**Poly(propylene imine) dendrimer-grafted nanocrystalline cellulose: doxorubicin loading
and release behavior**

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Abstract

4th-generation poly(propylene imine) (PPI)-grafted nanocrystalline cellulose (NCC) is prepared via iterative Michael addition of acrylonitrile onto amine-functionalized NCC as initial core and homogeneous hydrogenation of nitrile groups to primary amines. Successful grafting of dendrimers onto NCCs is confirmed by TEM, FT-IR, XPS, TGA and elemental analyses. Also, peripheral primary amines of dendrimer-grafted NCCs are conjugated with folic acid (FA) to investigate bioconjugation effect on drug release behavior of nanostructures. FA-conjugated and nonconjugated nanostructures are loaded with DOX and exposed to environments with different pH values to examine release behavior. Also, drug release kinetics is studied by fitting of experimental data with release models. At different pH values, nonconjugated nanostructures show higher cumulative drug release than FA-conjugated systems while loading content was higher for conjugated systems. Drug release from nonconjugated nanostructures is modeled using Korsmeyer-Peppas model that describes release from polymeric matrix. However, in conjugated system, Higuchi model is the best one due to the better solubility of DOX in release media than cavities of grafted dendrimers.

Keywords: Nanocrystalline cellulose, Poly(propylene imine) dendrimer, Folic acid, Bioconjugation, Drug release kinetics

1. Introduction

Cellulose as natural linear carbohydrate polymer is the most abundant biopolymer found in wood, cotton, and hemp [1]. Cellulose nanocrystals or nanocrystalline cellulose (NCC) is a highly crystalline nanometer-sized rod-like particle [2] and can be produced from cellulose via a controlled acid hydrolysis process [3] that may be assisted with other methods such as high pressure homogenization [4] and low-intensity ultrasonication [5]. Due to a very high modulus of elasticity, high aspect ratio, specific surface area in the range of 150-300 m²/g, low density, easy production, nonabrasive nature, renewability and recyclability, NCC has attracted enormous attention for different applications [6-8].

Among different polymeric structures, dendrimers as three-dimensional, highly-branched and monodispersed nanostructures are synthesized via an iterative sequence of reactions [9-10]. Among different dendrimers, poly(propylene imine) (PPI) dendrimer as an amine-terminated dendrimer is synthesized via iterative Michael addition of acrylonitrile to an amine-capped core and subsequent reduction of nitrile groups by reduction catalysts [11-12]. PPI dendrimers have been applied in different applications such as dye adsorption [13], drug delivery systems [14], etc. Although grafting PPI dendrimers onto different surfaces has been investigated recently [15-18], no exemplary study has been reported about surface modification of NCC with PPI dendrimers. In other words, PPI-dendrimer-grafted NCC nanoparticles are attractive especially as drug carriers but there is no report about synthesis, characterization and application of such a structure.

In this work, NCC nanoparticles have been modified with (3-aminopropyl)triethoxysilane (APTES) and used as initial core to synthesize PPI-dendrimer-grafted NCC nanoparticles up to 4th generation. To prepare half generations, acrylonitrile was added to amine groups via Michael addition. Then, nitrile groups were reduced via a homogeneous hydrogenation by

lithium aluminum hydride (LAH). Due to cationized peripheral groups [19-20], dendrimers are known as toxic materials. To decrease cytotoxicity of PPI-dendrimer-grafted NCC nanoparticles, they were conjugated with biocompatible folic acid (FA) which is one of the most promising candidates with the potential for cancer-cell specific targeting due to its high affinity for folate receptors (FRs) [21-23]. Finally, doxorubicin (DOX) as an anti-cancer drug was loaded onto synthesized dendrimer-grafted NCC nanoparticles and its release behavior from FA-conjugated and nonconjugated nanoparticles has been studied in different conditions. Also, release kinetics has been studied by fitting of release data with different drug release models.

