Available online at www.ijddt.com
International Journal of Drug Delivery Technology 2010; 2(2) 35-41

ISSN: 0975 4415

Research Article

Synthesis, Characterization and in-vitro drug release studies of a macromolecular prodrug of Didanosine.

Neeraj Agrawal[*1], M.J.N Chandrasekar[1], U.V.S. Sara[2] , Rohini A[1]

J.S.S College of Pharmacy, Ooty, 643001, Tamil Nadu. (Off campus of JSS University,Mysore) India.
[2]D.J College of Pharmacy, Modinagar, U.P, India.

ABSTRACT
A macromolecular prodrug of didanosine (ddI) for oral administration was synthesized and evaluated for in-vitro drug release profile. Didanosine was first coupled to 2-hydroxy ethyl methacrylate (HEMA) through a succinic spacer to form HEMA-Suc-ddI monomeric conjugate which was subsequently polymerized to yield Poly(HEMA-Suc-ddI) conjugate. The structures of the synthesized compounds were characterized by FT-IR, Mass and ^{1}H-NMR spectroscopy. The prodrug was subjected for in-vitro drug release studies in buffers of pH 1.2 and 7.4 mimicking the upper and lower GIT. The results showed that the drug release from the polymeric backbone takes place in a sustained manner over a period of 24 h and the amount of drug released was comparatively higher at pH 7.4 indicating that the drug release takes place predominantly at the alkaline environment of the lower GIT rather than at the acidic environment of the upper GIT. This pH dependent sustained drug release behavior of the prodrug may be capable of reducing the dose limiting toxicities by maintaining the plasma drug level within the therapeutic range and increasing $t_{1/2}$ of ddI. Moreover, the bioavailability of the drug should be improved as the prodrug releases ddI predominantly in the alkaline environment which will reduce the degradation of ddI in the stomach acid.

Key words: AIDS, Didanosine, Polymeric prodrug, sustained release, succinic spacer.

INTRODUCTION

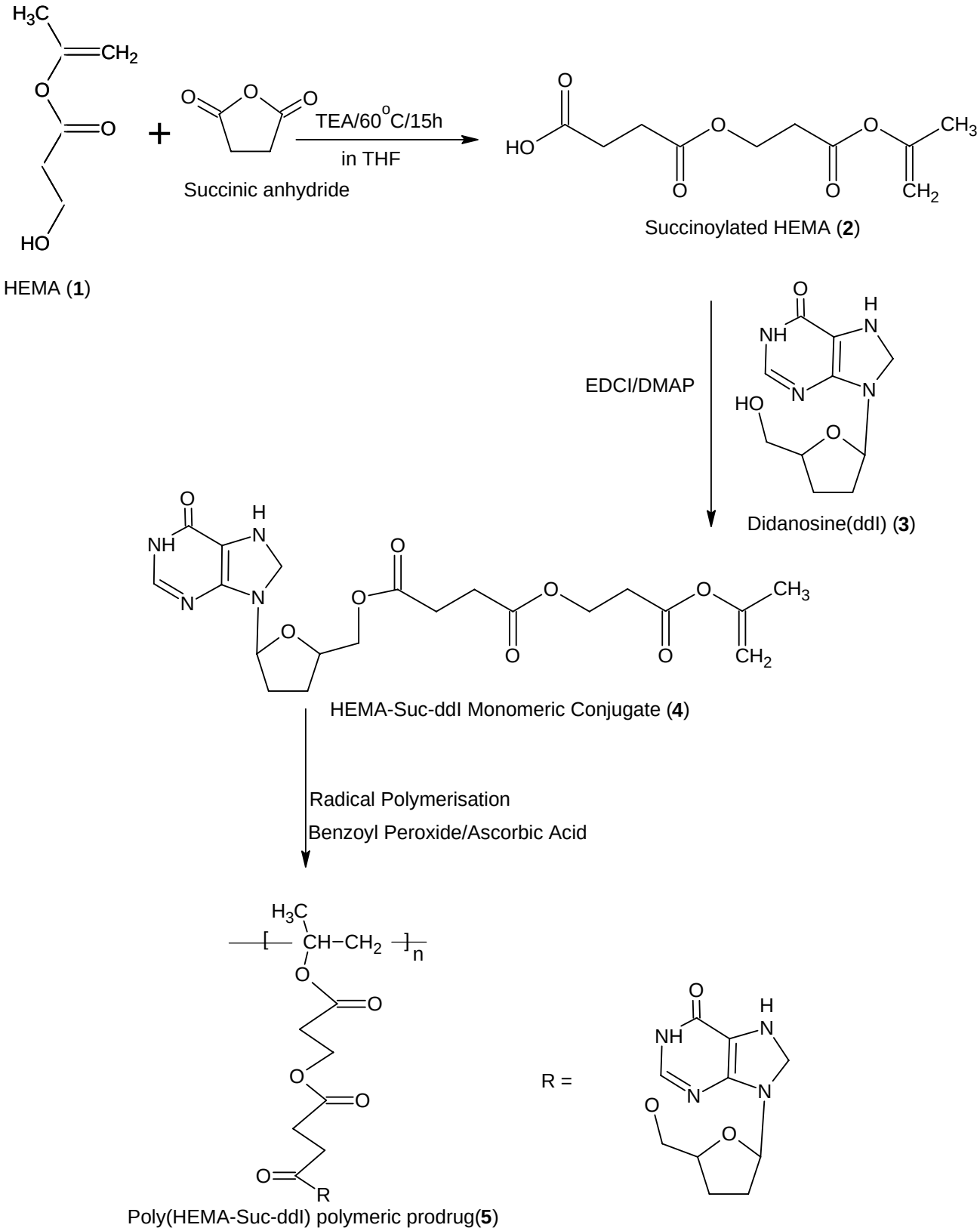
The Acquired Immunodeficiency Syndrome (AIDS) has been a major public health problem, since it was first recognized in 1981 [1]. Didanosine (5'-O-2',3'-dideoxydidanosine, ddI) is a nucleoside analog reverse transcriptase inhibitor (NRTIs) effective against HIV and used in combination with other antiretroviral drug therapy as part of highly active antiretroviral therapy (HAART) [2].

