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A Simple Quaternization Method of Hyperbranched Polyamidoamine Polymer and Antimicrobial Activity Evaluation of Cationic Hyperbranched Polyamidoamine Polymer

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A series of cationic hyperbranched polyamidoamine (cationic PAMAM) containing terminal methyl ester end group (G-0.5, G0.5, G1.5, G2.5 and G3.5) were prepared by repetitive reactions between Michael addition and amidation to obtain the methyl ester group terminated hyperbranched PAMAM and then followed by a simple methylation of thus obtained PAMAM with dimethyl sulphate to obtain a series of cationic PAMAM. Characterizations by FTIR and ^{1}H NMR analysis were performed to investigate the chemical structure. Thus obtained cationic PAMAM was padded into cotton fabric and followed by batching (24 h) and washing to remove all impurities. Following that the antibacterial activity of cationic PAMAM against *S. aureus* was evaluated by a quantitative test method according to AATCC test method 100 (antibacterial Finishes on textile material). As expected, the antimicrobial activity of cationic PAMAM increased with an increase in the molecular weight of PAMAM namely in the following order G-0.5-G0.5 < G1.5 < G2.5 < G3.5, respectively due to the increase in the cationic moieties in cationic PAMAM.

Key Words: Cationic hyperbranched polyamidoamine, Simple methylation, Antimicrobial activity, *S. aureus*.

INTRODUCTION

Starburst polyamidoamine (PAMAM) dendrimers are a new class of highly branched spherical polymers that are highly soluble in aqueous solution and have a plurality of terminal groups (methyl ester group or amino group). These end groups could be further functionalized to achieve a variety of functional groups which inspire many potential applications. These types of unique polymers have attracted considerable interest due to their novel functionalities including nanoscopic containers, delivery devices, ultrafine colloid stabilizers and nanocomposite materials[1-4]. Hyperbranched polyamidoamine structure is similar to PAMAM dendrimer except that its branches are imperfect (irregular). Advantageously, the synthesis of hyperbranched polymers is far less complicated and faster than the synthesis of dendrimer. Typically, the synthesis method involves two-step iterative reaction sequence that produces intermediate branches (generation) around a central initial core.

In the case of hyperbranched PAMAM, the initiator core is an ethylenediamine. Synthesis is achieved by the consecutive Michael addition and amidation between ethylenediamine and methyl acrylate as shown in Fig. 1[1]. Currently, these versatile hyperbranched polymers have attracted considerable interest in a variety of applications involving combinatorial chemistry,

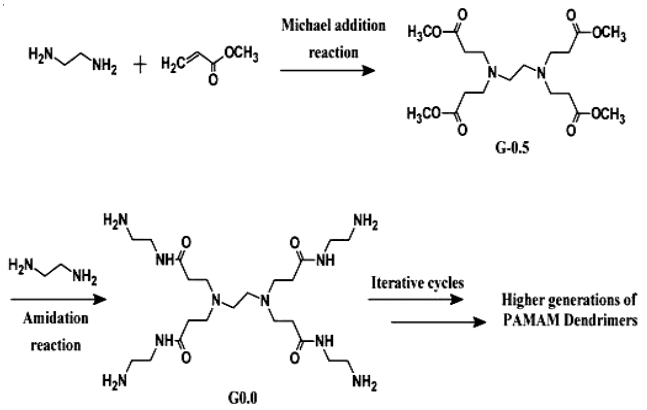


Fig. 1. Divergent growth for the synthesis of (polyamidoamine) dendrimers

surface coating, photoactive system and, particularly, gene or drug delivery. Recent researches on nonviral gene delivery have been focused on cationic hyperbranched PAMAM polymers as effective gene transfer molecules[5,6]. In another interesting aspect, cationic polymers including hyperbranched PAMAM polymers are able to exhibit an antimicrobial activity. At present, researches on the antimicrobial study of cationic PAMAM dendrimers as well as cationic hyperbranched PAMAM polymers are reported[7-9].