2. Experimental Methods

2.1. Modification of NCC by APTES

Surface of NCC nanoparticles bears numerous hydroxyl functions which can be used for further modification reactions. Herein, to surface modification of NCC nanoparticles, a previously described protocol for silane coupling agents was used [24]. APTES grafting onto the surface was carried out through the hydroxyl functional groups. NCC (5.0 g) was dispersed in ethanol/water mixture (80 mL, 50/30) and ultrasonically agitated for 1 h to reach a homogeneous dispersion. Then, ammonia (30 mL) was added and stirring was continued for 1 h. APTES/ethanol solution (10 mL/30 mL) was added dropwise during 6 h and stirring was continued at room temperature overnight. The obtained suspension was centrifuged (10000 rpm) and washed with ethanol three times. Finally, the obtained product (NCC-NH₂) was diluted to 500 mL and after sonication for 30 min, freeze-dried and weighted 5.90 g.

2.2. Synthesis of PPI-dendrimer-grafted NCC

To graft nitrile-terminated first generation of PPI dendrimer (G1N) onto surface of **NCC nanoparticles**, a previously described divergent method with some modifications was used [11]. To this end, NCC-NH₂ (1.0 g) was used as an initial core and dispersed in deionized water (10 mL) via ultrasonication (30 min). ACN (1 mL, 15.3 mmol) was added dropwise at room temperature and dark room condition during 1 h to participate in Michael addition reaction. Reaction was continued for 1 h and then, media was heated to 80 °C and reaction was continued for 1 h to complete the addition reaction. The obtained suspension was centrifuged (10000 rpm) and washed with water three times. Finally, the obtained product (NCC-G1N nanoparticles) **was** diluted to 100 mL and after sonication for 30 min, freeze-dried.

To obtain amine-terminated first generation of PPI dendrimer (G1A)-grafted **NCC nanoparticles**, nitrile groups should be reduced to primary amines. Due to high pressure of hot **hydrogen in heterogeneously hydrogenation of primary amines using Raney nickel catalyst** [25], we used a homogeneous reduction method via LAH. Accordingly, NCC- G1N (1.0 g) was dispersed in diethyl ether (20 mL) via ultrasonication (30 min) and a mixture of LAH (0.19 g) in diethyl ether (60 mL) was added dropwise and reaction was continued for 4 h at 34 °C. Then, reaction mixture was placed in ice-water bath and deionized water (10 mL) was added dropwise and stirring was continued for 1 h. After decanting diethyl ether, an aqueous solution of HCl (pH = 3) was added to aqueous media to dissolve salts produced during reaction. The reaction mixture was then filtered via 0.22 µm PTFE membrane filter, diluted to 100 mL and after sonication for 30 min, freeze-dried to obtain NCC-G1A. Scheme 1 shows the reaction route to achieve NCC-G1A nanoparticles. **PPI-dendrimer-grafted** NCC nanoparticles up to fourth generation were prepared by repetition of all the above steps consecutively, with increasing quantity of ACN and subsequently LAH. Also, reaction time

of Michael addition was increased 1 h per each generation increase. The detailed reaction conditions in each step are presented in Table 1.

Scheme 1: The reaction route to achieve NCC-G1A nanoparticles

Table 1: Reaction conditions in each step to synthesize NCC-G4A

Sample name	Reactants	Temperature (duration)
NCC-G2N	NCC-G1A (1.0 g) in water (10 mL) + ACN (2 mL, 30.5 mmol)	25 °C (1 h) + 80 °C (2 h)
NCC-G2A	NCC-G2N (1.0 g) in diethyl ether (20 mL) + LAH (0.38 g) in diethyl ether (120 mL)	34 °C (4 h)
NCC-G3N	NCC-G2A (1.0 g) in water (10 mL) + ACN (4 mL, 61.1 mmol)	25 °C (1 h) + 80 °C (3 h)
NCC-G3A	NCC-G3N (1.0 g) in diethyl ether (20 mL) + LAH (0.76 g) in diethyl ether (240 mL)	34 °C (4 h)
NCC-G4N	NCC-G3A (1.0 g) in water (10 mL) + ACN (8 mL, 122.1 mmol)	25 °C (1 h) + 80 °C (4 h)
NCC-G4A	NCC-G4N (1.0 g) in diethyl ether (20 mL) + LAH (1.52 g) in diethyl ether (480 mL)	34 °C (4 h)

2.3. Conjugation of FA with surface of NCC-G4A

Firstly, NCC-G4A was reacted with glycidol to achieve NCC-G4OH via previously described method [26]. NCC-G4A (0.2 g) was dispersed in methanol (10 mL) via ultrasonication (30 min). A solution of glycidol in methanol (0.14 mL in 10 mL) was added dropwise to reaction media and the mixture was stirred at 60 °C under nitrogen for 24 h. The mixture was centrifuged and obtained powder was washed with methanol and centrifuged three more times. Finally, product diluted to 20 mL and after sonication for 30 min, freeze-dried.

