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Macromolecular Co-Conjugate of Bisphosphonate: Synthesis and Kinetic Drug Release Study.

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Abstract

Cancer chemotherapy often results in side effects such as high toxicity and drug resistance. Sequential and simultaneous delivery of drug combinations have been found to reduce the side effects associated with systemic delivery of anticancer drugs. An alternative approach to systemic delivery of anticancer drugs is the co-conjugation of two or more these agents to a single polymeric carrier *via* biofissionable linkages. In this study, macromolecular co-conjugates of bisphosphonate and ferrocene were synthesized and the kinetic drug release studied. Phosphorus and proton NMR and FTIR were used to characterize the co-conjugates. The mass percentages incorporation of ferrocene analogue were found to be between 4-5 % and 10-12 % for bisphosphonate. Kinetic drug release results at selected pH (1.2, 5.5 and 7.4) were fitted in different mathematical drug release model to determine the mechanism of release of ferrocene and bisphosphonate from a co-conjugate pro-drug. The best model describing the release of ferrocene were found to be Korsmeyer-Peppas model at pH 1.2 and zero order model at pH 5.5 and 7.4. For bisphosphonate, Korsmeyer-Peppas, Higuchi and zero order models were found to best fit the release mechanism at pH 1.2, 5.5 and 7.4 respectively.

Keywords: Ferrocene . Bisphosphonate . Co-conjugate . Macromolecule . Drug release.

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"This article is dedicated to the memory of our colleague Professor Eberhard W. Neuse."

1 Introduction

Bisphosphonates constitute a class of bioactive compounds which exhibit an exceptional affinity with bone tissue [1,2]. They have been used in the treatment of paget's diseases and osteoporosis [3,4]. Bisphosphonates have been extensively studied, and they have received more attention following the discovery of their antimetastatic activity in cancer patients [5] and in the control of tropical diseases such as malaria [6] and sleeping sickness [7]. However, bisphosphonates are large, hydrophilic molecule that cannot cross the gastrointestinal epithelium via the transcellular route and inefficiently utilize the paracellular route of absorption due to their large size and negative charge when administered orally [8]. Thus, despite the physiochemical advantage, the oral bioavailability of bisphosphonates remains low at 0.6% to 1.5% of the admistered dose [9].

The use of ferrocene for medicinal applications has grown fast since its discovery [10]. Many reports have shown that some ferrocene derivatives are highly active *in vitro* and *in vivo*, against several diseases including fungal and bacterial infections [11,12], malaria [13-15] and cancer [16-20].

During the past decade, many research studies focussed on the sequential and simultaneous delivery of drug combinations, to reduce the side effects associated with the systemic delivery of cancer agents [21]. Several drugs have been found to amplify the anticancer activity of others [22]. This synergistic effect could lead to reduced doses for each drug administered [23], thereby reducing the side effects caused by the high doses of a single drug, and preventing the development of multi-drug resistance [24]. A possible route for the simultaneous delivery of anticancer drugs is the co-conjugation of two or more of these compounds to a single polymeric carrier. Reports covering this approach are rare. However, the literature extensively reported the targeted delivery of a drug system in which a targeting moiety together with a drug is anchored to the same polymer [25,26].

The water soluble, biocompatible and biodegradable macromolecular-drug co-conjugate, administered intravenously, offers significant advantages over the free drug, these include: (i) Utilization of the enhanced permeability and retention effect [27], which serves to provide preferential accumulation of macromolecules (as compared to low –molecular compounds) in tumorous tissue. (ii) Temporary protection, while in transit, from premature catabolism, enzymatic attack, or serum protein binding, thus increasing circulation life time and bioavailability. (iii) Facilitated endocytotic cell entry, thus overcoming problems of drug charge and polarity, as well as resistance caused by accelerated drug efflux from affected cell.

The overall consequence of these features is a widened therapeutic window and enhanced drug effectiveness [28].

The synthesis and utilization of macromolecules of the poly(amidoamine) type in biomedicine, pioneered by Ferruti [29,30], has laid fertile ground for pharmaceutical research. Amidoamine macromolecules, in which both carboxamide and secondary or tertiary amino groups appear in ordered fashion as main-chain constituents, biodegradable, biocompatible (*i.e.* non-toxic and non-immunogenic), and, if properly designed, dissolve readily in aqueous medium. Poly(amidoamine) type macromolecules have been used extensively as drug carriers [31,32]. In this study, we describe the preparation of poly(amidoamine) co-conjugates containing ferrocene analogue and bisphosphonate, and the kinetic drug release study of both drugs from the macromolecule.

2 Experimental

2.1 General Procedure

Dialysis processes were performed with the aid of cellulose membranes tubing (Spectrum Industry Inc., Los Angeles, USA) of type Spectra/Por 4 (12 000-14 000 molecular mass cut-off limit) against several batches of distilled water. Freeze-drying operations were performed with the aid of a Virtis Bench Top 3 freeze drier (operating at a temperature of -58^{o}C and a pressure of 20-30 mT). The freeze dried polymers were routinely subjected to a post drying in an Abderhalden woven at 40^{o}C under vacuum ($CaCl_2$ as drying agent). Inherent viscosities, η_{inh}, were determined at 30.0 ± 0.5^{o}C in cannon-Fenske viscosimeter tubes. Distilled water was used as solvent at a concentratgion of 0.2 g/100 mL. The results, average of three runs, are given in units of mL/g. ^{1}H and ^{31}P NMR spectra (400 MHz, Bruker, Germany) were recorded using deuterium Oxide (D_2O) solution with chemical shifts (δ) in ppm, (integration error limits ±10%). All samples were adjusted to pH 10-11 with NaOH in order to eliminate protonation effects.