In this research, a simple quaternization method of hyperbranched PAMAM was presented. It has been well understood that mode of action is dependent on a highly cationic system that promotes the adsorption, causing cell disruption. Therefore, it was interesting in synthesizing a series of cationic hyperbranched PAMAM polymers (G-0.5, G0.5, G1.5, G2.5 and G3.5) polyamidoamine polymers; the higher the generation, the more the multivalent cations. Then, antimicrobial activity evaluation on cotton fabric according to AATCC Test Method 100-2004 was conducted in order to explore its potential as an antimicrobial agent for textiles.

EXPERIMENTAL

Ethylene diamine (EDA) and methyl acrylate (MA) were purchased from Fluka (Switzerland). Commercial-grade methanol was purchased from TSL Chemical Co., Ltd (Thailand). Dimethyl sulphate (commercial grade) was kindly provided by Modern Dyestuff & Pigment Co., Ltd. (Thailand). Sodium carbonate (anhydrous) was purchased from Ajax Finechem. Bleached cotton knit fabric was purchased from Boonchuay Industrial Co., Ltd (Thailand).

Synthesis of hyperbranched PAMAM: Hyperbranched PAMAM polymers were prepared by a divergent synthesis starting from Michael addition reaction. Methyl acrylate (350 g, 4.069 mol) was dissolved in methanol (200 mL) which was kept in an ice bath. Then ethylene diamine (50 g, 0.833 mol) in methanol (200 mL) was added dropwise under continuous stirring over a period of 2 h. The mixture temperature was then allowed to rise to room temperature (*ca*. 25 ºC) and the mixture was continuously stirred for 48 h. The excess of methyl acrylate and solvent were removed using a rotary evaporator under reduced pressure at 45 ºC. In this step, methyl ester terminated hyperbranched PAMAM (G-0.5) was achieved.

The amidation step was carried out as follows: a solution of G-0.5 hyperbranched PAMAM (100 g) in methanol (100 mL) was carefully added dropwise to a stirred solution of ethylene diamine (60 g, 1 mol or four times higher than that of the methyl ester content) in methanol (300 mL) at 0 ºC. The rate of addition was carefully controlled to assure that the temperature did not rise over room temperature. After completion of ethylene diamine addition, the mixture was stirred for 72 h at room temperature. The solvent and unreacted ethylene diamine were removed under reduced pressure at a temperature below 50 ºC. The excess ethylene diamine was removed to completion by detecting ethylene diamine vapor using pH test paper. G0.0 hyperbranched PAMAM was obtained.

The next round syntheses were then repeated as above except that a two times higher methyl acrylate was used than that in the first round synthesis, to yield generation 0.5, 1.0, 1.5, 2.0 and 2.5 designated to G0.5, G1.0, G1.5, G2.0, G2.5 and G3.5, respectively.

Methylation of methyl ester terminated hyperbranched polyamidoamine: A typical method was as follows: to a solution of G2.5 hyperbranched PAMAM-ester (20 g, 0.0033 mol) in methanol (20 mL), 13 g (0.1 mol) of dimethyl sulphate was slowly added. The methylation reaction proceeded immediately at room temperature. The solution was continuously stirred 3 h and then left standing in a dessicator to allow the successive reaction under the absence of moisture atmosphere. The resultant cationic hyperbranched PAMAM was achieved. The ideal structure of G2.5 cationic hyperbranched PAMAM is illustrated in Fig. 2.

