Poly(amidoamine)(PAMAM) /CMS Dendritic nanocomposite for controlled drug delivery

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ABSTRACT: Dendrimers are one of the emerging delivery systems with the capability to present such hydrophobic agents in a formulation with better prospective[1] .These dendritic macromolecules with a large number of suface terminal groups and interior cavities offer a better opportunity for delivery by becoming charged and acting as static covalent micelles.These are biocompatible, nonimmunogetic , and water- soluble and possess terminal functional groups for binding various targeted or guest molecules[2]. The host – guest properties of dendrimers based on hydrophobic and ionic interactions apart from physical entrapment have been thoroughly studied .In the present study , the conjugate carboxymethyl starch (CMS) /PAMAM dendrimer were exhaustivly studied as controlled – release systems for parenteral administration of a model drug 5- aminosalicyclic acid (mesalamine) and analyzed using various release kinetic studies. The synthesized nanocomposite have been characterized by Fourier transfer infrared(FTIR) spectrophotometer. The morphology of these composites was studied by scanning electron microscopy. [The Journal of American Science. 2008;4(1):48-52]. (ISSN: 1545-1003).

KEYWORDS: PAMAM dendrimer , CMS, 5-amino salicyclic acid(5-ASA), nanocomposite.

INTRODUCTION

The invention of the dendrimer is attributed to Donald Tomalia, who first published his report of poly(amidoamine) dendrimer synthesis in 1979 from his laboratory at the Michigan Molecular Institute in Midland, Michigan (Tomalia 1995)[3].His first poly(amidoamine) dendrimer was the result of reacting three methylacrylate molecules to an ammonia core, followed by the addition of three ethylenediamine molecules to form the G0 amidoamine. By continuing this two-step process of methylacrylate/ethylenediamine addition, successive amidoamine generations are produced, doubling the number of terminal amine groups each time[4] .

Concurrently, Fritz Vögtle of the University of Bonn published his own dendrimer synthesis consisting of ammonia derivatives with acrylonitrile. Later, George Newkome would publish an alternative synthesis of similar molecules that he called “arborols,” after the tree-like symmetry, but his 1985 discovery would be largely overshadowed four years later when a collaboration between Cornell University and AT&T Laboratories would revolutionize dendrimer synthesis, setting off an explosion of dendrimer research around the globe[5] .

In 1989, Jean Fréchet of Cornell University and Timothy Miller of AT&T Laboratories jointly developed a convergent synthesis for producing dendrimers. Rather than иeginning with a core molecule and building each generation onto the core outwardly, Fréchet and Miller were able to begin with the dendrimer periphery and inserted the molecular core as the last step[6]. In this manner, high-purity dendrons of the desired generation could be synthesized, and then by reacting these dendrons with the core molecule, dendrimers could be produced with the same high purity[7]. Prior to the development of convergent dendrimer synthesis, only a handful of scientific papers had been published on dendrimer research; in the five years that followed, dendrimer research literally exploded within the scientific community (Tomalia 1995)[8].

Because of their early discovery and thus the amount of research that has been conducted with them (Bosman, Janssen et al. 1999), PAMAM dendrimers are among the very few commercially available dendrimers, available in generations 0 to 10 from Aldrich, Inc. The PAMAM dendrimers available from Aldrich, Inc., are prepared by a divergent synthesis[9].

Dendrimers are one of the emerging delivery systems with the capability to present such hydrophobic agents in a formulation with better prospective .These dendritic macromolecules with a large number of suface terminal groups and interior cavities offer a better opportunity for delivery by becoming charged and acting as static covalent micelles.These are biocompatible, nonimmunogetic , and water-soluble and possess terminal functional groups for binding various targeted or guest molecules[10]. The host – guest properties of dendrimers based on hydrophobic and ionic interactions apart from physical entrapment have been thoroughly studied[11,12] .

In the present study , the PAMAM dendrimers were exhaustivly studied as controlled – release systems for parenteral administration of a model drug 5- aminosalicyclic acid (mesalamine) and analyzed using various release kinetic studies[13,14].

Experimental Methods

Materials

All reactions were performed under an atmosphere pressure . All reagents and solvents , unless otherwise specified,were obtained from Merck Chemical Company.

