5-FLUORORACIL LOADING AND RELEASING BEHAVIOR FROM ALKYLATED POLYAMIDOAMINE G3.0 DENDRIMER-FOLATE

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ABSTRACT

In recent years, many researches have been developing towards biocompatible improvement and cellular penetrating ability of dendrimers in order to use in diagnosis and treatment therapy for several type of cancer. In this study, to enhance biocompatibility, drug loading efficiency and cellular uptake of the polyamidoamine dendrimer, the dendrimer generation 3.0 (G 3.0) was conjugated with hexanoyl chloride and targeted with acid folic (G3.0-C6-FA). The structure of the G3.0-C6-FA was determined by ^{1}H-NMR. According to ^{1}H-NMR spectra, 9 hexanoyl groups and 2 folate groups were attached to the G3.0 dendrimer. TEM image of G3.0-C6 dendrimer exhibited spherical shape and nano sizes ranging from 3 to 4 nm and TEM image of the G3.0-C6-FA indicated a size distribution ranging from 5 to 7 nm. In addition, Fluorouracil (5-FU)-loaded G3.0 and 5-FU-loaded G3.0-C6-FA were also prepared to evaluate drug loading efficiency was using High-performance liquid chromatography (HPLC). The obtained results indicated that drug loading efficiency of G3.0-C6-FA (13,8% of 5-FU) is higher than G3.0 (11% of 5-FU). 5- G3.0-C6-FA also showed a slow release profile of the drug. These positive results show a potential of the drug-nanocarrier system in practical application.

Keywords: *Alkylated dendrimer, 5-FU, drug delivery.*

1. INTRODUCTION

5-Fluorouracil (5-FU) is highly effective drugs for chemotherapy treatment of breast cancer. However, it has many side effects to patients due to their non-specific interaction with abnormal and normal cells. Many studies had showed that using drugs-loaded nanoparticles could reduce side effects of 5-FU. It can also prolong plasma circulation and enhance drug accumulation in cancer tumors by increased permeability-retention (EPR) effect. One type of nano-carrier is dendrimer, which is one of the most studied dendritic nanopolymers with internal cavities that can be ultilized as a novel nanocarrier for dilivering anticancer drug, because drugs can be encapsulated via (non-) covalent interactions resulting in decreasing its side effects [2-3].

Polyamidoamine (PAMAM) dendrimer is the most well known nanopolymer that can be synthesized with controlled size and predetermine molecular weight. Many generations and derivatives of PAMAM have

been being studied and applied in biomedical fields such as drug/gene delivery nanocarriers, therapeutic/diagnostic nanodevices etc. [4-7]. However, there are a few disadvantages accompanied with PAMAM dendrimer drug-delivery system including hemolytic toxicity and cell lysis, which happen due to strong interactions of the positively charged dendrimer and the negatively charged cell membrane resulting in membrane disruption [8-10]. Chemical modification of the surface is an important strategy to overcome the toxicity problems of the dendrimers, for example pegylation, acetylation, carbohydrate and peptide conjugation… The conjugation may lead to increase the inner cavity space of dendrimers that contribute to the increment of drug-loading capacity. Moreover, the drug nanocarriers can also increase the residence time of the drug in blood circulation by its stealth properties in the blood plasma [11, 14].

In this study, denrimers G3.0 are modified with FA and hexanoylchloride. This modify can improve the biocompatibility of PAMAM G3.0 such as reduced toxicity, increased ability to carry drugs and target cancer cells. The PAMAM G3.0 dendrimer and PAMAM G3.0-C6-FA dendrimer were evaluated ability to carry and release the drug 5-FU using HPLC assay.

2. MATERIALS AND METHODS

2.1. Materials

5-FU, methyl acrylate (MA), ethylendiamine (EDA), toluen, Hexanoyl chloride, Dimethylformamide (DMF), dimethylsulfoxide (DMSO) and 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) were purchased from Acros Organics. Methanol (MeOH, China). Regenerated Cellulose MWCO 3500-5000D dialysis bags were purchased from Spectrum Laboratories Inc.