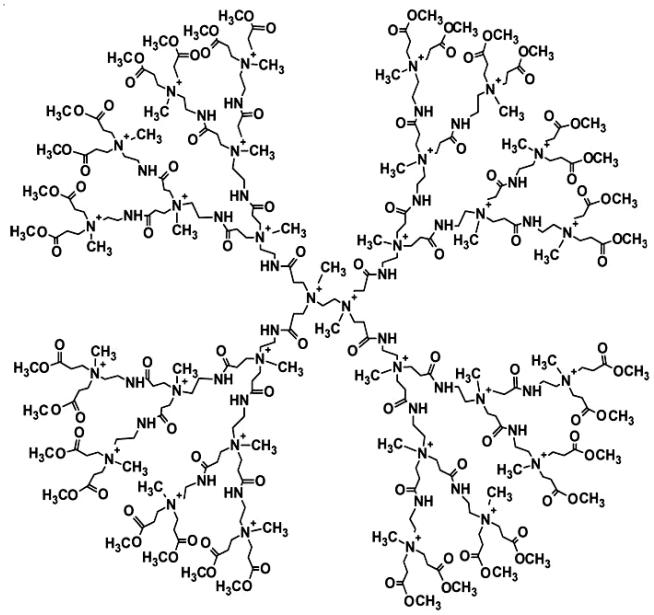


Fig. 2. Ideal structure of G2.5 cationic hyperbranched PAMAM

Application of cationic hyperbranched polyamidoamine onto cotton fabric: A series of cationic PAMAM were dissolved in distilled water to obtain 50 g/L cationic PAMAM solution. A cotton fabric sample was padded (100 % pick-up) with the solution of cationic PAMAM and batched for 24 h and then finally rinsed in distilled water to neutralize the treated fabric and to assure that only cationic PAMAM was present. The presence of cationic PAMAM on fabric surface was analyzed using ATR/FTIR techniques.

Antimicrobial activity of cationic PAMAM on cotton fabric samples: The antimicrobial activity of cationic PAMAM treated cotton fabrics was evaluated against gram-positive bacteria *S. aureus* by using quantitative method according to AATCC Test Method 100-2004 (Antibacterial Finish on Textile Material) the microbial reduction was calculated according to the following equation,

$$\text{Reduciton (\%)} = \frac{C - A}{C} \times 100 \quad (1)$$

where A is the number of bacteria recovered from the inoculated treated test specimen swatches in the jar incubated over the desired contact period and C is the number of bacteria recovered from the inoculated untreated control specimen swatches in the jar immediately after inoculation (at "0" contact time). Visual detection was recorded at 24 h contact time using a digital camera.

FTIR analysis: The functional group of cationic PAMAM was characterized by Nicolet 6700 FTIR spectrometer. A sample was scanned at the frequency range of 4000-400 cm^{-1}.

^{1}H NMR Analysis: The chemical structure of D_2O dissolved cationic PAMAM was investigated by ^{1}H NMR spectroscopy. Chemical shifts were reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal

standard. The measurement was carried out using a Bruker DPX-300 spectrometer.

RESULTS AND DISCUSSION

The hyperbranched PAMAM polymers obtained by Michael addition (G-0.5, G0.5, G1.5, G2.5 and G3.5 products) and amidation (G0.0, G1.0 and G2.0) were characterized using FTIR spectroscopy. FTIR spectra of representative samples (G-0.5, G1.0 and G1.5) are shown in Fig. 3.

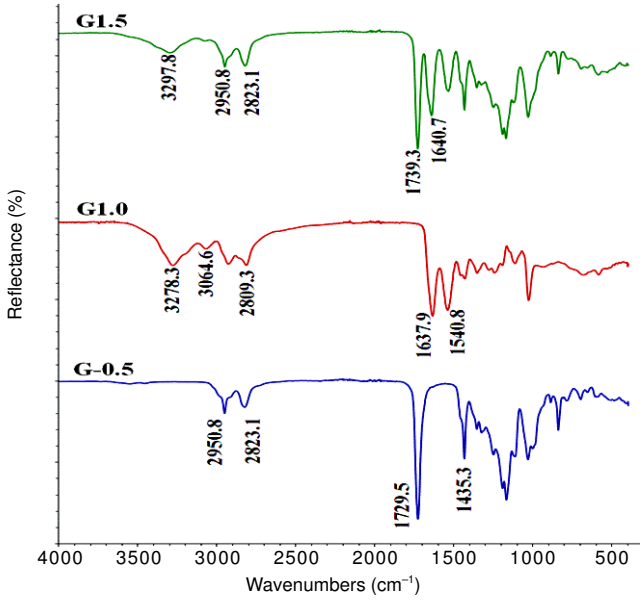


Fig. 3. Representative FTIR spectra of Michael addition adduct (G-0.5, G1.5) and amidation adduct (G1.0)