Didanosine is associated with several limitations like short plasma half-life (1-1.5 h), relatively low bioavailability (42 %) and severe dose dependent cellular toxicities. Didanosine is a highly acid labile compound which is quite stable in alkaline environment. It is easily damaged by stomach acid which is the major reason for its low bioavailability. The major clinical toxicities of ddI are peripheral neuropathy, pancreatitis, hepatitis, optic neuritis and alteration of liver functions [3] necessitating dose reduction or discontinuance of treatment. The short $t_{1/2}$ and poor bioavailability necessitates frequent administrations to maintain therapeutic drug doses, thus increasing the incidence of unwanted side effects, which usually compromises the adherence of the patient to the anti-HIV treatment. Thus, there is still a need to improve several properties of this anti-HIV agent, mainly focusing in enhancing its elimination half life, bioavailability and maintaining the plasma concentration of the drug within the therapeutic range to avoid dose limiting toxicities. Literature reveals that many efforts had been made to develop ester prodrugs of didanosine by esterification of hydroxyl group of the drug and evaluated in vitro and in vivo to overcome some of these problems [4, 5, 6, 7], this is the first report on macromolecular prodrug of ddI for sustained and pH dependent drug release behavior.

It was proposed therefore, to synthesize a macromolecular prodrug of ddI for oral administration by coupling the drug to Poly(HEMA) through a succinic spacer by ester linkages which would undergo pH dependent hydrolysis to cleave the parent drug in a sustained manner in the alkaline environment of the lower GI tract rather than the acidic environment of the stomach. This pH dependent and sustained release of ddI may result in increasing the bioavailability, $t_{1/2}$ and maintaining the plasma drug level within the therapeutic range.

In spite of the low bioavailability from the oral route of administration in comparison to i.v route, the oral route is the most convenient for AIDS patients as they require a long term treatment regimen. The spacer was used to link the drug to the polymer and separate the drug molecules from the polymeric backbone for better enzyme accessibility for ester hydrolysis and for controlling the site and rate of release of the active drug from the drug-polymer conjugate [8].

