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Synthesis of New Functionalized Citric Acid-based Dendrimers as Nanocarrier Agents for Drug Delivery

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ABSTRACT

Introduction: Citric acid-polyethylene glycol-citric acid (CPEGC) triblock dendrimers can serve as potential delivery systems. ***Methods:*** In this investigation, CPEGC triblock dendrimers were synthesized and then imidazole groups were conjugated onto the surface of the G_1, G_2 and G_3 of the obtained dendrimers. In order to study the type of the interactions between the functionalized dendrimers and a drug molecule, Naproxen which contains acidic groups, was examined as a hydrophobic drug in which the interactions would be of the electrostatic kind between its acidic groups and the lone pair electrons of nitrogen atom in imidazole groups. The quantity of the trapped drug and also the amount of its release were measured with UV spectrometric method in pH 1, 7.4 and 10. The average diameter of the nanocarriers was measured by Dynamic Light Scattering (DLS) technique ***Results:*** The size range of particles was determined to be 16-50 nm for different generations. The rate of the release increased in pH=10 in all generations due to the increases in Naproxen solubility and the hydrolysis of the esteric bonds in the mentioned pH. The results showed that the amount of the trapped drug increased with the increase in the generation of the dendrimer and pH. ***Conclusion:*** Based on our findings, we suggest CPEGC triblock dendrimers possess great potential to be used as drug/gene delivery system.

Introduction

Dendrimers are a new class of polymeric belongings. Dendrimer chemistry is one of the most attractive and hastily growing areas of new chemistry (Klajnert and Bryszewska, 2001, Tomalia *et al.*, 1985, Spataro *et al.*, 2010). The structure of these resources has a great contact on their physical and chemical possessions (Didehban *et al.*, 2009b, Svenson and Tomalia, 2005, Haririan *et al.*, 2010). Medical application is one of the private behaviors of the dendrimers (Didehban *et al.*, 2009a). A choice of biologically active molecules, such as antibodies (D'Emanuele and Attwood, 2005) and sugar molecules (Ganta *et al.*, 2008, Torchilin, 2001, Paleos *et al.*, 2004) have been conjugated to the chain ends of the dendrimers. For example: Zanini and Roy (Schenning *et al.*, 1998) organized a series of thiosialodendrimers by coupling a-thiosialoside to dissimilar generations of the dendrimers and investigated their binding to the silica acid- specific lectin from Limaxflavus. These structures are highly branched macromolecules with low polydispersity that supply many exciting opportunity to design novel drug-carriers, gene delivery systems and imaging agents (Basavaraj *et al.*, 2009, Crampton and Simanek, 2007). There are attempts to use dendrimers in the targeted delivery of drugs and other beneficial agents (Namazi and Jafarirad, 2011). Drug molecules can be overloaded both inside the dendrimers as well as attached to the exterior groups (Jansen *et al.*, 1995). Sialylated dendrimers, called sialodendrimers, have been shown to be effective inhibitors of the haemagglutination of human erythrocytes by influenza viruses. The first step in the infection of a cell by influenza virus is the attachment of the virus to the cell membrane. The attachment occurs through the interaction of a virus receptor haemagglutinin with sialic-acid groups presented on the surface of the cell (Wang *et al.*, 2003). Sialodendrimers bind to haemagglutinin and thus prevent the attachment of the virus to cells. They can be useful curative agents