For the Michael addition steps (G-0.5, G0.5, G1.5 and G2.5) the absorption band for carbonyl in the range 1750-1730 cm^{-1} is observed, contributing to the methyl ester group. This peak completely disappears from the spectra of G 0.0, G1.0 and G2.0 as a result of amidation reactions. In this step, the amide linkage was formed, the corresponding carbonyl shifted to 1650-1550 cm^{-1}. In addition, the terminal amine group was obtained which corresponds to the appearance of the strong absorption intensity of the N-H band in the region of 3350-3000 cm^{-1}. Its absorption intensity remarkably increases with an increase in PAMAM generation, reflecting that the number of terminal amine groups also significantly increases with an increase in the synthesis rounds. It should be noted that the intensity of amine groups in the spectrum of G1.5 hyperbranched PAMAM significantly decreases as a result of the Micheal addition which converted the amine group of G1.0 hyperbranched PAMAM to terminal methyl ester group.

^{1}H NMR analysis: The representative ^{1}H NMR spectrum of cationic PAMAM (cationic G2.5 hyperbranched PAMAM-ester) is presented as shown in Fig. 4. The signal which is associated to the presence of terminal methyl ester group (CH_3-O-C=O) strongly appear at 3.61 ppm, indicating that this group was intact during methylation step. Following the methylation reaction, the methyl group (CH_3-) was anticipated to be incorporated into tertiary amine, resulting in a quaternary ammonium moiety being introduced. As a result, cationic hyperbranched

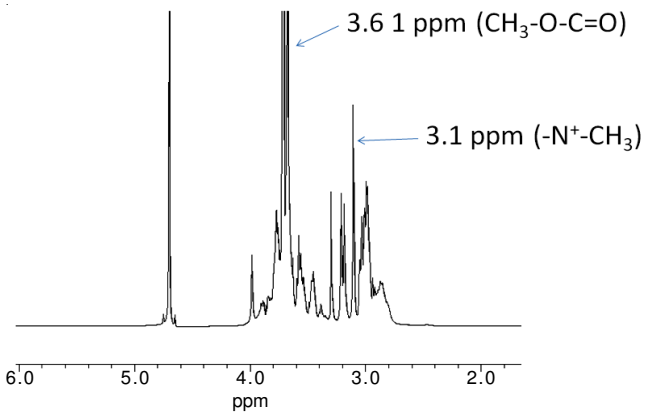


Fig. 4. ^{1}H NMR spectrum of cationic G2.5 hyperbranched PAMAM-ester

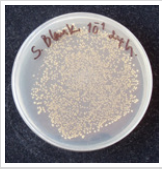
PAMAM-ester was achieved. It was expected that the signal representing methyl proton should appear at around 3 ppm in vicinity of methylene proton. As seen in the Fig. 4. , it is likely that the strong signal appearing at 3.1 ppm arises from signal overlapping between methyl (N^+-CH_3) and methylene (CH_2) protons.

Antimicrobial evaluation: The antimicrobial activity of cationic hyperbranched PAMAM containing methyl ester end group was treated on cotton fabric. Then cationic hyperbranched PAMAM-cotton fabric was tested for the antimicrobial activity against *S. Aureus* in terms of cell viability and per cent microbial reduction using a single application dose. The reason for choosing of PAMAM containing methyl ester end group instead of PAMAM containing amino end group is that PAMAM-ester is capable of reacting with cellulose hydroxyl group, resulting cationic PAMAM bonded cellulose. On the other hand, cationic PAMAM-NH_2 is highly soluble, resulting in no affinity on cotton fabric (no immobilization). The microbial reduction is presented in Table-1.

