PREPARATION AND BIOPHYSICAL CHARACTERIZATION OF NOVEL PAMAM DENDRIMER-POLY (ACRYLIC ACID) GRAFT

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Abstract:

A series of PAMAM dendrimer generation 5-poly (acrylic acid) grafts were prepared to evaluate the potential use of dendritic grafts as a drug encapsulated nanocarrier. The structural features of the synthesized polymer graft were identified by FT-IR and ^{1}H-NMR spectra and the biophysical properties were characterized by measuring its particle size and zeta potential. The prepared dendrimer G5–PAA grafts have a particle size in the range of 600 to 900 nm and the size gets bigger as the number of PAA on the dendrimer surface increases. The electrostatic property of the dendrimer G5-PAA, carried out by HPLC reversed phase column analysis and the measurement of zeta potential, revealed that both migration time and zeta potential were dependent on the number of grafted PAA. The number of free amino groups on dendrimer G5-PAA, determined quantitatively by fluorescamine assay, was in a reverse order with the reaction mole ratio of dendrimer to PAA. In addition, dendrimer G5-PAA had a pH-dependent solubility in aqueous solution with low solubility at pH 4.7 to 7.8.

Keywords: Dendrimer, poly(acrylic acid), dendrimer G5-PAA, graft.

1. INTRODUCTION

Dendrimers, spherical macromolecules with numerous inner branches, are attractive polymer for the delivery of various types of drugs including nucleic acids into cells or tissues [1,2]. However, cellular delivery of drugs by dendrimer has been limited in its effectiveness as well as *in vivo* efficacy due to cytotoxicity and non-specific interaction with serum proteins. To overcome these problems, various modification works have been made on dendrimer surfaces such as pegylation, grafting with other polymers and conjugating with ligands [3,4].

Poly (acrylic acid) (PAA) is an anionic polymer consisting of acrylic acid as a monomer. Carboxylic groups of PAA in aqueous solution at neutral pH lose their protons, making PAA a polyelectrolyte with a high negative charge density. In this study, we prepared a series of PAMAM dendrimer generation 5-poly(acrylic acid) grafts (PAMAM dendrimer G5-PAA graft) to evaluate the potential use of dendrimer–PAA as a drug encapsulated nanocarrier [5,6]. The negative charge of PAA on the grafted dendrimer is expected to neutralize the positive charge of surface amino groups and thus shield from non-specific interaction with cell surface proteins or serum proteins in blood, reducing cellular toxic effects and increasing circulation time. We also investigated its structural features and biophysical properties to examine whether this dendritic graft is suited as a drug-carrier.

2. EXPERIMENTAL

2.1. Materials

PAMAM dendrimer G5 (Mw 28.826)

and poly(acrylic acid) (Mw 1.800) were purchased from Aldrich (Milwaukee, WI, USA). DMEM was from Life Technologies (Gaithersburg, MD). All other chemicals including fluorescamine were from Sigma Chemical (St. Louis, MO, USA).

2.2. Synthesis of Dendrimer G5-poly(acrylic acid) grafts

To a flask containing 5 mg of dendrimer G5 in 10 ml buffer HEPES, pH 8.3, 200 µl of 10% 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) in distilled water was added as a catalyst. PAA in distilled water was dropped slowly while stirring at 40°C, and pH was adjusted at 7.5 with 1 N aqueous NaOH. Purified products were further characterized for their chemical and physical properties by FT-IR and ^{1}H-NMR spectrum, HPLC mobility, particle size and zeta potential.

2.3. Structural Characterization by ^{1}H-NMR and FT-IR

^{1}H-NMR spectra were taken of dendrimer G5, poly(acrylic acid) and dendrimer G5-PAA graft (mole ratio, 1:40) in D_2O at a concentration of 10 mg/ml, using 500 MHz NMR spectrometer (Bruker, DRX-500 MHz). FT-IR spectra were collected with a Shimadzu FT-IR 8700 instrument using KBr disc.

2.4. Fluorescamine Assay

A series of dendrimer G5-PAA solutions was prepared in HEPES solution, pH 7.8. A 40 µl sample of each solution was transferred into a black 96-well microplate including a control well containing HEPES only. A 50µl of 0.1% fluorescamine in acetone was added immediately using a multi-pipette. The fluorescence intensity was measured with the excitation wavelength at 360 nm and the emission wavelength at 485 nm (BioTek FL600 fluorescence microplate reader). Data were collected by KC4 data reduction software.