***Corresponding author: Neeraj Agawal**
JSS College of Pharmacy, Ooty, India
E-mail: neeraj_pharm@rediffmail.com
Fax No.: +91-022-26445882



Scheme 1. Synthesis of Macromolecular prodrug of Didanosine.

Poly(HEMA) was used as the drug carrier as it is known to exhibit low interfacial energies with aqueous solutions and has a weak tendency to absorb biological species such as blood cells and proteins. It is a typical hydrogel which swells in water, facilitating the easy penetration of water and release of the drug by hydrolysis. Moreover, it is not absorbed by mucosal surfaces and is excreted unchanged. Ester linkage, which is formed between the drug and the poly(HEMA) has relatively low stability and can hydrolyze easily in the physiological medium [9, 10].

The polymer-drug conjugate obtained was subjected to *in-vitro* hydrolysis studies in order to evaluate its ability

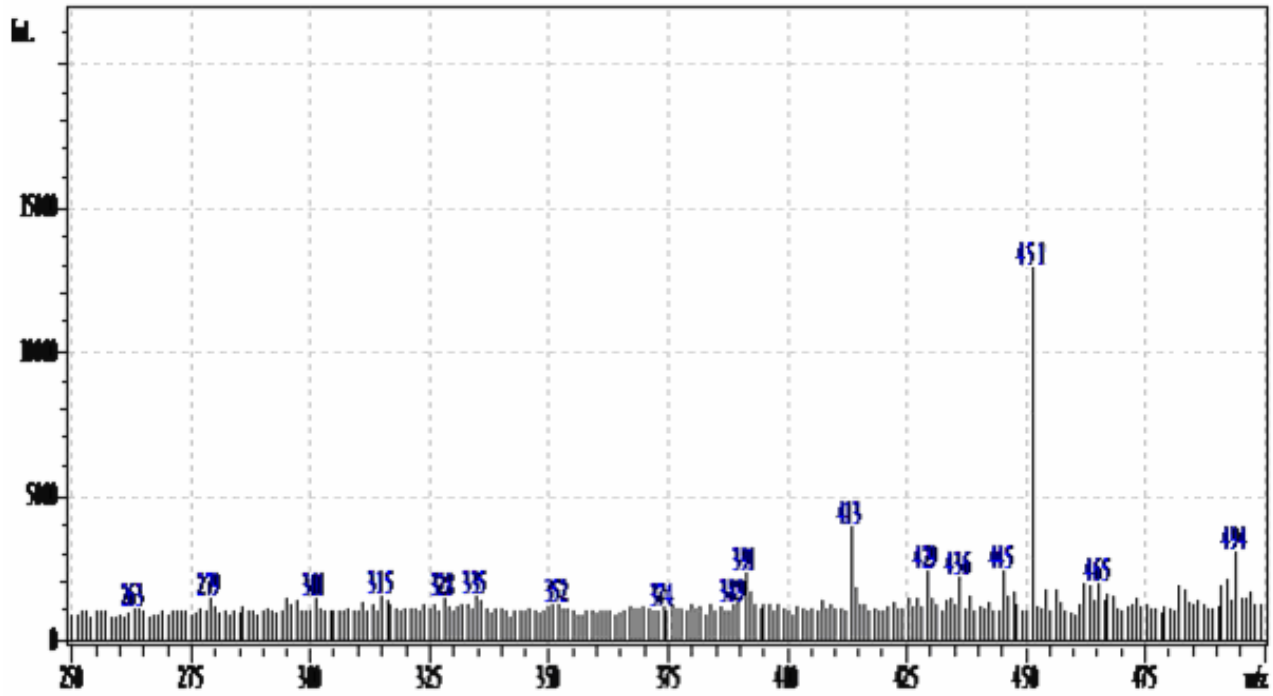


Figure 1. Mass spectrum of HEMA-SUC-DDI monomeric Conjugate

to release drug molecules in buffers of pH 1.2 and pH 7.4 mimicking the pH of upper and lower GIT respectively. The prodrug was proposed to be synthesized by first synthesizing the polymerisable drug derivative (monomer) and polymerizing it by adopting suitable polymerization technique. This procedure is preferred because it should result in a polymeric prodrug with 100 % degree of substitution which is required for higher yields of drug release. This is the first report on macromolecular prodrugs of ddI for oral administration involving the polymerization of the monomeric drug conjugate to get 100 % attachment of drug molecules to linking sites.