FA-conjugated nanoparticles (NCC-G4F) were also synthesized using a previously described method with some changes [27]. FA (0.28 g), DCC (0.133 g) and DMAP (0.026 g) were dissolved in DMSO (10 mL) and NCC-G4OH (0.2 g) was dispersed in media via ultrasonication (30 min). Then, the mixture was stirred for 72 h at 50 °C and centrifuged to obtain NCC-G4F. To further purification, product was washed with water and centrifuged three more times. Finally, product diluted to 20 mL and after sonication for 30 min, freeze-dried. Scheme 2 shows the route that we used to achieve NCC-G4F nanoparticles.

Scheme 2: The route to achieve NCC-G4F nanoparticles

2.4. Encapsulation of DOX in NCC-G4A and NCC-G4F nanoparticles

Aqueous solution of DOX (1 mg/mL, 6 mL) was mixed with NCC-G4A or NCC-G4F nanoparticles (24 mg) at desired pH with subsequent shaking for 48 h in dark conditions. The DOX-loaded nanoparticles were separated by centrifugation and the loaded drug content was obtained from the drug concentrations before and after loading by means of UV-visible absorption at 480 nm. To measure concentrations, a calibration curve was used in that concentration of DOX was plotted versus UV absorption.

2.5. *In vitro* drug release

The DOX-loaded NCC-G4A or NCC-G4F aqueous solutions (4 mg/mL, 5 mL) in cellulose dialysis bag (MWCO = 12000) were submerged in phosphate-buffered saline (PBS, 100 mL) stirring at 200 rpm (37 ± 2 °C) for 48 h at two pH values (5.3 and 7.4). At predetermined intervals of time, 1-mL aliquots were withdrawn and replaced with an equal volume of fresh medium. The DOX concentrations were determined by UV-visible absorption at 480 nm according to a pure DOX calibration curves at different pH values.

3. Results and discussion

3.1. Synthesis of dendrimer-grafted NCC nanoparticles

Herein, MCC was hydrolyzed to synthesize NCC via separation of small crystalline blocks of cellulose from amorphous parts. Then, PPI dendrimers up to 4th generation were grafted onto the surface of NCCs. Figure 1 shows the TEM images of NCC and NCC-G4A samples. As depicted in Figure 1, NCC nanoparticles are rod-like with length of 100-200 nm and diameter of 10-20 nm. According to the TEM image of NCC-G4A nanoparticles, a layer of spherical-like dendrimers with sizes < 10 nm has covered the surface of NCCs.

Figure 1: TEM images of NCC and NCC-G4A nanoparticles

Direct information about changes in chemical functionality can be obtained by FT-IR spectroscopy before and after chemical treatments. Figure 2 shows the FT-IR spectra of NCC, NCC-NH₂, NCC-G4N, NCC-G4A, and NCC-G4F. The broad spectral band at around 3400 cm⁻¹ is ascribed to O-H stretching of intramolecular hydrogen bonds for cellulose I [28]. Also, characteristic peaks at 2880 and 2930 cm⁻¹ are related to C-H stretching of alkyl groups in NCC structure [29]. Peak at 1640 cm⁻¹ is originated from -OH stretching of water molecules absorbed to the structure of NCC nanoparticles [30] and peaks appearing from 1310 to 1370 cm⁻¹ are due to the bending vibration of the C-H and C-O groups in the polysaccharide rings in cellulose [31]. Also, stretching of C-O and O-H is reflected by peaks appearing in 1050-1070 cm⁻¹ and the peak at 1056 cm⁻¹ is due to the bending vibration of the C-O-C pyranose ring [32]. After modification of NCC nanoparticles with APTES, new