2.5. HPLC Analysis of Dendrimer G5-PAA grafts

HPLC analysis was carried out with a high performance liquid chromatography system from Beckman (Beckman Instruments, Inc., Fuller-ton, CA, USA) consisting of a DAD detector A C_{18} reversed-phase column (4.6 x 250 mm) was used with a guard column (15 x 3.2 mm). Analysis conditions were done with isocratic of 80% acetonitrile in DW at 30°C with flow rate 1 ml/min.

2.6. Determination of Zeta Potential and Particle Size

Particle size and surface charge of the dendrimer G5-PAA grafts (at mole ratios of 1:10, 1:40, 1:80 and 1:130) were determined on a Malvern Zetasizer 3000 (Malvern, UK). Zeta potential of dendrimer G5-PAA grafts was measured in capillary cell swith analysis mode at 50 mV of voltage and wavelength of 633 nm. Each sample was automatically calculated on ten times prior to the record of mean value.

3. RESULTS AND DISCUSSION

A series of PAA grafted PAMAM dendrimer generation 5 with varying degrees of substitution of the surface amino group by PAA were prepared by the reaction between the amino groups of dendrimer surface and the carboxylic groups of PAA, as illustrated in Fig 1.

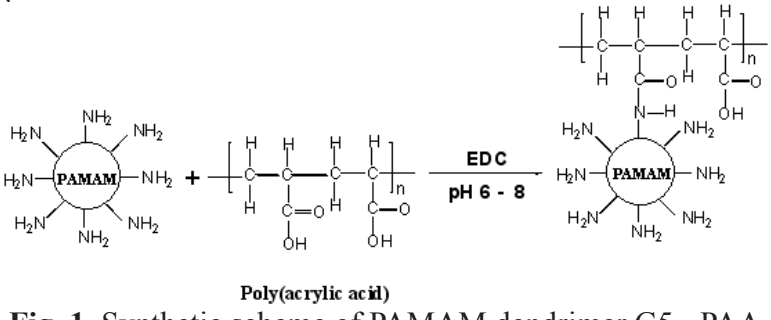

Fig. 1. Synthetic scheme of PAMAM dendrimer G5 - PAA graft. PAMAM dendrimer G5-PAA grafts (mole ratios of 1:10, 1:40, 1:80, 1:130) were synthesized

^{1}H-NMR and FT-IR spectra were taken of G5-PAA graft (mole ratio, 1:40). NMR and FT-IR analyses confirmed that the reaction was accomplished between amino groups of the dendrimer surface and the carboxylic acid groups of PAA via carbodiimide activation (Fig 2).

Electrophoretic properties of dendrimer-PAA grafts was investigated by HPLC, using isocratic conditions of 80% acetonitrile in DW at 30^{o}C with flow rate 1 ml/min (Fig 3). Dendrimer G5-PAA migrated as a single peak. The mobility was proportionally increased to the number of mole ratio of dendrimer to PAA.

Free surface amine groups on G5-PAA grafts were determined by a fluorescamine assay which measures the fluorescence intensity at 485 nm, after the reaction of free amino groups on dendrimer G5-PAA with fluorescamine (Fig 4A).