MATERIALS AND METHODS

Apparatus and materials

Infrared spectra were obtained using a Shimadzu-FT-6300 FT-IR spectrophotometer in KBr disks. The ^{1}H - NMR spectra were recorded in DMSO using Brucker AV-III 400 MHz instrument. The Mass spectrum was

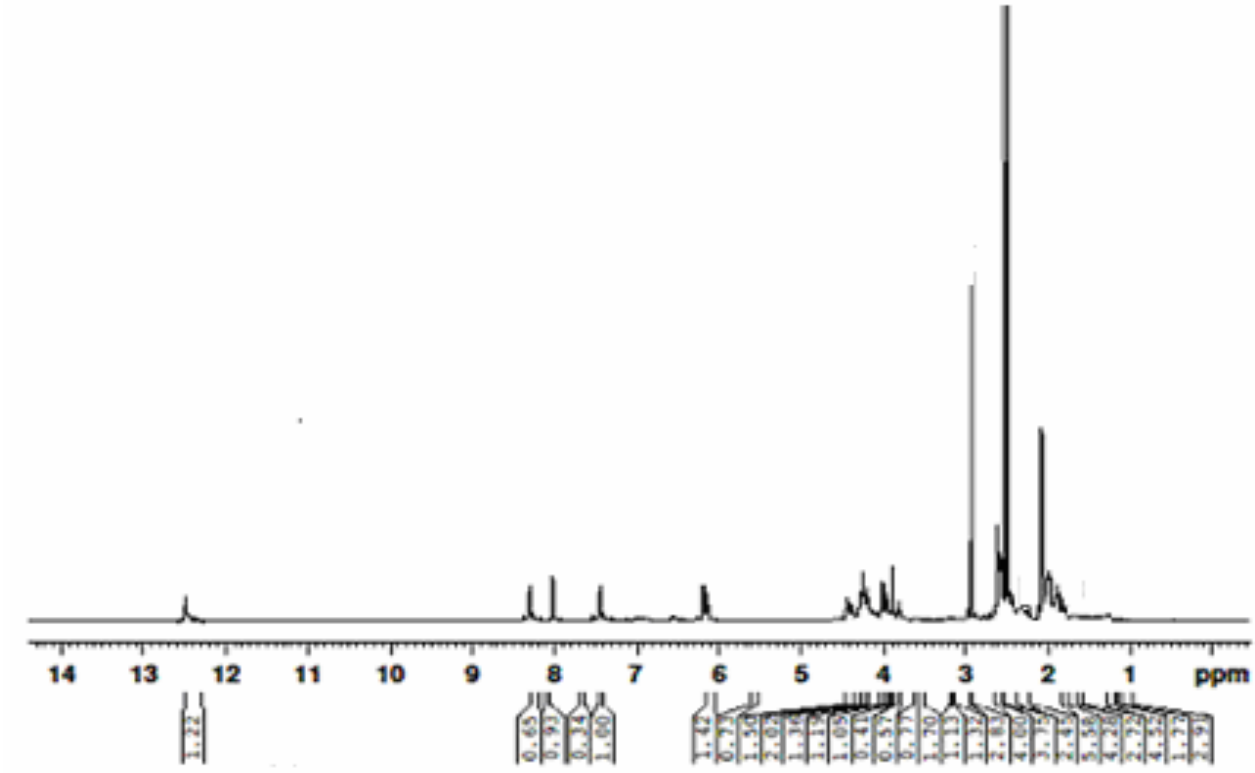


Figure 2. ^{1}H-NMR spectrum of Poly(HEMA-SUC-DDI) Conjugate

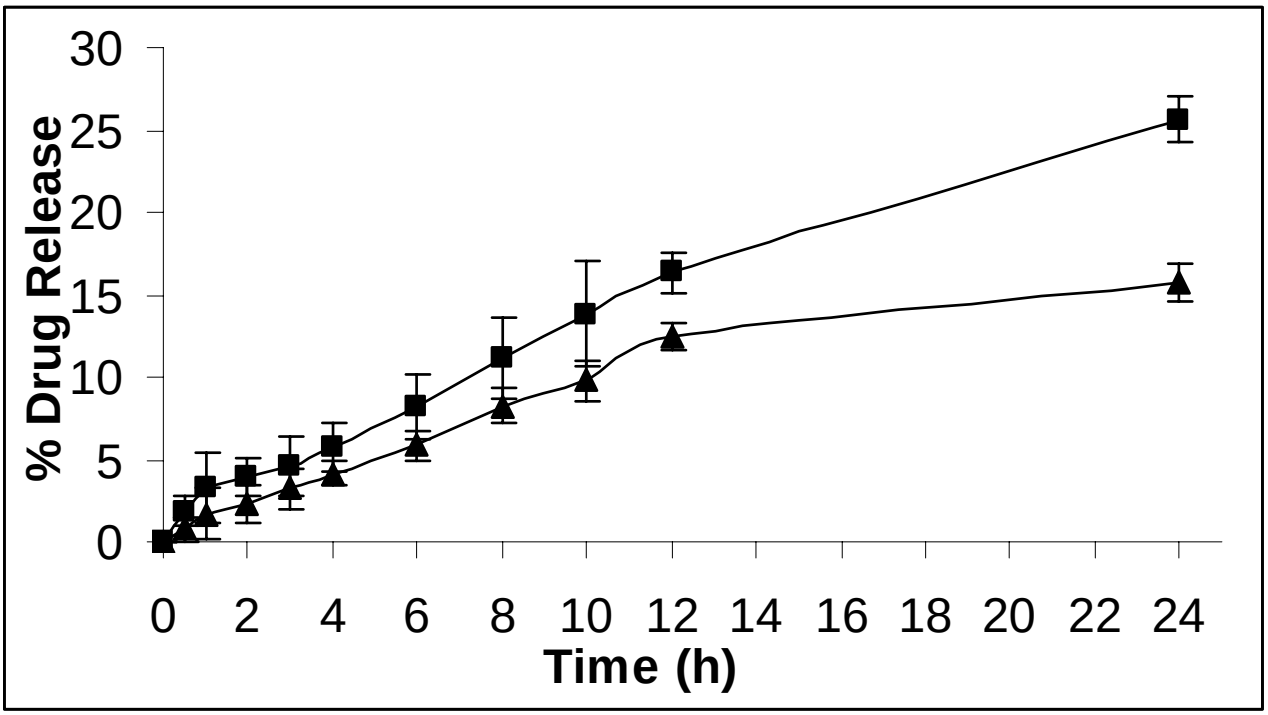


Figure 3. Release of ddI (■) and succinylated ddI (▲) from prodrug in pH 1.2 buffer solution at 37±0.1 ºC. Each value is mean ± S.D, n = 3

recorded on Shimadzu-2010A mass spectrophotometer. HPLC analyses were carried out with a Waters Breeze HPLC, column Princeton SPHER $^{®}C_{18}$ (250 cm x 4.6 mm i.d, 5μ). The absorbance maxima (λmax) were determined on a Shimadzu UV-1700 UV-Visible double beam spectrophotometer using 10 mm matched quartz cells. The purity of the compounds was ascertained by TLC on pre-coated silica gel-60 F_{254} plates (Merck, Mumbai).

2-Hydroxyethyl methacrylate (HEMA), 4-dimethylaminopyridine (DMAP), EDCI (N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride were purchased from Fluka. Benzoyl peroxide, tetrahydrofuran (THF), triethylamine (TEA) and ascorbic acid, were purchased from SD Fine Chemicals Ltd. All other chemicals and solvents were of analytical grade. Didanosine was a gift sample from Hetero Drugs Limited (Hyderabad, India).