2.2. Synthesis of the hexanoyl-PAMAM G3.0 dendrimer (G3.0)

2.2.1. Materials

PAMAM G3.0 dendrimer (Fig. 1) was synthesized from ethylendiamin (EDA) methyl acrylate (MA) via step-wise Michael addition reaction, and amidation of multifunctional groups as reported of Donald Tomalia [4]. Structure and morphology were characterized using ^{1}H-NMR Spectrometer and TEM. [1]

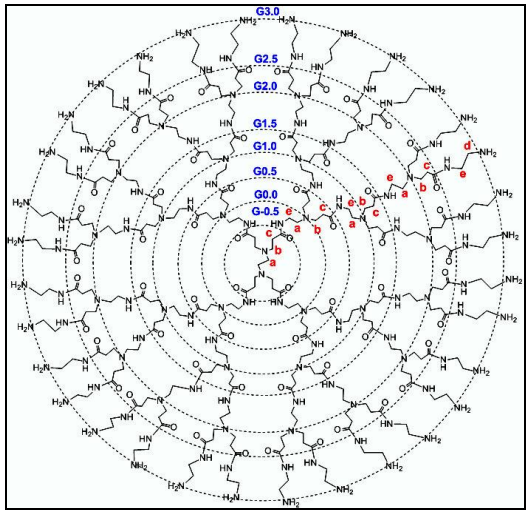
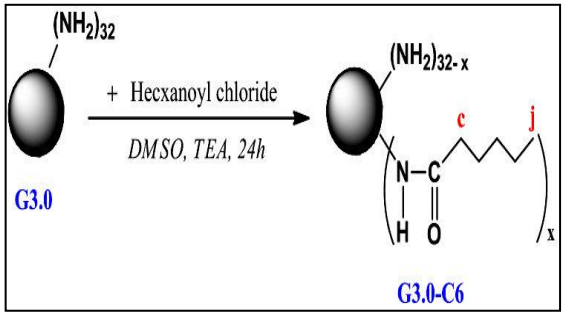


***Figure 1**: Illustration of PAMAM G3 dendrimer*

2.3. Synthesis of the hexanoyl-PAMAM G3.0 dendrimer (G3.0-C6)

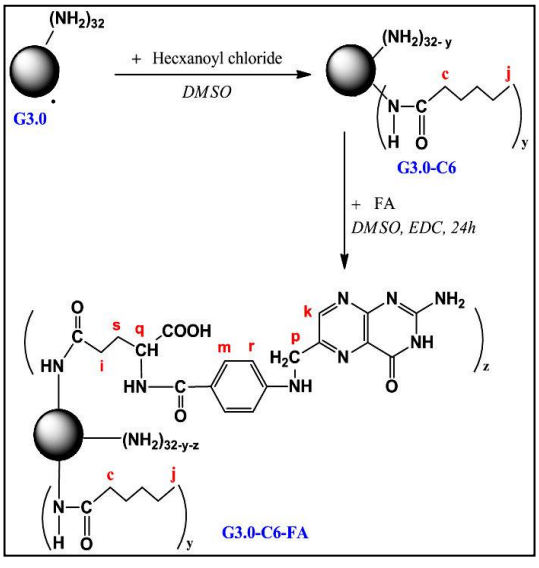
1 mmol G3.0 PAMAM dendrimer and 16 mmol TEA were completely dissolved in 10 ml DMSO and then 16 mmol hexanoyl chloride in 10 ml DMSO was added dropwise to the dendrimer solution (scheme 1). The mixture was stirred for 24 hours at room temperature. The crude product was dialyzed (MWCO 3,500-5,000 D dialysis bags) with MeOH in 48 h, and dried *in vacuo* to obtain the G3.0-C6 product. The structure and properties of the product were determined by ^{1}H-NMR, TEM.



***Scheme 1**: Synthetic scheme of the hexanoyl-PAMAM G3.0 dendrimer (G3.0-C6)*

2.4. Synthesis of the hexanoyl-folat-PAMAM G3.0 dendrimer (G3.0-C6-FA)

0.4 mmol FA was fully dissolved in 9 ml of DMF and 3 mL of DMSO and added to 0.8 mmol EDC. This mixture was then added dropwise to the solution of 1 mmol PAMAM and 16 mmol hecxanoyl chloride in 10 ml of DMSO. The result mixture was stirred for 24 hours at room temperature (Scheme 2). The crude product was dialyzed (MWCO 3,500-5,000 D dialysis bags) with MeOH in 48 h, and dried *in vacuo* to obtain the G3.0-C6-FA product. The structure and properties of the product were determined by ^{1}H-NMR, TEM



***Scheme 2**: Synthetic scheme of the hexanoyl-folat-PAMAM G3.0 dendrimer (G3.0-C6-FA)*

2.5. 5-FU loading and in vitro release evaluation

0,3 mmol 5-FU was dissolved in 10 ml of distilled water, then added to 0,025 ml (G3.0 or G3.0-C6-FA) sample under slow stirring (50 rpm) for 24 h. The mixture was sonicated for about 30 minutes in order to increase the drug encapsulated. After that, it was dialyzed twice in 40 min to remove the amount of insoluble 5-FU. The dialyzed solution was lyophilized and used for further studies. The drug loading capacity of 5-FU in samples were calculated by comparing the weight of 5-FU in nanocarrier with the weight of original 5-FU and nanocarrier by HPLC method.

