm^{-1}, respectively.[22] During the process of coprecipitation, the surface of the Fe_3O_4 particle was easily covered by a hydroxyl group in a watery environment.24[23] This is shown by the peak wave numbers 1633.7 cm^{-1} and 3279.3 cm^{-1} (Fe_3O_4), 1634.2 cm^{-1} and 3313.4 cm^{-1} (Fe_3O_4-Cur), and 1633.5 cm^{-1} and 3314.9 cm^{-1} (Fe_3O_4-AMPTS-Cur).[5]

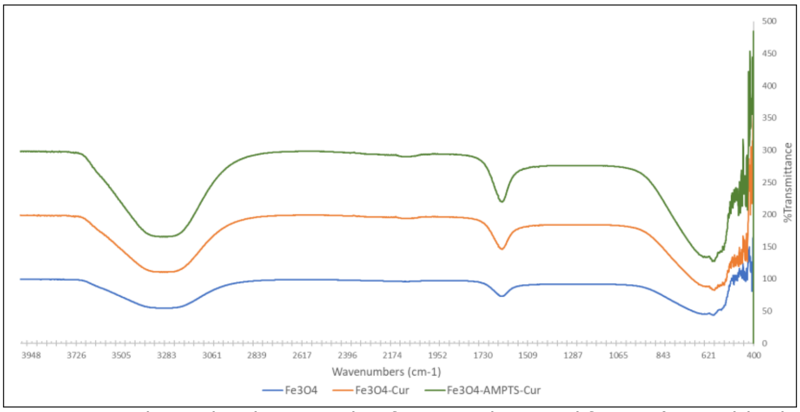
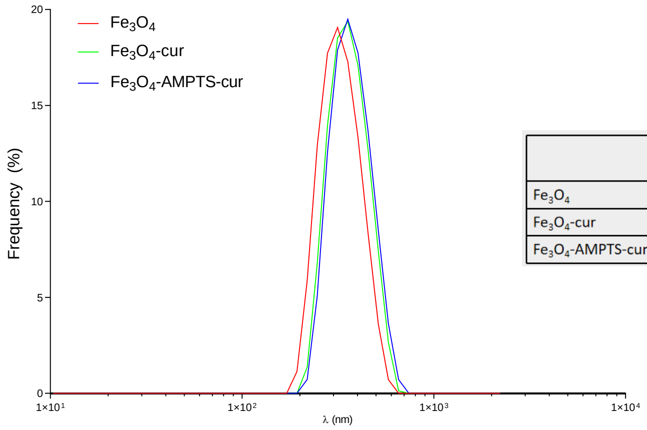


Figure 1. FTIR spectra shows absorbance peaks of Fe_3O_4 without modification (Fe_3O_4, blue line), following to modification by curcumin (Fe_3O_4-Cur, orange line), and following to modification by AMPTS and curcumin (Fe_3O_4-AMPTS-cur, green line).



	mean (nm)	SD	PDI	z-average (nm)
Fe_3O_4	312.7	73.5	2.394	226.5
Fe_3O_4-cur	345.3	78.6	2.898	250.7
Fe_3O_4-AMPTS-cur	355.5	82.0	3.141	262.4

Figure 2. Dynamic light scattering showing size distributions of Fe_3O_4 without modification (Fe_3O_4, red line, 226.5 nm), following the modification by curcumin (Fe_3O_4-Cur, green line, 250.7 nm), and following the modification by AMPTS and curcumin (Fe_3O_4-AMPTS-cur, blue line, 262.4 nm).

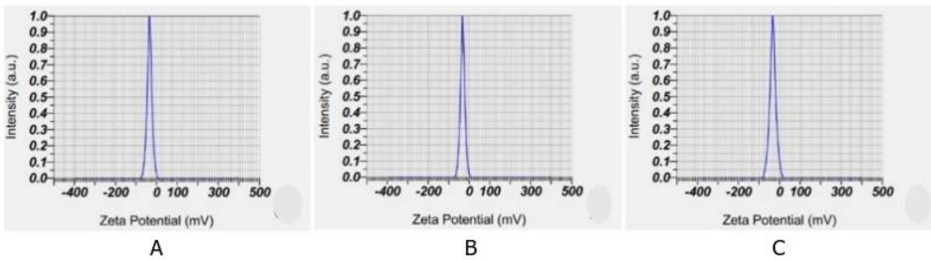


Figure 3. Zeta potential distributions of Fe_3O_4 without modification (Fe_3O_4, -33.9 mV), following modification by curcumin (Fe_3O_4-Cur, -31.9 mV), and following modification by AMPTS and curcumin (Fe_3O_4-AMPTS-cur, -32.9 mV).