2.2 Solvent and Reagents

Distilled water was used for all preparative work. All other solvents were laboratory grade, received from commercial sources, and all reagents were used as received (Aldrich Chemie, Fluka AG). These included: ferrocene, N,N'-methylenebis(acrylamide) (MBA), 3,3dimethylpropylamine (DMP), 2-(2-aminoethoxy)ethanol (AEE), ethanolamine (EA), 1,3-

diaminopropane (PDA), phosphorous acid (99%), methanesulfonic acid (99%), phosphorous trichloride, 6-aminocaproic acid.

2.3 Synthesis of 4-ferrocenylbutamidopropylamine (Fc-PDA)

In the first step, ferrocenylbutanoic acid (Fc) was prepared. This ferrocenylation agent was prepared according to the procedure described in literature [33]. The isolated Fc was used to prepare Fc-PDA (Fig. 1b)according to the following procedure:

Fc (2.72 g, 10 mmol) was dissolved in 20 mL of THF. HSU (1.38 g, 12 mmol) was added in small portions while stirring at room temperature (RT), then in ice-bath. N,N'-dicyclohexylcarbodiimide (DCC) (2.48 g, 12 mmol) in THF (5 mL) was added drop-wise over a period 30 min period. Stirring continued in ice-bath for 4 h, then 48 h at RT. The solid was filtered off and washed with THF. The filtrate and washings were combined and added drop-wise to a stirred solution of PDA (2.99 g, 40 mmol) in THF (20 mL) at ice-bath temperature. Stirring continued at ice-bath temperature for 24 h and for another 6 h at RT. The solid was removed and washed with THF. The filtrate and washes were combined and spun to an oily viscous liquid by rotating evaporation (bath temperature 65 ^{o}C). The oily liquid was dissolved in MeOH (4 mL) and acidified to pH 3-4 with conc. HCl, then purified by flash chromatography (silica gel Column) with MeOH as eluent. The solvent was removed by rotating evaporation. The product was viscous at RT and obtained in yield of 1.92 g (58.7 %).

^{1}H NMR : δ/ppm (expected proton counts in parentheses): 1.9-1.7, 4H (4H, CH_2**CH_2**CH_2); 2.4-2.2, 4H (4H, Fc-**CH_2** CH_2, **CH_2**NH_2); 2.8-2.9, 2H (2H, **CH_2**$CONH_2$); 3.25-3.1, 2H (2H, CONH**CH_2**); 4.1-4.25, 9H (9H, **CH**, Ferrocenyl).

2.4 Synthesis of bisphosphonate

BP (Fig. 1a) was prepared according to general method reported by Kieczykowski *et* al [34]. By the reaction of 6-aminocaproic acid with phosphorous acid and phosphorus trichloride in methane sulfonic acid as solvent. This compound was characterized by ^{1}H NMR, ^{31}P NMR and FTIR to confirm its isolation.

^{1}H NMR (D_2O/NaOH, 400 MHz): 1.34-1.26 ppm (m, 2H), 1.51-1.43 ppm (m, 2H), 1.62-1.53 ppm (m, 2H), 1.94-1.82 ppm (m, 2H), 2.61 ppm (t, 7.0 Hz, 2H); ^{31}P (D_2O/NaOH, 400 MHz): 18.98 ppm (s).

2.5 Synthesis of polyamidoamines co-conjugates

The conventional work-up involved the concentration of the reaction solution on the rotary evaporator (bath temperature 50-55 ^{o}C) to approximately 7 mL, followed by precipitation with a mixture of ethanol/hexane, 1:1 by vol. (20 mL), then washing of the precipitate with hot acetone and dissolving the precipitate in distilled H_2O (15 mL), and adjusting to pH 8.0 with concentrated HCl.

*2.5.1 Co-conjugate **1***

To MBA (1.5417 g, 10 mmol) dissolved in boiling H_2O (10 mL), then cooled to room temperature (RT) was added bisphosphonate (0.297 g, 1.0 mmol) in H_2O (1 mL). The mixture was purged and saturated with argon gas. While stirring the mixture was allowed to react for 8h at 55^{o}C. Thereafter ferrocene (0.272 g,1.0 mmol) was added to the reaction mixture. The mixture was resaturated with argon gas and stirring continued for another 12h at 55^{o}C. For the last step, DMP (0.8174 g, 8 mmol) was added to the reaction mixture, then resaturated with argon gas and stirred for another 48h at 55^{o}C. The conventional work-up was performed and the solution was dialyzed in spectra/Por 4 membrane tubing for 2d. The water-soluble conjugate was freeze-dried, then post dried at 40^{o}C under vacuum. Co-conjugate **1** was obtained as yellow water soluble solid in a yield of 1.330 g (46.3 %) and inherent viscosity of 15.6 mL.g^{-1}.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.1-1.0 ppm 2H (2H, **C**$\mathbf{H_2}$ BP), 1.45-1.25 ppm 4H (4H, **C**$\mathbf{H_2}$ BP), 1.75-1.45 ppm 23H (22H, CH_2**C**$\mathbf{H_2}$$CH_2$, **C**$\mathbf{H_2}$ BP, **C**$\mathbf{H_2}$ Fc), 2.7-2.2 ppm 164H (160H, **C**$\mathbf{H_3}$N, **C**$\mathbf{H_2}$N, NHCO**C**$\mathbf{H_2}$, **C**$\mathbf{H_2}$ BP, **C**$\mathbf{H_2}$ Fc), 3.2-3.0 ppm 2H (2H, **C**$\mathbf{H_2}$ Fc), 4.1-4.0 ppm 4.6H (4.5H, Ferrocenyl), 4.4-3.35 ppm 20H (20H, NH**C**$\mathbf{H_2}$NH); ^{31}P (D_2O): 18.74 ppm (s).