For release study, 5-FU-loaded sample (4,5 ml) and 10 mL deionized water were added to dialyzer membrane (MWCO 3,500D) to dialyze against 1000 mL deionized water. At a predetermined time interval, 10 mL of dialyzed solution was drawn to determine 5-FU release by absorbance measurement at wavelength 265.5 nm. After that another 10 mL of deionized water was added to the dialyzed solution to compensate for the withdrawn volume. Similar concentration of dendritic solution without drug loading was dialyzed in the same condition to serve as control.

3. RESULTS AND DISCUSSION

3.1. Characterization of the PAMAM G3.0 dendrimer (G3.0)

1H NMR (PAMAM G3.0, MeOD, ppm) (Fig. 2): 2.605-2.618 (a), 2.804-2.831 (b), 2.379-2.404 (c), 2.735-2.760 (d) and 3.261-3.334 (e). Using method to calculate molecular weight of PAMAM dendrimer [1], we calculated the molecular weight of PAMAM G3.0 dendrimer Mw appro-ximately 6529. TEM image of PAMAM G3.0 dendrimer

showed that the synthesized nanoparticles were formed with spherical shaped ranging from 3 nm to 4 nm [1], [4].

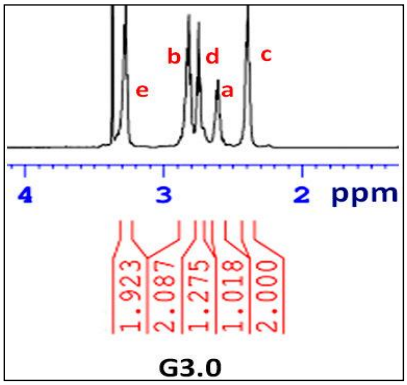


***Figure 2**: [1]H-NMR spectra of the PAMAM G3.0 dendrimer (G3.0))*

3.2. Characterization of the hexanoyl dendrimer G3.0 (G3.0-C6)

Amide reaction between PAMAM G3.0 dendrimer and hexanoyl chloride easily occur in the presence of TEA. 1H-NMR spectra of G3.0-C6 derivative of PAMAM dendrimer (500 MHz, D2O, δppm) showed typical peaks of the synthesized dendrimer (Fig. 3): 2.589 (a), 2.780-2.793 (b), 2.379-2.415(c), 2.974 (d), 3.260 (e) and 0.808-0.836 (j). Based on 1H NMR spectra, degree of activation is 35,71% (number of alkylated group (z, 11); Mw, 8040) [1].

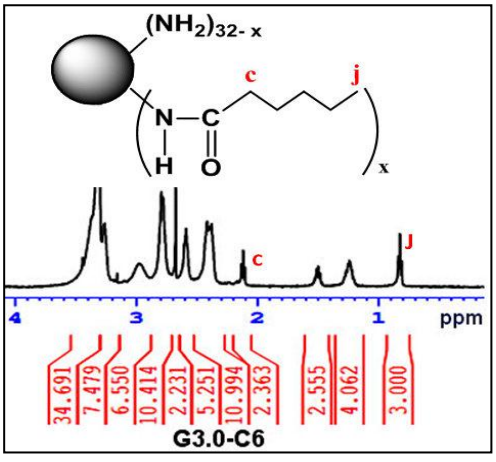


***Figure 3**: 1H-NMR spectra of the hexanoyl dendrimer G3.0 (G3.0-C6)*

Compared with TEM image of PAMAM G3.0 dendrimer (spherical shaped ranging from 3 nm to 4 nm), TEM image of the G3.0-C6 (Fig. 5a) has in size from 4 to 6 nm. This results are consistent with reports of (name of author, publishing date) [1, 4], [17-19].

