Comparison Characteristics of Different Synthesis Methods for Production of Gadolinium Diethylenetriaminepentaacetate-Folate

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***Abstract*—Gadolinium diethylenetriaminepentaacetate-folate (Gd-DTPA-Folate) has been proposed as a novel Magnetic Resonance Imaging contrast agent for diagnosing cancer that overexpressed folate reseptor. Gd-DTPA-Folate has been synthesized succesfully via γ-methyl esther EDA-folate through indirect synthesis methods with the complicated routes of synthesis. This continuing study is aimed to compared the characteristics of Gd-DTPA-Folate which has been synthesized in our previous study with Gd-DTPA-Folate wich has been synthesized directly via EDA-Folate. The characteristics of structures will be compared using Ultraviolet, Infrared and Mass Spectroscopies. From the result, it can be concluded that Gd-DTPA-Folate which have been synthesized via two methods have similar characteristics. The experimental data from ultraviolet showed absorption peak at 274 nm (from the C=C bond of benzene rings) and 362 nm (from C=O bonds), the infrared spectras showed that the presence of absorption at similar wavenumer in the functional groups (4000-1400 cm^{-1}), and the mass spectra showed that a parent Peak at m/z 1011 which indicated a molecular weight of Gd-DTPA-Folate.**

***Keywords*—Gadolinium, Gd-DTPA-Folate, Magnetic Resonance Imaging, Synthesis Methods.**

I. Introduction

Magnetic Resonance Imaging (MRI) is able to detect cancer more rapidly than other diagnosis techniques can do. Furthermore, there are some more advantage of MRI than those other methods such as Computer Tomography (CT), Ultrasonography (US) or PET/SPECT, those are much smaller hazardous effect of radiation and the non-invasives techniques [1].

The quality of cancer imaging using MRI will improve if we use contrast agents. Contrast agents which has been recommend by FDA USA in 1988 is gadolinium diethylenetriaminepentaacetate (GD-DTPA) which the commercial name is Magnevist, and widely, has been used in many countries in the world. However, Gd-DTPA cannot detect cancer specifically [3],[6].

For this purposes, we develope a novel MRI contrast agent which can detect cancer specifically. Folic acid has a high affinity to the folate receptor on the surface of cancer cell, therefore folic acid will be bring Gd-DTPA directed to the cancer which are overexpressed folate receptor. Gd-DTPA-Folate will entrance the cancer cell via receptor mediated endocytosis process [9].

Ethylenediamine-Folate (EDA-Folate) as a precursor of DTPA-Folate ligand has been synthesis throught derivate of folic acid steps [4],[5],[7]. However this method is very complicated and non economically. For the alternative procedurs, we develop a new method synthesis through folic acid activation via directed method which were adopted from the Wang methods [8].

The synthesis methods of EDA-Folate via direct and indirect methos has been compared and showed there was no significant differences between two methods [2].

In this research, we will produces two diethylentriaminepentaacetet-folate (DTPA-Folat) from its precursor EDA-Folate which has been synthesis via two methods. Then DTPA-Folate will conjugated to gadolinium and forming Gd-DTPA-Folate. The characteristic of two Gd-DTPA-Folate will be compared using spectroscopies methods, such as ultraviolet, infrared and mass spectrocopies.

II. Materials and Methods

A. Materials

Chemicals for this research were EDA-Folate which were synthesis via direct and indirect method in our preeliminary studies [4], dimethylsulfooxida (DMSO) purchased from Sigma Aldrich, diethylenetriaminepentaacetate–dianhydride (DTPA dianhydride) purchased from Chematech, gadolinium chloride ($GdCl_3$) which were the source of gadolinium ion from Sigma Aldrich, potasium hydroxide (NaOH) and hydrochloric acid (HCl) were from Merck for adjusting pH.

B. Synthesis of Gadolinium Diethylenetriaminepentaacetate-Folate (Gd-DTPA-Folate) via direct and indirect methods.

EDA-Folate (a) was synthesis from folic acid via direct

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method through two step reaction. (1) activated folic acid as N-hydrosucsinimide-Folate (NHS-Folate), and (2) the formation of EDA-Folate from reaction between NHS-Folate and EDA [2].