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in the prevention of bacterial and viral infections (Sigal *et al.*, 1996). In addition, water soluble dendrimers are capable of binding and solubilising small acidic hydrophobic molecules with antifungal or antibacterial properties. The bound substrates may be unconfined upon contact with the target organism. Such complexes may be considered as potential drug delivery systems (Boas and Heegaard, 2004, Tomalia *et al.*, 1990). An area that has concerned great notice is the contact between drugs and dendrimers (Liu *et al.*, 2000). Several types of interactions have been explored, which can be broadly subdivided into the trap of drug molecules within the dendritic design (involving electrostatic, hydrophobic and hydrogen bond interactions) and the interaction between a drug and the surface of the dendrimers (electrostatic and covalent interactions) (Namazi and Adeli, 2005, Veronese *et al.*, 1998). The applications of such systems have been several-fold, counting the use of dendrimers to improve drug solubility and bioavailability, and to act as release modifiers and platforms for drug targeting (Ambade *et al.*, 2005, Kurtoglu *et al.*, 2009, Qiu and Bae, 2006). The synthesis of the dendrimers from citric acid, and poly (ethylene glycol) (G_1, G_2 and G_3) was already reported from our laboratory (Namazi and Adeli, 2003). In this work, we conjugated imidazole groups onto the surface of the dendrimers and investigated how the guest molecules get trapped in the dendritic compounds. Here, we report the preparation of the drug/dendrimer complexes containing G_1-Imz, G_2-Imz and G_3-Imz of dendritic citric acid. Also the quantity of loaded drug and the controlled release of naproxen drug molecule from the synthesized dendritic compounds were investigated in pH 1, 7.4 and 10.

Materials and methods

Materials

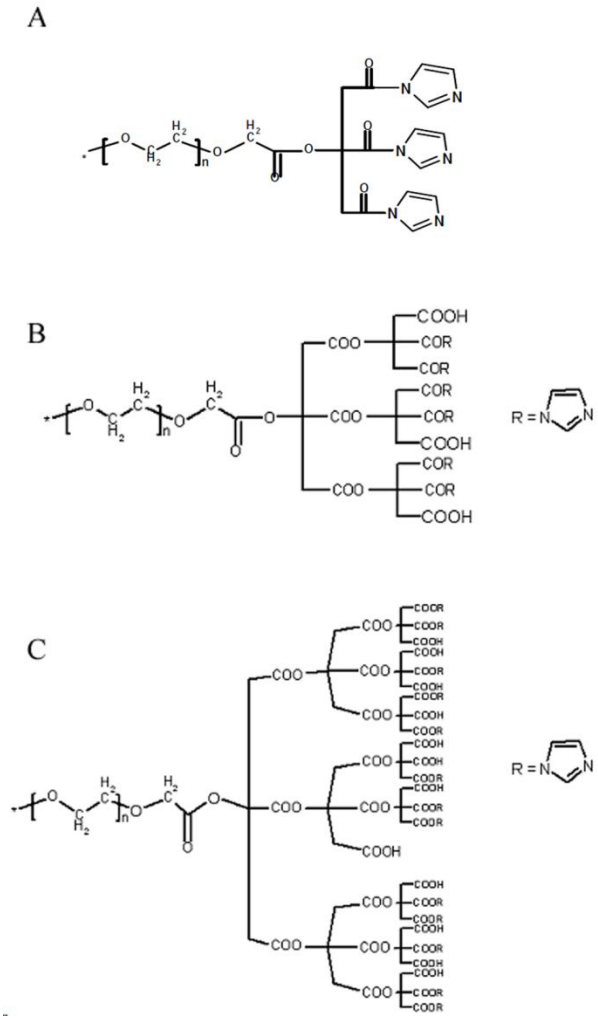
Poly (ethylene glycol) 600 diacid (acid number 175, 96–98%, from Fluka) was dehydrated over Na_2SO_4. Citric Acid and pyridine (purified with refluxing over NaOH for 2 h and succeeding distillation) were obtained from Merck. Thionylchloride (from Merck) was purified by refluxing a mixture of 10 wt% linseed oil in thionylchloride for 2h and subsequent distillation. Dicyclohexyl carbodiimide (DCC) was purchased from Merck. Imidazole and Naproxen were purchased from Sigma Aldrich and Merck, respectively.

Instrumental measurement

FT-IR spectra were measured on a Shimadzu Model FT-IR-8101M spectrometer. ^{1}H NMR spectra was recorded on FT-NMR (400 MHz) Brucker in DMSO-d_6. For the investigation of dendrimer/drug complex compounds UV 2100 Shimadzu spectrophotometer was applied.

Methods

The synthesis of G_1- Imz, G_2-Imz and G_3-Imz



Scheme 1. The dendrimer/imidazole conjugates. (A) G_1-Imz conjugates. (B) G_2-Imz conjugates. (C) G_3-Imz conjugates.