3.3. Characterization of the hexanoyl-folat-PAMAM G3.0 dendrimer (G3.0-C6-FA)

1H-NMR spectra of G3.0-C6-FA compounds have proton signals of characteristic groups: -NHCO-CH2(CH2)3CH3 (0,823ppm, peak j) and -NHCO-CH2(CH2) 3CH3 (1,242-2,131 ppm). When replacing proton H of the -NH2 groups in PAMAM G3.0 dendrimer by -CO-CH2(CH2)3CH3 groups, 1H-NMR spectra of G3.0-C6-FA derivative also display the characteristic proton signals of the atom groups in folic acid: k (8,543 ppm), m (7,614-7,631 ppm); r (6,711-6,728 ppm); p (4,700 ppm); q (4,488 ppm); i, s (2,102-2,348 ppm) and in the PAMAM molecule: a (2,524 ppm); b (2,815 ppm); c (2,102-2,348 ppm), d (2,734 ppm); e (3,208 ppm) (Fig. 4).

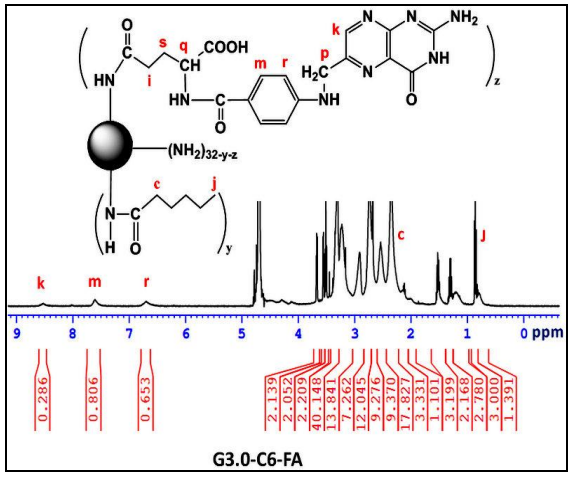


***Figure 4**: 1H-NMR spectra of the hexanoyl-folat-PAMAM G3.0 dendrimer (G3.0-C6-FA)*

Formula 1: Method to calculate degree of PAMAM dendrimer derivatives.

$$x\% = \frac{\frac{S_{H(-C\overset{(j)}{H_3})}}{S_{H(-C\overset{(a)}{H_2}-)}}}{\frac{\sum H_{(-C\overset{(j)}{H_3})}}{\sum H_{(-C\overset{(a)}{H_2}-)}}}.100\%$$

$S_{H(-C\overset{(j)}{H_3})}$, $S_{H(-C\overset{(a)}{H_2}-)}$: The peak area of protons in (j) and (a) positions were appeared in the ^{1}H-NMR spectrum.

$\sum H_{(-C\overset{(j)}{H_3})}$ $\sum H_{(-C\overset{(a)}{H_2}-)}$: The sum of protons in (j) and (a) positions in the molecular formula of the PAMAM dendrimer'

x%: Degree of alkylation derivatives.

The conversion (x%), conversion groups (y, z) and molecular weight (Mw) of the hexanoyl-folate-PAMAM G3.0 dendrimer product are identified by ^{1}H NMR (Table 1). [1]

***Table 1**: The conversion (x%), conversion groups (y, z) and molecular weight (Mw) of the hexanoyl-folat-PAMAM G3.0 dendrimer derivative*

Dendrimers derivative		x% (y)	x% (z)	M% (w)
G3.0-C6-FA	G3.0-$(C6)_y$-$(FA)_z$	29% (9)	6% (2)	638

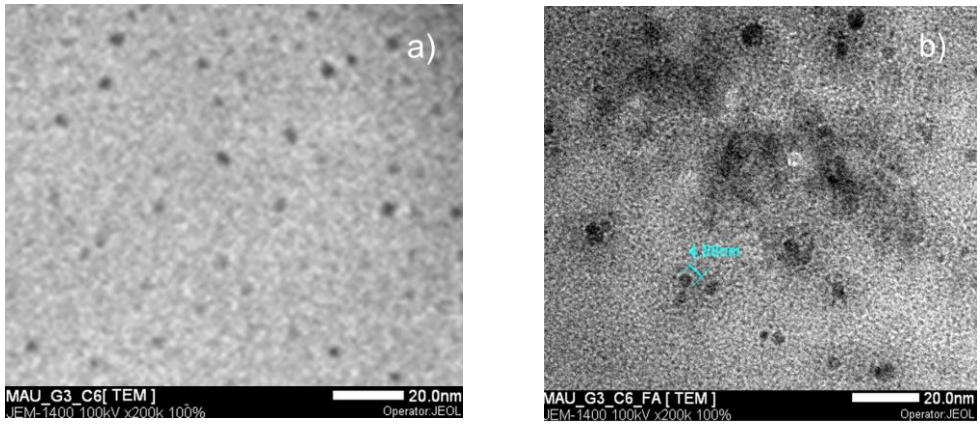


***Figure 5**: Morphologies of dendrimers indicate these spherical shaped nanoparticles ranging from 4 nm to 5 nm for G3.0-C6 (a) and 5 to 8 nm for G3.0-C6-FA (b)*