*2.5.2 Co-conjugate **2***

By the procedure leading to co-conjugate **1**, co-conjugate **2** was prepared from MBA (1.5417 g, 10 mmol), bisphosphonate (0.2971 g, 1.0 mmol), ferrocene (0.272 g, 1.0 mmol) and AEE (0.8410 g, 8 mmol). The solution was treated and worked up as before, giving 1.211 g (41.8 %) of **2** as yellow water soluble solid with inherent viscosity of 12.8 mL.g^{-1}.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.15-1.05 ppm 2H (2H, **C**$\mathbf{H_2}$ BP), 1.45-1.25 ppm 4H (4H, **C**$\mathbf{H_2}$ BP), 1.75-1.6 ppm 4H (4H, **C**$\mathbf{H_2}$ BP, **C**$\mathbf{H_2}$ Fc), 2.35-2.2 ppm 46H (44H, $COCH_2$**C**$\mathbf{H_2}$N, **C**$\mathbf{H_2}$ Fc), 2.7-2.5 ppm 61H (62H, N**C**$\mathbf{H_2}$$CH_2O$, NHCO**C**$\mathbf{H_2}$, **C**$\mathbf{H_2}$

BP), 3.1-3.0 ppm 2H (**CH$_2$** Fc), 3.5-3.4 ppm 34.5H (36H, **CH$_2$**O**CH$_2$**), 3.6-3.5 ppm 17H (18H, **CH$_2$**OH), 3.9-4.0 ppm 4.5H (4.5H, Ferrocenyl H), 4.4 ppm 20H (20H, NH**CH$_2$**NH); ^{31}P (D_2O): 18.94 ppm (s).

*2.5.3 Co-conjugate **3***

An experiment conducted as in the forgoing, MBA (1.5417 g, 10 mmol), BP (0.2971 g, 1.0 mmol), ferrocene (0.272 g, 1.0 mmol) and EA (0.4886 g, 8 mmol) were used. Co-conjugate **3** was obtained as yellow water soluble solid in yield of 1.227 g (48.6 %) with inherent viscosity of 13.4 mL.g^{-1}.

^{1}H NMR (D_2O), δ/ppm (expected proton counts in parentheses): 1.15-1.05 ppm 2H (2H, **CH$_2$** BP), 1.45-1.30 ppm 4H (4H, **CH$_2$** BP), 1.75-1.65 ppm 2H (2H, **CH$_2$** BP), 2.35-2.15 ppm 44H (42H, COCH$_2$**CH$_2$**N, **CH$_2$** Fc), 2.5-2.4 ppm 21H (20H, N**CH$_2$**CH$_2$OH), 2.7-2.55 ppm 43H (42H, NHCO**CH$_2$**, **CH$_2$**), 3.1-3.0 ppm 2H (2H, **CH$_2$** Fc), 3.5-3.45 ppm 17H (17H, **CH$_2$**OH), 4.0-3.95 ppm 4.5H (4.5H, Ferrocenyl H), 4.3 ppm 20H (20H, NH**CH$_2$**NH); ^{31}P (D_2O/NaOH, 400 MHz): 18.91 ppm (s).

2.6 In vitro release studies

In vitro release mechanism of ferrocene analogue and (6-Amino-1-hydroxyhexylidene)bisphosphonic acid from polyamidoamine, exemplified by co-conjugate **1**, was studied at 37 ^{o}C. 20 mg of **1** was dissolved in 10 mL of different selected buffer solutions with pH values of 1.2, 5.5 and 7.4 and placed in 12000 – 14000 molecular mass cut off tubing. The tubing were placed in corresponding buffers (50 mL). The study was performed using a shaking water bath WBM-SPL25 supplied by Labcon, South Africa, at 100 RPM. 4 mL of sample was collected and replaced with a corresponding volume of the corresponding buffer solution in intervals of time. Five standard solutions with concentration raging between 18.6-371 mM were prepared by dilution of corresponding stock solution of ferrocenylbutanoic acid in distilled water. The absorbance of these standard solutions was measured at 400 nm and the calibration curve generated. Thereafter, using the same wavelength the absorbance of the ferrocene complex released was measured and the corresponding amount determined. In the same manner six standard solutions of BP with concentration ranging between 1.25-49.94 mM were prepared by dilution of the corresponding stock in perchloric acid solution (2 M). The standard solutions were mixed with a solution of Fe(III) chloride hexahydrate (4.99 mM) in perchloric acid (2 M) and the absorbance of the complex was measured at 300 nm immediately after mixing for the calibration curve. The amount of BP released was determined *via* complex

formation with Fe(III) in perchloric acid. The absorbance of the complex formed was measured at 300 nm and converted to concentration.