A solution of G_1, G_2 and G_3 (2 g) in 15 mL of dry THF was added to a round-bottom flask equipped with reflux condenser, argon inlet, dropping funnel and magnetic stirrer 0.2 mL of dry pyridine was added to this solution by the dropping funnel in 15 min. The mixture was stirred vigorously for 10 min. Then a solution of DCC in 10 mL of dry THF was added to the mixture at 0 °C by the dropping funnel and was stirred for 20 min. After a drop wise addition of a solution of imidazole in 10 mL of THF, the mixture was stirred at 0 °C for 1 h, then under argon at room temperature for 24 h and for 48 h at 50°C. The solution was filtered off and was placed at 5 °C for 24 h and again it was filtered off. The product was precipitated in diethyl ether which was repeated for several times. The product was dissolved in 5 mL of

water and stirred for 24 h at room temperature. The solution was filtered off and poured in 5 mL of water at 25 °C. The mixture was conducted into a cellophane membrane dialysis bag. The bag was closed and transferred into a flask containing 100 mL of water maintained at 25 °C. The external water was removed after 24 h and 100 mL of fresh water was added instead. The product was removed from the dialysis bag and dried under vacuum at 50 °C as an amorphous compound. The structure of G_2-Imz and G_3-Imz are shown in Scheme 1A-B.

The preparation of $G_{n\ (n=1-3)}$ -Imz/Naproxen complexes

For the preparation of $G_{n(n=1-3)}$-Imz/Naproxen complexes, the dendrimers were dissolved in 20 mL of THF and were added to a round-bottom flask containing a solution of Naproxen drug molecule in 20 mL of THF and equipped with a reflux condenser and a magnetic stirrer. Then the solutions were stirred for 2h at 30°C. Complexes were precipitated in n-hexane and then dissolved in dichloromethane and again precipitated in diethyl ether to yield yellow compounds. The absorbed bonds in IR spectra for $G_{n\ (n=1-3)}$-Imz/Naproxen complexes are: IR (KBr, cm^{-1}): 1452- 1605(CO_2^-), 1748 (C=O), (2611) R_3NH^+, 1065 and 1107 (C-O), 2926 (CH_2 citric acid), 3132 (CH Imidazole).

Results

Compound G_1 was synthesized through the reaction of ClOC–PEG–COCl with anhydrous citric acid using slightly modified literature method (Namazi and Adeli, 2003). ClOC–PEG–COCl was already produced via the chlorination reaction of diacid poly (ethyleneglycol) using thionylchloride with the yield of 100%. DCC was used for the synthesis of G_2. For the preparation of G_3 the reaction of G_2 with citric acid was carried out using DCC in THF and the product was cut off (Namazi and Adeli, 2003). The compounds G_1-Imz, G_2-Imz, G_3-Imz were synthesized via the reaction of G_1, G_2 and G_3 with imidazole in the presence of dicyclohexylcarbodiimide, as shown in Fig. 1A-C representing the ^{1}H NMR spectra of these compounds. In all spectra the aromatic protons of imidazole groups appear at 7.1-8.1 ppm and the peak at 2.5 ppm is related to DMSO-d_6 as the solvent.

The integral ratio of the aliphatic protons of PEG to the aromatic ones of imidazole was used to account the amount of imidazole groups that were conjugated onto the surface of the dendrimers. These ratios show 33% of functionalization for G_1-Imz (see Fig. 1A) which is lower in comparison to the integral ratio of G_2-Imz in Fig. 1B, it indicates higher amount of functionalization for G_2-Imz which is 42% and for G_3-Imz that was calculated to be 55%. The presence of signals at 8.8-10.1 ppm indicates that some of the acidic groups in the third generation of the dendrimer have not been reacted with imidazole groups also the protons of citric acid were appeared at 1-2.3 ppm.

The size of the nanocarriers

The size of nanocarriers was determined by the means of dynamic light scattering (DLS) technique which are 16, 20 and 45 nm, respectively for G_1-Imz, G_2- Imz and G_3-Imz and are shown in Fig. 2.