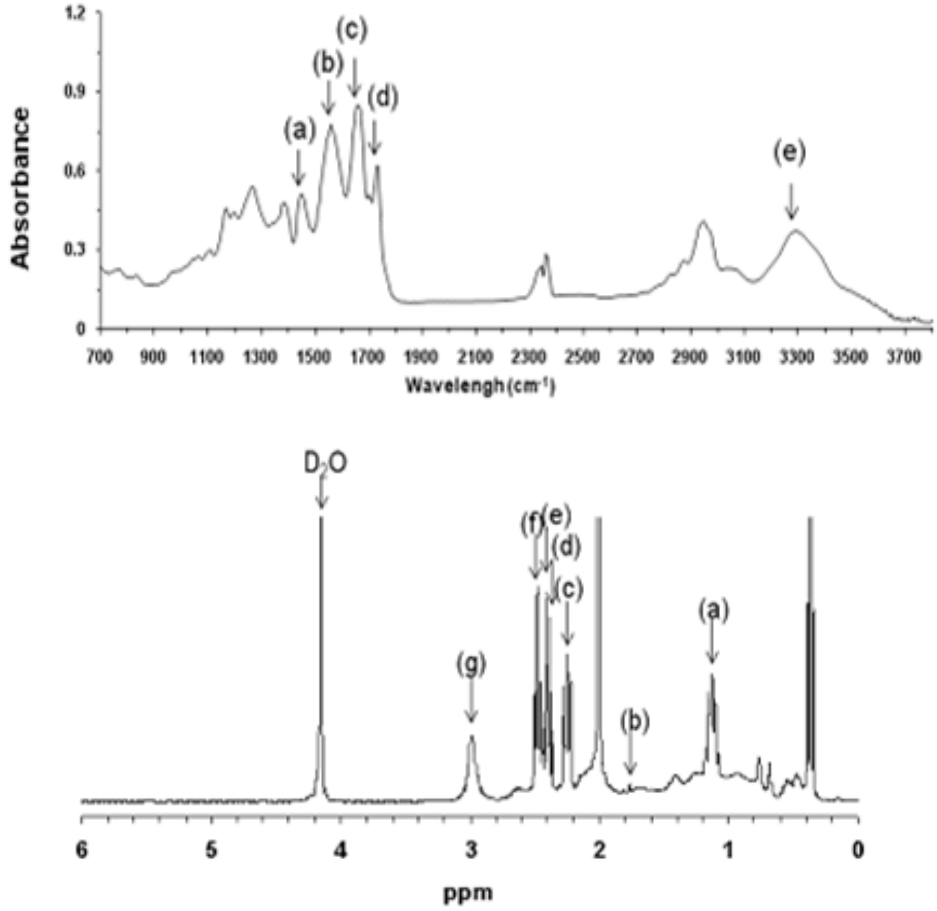

Fig. 2. FT-IR and ^{1}H-NMR spectra for PAMAM dendrimer G5-PAA graft (1:40 mole ratio).
FT-IR spectrum: (a) stretch of CH_2, 1430 cm^{-1}; (b) bend of amine, 1540 cm^{-1};
(c) bend of amide, 1670 cm^{-1}; (d) bend of carboxyl, 1770 cm^{-1}; (e) stretch of amine, 3350 cm^{-1}.
^{1}H-NMR spectrum in D_2O: (a) CH_2- at 1.1 ppm; (b) CH- at 1.78 ppm;
(c) -CH_2CH_2CONH- at 2.20 ppm; (d) -CH_2CH_2N- at 2.35 ppm; (e) -NCH_2CH_2CO- at 2.38 ppm;
(d) -$CH_2CH_2NH_2$- at 2.50 ppm; (e) -$CONHCH_2CH_2$- at 3.0 ppm.

The fluorescence intensities of dendrimer G5-PAA grafts (1:10, 1:40, 1:80 and 1:130) was decreased as the mole ratio of dendrimer to PAA increased, indicating that PAA substitution reduces the available free amino groups on the surface of the dendrimer for the reaction with fluorescamine.

The biophysical properties of dendrimer G5-PAA were further investigated by measuring its particle size and zeta potential as described in Experimental details. The relative comparison of molecular weight, nanoparticle size and zeta potential of the prepared dendrimer grafts is shown in Fig 4B and the characteristic biophysical features are listed in Table I. Particle size of the dendrimer G5-PAA graft increased from 600 to 900 nm as the mole ratio of dendrimer to PAA (1:10, 1:40, 1:80 and 1:130) increases. The relative zeta potential, reflecting the ratio of PAAs, also increased as the number of substituted PAAs increased.

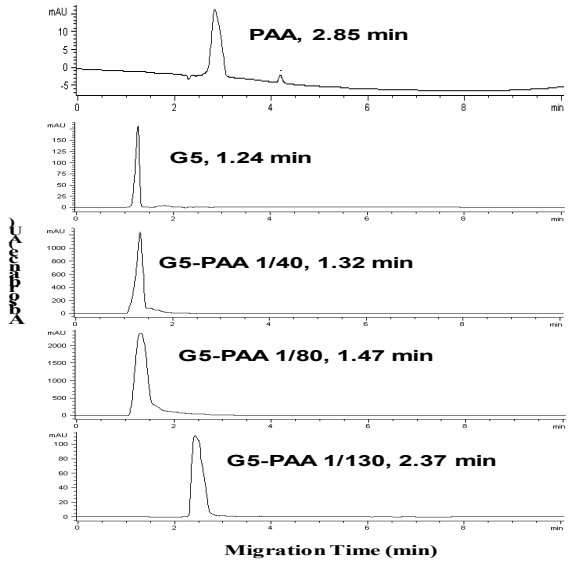

Fig. 3. Reversed phase HPLC electropherograms of PAMAM dendrimer G5 -PAA grafts prepared at various mole ratio reactions.