EDA-Folate (b) was synthesis from folic acid via indirect method through five step reaction. (1) Cyclization of one parit of folic acid (pyrofolic acid), (2) formation of pteroyl hydrazide, (3) formation of pteroyl azide, (4) formation methyl Esther folic acid, and (5) formation of EDA-Folate [4].

The next step were the formation of DTPA-Folate (for each of EDA-Folate which were synthesized via direct and indirect method). The two solution of EDA-Folate (EDA-Folate and DMSO) were reacted with the suspension of DTPA dianhydride (DTPA dianhydride and DMSO) into erlenmeyer flask, then mixing solution was stirred buntil perfectly reacted. pH was Measures and adjusted using NaOH/HCl 0.5 M. Yellow solid of DTPA-Folate was separated using sentrifugator and Then washed using acetonitrile/Water. DTPA-Folate were dryed 2-3 days (c) and (d).

The final process was formation of Gd-DTPA-Folate (e) and (f). DTPA-Folate (c) and (d) were reacted with $GdCl_3.6H_2O$ (1:2) in the reflux flask using Water as the solven. Each of mixing solutions were reacted for two hours, then pH was adjusted using NaOH/HCl 0.5 M. Yellow Ale solid of Gd-DTPA-Folate (e) and (f) were dryed under vacum pressure for 2-3 days.

C. Characterization of Gadolinium ***Diethylenetriaminepentaacetate-Folate using Spectroscopic Method***

Product of Gd-DTPA-Folate (e) and (f) were characterized using Ultraviolet, Infrared and Mass spectroscopies method. The result of characterization of Products Gd-DTPA-Folate (e) using Ultraviolet, Infrared and Mass spectroscopies method was compared with Products of Gd-DTPA-Folate (f).

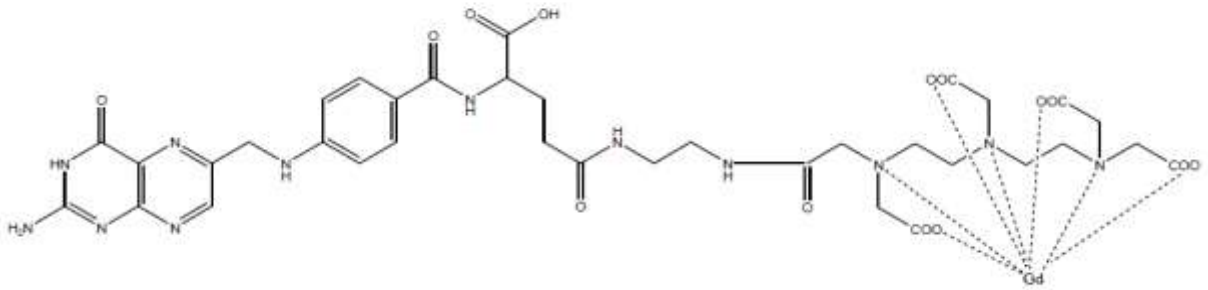

Fig.1 A proposed structure of (γ)-Gd-DTPA-Folate

III. Results and Discussion

A. Synthesis of Gadolinium ***Diethylenetriaminepentaacetatet-Folate via direct and indirect methods.***

EDA-Folate as a precursor of ligand DTPA-Folate has been synthesized through two methods, via direct and indirect methods [2],[4].

Structure (γ)-EDA-Folate was more preferable than (α)-EDA-Folate, because for the continue step reaction Gd-DTPA-Folate in gama position will conjugated to folate reseptor which is overexpressed on the surface of cancer cells and for future application (γ)-Gd-DTPA-Folate will used as a targeted contrast agent in Magnetic Resonance Imaging for cancer diagnosis which positive of folate reseptors.