Poly(amidoamine)(PAMAM) generation 4 contains 64 surface primary amino groups was obtained from Dendritech ,Inc. , Aldrich company.Melting points were obtained on a Mel-Temp melting point apparatus . Analytical TLCs were run on commericial Merck plates coated with silica gel GF250(0.25mm thick). Fourier transfer infrared(FTIR , Bruker)spectroscopy was used to identified the polymer surface.Specta were obtained in the wave number range of 400-4000cm^{-1} Spectra of samples were recorded from KBr in 1:10 (wt/wt) ratio.

Firstly ,the 0.5 g cornstarch and 120 ml 2-propanol were placed in a 500 ml vessel and stirred for 2 h . The 5 g sodium hydroxide was added and reacted for 1 h at 78-80 ˚C .After thet , the 10 g chloroacetic acid was added to the vessel and stirred for another 2 h at 50 ˚C .The product was filtered and washed several times with ethanol , then dried under vacuum . The resulting carboxymethyl starch (CMS) was crused in a mortar[degree of substitution (DS) = 0.49].

The condensation reaction between poly(AmidoAmnie)(PAMAM) and the activated carboxymethyl starch (CMS) was carried out in a borate buffer (PH=8.5). The solvent(methanol) was vacuum – evaporated from PAMAM prior to reaction.Then, 1.62mM, 23 mg PAMAM dendrimer 11.6 mM , 38.9 mg CMS dissolved in 50 cc borate buffer (PH= 8.5) and incubated at the room temperature for 24 h with shaking .Crude reaction mixtures of conjugates were dialyzed against distilled water using dialysis tubing for 24h.

Then, 5-aminosalicylic acid(5-ASA) was dissolved in methanol following which the dendrimer was added .The reaction mixture was stirred for 24 h in the dark ,then evaporated using rotaevaporator to remove methanol . The traces were dried under vacuum in order to remove methanol completely .To these traces , deionized water was added . This solution was stirred in the dark for 24 h. This was extract the drug – dendrimer complex ,as dendrimer is soluble in water while 5-ASA is not. The solution was then filtered through PTFE membrane(Millix Millipore) of pore size 200nm ,and then lypophilized to remove water.After approximately 180 min, the sample were sprayed into a liquid nitrogen bath cooled down to 77° K, resulting in frozen droplets. These frozen droplets were then put into the chamber of the freeze-dryer. In the freeze-drying process, the products are dried by a sublimation of the water component in an iced solution.The drug –CMS/dendrimer complex obtained was in the form of a brown powder.

RESULTS AND DISCUSSION

Figure 1 shows the SEM of PAMAM(generation 4)dendrimer/CMS / Mesalamine(5-ASA) nanocomposite that synthesized by chemical reaction. This nanocomposite is very sensitive to the temperature that due to the intraction electron and sample . Scanning electron micrography images were

obtains from a diluted solution of the nanocomposite particle . The white spots are drug nano particles .The SEM image shows the presence of 5-ASA spherical particles in polyfunctional dendrimeric matrix, which are homogenenously distributed throughout the composites ,which is also confirmed from ^{1}H-NMR studies. The ability of the dendrimer to form a complex with drugs depends on the core- suface groups of dendrimer ,electrostatic interactions between the dendrimer and the drug , and the ability of the drug to form a conjugate with the dendrimer through chemical bonding . Therefore , it is possible to manipulate the incorporation process for a given drug by appropriate selection of the dendrimer and the surface functionality.One might expect that the mesalamine with the carboxylic group may form a complex with surface NH_2 groups of PAMAM dendrimer.

Fig. 2a shows the FT-IR spectrum of pure carboxymethyl starch (CMS) , where the % of transmittance is plotted as a function of wave number (cm^{-1}) . The wide peak around 3411 cm^{-1} is attributing to the O-H stretching vibrations of CMS.The peaks at 1597 and 1417 cm^{-1} attribute to the COO^- unsymmetrical and symmetrical stretching vibration respectively .The peaks from the FTIR spectrum of CMS-PAMAM 4.0G complex at 3250 cm^{-1} showed the presence of terminal primary amino groups (figure 2b).In case of drug – dendrimer complex , the broad peak at 3200 cm^{-1} is the strong evidence of the presence of NH_3^+ showing the electrostatic association of drug with dendrimer (figure 2c).For learn of effect of the nature and size of the drug in drug delivery, we study drug release of the polymers containing 5-ASA as a pharmaceutically active compound as a function of time is shown in figures 3. The concentration of 5-ASA released at selected time intervals was determined by UV spectrophotometry at 205 and 235 nm, respectively. In order to study potential application of nanocomposite containing 5-aminosalicylic acid as pharmaceutically active compounds, we have studied the drug release behavior of the polymers under physiological conditions. The concentration of drugs released at selected time intervals was determined by UV spectrophotometry. Important parameter for increasing of diffusion coefficient is decreased of particle size. It appears that the degree of drug release polymers depends on their particle size. In odder hand, the chemical structure of the drug too is an important factor in hydrolytic behavior of polymeric prodrugs. 5-ASA contains both amine (basic) and carboxylic acid (acidic) functional groups. This factor ultimately result in an increase hydrophilicity of 5-ASA in acidic media.