3 Results and Discussion

The synthetic strategy chosen for the preparation of the target co-conjugates **1**, **2** and **3** involved the Michael addition copolymerization of methylenebisacrylamide (MBA) with primary amines (Scheme 1). The diamine comonomers were 3,3dimethylpropylamine (DMP), 2-(2-aminoethoxy)ethanol (AEE), ethanolamine (EA), BP and Fc-PDA. The first three amines were used as a means of introducing different hydrosolubilizing moieties; and the last two amines were used as co-drugs. Both ferrocene derivative and bisphosphonate contained a terminal amino group. Under the experimental conditions chosen, each monomer bearing a terminal primary amino group was expected to react difunctionally in the polyaddition reaction. In the first addition step, the primary amino group reacted with the vinyl group to generate a secondary amino group. Though in principle the secondary amino groups are less reactive than the primary amino groups, because of the availability of vinyl groups, the secondary amino groups were susceptible to further the polyaddition. Typically the reactions were performed in water by allowing MBA and bisphosphonate to copolymerize first, then with Fc-PDA and at the end the solubilizing moiety was added. The advantage of the route chosen was that the two drugs were incorporated directly into the polymer during polymerization. In this case good incorporation are recorded. In another study [35] using MBA and aspartic acid for platinum chelation, it was notice that during drug coupling some of the ligands were not anchored to the drug and the incorporation was always below 100 %. Sometimes there were a need of retreatment of the conjugate with platinating agent. Similar findings were also reported by Komane *et al* [36]. The macromolecular co-conjugates were fractionated and purified by dialysis in membrane possessing molecular mass cut off limit of 12000-14000. The yields were in the range of 41-48 % and the inherent viscosities were in the range of 12-16 $mL.g^{-1}$ for an average of three measurements. The drug contents in the co-conjugates were determined by both ^{1}H NMR and UV-vis spectroscopy and were found to be in the range of 4-5 % for ferrocene and 10-12 % for bisphosphonate which represent 100 % incorporation for both drugs based on the structures in scheme 1. Both methods of analysis gave almost similar results with a small difference not exceeding 2 %. The ^{1}H NMR spectra of co-conjugates **1**, **2** and **3** showed the proton signals of the MBA methylene bridge (4.5-4.4 ppm), protons of ferrocenyl (4.1-4.0 ppm), protons of the bisphosphonate (1.45-1.05 ppm) and the remaining proton groups (3.6-2.0 ppm) in the expected area ratios. The ^{31}P NMR spectra showed a phosphorus signal (18.98

ppm) as a singlet. From ^{1}H NMR data, the x/y/z ratios of hydrosolubilizing to bisphosphonate to ferrocene groups were deduced and this correlated with the proposed structures. Further corroboration was obtained by using FTIR recorded by using the KBr pellet.

The FTIR spectrum of BP revealed characteristic peaks at wavenumber 802, 1180 and 1140 cm^{-1}, corresponding to P-C, P-OH and P=O stretching respectively and these peaks were also recorded in the spectra of the co-conjugates. Likewise, the FTIR spectrum of Fc-PDA showed characteristic peaks at 1705 cm^{-1} corresponding to C=O band as well as CH bending for ferrocenyl at 810 cm^{-1} which were also revealed in the co-conjugates. The broad band in the region of 3400-3200 cm^{-1} was assigned to OH group, while the presence of amide band was noticeable at 1530 cm^{-1}. A tertiary amine band was noticed at 1400-1340 cm^{-1} as well as the ether (C-O-C) band at 1060 cm^{-1}. These findings confirmed the successful preparation of the co-conjugates.

The kinetic drug release of co-conjugate 1 was performed at pH 1.2, 5.5 and 7.4, simulating gastric pH, cancer cells pH and blood serum pH respectively. Co-conjugate **1** was used as to demonstrate the mechanism of release of two different drugs from the same carrier. The study was performed over a period of 72 h and the percentage drug release was calculated using equation 1:

$$DR = \frac{Mt}{Mo}\ x\ 100 \qquad (1)$$

Where DR is the % of a specific drug released, Mt is the mass of a specific drug in the buffer solution at time t and Mo is the initial mass of specific drug in the co-conjugate. The drug release trend was similar for both ferrocene and bisphosphonate at different pH. After 24 h, around 72 %, 18 % and 2 % of ferrocene were release at pH 1.2, 5.5 and 7.4 respectively, while 63 %, 13 % and 3 % of bisphosphonate were released after the same period. The rate of release was found to be faster at pH 1.2 and slower at pH 7.4 for both drugs, with 100 % drug release at pH 1.2 after 72 h and only 11 % of ferrocene and 14 % of bisphosphonate were released at pH 7.4 after 72 h. Since the release was *via* the amide bond which is sensitive to pH, these observations support the suggestion by Neuse [37], that the polymer conjugate will circulate in blood serum and will only deliver the active compound at the site of action, and the release can be done enzymatically or hydrolytically.

In order to understand and determine the kinetic release mechanism of ferrocene and bisphosphonate from the pro-drug, selected mathematical release model (zero order, first order,

Higuchi and Korsmeyer-Peppas) were used and the release data obtained at different pH were fitted in the selected equations.