peaks appear at 3320 and 3290 cm^{-1} related to stretching and at 1564 cm^{-1} due to bending vibrations of $-\text{NH}_2$ groups [32]. Michael addition reaction of ACN and $-\text{NH}_2$ groups results in disappearance of characteristic peaks of amine groups at 3320, 3290 and 1564 cm^{-1} and appearance of a new peak at 2250 cm^{-1} related to nitrile groups of half-generation PPI dendrimer (NCC-G4N) [33]. After reduction of nitrile groups to primary amines, peak at 2250 cm^{-1} disappears and peaks at 3346 and 3294 cm^{-1} related to primary amines appear again. Conjugation of FA onto the surface of nanoparticles leads to appearance of peaks at 1690 and 1626 cm^{-1} related to amide bonding within FA structure [34] and peaks at 3050 and 1576 cm^{-1} due to aromatic rings of structure [35]. Also, peak at 3330 cm^{-1} is ascribed to the amine groups within the structure of FA [36].

Figure 2: FT-IR spectra of NCC, NCC-NH₂, NCC-G4N, NCC-G4A, and NCC-G4F nanoparticles

Elemental analysis was performed to prove the presence of grafted dendrimers on surface of NCCs as depicted in Table 2. The results show that there is an increment in carbon, hydrogen and nitrogen contents after each modification step or generation progression whereas oxygen

content decreases drastically. This is ascribed to introduction of hydrogenated ACN molecules those compose of carbon, hydrogen and nitrogen.

Table 2: Elemental analysis of NCC, NCC-NH₂, NCC-G1A, NCC-G2A, NCC-G3A, and NCC-G4A nanoparticles

Sample	C (wt. %)	H (wt. %)	N (wt. %)	S (wt. %)	O (wt. %, calculated)
NCC	41.65	6.88	-	0.08	51.39
NCC-NH₂	44.79	8.01	1.60	0.06	45.54
NCC-G1A	47.11	8.66	4.53	0.04	39.66
NCC-G2A	50.63	9.48	8.92	0.03	30.94
NCC-G3A	55.24	10.55	14.70	0.02	19.49
NCC-G4A	59.42	11.51	19.92	-	9.15

XPS was used to investigate chemical composition of dendrimer-grafted NCC nanoparticles. Figure 3 shows survey data of NCC, NCC-NH₂, NCC-G1A, NCC-G2A, NCC-G3A and NCC-G4A. NCC nanoparticles show C 1s peak centered at 284.5 eV with content of 64.76% and O 1s peak centered at 531.2 eV with content of 35.24%. After surface modification with APTES, new peaks appear at 102.0, 152.8 and 399.6 eV related respectively to Si 2p, Si 2s and N 1S [37-38] with Si content of 2.11% and N content of 2.21% (Table 3). This shows that APTES successfully is grafted on the surface of NCC nanoparticles. After generation progression on surface of NCC nanoparticles, content of nitrogen increases whereas oxygen content decreases drastically. This is due to presence of carbon and nitrogen atoms and lack of oxygen atom in the structure of PPI dendrimers. Also, after grafting of 2nd generation, Si content cannot be calculated by XPS software as its content decreases dramatically.

Figure 3: XPS survey data of NCC, NCC-NH₂, NCC-G1A, NCC-G2A, NCC-G3A and NCC-G4A nanoparticles

Table 3: Surface elemental composition of NCC, NCC-NH₂, NCC-G1A, NCC-G2A, NCC-G3A, and NCC-G4A nanoparticles obtained by XPS

Sample	C (at. %)	O (at. %)	N (at. %)	Si (at. %)
NCC	64.76	35.24	-	-
NCC-NH ₂	60.87	34.81	2.21	2.11
NCC-G1A	63.91	28.57	5.73	1.79
NCC-G2A	65.66	22.95	10.31	1.08
NCC-G3A	68.69	15.33	14.98	-
NCC-G4A	70.13	10.11	19.76	-