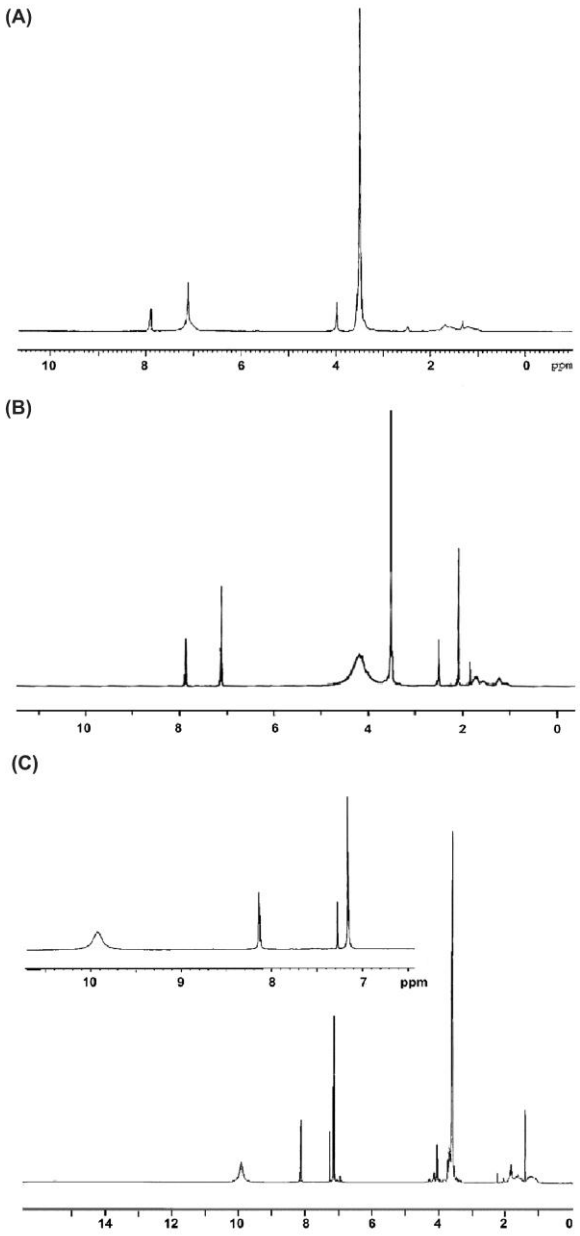


Fig. 1. ^{1}H NMR (400MHZ) spectra of the dendrimer/imidazole conjugates. (A) G_1-Imz in $CDCl_3$. (B) G_2-Imz in $CDCl_3$. (C) G_3-Imz in $CDCl_3$.

Loading Naproxen drug molecule into the dendrimers

The formation of the water-soluble complexes of guest molecules with G_1-Imz, G_2-Imz and G_3-Imz as biocompatible compounds is described here as well. When the guest molecules are drugs, the resulted drug/dendrimer complexes could be used as ideal candidates for carriers and in drug release. Herein, we used Naproxen as the guest molecule that is lipophile and has an acidic group in its structure; its structure is shown in Scheme. 2.

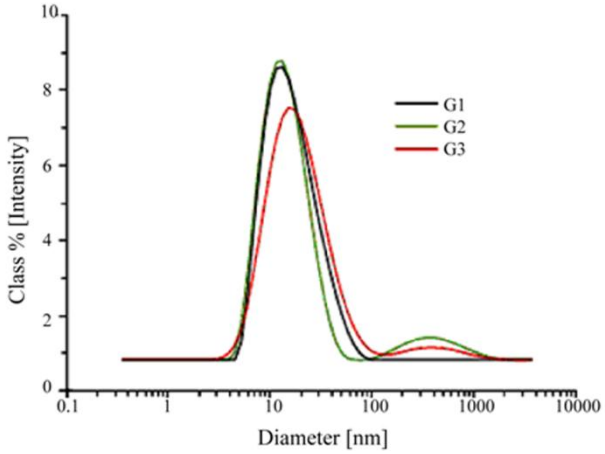
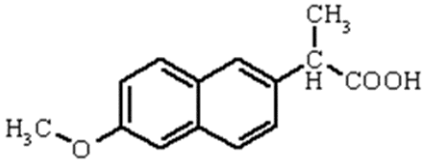


Fig. 2. Dynamic Light Scattering diagrams of G_1–, G_2–, and G_3–imidazole conjugates.