B. Comparison Characteristics of Gadolinium Diethylenetriaminepentaacetate-Folates using Spectroscopic Method

Characterization using ultraviolet spectroscopy

The resulted synthesis of two products were characterized using ultraviolet spectroscopy method and showed a similar characterization of absorption peak at two wavelengths (Fig.2), 274 nm and 362 nm (for two products).

The first wavelength 274 nm is from the electronic transition of π-π* which came from benzene rings at the structure of Gd-DTPA-Folate.

The second wavelength 362 nm is from the electronic transition of n-π* which came from conjugated C=O at the structure of Gd-DTPA-Folate.

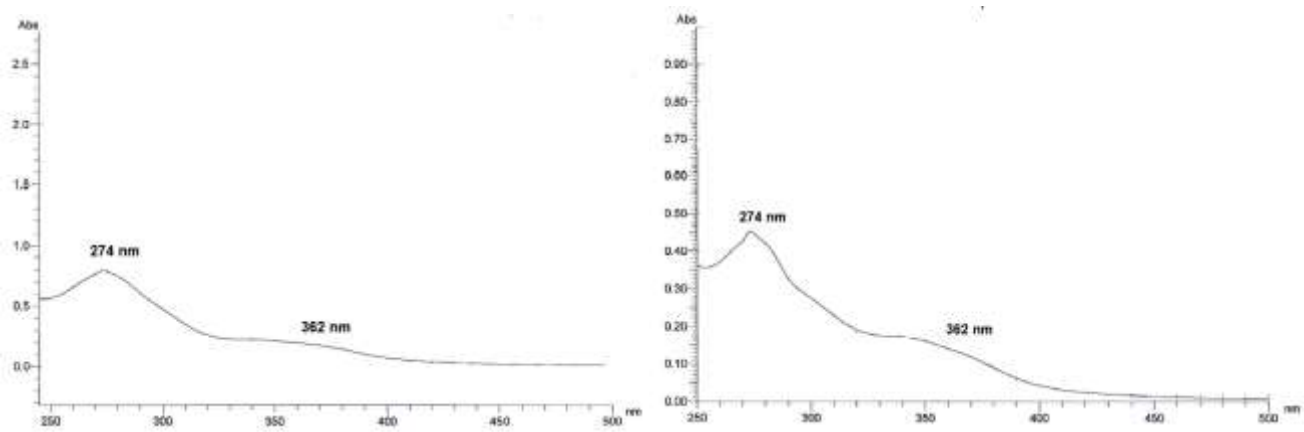

Fig.2 Ultraviolet spectrum of Gd-DTPA-Folate which were synthesized via direct (left) and indirect (right) methods.

Characterization using infrared spectroscopy

The resulted synthesis of two products were characterized using infrared spectroscopy method and showed the presence of absorption at similar wavenumber (Fig.3) in the functional groups (4000-1400 cm^{-1}).

The absorption peaks are: 3317,99-3332,03 cm^{-1}, which were originating from stretching O-H and N-H; at 2829,31-2829,74 cm^{-1} from the stretching vibration of C-H groups; at 1587,90-1633,00 cm^{-1} from the stretching of C=O and at 1025,43-1026,33 cm^{-1} from C-O and C-N groups.