Synthesis of Succinoylated HEMA (2):

A solution of 5 mmol of HEMA (**1**), (8 mmol) of succinic anhydride and 0.84 ml of triethylamine (6.04 mmol), in 50 ml of dry THF was refluxed at 60º C for 15 h. The progress of the reaction was followed by TLC (CH_3OH:$CH_3COOC_2H_5$, 7:3, v/v) and after 6 h, an additional 8 mmol of succinic anhydride and 0.4 ml of triethylamine were added. A semisolid residue was separated. The solvent was evaporated in vacuo and the semisolid residue was washed three times with fresh dry THF. The residue was purified by column chromatography using CH_3OH:$CH_3COOC_2H_5$, 7:3, v/v as the mobile phase.

IR (CH_2Cl_2) spectrum showed bands at: 1637 cm^{-1} (-C=C- stretching), 1080 cm^{-1} (-OH bending of –COOH), 1720 cm^{-1} (-C=O stretching of ester group), 1298 cm^{-1} (-COO^{-} Carboxylate anion stretching).

^{1}H-NMR (DMSO): δ 1.82 (s, 3H, CH_3 of HEMA), 5.62 (s, 2H, -C=CH_2), 2.80 (m, 8H, -OCO(CH_2)$_2$OCO-(CH_2)$_2$-), 10.51 (s, 1H, -COOH).

Synthesis of HEMA-Suc-ddI monomeric conjugate (4):

A mixture of ddI (**3**, 10 mmol), DMAP (5 mmol), Succinoylated HEMA (**2**, 10 mmol) in 100 ml DMF was stirred at room temperature. N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride, EDCI (12 mmol) was then added slowly to the mixture. The mixture was stirred for 14 h at room temperature. Then the clear solution was concentrated in vacuo. The pale yellow liquid obtained with a 84% yield was passed through a silica gel column using MeOH : $CH_3COOC_2H_5$ (6:4, v/v) to give the HEMA-Suc-ddI monomeric conjugate.

IR (CH_2Cl_2) spectrum showed bands at: 1099 cm^{-1} (-C-O-C- asymmetric stretching), 1701 cm^{-1} (the C=O stretching of –CO-NH-), 1743 cm^{-1} (-C=O stretching of ester group), 1636 cm^{-1} (-C=C- stretching).

^{1}H-NMR (DMSO): δ 2.0-2.6 (m, 10H, 4H 2',3' and 6H α carbonyl), 2.90 (s, 3H, $C\underline{H}_3$-C=CH_2), 4.21-4.26 (m, 2H-5' and 2H –O$C\underline{H}_2CH_2CO$,), 4.30 (m, 1H, H-4'), 6.22 (t, 1H, H-1'), 6.57 (m, 2H, -C=$C\underline{H}_2$), , 8.02(s, 1H, H-8), 8.28 (s, 1H, H-2), 12.52 (s, 1H, H-1).

Disappearance of proton peak of carboxyl group indicates its esterification.

Mass spectrum (Figure 1): showed M+1 peak at m/z 451

Synthesis of Poly(HEMA-Suc-ddI) polymeric conjugate (5):

The monomeric drug derivative (**4**, 5 mmol) was dissolved in DMSO (100 ml). Benzoyl peroxide (0.8257 mmol) and ascorbic acid (0.8517 mmol) were added and the contents were refluxed at 70^{0}C for 6 h with continuous stirring. The reaction mixture was then