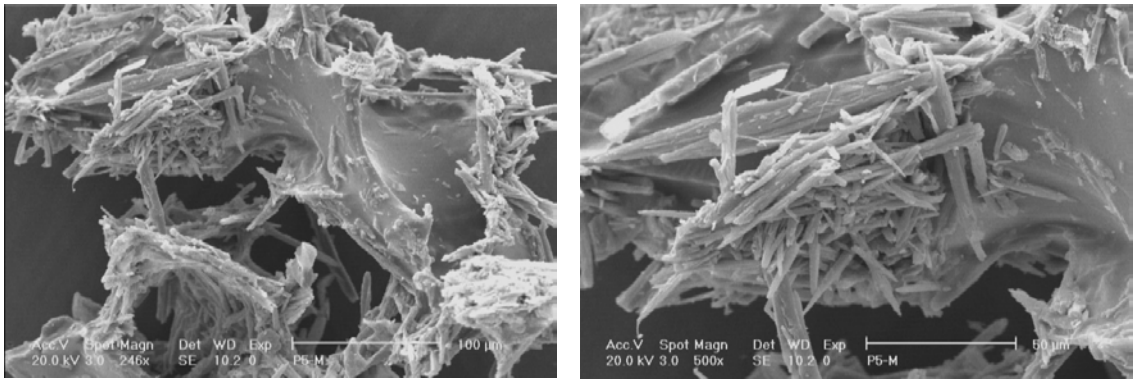

Fig.1 – SEM of CMS-PAMAM(G4)–(5-ASA) nanocomposite

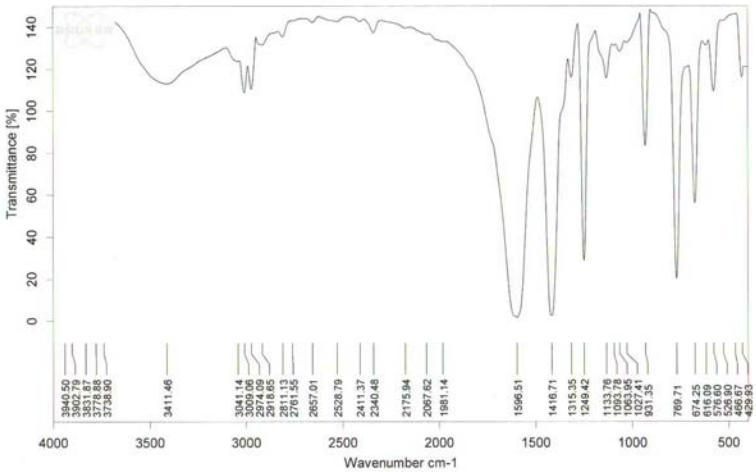

Fig.2a – FT- IR spectrum of pure CMS

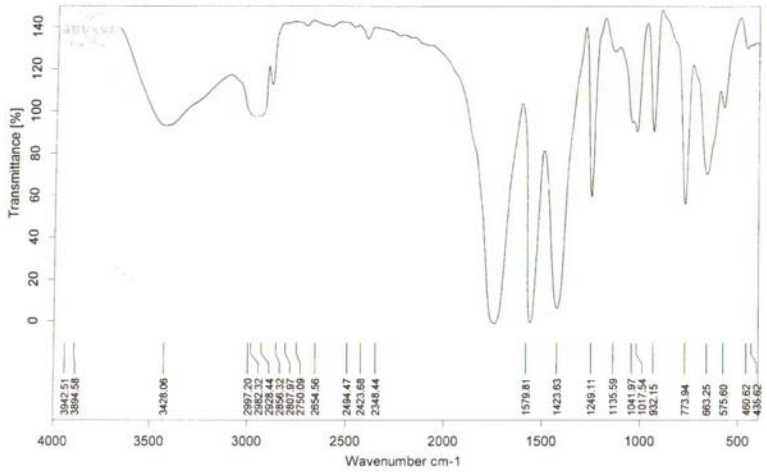

Fig.2b – FT- IR spectrum of CMS-PAMAM complex

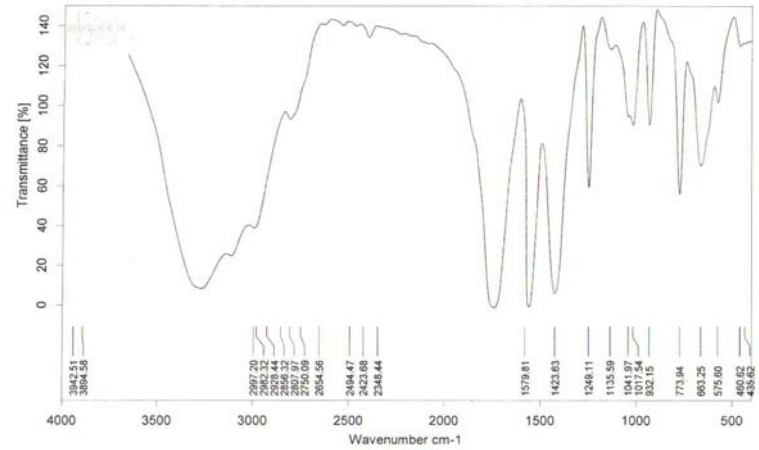

Fig.2c – FT- IR spectrum of CMS-PAMAM-Drug complex

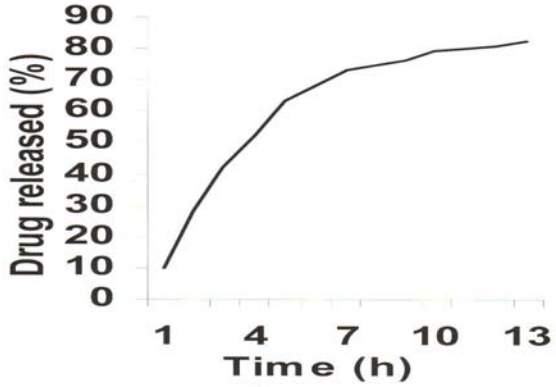

Fig. 3 – Drug release percentage from CMS-PAMAM- (5-ASA) nanocomposite

CONCLUSION

The present study reveals that the conjugate CMS-PAMAM dendrimer interact with hydrophobic 5-ASA molecules to bring it in its ionized state and hence enhace solubility.At the same time dendrimers can localize the drug at the site of inflammation and the drug can provide effective pharmacological action .However , the potential role of our system in various other categories of the drugs for drug delivery is still under investigation.

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REFERENCES

1. Saboktakin,M.R.,A., Maharramov, M.,A.,Ramazanov, *Nature and Science*,2007,5(3), 30-36.