Figure 4 illustrates **XRD** patterns of different samples. NCC displays four diffraction peaks at 15.1 (110 diffraction), 16.5 (110 diffraction), 22.7 (200 diffraction), and 34.6° (004 planes) those are typical peaks of cellulose [39-40]. Peaks at 15.1 and 16.5° are the peaks of the cellulose-I polymorph structure and the peak at 22.7° is attributed to the crystalline parts. **Crystallinity** index (I_c) of **samples** was calculated according to amorphous subtraction method [41] via Equation (1) as follow:

$$I_c (\%) = \frac{I_{(200)} - I_{am}}{I_{200}} \times 100 \quad (1)$$

where, $I_{(200)}$ represents **combined** crystalline and amorphous parts of cellulose (peak intensity at around 22.7°) and I_{am} represents the amorphous part of cellulose (peak intensity at around 18°). According to the results, crystallinity of NCC, NCC-NH₂, NCC-G1A, NCC-G2A, NCC-G3A, and NCC-G4A was calculated 78.7, 67.3, 67.1, 65.8, 65.4, and 61.9% respectively. Decrease of crystallinity index of modified-NCC nanoparticles is ascribed to increasing the amount of amorphous modifier molecules comprising APTES molecules and PPI dendrimers. However, XRD peaks of different modified samples **show** that the structure of NCC nanoparticles is retained and different processes including modification and acid washing did not disturb the crystalline structure.

Figure 4: XRD patterns of NCC, NCC-NH₂, NCC-G1A, NCC-G2A, NCC-G3A, and NCC-G4A nanoparticles

For further proving the success of the modification process, different samples were analyzed by TGA. TGA thermograms of NCC, NCC-NH₂, NCC-G1A, NCC-G2A, NCC-G3A, and NCC-G4A are depicted in Figure 5-A. Also, TGA results are summarized in Table 4. According to the results, decomposition of NCC started at about 230 °C. This low temperature of degradation is due to small particle size and high specific surface area **those result** in higher number of free end chains in the surface. Also, amorphous chains on the surface of NCC degrade at low temperatures and result in lower thermal stability of NCC [42]. Degradation of NCC **takes** place at one step without any degradation step at temperatures between 150 and 200 °C which usually takes place in sulfate-containing NCC nanoparticles due to presence of sulfate groups [42]. Thus, repeated centrifugation and ultrasonic processes in combination with base treatment with NaOH **result** in sulfate free NCC product [24]. Also, $T_{d,max}$ (peak location of DTG curve) was obtained 289.7 °C with char of 15.90 wt. % for NCC. After modification with APTES, $T_{d,max}$ increases to 359.5 °C

with char of 22.45 wt. % for NCC-NH₂. These results coincide the results obtained by Zhang et al. [32]. This improvement in thermal stability originates from higher thermal stability of silane coupling agents due to presence of Si-O bands in their structures [43] and also, occurring crosslinking of APTES molecules on the surface of nanoparticles [44]. To calculate grafting density (GD) of APTES on the surface of NCC, gravimetric method was used via Equation (2) as follow:

$$GD\left(\frac{\text{mol}}{\text{g of NCC}}\right) = \frac{w_{NCC-NH_2} - w_{NCC}}{M} \quad (2)$$

where, w_{NCC} is **normalized** weight of NCC sample used in reaction (1.0 g) and w_{NCC-NH_2} is **weight** of NCC-NH₂ sample obtained after reaction per 1.0 g of NCC (1.18 g). Also, M is **molecular** weight of hydrolyzed APTES (134.2 g/mol). Conclusively, GD of APTES molecules on the surface of NCC nanoparticles was calculated 1.3 mmol/g of NCC. To calculate the GD of amine moieties on the surface of NCC with dendrimer generation progression, Equation (3) was used as follow [45]:

$$GD\left(\frac{\text{mol}}{\text{g of NCC}}\right) = \frac{\left(\frac{W_i - W_{i-1}}{100 - W_i}\right)}{M} \quad (3)$$

where, W_i , W_{i-1} and M are mass losses (in the range of 120-700 °C) and molecular weight of degradable moiety added in each modification step respectively. According to the results, GD of amine groups was obtained 2.9, 4.9, 10.4, and 20.1 mmol/g of NCC for NCC-G1A, NCC-G2A, NCC-G3A, and NCC-G4A respectively. Also, by increasing the generation of attached dendrimers onto the surface of NCC, $T_{d,max}$ shifted to **lower** temperature from 354.0 °C for NCC-G1A to 317.0 °C for NCC-G4A. This is attributed to **increment** the amount of amorphous dendrimer chains on the surface of crystalline nanoparticles. As shown in Figure 5-B, an exponential increase of the weight losses with increasing dendrimer generation ($y =$

$76.9537e^{0.0533x}$, where y is the weight losses (%) and x is the number of the generation, $R^2 = 0.9938$) is the best proof of successful grafting of PPI dendrimers onto the surface [46-47]. Moreover, when GD of amine groups is plotting versus dendrimer generation, an exponential increment of GD versus generation progression is observed ($y = 1.3683e^{0.6704x}$, where y is the GD (mmol/g of NCC) and x is the number of the generation, $R^2 = 0.9977$). In this case, exponent of the equation is 0.6704 that is so close to the ideal exponent (0.6931). Conclusively, dendrimer growth from the surface of NCCs has been occurred ideally and synthesis has been progressed successfully.