Zero order release mechanism is applicable to system in which the rate of drug release is independent of the concentration of the drug within a pharmaceutical dosage and time, and equation 2 is used [38].

$$Q = Q_o + K.t \quad (2)$$

Where Q is the cumulative amount of drug released at time t, Q_o is the initial amount of drug in the solution (most times $Q_o = 0$), K is the zero order release constant and t is the time in hours.

Higuchi release mechanism is applicable to system in which the drug release is by diffusion, and equation 4 is used [39].

$$Q = K.\sqrt{t} \quad (4)$$

Where Q is the cumulative amount of drug released at time t, K is the Higuchi released constant and t is the time.

Korsmeyer-Peppas release model is used to describe drug release from polymeric system, and equation 5 is used [40].

$$Q = K.t^n \quad (5)$$

Where Q is the fraction of drug released at time t, K is the release rate constant, n is the release exponent and t is the time. The diffusion exponent (n) is used to determine the release mechanism[40]. In this model, $0.45 \leq n$ corresponds Fickian diffusion mechanism, $0.45 < n < 0.89$ corresponds anomalous or non-Fickian transport and $n = 0.89$ corresponds to case II (relaxational) transport while $n > 0.89$ corresponds to super case II transport [41]. The release exponent n was determined from the plot Log M_t/M versus Log t for the first 60 % drug release.

The analysis of Figs 5-10 shows that, the kinetic release mechanism is highly pH dependent. For both ferrocene and bisphosphonate the release was faster at pH 1.2 and pH 5.5, and slower at pH 7.4. 100 % of both ferrocene and bisphosphonate were released after 72 h at pH 1.2, and 11 % of ferrocene and 14 % of bisphosphonate were released after the same period of time at pH 7.4. For 60 % of drug to be released it needed 18 h at pH 1.2 and 50 h at pH 5.5. The mechanism of release of ferrocene from the co-conjugate followed Korsmeyer-Peppas model at pH 1.2, with a goodness of fit of $R^2 = 0.9979$ and a diffusion exponent of $n = 0.46$. In light

of these findings, the release of ferrocene corresponded to anomalous or non-Fickian transport. At pH 5.5 and 7.4 the best fit corresponded to a zero order release mechanism with R^2 of 0.9583 and 0.9026, and K of 0.0184 and 0.0026 respectively. With regards to bisphosphonate, the release was a Fikian diffusion mechanism, with n = 0.45 and R^2 = 0.9875 at pH 1.2 and the other model did not indicate a good fit. The Higuchi release mechanism which is a diffusion drug release was noticed at pH 5.5 with K = 0.91 and R^2 = 0.9886. At pH 7.4 only 13.4 % of drug was released after 72 h. R^2 was 0.9195 for zero order release mechanism and the other investigated models did not indicate good linearity (see Table 1).

4 Conclusion

Macromolecular co-conjugates containing bisphosphonate and ferrocene complex were synthesized. The mass percentages incorporation of ferrocene complex and bisphosphonate were found to be in the range of 4-5 % and 15-18 % respectively. Both ferrocene complex and bisphosphonate were released during the study. The release of both drugs was found to be faster pH 1.2, moderate at pH 5.5, and slower at pH 7.4. The best model describing the kinetic release of ferrocene complex were Korsmeyer-Peppas model indicating an anomalous or non-Fickian transport at pH 1.2. At pH 5.5 and 7.4, the results suggested a zero order release mechanism. With bisphosphonate, Korsmeyer-Peppas model was also noticed at pH 1.2, but a Fikian diffusion mechanism. At pH 5.5 it was the Higuchi release model indicating a diffusion mechanism and a zero order release mechanism was noticed at pH 7.4.