2. Saboktakin,M.R.,A., Maharramov, M.,A.,Ramazanov,*Journal of American Science*,2007, 3(4),40-45
3. Armstrong ,J.,A.,N., Bloembergen, et al., *Physical Review*,127(6),1918-1939.
4. Betley,T.,A.,M.,M., Holl, et al. , *Langmur*,2001,17(9),2768-2773.
5. Bloembergen, N.,and Pershan, P.,S.,*Physical review*,1967,128(2),606-622.
6. Bloembergen, N.*, Applied physics,B,Lasers and optics* , 1999,68(3),289-293.
7. Chen,H.,G., and P. Knochel,*Tetrahedron Letters*,1988,29(51),6701-6702.
8. Chen,W.,D.,A.,Tomalia, et. Al. , *Macromolecules*,2000,33(25),9169-9172.
9. dvornic, P.,R.,A. M. ,de Leuze-Jallouli,et.al., *Macromolecules,* 2000,33(15), 5368-378.
10. Esfand , R., D.,A.,Tomalia ,*Drug Delivery Today*,2001,6(8),427-436.
11. ghosh,S. and A.K. Banthia ,*Tetrahedron Letters* , 2001,42(3),501-503.
12. Kolhe, P., E.,Misra,R.,M., Kannan,*Int. J. Pharm*.,2003,259,143-160.
13. Ahmed,S.M.P.M. Budd,et al.,*polymer*,2001, 42(3) ,889-896
14. Antonovic,D.,G.,N.D.,Stojanovic,et al. ,*journal of Molecular structure*,1997,408/409(1), 421-423.