Figure 5: A) TGA thermograms of NCC, NCC-NH₂, NCC-G1A, NCC-G2A, NCC-G3A, and NCC-G4A nanoparticles; B) Weight loss and GD versus generation progression for modified NCC nanoparticles

Table 4: Summarized results of TGA for NCC, NCC-NH₂, NCC-G1A, NCC-G2A, NCC-G3A, and NCC-G4A nanoparticles

Sample	T _{d,max} (°C)	Weight loss (%)	GD (mmol/g of NCC)
NCC	289.7	84.10	-
NCC-NH ₂	359.5	77.55	1.3
NCC-G1A	354.0	80.71	2.9
NCC-G2A	340.8	84.91	4.9
NCC-G3A	340.0	90.52	10.4
NCC-G4A	317.0	95.59	20.1

3.2. Drug loading

Drug loading (DL) content of NCC-G4A and NCC-G4F were calculated according to **Equations (4)** [48]:

$$DL(\text{mg} / \text{mg}) = \frac{\text{Weight of the drug in nanoparticles}}{\text{Weight of the nanoparticles}} \quad (4)$$

DL was calculated **0.009 ± 0.001** and **0.011 ± 0.001** for NCC-G4A and NCC-G4F respectively. DL was obtained higher for NCC-G4F compared with NCC-G4A. Better interaction of DOX with surface groups because of FA conjugation can be the main reason of this phenomenon [12]. Also, noncovalent interactions such as hydrogen bonding and hydrophobic interaction between DOX and PPI dendrimers lead to physical binding of drug molecules inside the core and surface-engineered groups of dendrimer [49].

3.3. *In vitro* drug-release studies

To investigate the ability of synthesized dendrimer-modified NCC nanoparticles as anti-cancer drug carriers, the *in vitro* release behavior was investigated at two different pH values comprising physiological condition (pH = 7.4) and acidic environment (pH = 5.3) at 37 °C. As results depicted in Figure 6, NCC-G4A and NCC-G4F show no burst release in each condition due to encapsulation of the DOX in the cavities of the dendrimers those act as sink to retain the drug molecules for a long duration. However, free drug showed monophasic rapid release (97.2% in 4 h at pH = 5.3 and 83.1% in 7 h at pH = 7.4). This confirms that synthesized nanocarriers act as drug-retentive structures for DOX drug. Also, at different pH values, nonconjugated structures show higher cumulative drug release with respect to FA-conjugated systems. The possible reason for this behavior is originated from steric hindrance on the dendrimer surface due to FA conjugation that prevents the DOX molecules from leaving the nanoconjugate system. However, by decreasing the pH value of release media, the DOX release was more rapid in conjugated and nonconjugated nanostructures. It should be noted that DOX is dissolved in water at low pH, but insoluble in an alkaline environment [50]. At low pH value, DOX becomes positively charged and more soluble in and diffuses more easily from the dendrimer structure to aqueous media. Also, at low pH values, dendrimer cavities become positively charged due to existence of tertiary amines. Conclusively, electrostatic repulsions between cavities and drug acts as driving force of drug release through cavities of dendritic structure.