The electrostatic interaction between the acidic group of Naproxen and the lone pair electrons of nitrogen atom of imidazole in the exterior of the dendrimers was investigated. When Naproxen is loaded, the lone pair electrons of imidazole are protonated with the acidic proton of Naproxen. Therefore, the stretch vibration of carboxylate group in Naproxen is observed at 1400 and 1600 cm^{-1} instead of 1700 cm^{-1} due to the red shift and the N-H of protonated nitrogen atoms in imidazole appear at 2600-3300 cm^{-1}.



Scheme 2. The structure of Naproxen drug molecule.

In Fig. 3A-C FT-IR spectra of $G_{n\ (n=1-3)}$-Imz/Naproxen complexes are shown. Also, other investigations proved that the guest molecules like drug molecule can be loaded in the inner part of the dendrimers.

Calculating the amount of the trapped and released drug molecule

The amounts of the trapped drug for each generation before and after the functionalization with imidazole are displayed in Table 1 which is calculated with two procedures. In the first method, the amount of the trapped drug was measured using the weight of the dendrimers before and after complexation (drug/dendrimer complex).

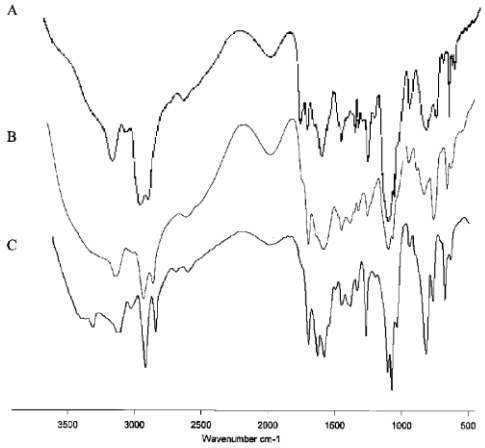


Fig. 3. . FT- IR spectra of the dendrimer/Naproxen complexes. (A) G_1-Imz/Naproxen complex. (B) G_2-Imz/ Naproxen complex. (C) G_3-Imz/ Naproxen complex.

The difference between the measured weights gave the quantity of the trapped drug in the dendritic compound. In the second method, UV spectrophotometer was used. For instance, the calculation of the amount of Naproxen in drug/G_2-Imz complex is as follows:

$$\text{Weight of dendrimer after complexation} = X$$

$$\text{Weight of dendrimer before complexation} = Y$$

$$X - Y = \text{Weight of trapped drug}$$

$$X = 0.0611\ \text{g};\ Y = 0.06\ \text{g and equal to } 3.013 \times 10^{-5}\ \text{mol of dendrimer}$$

$$0.0611 - 0.06 = 1.1 \times 10^{-3}\ \text{g weight of Naproxen in the complex} = 4.77\times10^{-6}\ \text{mol}$$

$$4.77 \times 10^{-6}\ \text{mol drug} \quad 3.013 \times 10^{-5}\ \text{mol dendrimer}$$

$$X \quad 100$$

$$X(\text{Mol\% of Naproxen}) = 15.5 mol\ \%$$

Discussion

Studying the controlled release of Naproxen from the nanocarriers

The controlled release of Naproxen from the nanocarriers was investigated in pH 1, 7.4, and 10. In

order to study the potential application of the first, second and third generations of the linear–dendritic dendrimers, they were functionalized with imidazole groups. Specific concentration of the dendrimer/drug complex was prepared in separate buffer media. Hydrolysis was carried out in a cellophane membrane bags permeable to low molecular weight of compounds and the amount of the drug release was determined in different pH values (pH 1, 7.4, and 10 all at 37 °C).

As it is known, HPLC and UV spectrophotometer are two common methods to study the release of the drug molecules from nanocarriers. In this work we used the latter method and the absorbance of the released drug at its λ_{max} was determined. Figures 4 and 5 show the release of the drug from G_1, G_2 and G_3 and G_1-Imz, G_2-Imz and G_3-Imz in different pHs, respectively.