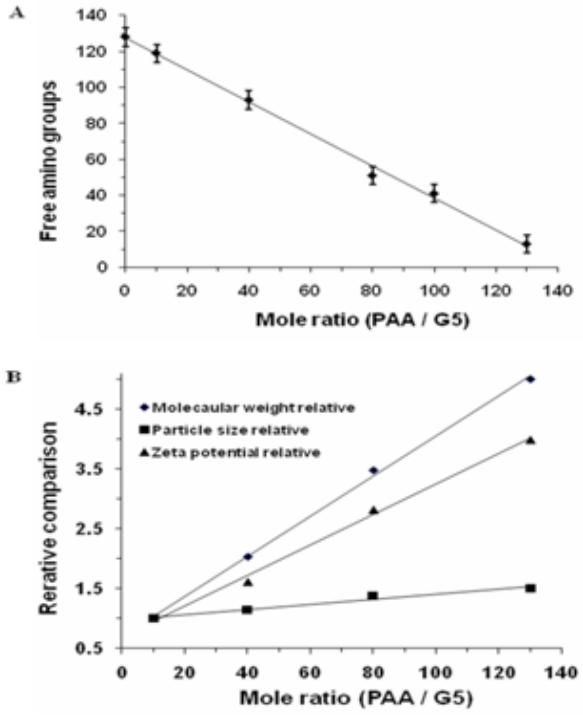

Fig. 4. Relative comparison of biophysical properties of PAMAM dendrimer G5 -PAA grafts at different mole ratios

Table I. Characteristic features of PAMAM dendrimer G5 - PAA grafts.

*Mole ratio	Number of amino groups	Number of conjugated PAA	Molecular weight	HPLC migration time (min)	Particle size (nm)	Zeta Potential (mV)
1 : 10	118	10	46646	1.26	596.2	-4.8
1 : 40	91	37	94760	1.32	668	-7.7
1 : 80	53	72	162476	1.47	865	-10.9
1 : 130	13	115	233756	2.37	929	-12.6

*Mole ratio indicates PAMAM dendrimer generation 5 to PAA in conjugate reaction.

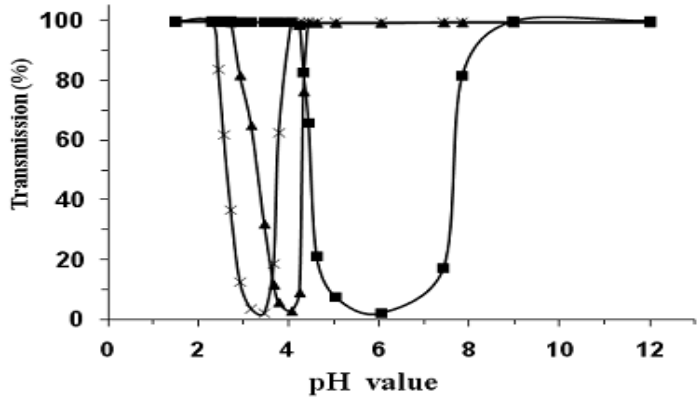

Fig. 5. pH-Dependent solution properties of dendrimer G5-PAA graft. The distribution of electrostatic point of dendrimer G5-PAAs was determined by measuring the percent transmission at the wavelength of 550 nm by using UV-Vis spectrometer. Percent transmission of dendrimer G5-PAA in aqueous solution was varied with distinguished range of pH region. Crosses, G1-PAA 1:10; filled triangles, G2-PAA 1:10; filled squares, G5-PAA 1:10.

The solubility of G5-PAA graft in aqueous solution was pH-dependent. Dendrimer-PAA (1:30 mole ratio) was found to have three distinguishable pH regions in UV-transmission: the low pH region, intermediate precipitation region and the high pH region. The lowest percent transmission of G5-PAA was found to be at pH 4.7 to 7.8, indicating that aggregation phenomena of G5-PAA grafts takes place in this pH range due to the charge neutralization.

4. CONCLUSION

A novel nanoparticle, PAMAM dendrimer G5– PAA graft, was prepared by the reaction of PAMAM dendrimer G5 with poly (acrylic acid). Dendrimer G5-PAA grafts showed the characteristic biophysical properties which are promising as a drug encapsulated nanocarrier.

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