The average diameter of the Fe_3O_4, F_3O_4-Cur, and Fe_3O_4-Cur-AMPTS particles is determined by the *particle size analyzer* (Figure 2). The preparation process yields an average diameter of 226.5 nm, 250.7 nm, and 262.4 nm for Fe_3O_4, F_3O_4-Cur, and Fe_3O_4-Cur-AMPTS, respectively. The yielded values of zeta potential measurement (Figure 3) from Fe_3O_4, F_3O_4-Cur, and Fe_3O_4-Cur-AMPTS are as follows: -33.9 mV, -31.9 mV, and -32.9 mV, respectively.

DISCUSSION

The FTIR spectrum in Figure 1 shows that the process of the Fe_3O_4 particle coating with AMPTS and curcumin coating agents has not been successfully done. We are unable to make precise comparisons in our study due to the limited equipment and, consequently, their own constraints. The wave peak at 3502 cm-1, which generally corresponds to curcumin, indicates OH from curcumin phenol group.[19] Besides that, the wave peak at 1427 cm^{-1} is related to the vibrations of C-O, C=O, and C-H from benzene ring in curcumin.[19] If the wave peaks are not observed, the curcumin conjugation are unable to be conducted. The red spectrum shows the FTIR spectrum from the Fe_3O_4 particles coated with AMPTS and conjugated with curcumin. The wave peak, which generally shows AMPTS, appears at 993 cm^{-1}, indicating the Si-O-Fe bond.[24] The absence of the wave peak showing AMPTS and curcumin makes the process of AMPTS coating and curcumin conjugation unfeasible. The FTIR results of the three samples only shows a Fe-O bond and hydroxyl group without the presence of a coating agent.[22,23]

The hydrodynamic diameter of the three samples in Figure 2 does not show significant differences in size. In contrast to more advanced processes (i.e. hydrothermal, sol-gel), we could not successfully synthesize SPION in nanometer range since the growth in precipitation is uncontrolled. As for the particle size, it does not illustrate the real size of the particle as the particle is covered by the hydroxyl group found in the liquid.26[25] Besides the hydrodynamic diameter, the zeta potential value of the three samples (Figure 3) does not show significant difference. The high negative load of the three samples shows that the particles contain numerous hydroxyl groups on their surface and thus are not easily aggregated; loads between ±30 and 40 mV are categorized as moderately stable.[26]

A study with a similar method by Darwesh *et al*,.[19] resulted in Fe_3O_4 nanoparticle curcumin conjugated without using AMPTS. However, after we tried to conduct conjugation using a similar method, we found weaknesses in the methodology. The method in the study by Darwesh *et al*.,[19] did not mention the amount of concentration of Fe^{3+} and Fe^{2+} masses dissolved in water. The lack of information, therefore, resulted in the inaccurate amount of the Fe concentration and the coating agents. Besides that, the Fe concentration was suspected to be too high, which led to the production of larger particles.[27] Another weakness was that it did not use atmospheric aid of *inert* gas of nitrogen and sonicator. In contrast to a study conducted by Rahimnia *et al*.,[5] which carried out curcumin conjugation using coprecipitation method with the help of nitrogen *inert* gas atmosphere. This method can increase particle dispersion, resulting in smaller and softer crystal grain, and enriches pore channel, so particles will find it easier to chemically bond with coating agents.[28] In addition, in regard to the functioning of AMPTS, a study conducted by Khosroshahi *et al*.,[24] carried out sonication of the Fe_3O_4 particles before they were functional with AMPTS. Sonication allows big particles to break down into smaller fragments, or particles that are uniform in size in the solution, so Fe_3O_4 particles will find it easier to chemically bond with AMPTS.[29]

Due to the lack of equipment and supporting materials including gas *inert* nitrogen and a sonicato*,* we chose the simplest method possible to obtain the Fe_3O_4 particles that were coated with curcumin and AMPTS coating agents. It is recommended that further studies are able to employ more advanced methods and equipment to perform AMPTS and curcumin function. Additionally, conduct reviews on the concentration of Fe precursor should be conducted in order to obtain data on the effect of the coating agent towards Fe_3O_4, particle physicochemical characteristics. The quantity of samples were as per the plan for the experiment, and no population sampling was conducted. The limitation of this research was the restricted availability of instruments.

CONCLUSION

Although FTIR showed no differences in peaks, DLS and zeta potential showed slight changes with different coatings, which makes it unclear to draw a conclusion. Ultimately, further analyses must be carried out to quantify and the conjugation yield and analyze its results.

Author Contribution: "Conceptualization, G.Y., N.D., and D.F.M. D.A; methodology, D.F.M.; investigation and data curation, G.Y.; resources: N.D. and D.F.M.; visualization, G.Y.; analyses and validation, G.Y., N.D., and D.F.M; writing—initial draft composing, G.Y.; writing-review and editing, G.Y., N.D., and D.F.M. D.A; project administration, D.F.M.

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Data Availability Statement: Research data availability will be provided with the permission of all researchers through email correspondence by considering ethics in the research.

Conflict of Interest: The authors stated that there is no conflict of interest in this research.

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