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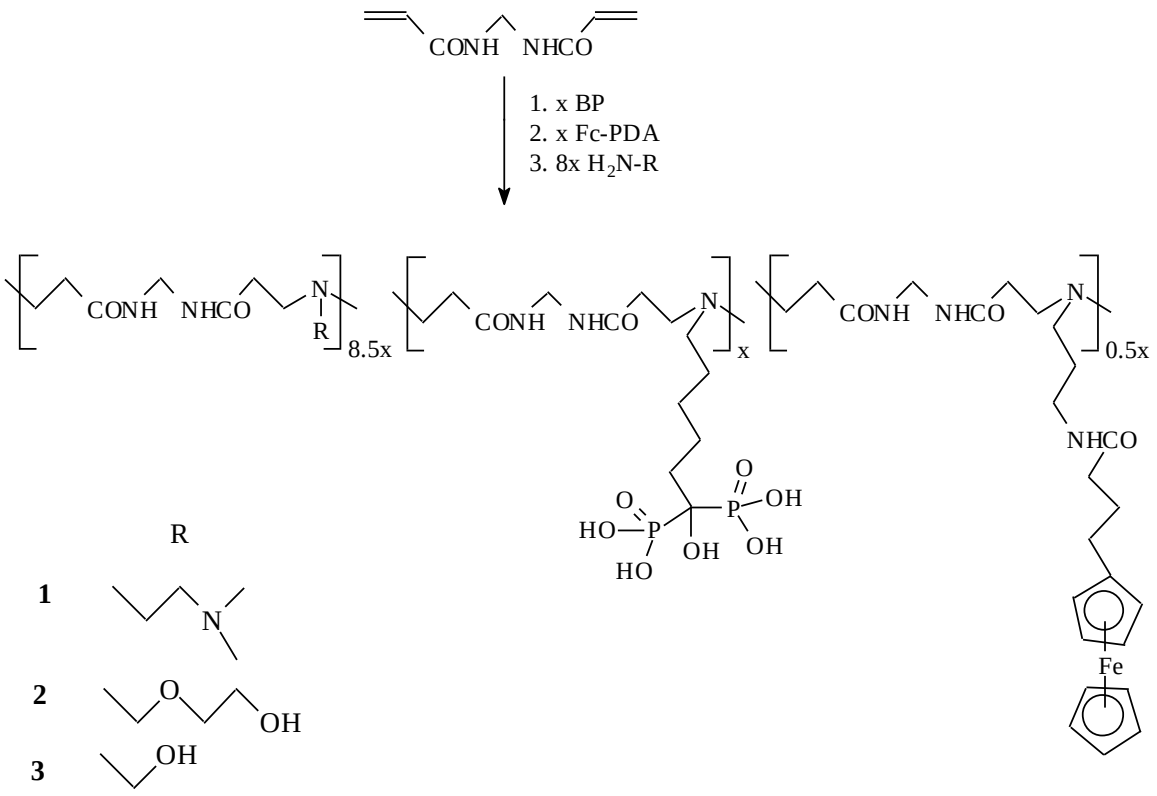
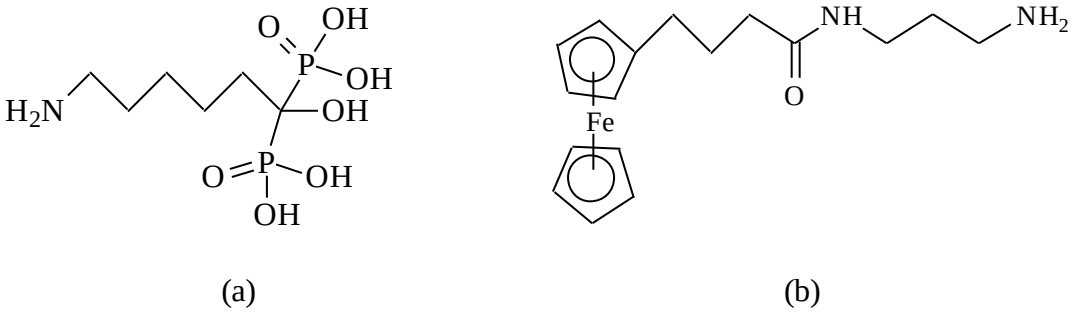


Scheme 1: Synthesis of co-conjugates **1**, **2** and **3**



(a) (b)

Fig. 1: (a) Structure of Bisphosphonate (6-amino-1-hydroxyhexylidene-1,1-bisphosphonate) (BP); (b) Structure of 4-ferrocenylbutamidopropylamine (Fc-PDA)