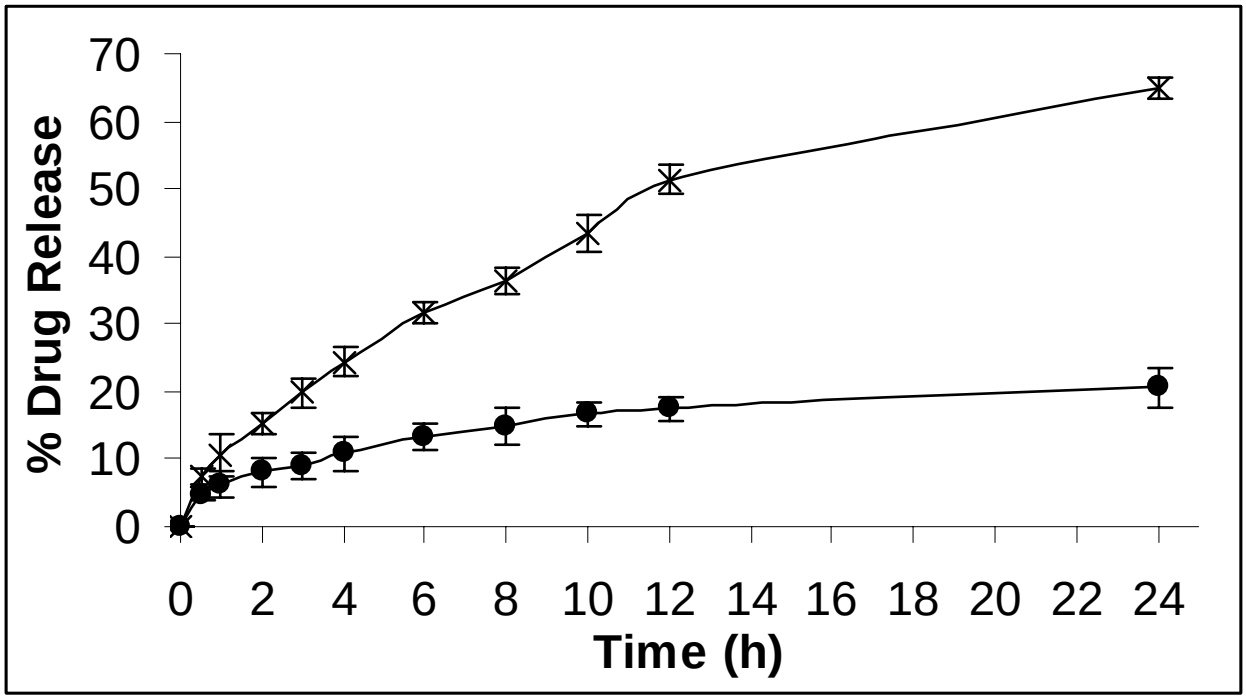


Figure 4. Release of ddI (×) and succinylated ddI (●) from prodrug in pH 7.4 buffer solution at 37±0.1 ºC. Each value is mean ± S.D, n = 3

cooled and poured into distilled water. A brown precipitate was formed which was filtered and dried to get the polymeric prodrug. It was purified by dissolving it in 20 ml acetone, reprecipitated from distilled water and dried at reduced pressure to a constant weight. The prodrug obtained with a yield of 78 % was further purified by column chromatography using benzene : methanol (9:1, v/v) as the solvent.

IR (KBr): 1101 cm^{-1} (-C-O-C- asymmetric stretching), 1706 cm^{-1} (the C=O stretching of –CO-NH-), 1737 cm^{-1} (-C=O stretching of ester group), 1450 (CH_3 bending) 1636 cm^{-1} (-C=C- stretching). Disappearance of -C=C- peak at 1636 indicates polymerization of HEMA.

^{1}H-NMR (DMSO) (Figure 2): δ 1.31 (m, 4H, -($C\underline{H}_2$-$C\underline{H}_2$)$_n$-), 1.9-2.6 (m, 10H, 4H 2',3' and 6H α carbonyl), 2.93 (s, 3H, $C\underline{H}_3$ of HEMA), 4.20-4.28 (m, 2H-5' and 2H –$OC\underline{H}_2CH_2CO$,), 4.36 (m, 1H, H-4'), 6.19 (t, 1H, H-1'), 8.06(s, 1H, H-8), 8.31 (s, 1H, H-2), 12.52 (s, 1H, H-1).

Disappearance of protons peak of –$C=C\underline{H}_2$- and appearance of peak of -($C\underline{H}_2$-$C\underline{H}_2$)$_n$ at1.31 δ indicates polymerization of HEMA.

Estimation of drug content

The amount of ddI attached to the polymer backbone was estimated by allowing 100 mg of the prodrug to get hydrolyze completely by keeping it in 1 N NaOH solution for 24 h. The solution was filtered and the filtrate was analysed for its spectrum by using UV-visible spectrophotometer. Then it was subjected for HPLC analysis to determine the amount of ddI attached to the polymer backbone. The experiment was repeated in triplicate.