The results indicate that cotton fabrics treated with cationic hyperbranched PAMAM solution have ability to inhibit the growth of *S.aureus*. In case of G-0.5, G0.5 and G1.5, the per cent reduction is found below 50 % which is considered that these generations show no antimicrobial activity against *S.aureus*. For cationic G2.5 and cationic G3.5 PAMAM which contain much more of cationic groups than lower generations, the significant per cent reduction is found *ca*. 84 and 97 % for G2.5 and G3.5. It is noted that the antimicrobial evaluation of cationic G3.5 PAMAM was carried out separately. From results obtained from G2.5 and G3.5, it is confident to say that a plurality of cationic moieties plays an important role in antimicrobial activity against *S. aureus*. as found elsewhere[8]. The action mode begins with the binding of PAMAM polycations onto the negatively charged bacterial cell surface, then diffusing through the cell wall and binding to the cytoplasma with subsequent disruption of the membrane.

Determination of cationic PAMAM on cotton fabric surface by ATR/FTIR: The application of cationic PAMAM onto beached cotton fabric was carried out by padding method. The treated fabric was rinsed thoroughly in deionized water to remove undesired chemicals. As a representative of cationic PAMAM treated fabrics, the 5 wt % G2.5 cationic PAMAM treated fabric was analyzed by ATR/FTIR spectroscopy to confirm the existence of cationic hyperbranched PAMAM

TABLE- 1
ANTIMICROBIAL ACTIVITY OF CATIONIC HYPERBRANCHED PAMAM ON COTTON FABRIC

Generation	Cell viability (24 h)	No. of bacteria CFU/Sample		Reduction (%)
		C (0 h.)	A (24 h.)	
Blank (reference for G-0.5-G2.5)		3.3×10^5	$>3.0 \times 10^6$	–
Blank (reference for G3.5)		6.0×10^5	$>3.0 \times 10^6$	–
G-0.5		–	2.1×10^5	36.6
G0.5		–	3.4×10^5	0
G1.5		–	2.0×10^5	39.9
G2.5		–	5.3×10^4	83.93
G3.5		–	1.5×10^4	97.5

on the fabric surface. The representative spectra are shown in Fig. 5. The spectrum of cotton fabric treated with cationic hyperbranched PAMAM (Fig. 5b) exhibits absorption peak at 1726.8 cm^{-1}, corresponding to C=O stretching of the methyl ester group which indicates the presence of cationic PAMAM on the fiber surface.

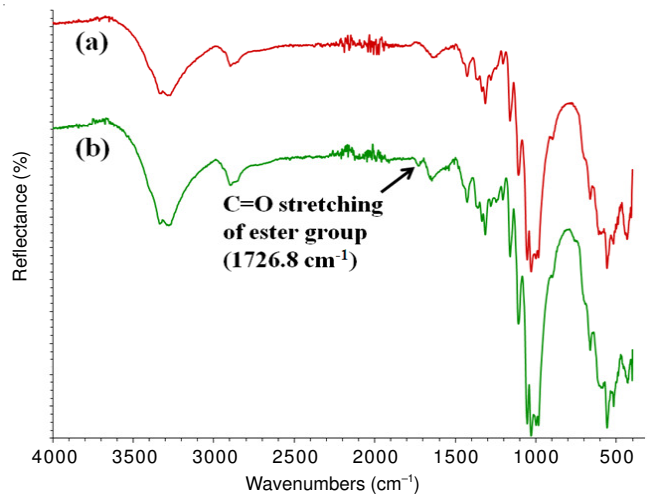


Fig. 5. ATR/FTIR spectra of (a) cotton fabric (b) cationic hyperbranched PAMAM-cotton fabric

Conclusion

A series of cationic hyperbranched PAMAM were successfully synthesized by the repetitive reactions between the Michael addition and amidation of ethylene diamine and methyl acrylate. Then, the hyperbranched PAMAM was methylated with dimethyl sulphate, yielding the cationic hyperbranched PAMAM. The antimicrobial activity of the cationic hyperbranched PAMAM was evaluated. The finding results showed that the antimicrobial activity was dependent on a plurality of cationic moities present in PAMAM.

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