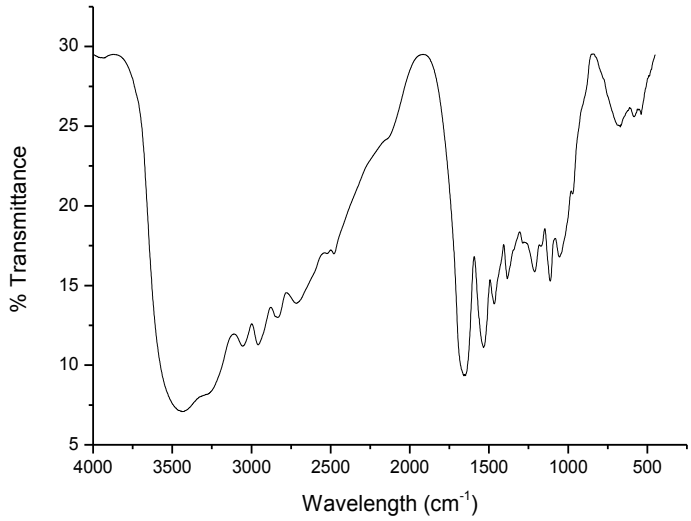


Fig. 2 FTIR spectrum of co-conjugate **1**

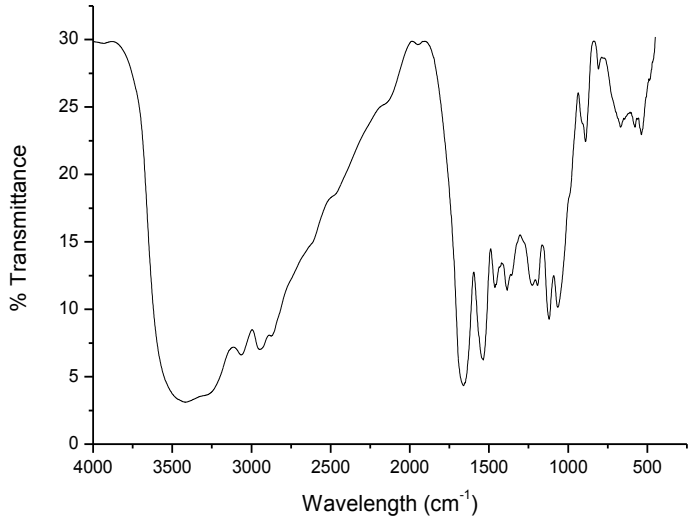


Fig. 3 FTIR spectrum of co-conjugate **2**

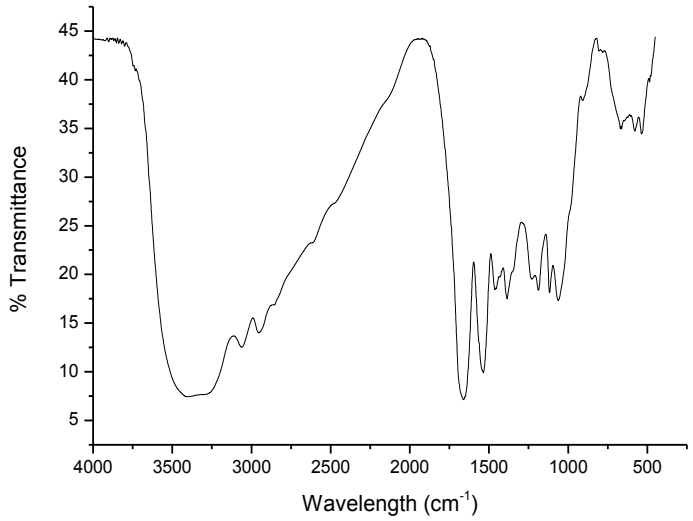


Fig. 4 FTIR spectrum of co-conjugate **3**

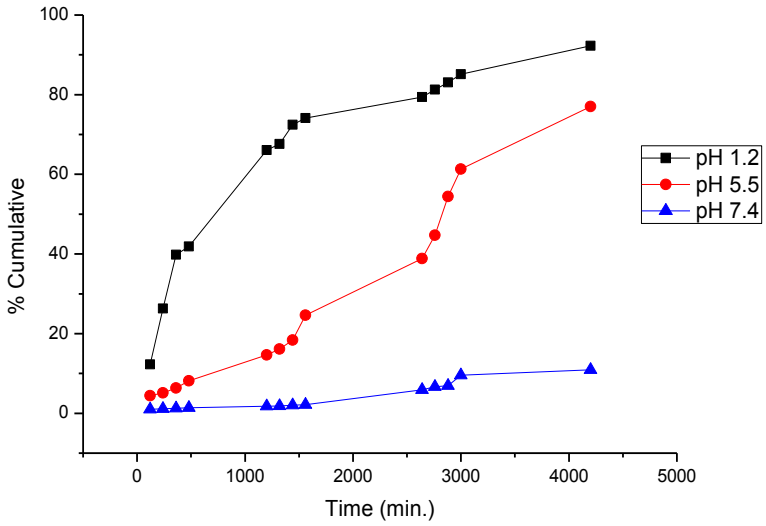


Fig. 5 Zero order cumulative release profile of ferrocene.

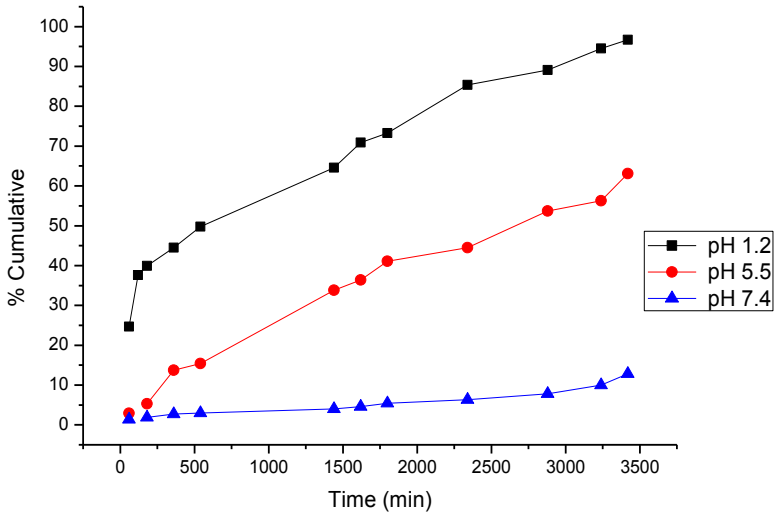


Fig. 6 Zero order cumulative release profile of bisphosphonate

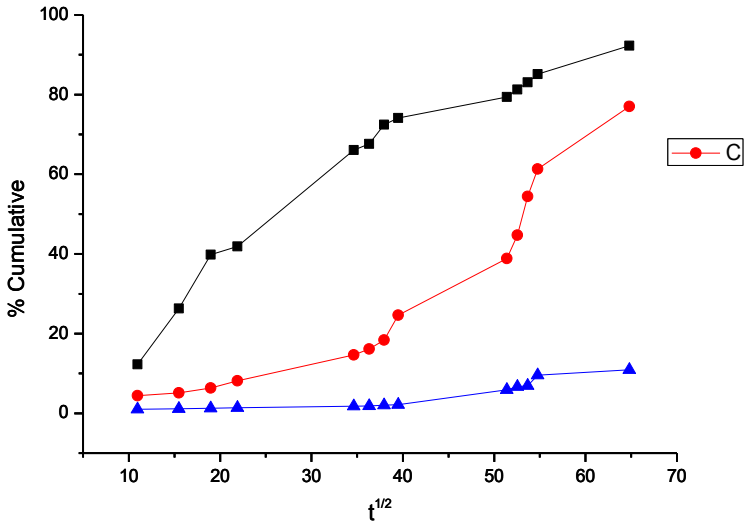


Fig. 7 Higuchi release profile of ferrocene.