Compared with TEM image of PAMAM G3.0-C6 dendrimer (spherical shaped ranging from 4 nm to 6 nm) (Fig. 5a), TEM image of the G3.0-C6-FA (Fig. 5b) has size ranging from 5 to 7 nm. This size corresponds to the molecular weight (8638 Da), which asserts that G3.0-C6-FA has been successfully synthesized.

3.4. 5-FU loading and in vitro release evaluation

Loading efficiency was about 11%, which means approximately 6 drug molecules were encapsulated within one G3.0 molecule structure. Moreover, optimal drug loading was also determined around 13,8 % for G3.0-C6-FA (9 drug molecules were encapsulated within one G3.0-C6-FA molecule structure).

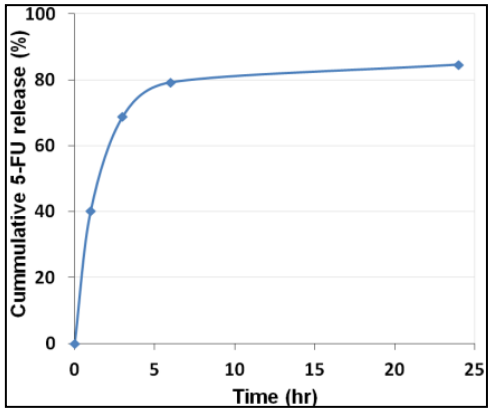


***Figure 6**: Release profile of 5-FU from drug-loaded dendrimer.*

Release profile of encapsulated drug molecules is shown in Fig. 6. drug was slowly released from the system and reached more than 84% after 24 hours. This is a significant improvement in prolonging drug bioavailability, since 5-FU anticancer drug was reported to have short remaining time in blood circulation.

4. CONCLUSION

In this work, dendrimer based drugs nanocarriers were successfully prepared and charaterized to determine their structure, morphologies, drugs loading capacity, slow release ability in vitro. Effect of drug-encapsulated nanocarrier has evaluated. These positive results show a potential of the drug-nanocarrier system in practical application. These obtained results may pave the way for further studies and development of dendrimer-based nanocarriers application in Vietnam.

ĐẶC TÍNH MANG VÀ NHẢ 5-FLUORORACIL CỦA ALKYLATED POLYAMIDOAMINE G3.0 DENDRIMER-FOLATE

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TÓM TẮT

Trong những năm gần đây, nhiều nghiên cứu đã được phát triển theo hướng cải thiện tương thích sinh học và khả năng thâm nhập vào tế bào của dendrimer để sử dụng trong chẩn đoán và điều trị cho một số loại ung thư. Trong nghiên cứu này, để tăng cường sự hấp thu của tế bào, tính tương thích sinh học và hiệu quả mang thuốc của dendrimer polyamidoamine, thế hệ dendrimer 3.0 (G3.0) đã được liên hợp với hexanoyl clorua và tác nhân hướng đích folic acid (G3.0-C6-FA). Cấu trúc của G3.0-C6-FA được xác định bằng 1H-NMR. Theo phổ 1H-NMR, 9 nhóm hexanoyl và 2 nhóm folate được gắn vào dendrimer G3.0. Ảnh TEM cho thấy hình thái cấu trúc cầu kích cỡ 3-4 nm của G3.0-C6 dendrimer và G3.0-C6-FA chỉ định một phân bố kích thước cầu từ 5-7 nm. Ngoài ra, G3.0 mang Fluorouracil (5-FU) và G3.0-C6-FA mang 5-FU cũng đã được chuẩn bị để đánh giá hiệu quả mang thuốc (sử dụng sắc ký lỏng cao áp - HPLC). Các kết quả thu được cho thấy rằng hiệu quả mang thuốc của G3.0-C6-FA (13,8% của 5-FU) cao hơn so với G3.0 (11% của 5-FU). G3.0-C6-FA mang 5-FU đã được chứng minh một khả năng nhả thuốc chậm. Những kết quả này cho thấy tiềm năng của hệ thống mang thuốc nano trong ứng dụng thực tế.

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