Figure 6: The DOX release behavior of synthesized NCC-based nanocarriers

3.4. Drug release kinetics

Mathematical modeling is a useful tool to investigate the release kinetics before the release systems are realized. Among different models, zero-order, first-order, Higuchi, Korsmeyer-Peppas and Hixson-Crowell models as the most investigated models [51-53] have been used here to predict the release behavior of nanocarriers. The release kinetics model and parameters of each model have been obtained by linear regression analysis, and the coefficients of correlation (R^2) were used to verify the accuracy of the fitting, as shown in Table 5 and Figures S1-S5. According to the results, no proper data fitting was obtained for the zero-order, first-order and Hixson-Crowell models due to the source of these models. The zero-order model is used in slow release kinetics. However, dendrimer-based formulations show more rapid drug release due to a rapid change in solubility in aqueous media especially at low pH values. Also, the first-order model is used to model drug release from porous matrices those are not soluble in release media. Moreover, Hixson-Crowell model can be used when the surface area and diameter of the drug matrix change with time. None of these

three models are the case of study in our work and our data show no proper fitting via these systems. In the case of free drug release systems, release kinetics conforms from both Higuchi and Korsmeyer-Peppas model at both pH values. In the case of nonconjugate systems, drug release from dendritic structures can be modeled using Korsmeyer-Peppas model. This model is a mathematical equation for release from polymeric matrix. However, in conjugated system, Higuchi model is the best one. Herein, the most important reason for DOX release from the cavities of dendrimers is the better solubility of DOX in release media than main structure of dendrimers. Conclusively, FA-conjugation changes the release mechanism from diffusion through matrix to dissolution of drug in release media.

Table 5: Mathematical models' correlation coefficients and release exponents for NCC-G4A and NCC-G4F dendritic nanostructures

Kinetics model	Equation [51-53]	Parameter	Value					
			pH = 5.3			pH = 7.4		
			Free drug	NCC-G4A	NCC-G4F	Free drug	NCC-G4A	NCC-G4F
Zero-order	$Q_t = Q_0 + K_0 t$	R^2	0.9530	0.8623	0.9734	0.9426	0.9264	0.9691
		K_0	22.924	1.275	0.688	9.526	0.813	0.421
First-order	$\log C = \log C_0 + \frac{K}{2.203} t$	R^2	0.8071	0.7186	0.8529	0.8208	0.8021	0.8407
		K	0.463	0.031	0.037	0.198	0.033	0.029
Higuchi	$f_t = K_H t^{1/2}$	R^2	0.9913	0.9647	0.9993	0.9936	0.9849	0.9934
		K_H	60.960	11.229	5.949	32.069	7.134	3.853
Korsmeyer-Peppas	$\frac{M_t}{M_\infty} = at^n$	R^2	0.9769	0.9755	0.9983	0.9943	0.9860	0.9864
		aM_∞	36.224	9.594	3.273	3.334	3.819	2.642
		n	0.805	1.118	0.637	0.523	0.582	0.587

Hixson-Crowell	$Q_t^{1/3} = Q_0^{1/3} + kt$	R^2	0.8698	0.7737	0.9062	0.8694	0.8536	0.8945
		k	0.567	0.037	0.033	0.244	0.032	0.024

4. Conclusions

A successful iterative sequence Michael addition of acrylonitrile and reduction reactions of nitrile groups to primary amines with amine-functionalized NCC as primary core was used to synthesize 4th-generation PPI dendrimer-grafted NCC nanoparticles. Then, peripheral amine groups of grafted dendrimers were bioconjugated with folic acid. TEM, FT-IR, CHNS elemental analysis, XPS, and TGA were used to approve the success of different processes. Results showed that dendrimers have grown ideally from surface of nanoparticles. NCC-G4A and NCC-G4F nanoparticles were exposed to pH variations to investigate the drug release behavior of dendrimer-grafted NCC nanoparticles. Drug loading content was found 0.009 ±0.001 and 0.011 ±0.001 (mg of drug/mg of nanoparticles) for NCC-G4A and NCC-G4F respectively. Higher amount of drug loading for NCC-G4F with respect to NCC-G4A was attributed to FA conjugation onto the dendrons' surface. However, at different pH values, nonconjugated structures show higher cumulative drug release with respect to FA-conjugated systems because of steric hindrance on the dendrimer surface due to FA conjugation that prevents the DOX molecules from leaving the nanoconjugate system. Additionally, by decreasing the pH value of release media, the DOX release was more rapid in both conjugated and nonconjugated nanostructures. In nonconjugate system, drug release was modeled using Korsmeyer-Peppas model that describes release from polymeric matrix. However, after bioconjugation, Higuchi model described the release behavior better than others due to the better solubility of DOX in release media than main structure of conjugated system.

Supplementary data: Materials, Preparation of NCC from MCC, Instrumentation, Drug release kinetics.

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