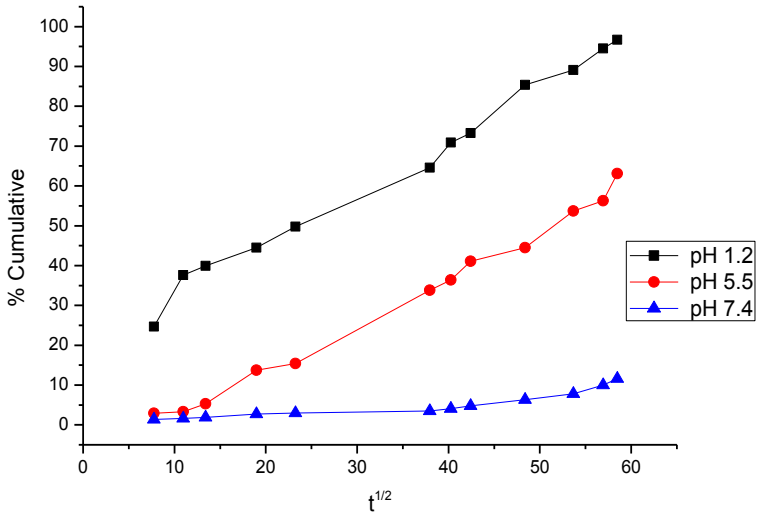


Fig. 8 Higuchi release profile of bisphosphonate

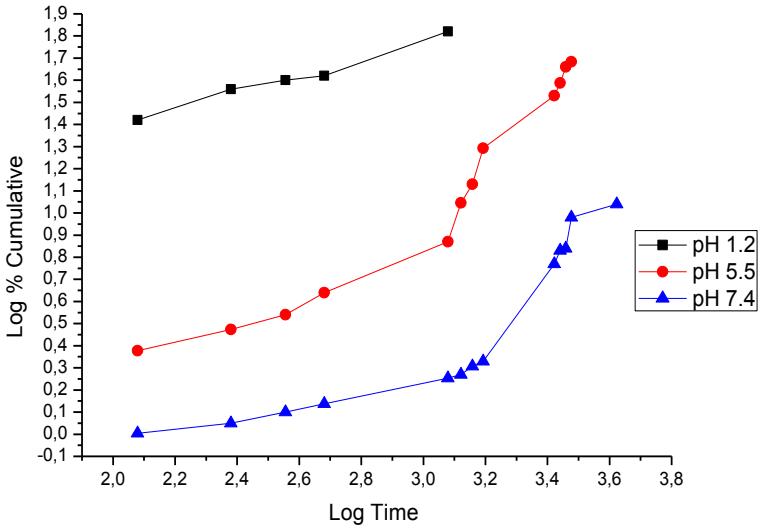


Fig. 9 Korsmeyer-Peppas release profile of ferrocene.

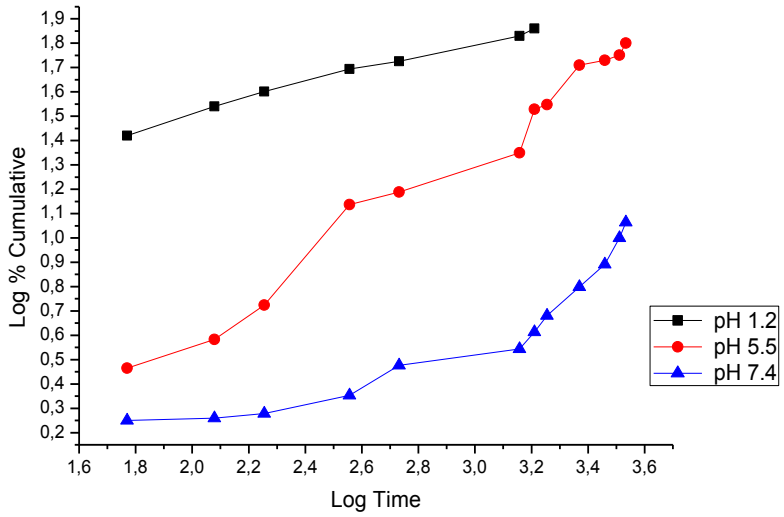


Fig. 10 Korsmeyer-Peppas release profile of bisphosphonate.

Table 1 K and n constants, and R^2 for bisphosphonate (BP) and ferrocene complex (Fc) at pH 1.2, 5.5 and 7.4 for different drug release model

		pH 1.2		pH 5.5		pH 7.4	
Drug release Model	Drug	R^2	K or n	R^2	K or n	R^2	K or n
Zero order mechanism	BP	0.9619	0.019	0.9778	0.0168	0.9112	0.0027
	Fe-c	0.7984	0.017	0.9583	0.020	0.9026	0.010
Higuchi mechanism	BP	0.988	1.31	0.9895	0.169	0.819	0.161
	Fe-c	0.9243	1.41	0.8728	1.32	0.7896	0.18
Korsmeyer-Peppas	BP	0.9922	0.45	0.9782	0.44	0.8281	0.44
mechanism	Fe-c	0.9779	0.46	0.9135	0.99	0.7822	0.69