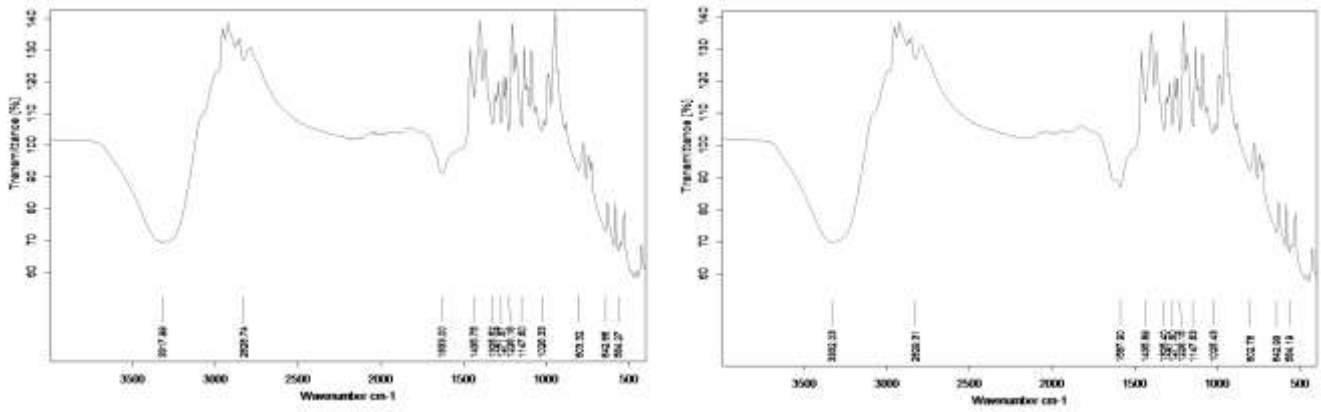

Fig.3 Infrared spectra of Gd-DTPA-Folate which were synthesized via direct (left) and indirect (right) methods.

Characterization using mass spectroscopy

The resulted synthesis of two products were characterized using mass spectroscopy method and showed the presence of parent peak at similar m/z which correspondence to the calculated of molecule compound of Gd-DTPA-Folate (Fig.4).

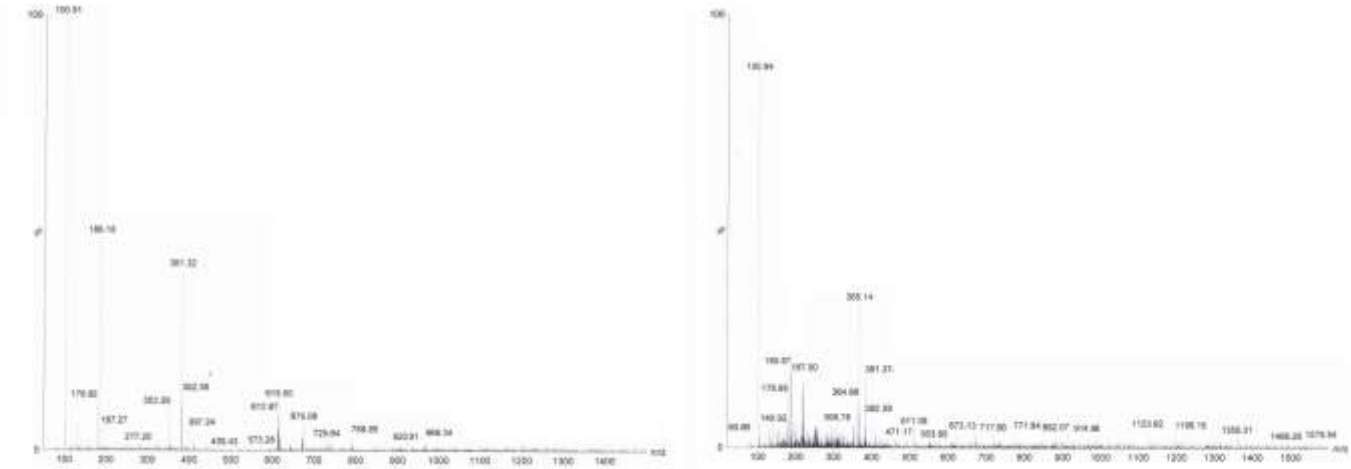

Fig.4 Mass spectra of Gd-DTPA-Folate which were synthesized via direct (left) and indirect (right) methods.

IV. Conclusion

Based on discussion above, we can concluded that Gd-DTPA-Folate which were synthesized via direct and indirect method have similar characteristics, those can be seen at ultraviolet spektrum which showed similar wavelength (274 nm and 362 nm), at infrared spectra whcih Shaw the presence of similar wavenumber in the functional groups (4000-400 cm^{-1}) and showed similar m/z at 101 which come from m/z of Gd-DTPA-Folate is 1011 (the tenth of 1011).

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