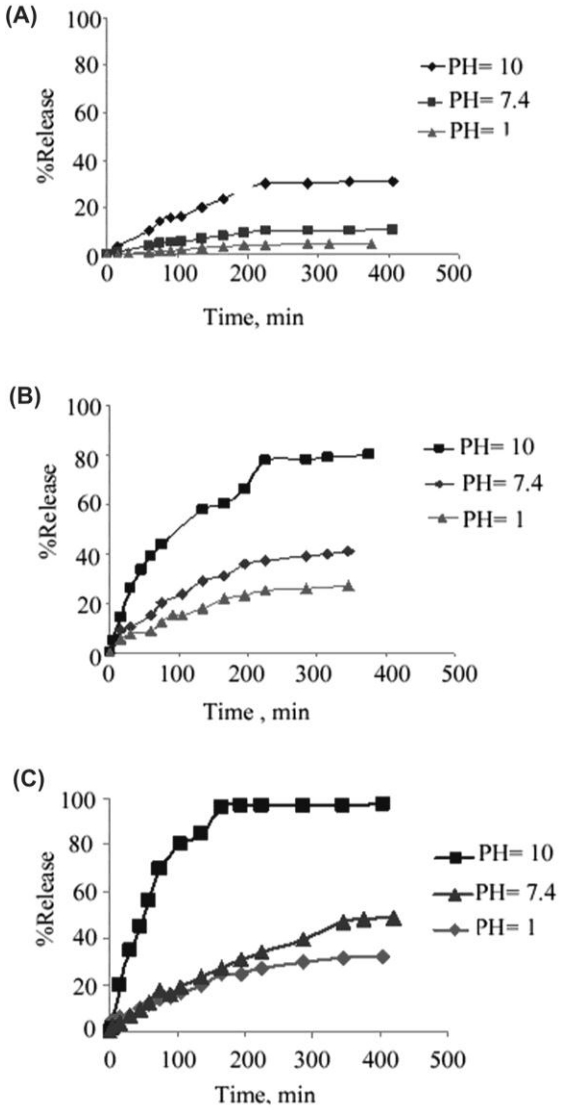


Fig. 4. Release of Naproxen from G_1 /drug complex (A), G_2 /drug complex (B) and G_3 /drug complex (C).

Table 1. The amount of the trapped drug in $G_{n\ (n=1-3)}$ /naproxen complex and $G_{n\ (n=1-3)}$ - imz /naproxen complex.

G_n (n=1-3) /naproxen complex	G_1 /drug	G_2 /drug	G_3 /drug	G_1 Imz /drug	G_2 Imz /drug	G_3 Imz /drug
Mol% of drug in complexes	10.2	5.5	25	38.4	39.7	45.8

Effective factors on the rate and the amount of the release of the drug molecule

As mentioned before, the release of Naproxen drug molecule was studied in three buffer media with pH 1, 7.4 and 10 which are the pH of body's biological fluids. The results displayed that both the rate and the amount of the release of the drug from dendrimer/drug complex is influenced by some factors such as the generation of the host molecule, biodegradability of the dendrimers, functional groups on the surface of the dendrimer, solubility of the drug, the interactions between the drug and the dendrimer in the complex and pH and the effects of each factor is considered as the following.

The generation of the host molecule

The release profiles show (Fig. 4. and Fig. 5.) that in both imidazole-functionalized dendrimers and unfunctionalized ones, since higher generations possess more cavities, the higher amounts of the loaded drug as well as the higher amounts of the release are observed. Thus, the amount of the release diminishes through the order of G_3>G_2>G_1 and G_3-Imz>G_2-Imz>G_1-Imz.

Biodegradability of the dendrimers

Esteric bonds are formed in the synthesis of CPEGC triblock dendrimers which are easily hydrolyzed in basic solutions and lead to the release of the loaded drug molecules. Hence the rate and the amount of the release diminishes through the order of pH=10>pH=7.4>pH=1.

Functional groups on the surface of the dendrimer

The lone pair electrons of nitrogen atom in imidazole has electrostatic interaction with the acidic group of Naproxen drug molecule which results in higher amount of loading and consequently in more release in comparison with the unfunctionalized dendrimers.

Solubility of the drug molecule

The solubility factor has a significant effect in the rate of the release. As mentioned, Naproxen is a lipophile molecule with acidic group which is less soluble in acidic media so, it shows fewer tendencies to release in acidic solutions while it is highly soluble in basic solutions which leads to higher amount of release. It is obvious that this factor along with the mentioned ones influence the amount and the rate of the release

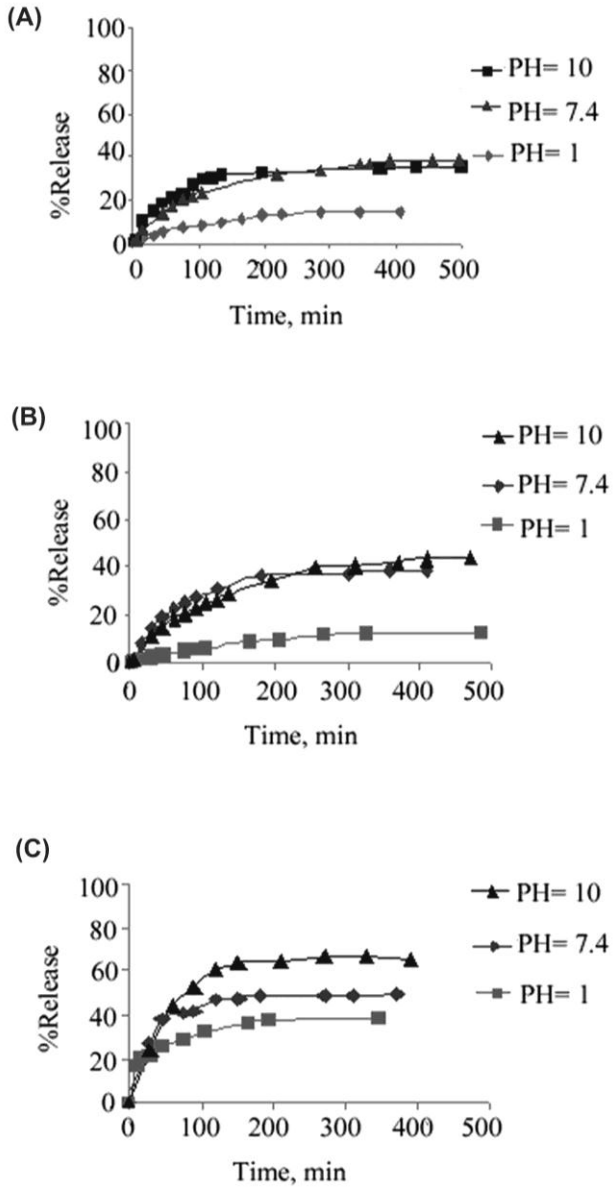


Fig. 5. Release of Naproxen from G_1-Imz/complex (A), G_2-Imz/complex (B) and G_3-Imz/complex (C).

Conclusions

In this research citric acid dendrimers were used since they are water soluble and biocompatible and low toxic. Imidazole groups were conjugated onto the surface of the dendrimers and the size of the nanocarriers in the distilled water was investigated using DLS technique which was 16, 20 and 45 nm for G_1-Imz, G_2- Imz and G_3-Imz, respectively. Naproxen, a lipophile drug molecule with an acidic group, was loaded into both functionalized and unfunctionalized dendrimers. The electrostatic interaction between the drug molecule and dendrimer in drug/dendrimer complexes was demonstrated with IR spectra. In-vitro release of Naproxen as the guest molecule was investigated in pH 1, 7.4 and 10. It was observed that the rate of the release increased in pH=10 in all generations which was related to the increase in Naproxen solubility and the hydrolysis of the esteric bonds in the mentioned PH. The effect of other factors such as the generation of the host molecule, biodegradability of the dendrimers, functional groups on the surface of the dendrimer and the interactions between the drug and the dendrimer in the complex were studied as well. The amount of the trapped drug in these nanocarriers was measured with two methods. The results showed that the amount of the trapped drug increased with the increase in the generation of the dendrimer and pH.

Ethical Issue

None to be declared.

Conflict of interest

Authors declare no conflict of interests.

Acknowledgements

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