In-vitro drug release studies.

The *in-vitro* drug release study of the polymeric pro-drug was carried out using USP dissolution apparatus (Type-2, Paddle assembly) (USP, 2005). 100 mg of the polymeric prodrug was placed separately in dissolution test apparatus containing 900 ml of dissolution media of pH 1.2 (HCl buffer) and pH 7.4 (Phosphate buffer) and stirred at 100 rpm at 37± 0.1° C. The buffer solutions were prepared as per mentioned procedure in I.P, 1996. An aliquot of 5 ml of the samples were withdrawn at suitable time intervals and replaced with equal volume of fresh dissolution medium. The samples withdrawn at each time interval were filtered and the filtrates were directly analysed by HPLC monitoring the released amount of didanosine (**3**) and 5' -O-succinyldidanosine (**6**). Each experiment was repeated in triplicate.

HPLC Analysis:

A Waters Breeze system was used for the analysis with Princeton SPHER C_{18}(250 cm x 4.6 mm i.d, 5μ) as stationary phase. A degassed mobile phase (CH_3OH : 0.2 M ammonium acetate buffer pH 7 (20:80, v/v %) at a constant flow rate of 1 ml/min at room temperature was employed. The detection wavelength of 249 nm and Stavudine as an internal standard was used.

Two different retention times were found for ddI and succinoylated ddI. Quantification of ddI and succinoylated ddI were done by using calibration curves obtained by plotting peak area ratios of ddI/stavudine or succinoylated ddI/stavudine and concentration of the standard solution.

RESULTS AND DISCUSSION

Preparation of Polymeric prodrug of Didanosine

A Poly(HEMA-Suc-ddI) conjugate was synthesized (Scheme 1) in order to study the role of Poly(HEMA) in drug delivery. A succinic spacer was used to link ddI to Poly(HEMA) through ester linkages. The hydroxylic groups of HEMA were activated to carboxylic groups by succinic anhydride [11] to get succinoylated HEMA. TLC showed only one spot and no succinic anhydride.

The product titrated with 0.01N NaOH yielded 100% of free carboxylic groups. The attachment of ddI to succinoylated HEMA was performed by means of EDCI and DMAP as coupling agents to get monomeric drug conjugate which was polymerized by using free radicals that are liberated during a redox reaction between a polymerization accelerator (benzoyl peroxide) and a polymerization initiator (ascorbic acid). The free radicals induce the aperture of the double bond of a HEMA molecule and a radical is transmitted to this unstable monomeric molecule which can in turn react with a new monomer molecule [12].

The purity of the synthesized compounds was verified by HPLC analysis. The analytical and spectral data were in agreement with the attributed structure.

Drug content

The spectrum of the solution obtained after keeping the prodrug for complete hydrolysis in 1N NaOH for 24 h, showed two λ_{max} at 248.8 nm and 210 nm representing the λ_{max} of ddI and succinic acid respectively. This showed that the polymer backbone is insoluble in aqueous solution but releases the free drug and the succinic spacer on hydrolysis. There are three ester groups present in the prodrug. The ester group present in the Poly(HEMA) does not undergo hydrolysis as Poly(HEMA) is known to exhibit low interfacial energies with aqueous solutions. The other two ester groups, one linking Poly(HEMA) and spacer and the other one linking spacer and the drug undergoes hydrolysis releasing 5'-O-succinyldidanosine (**6**) and free ddI (**3**). The released 5' -O-succinyldidanosine (**6**) in turn underwent complete hydrolysis of its ester linkage to release free ddI. The amount of ddI linked to poly(HEMA) as evaluated by HPLC and using standard calibration curve of ddI, was found to be 527.4 mg/g of the macromolecular prodrug showing 100 % attachment of drug molecules to the linking sites.

In vitro release studies

In order to obtain some preliminary information about the potential use of the macromolecular prodrug of ddI as a drug delivery system, the prodrug was subjected for in-vitro hydrolysis at 37 ± 0.1 ºC in buffer solutions at pH 1.2 and 7.4 mimicking the upper and lower gastro intestinal tract respectively. The hydrolysis studies were carried out in USP type-2 dissolution apparatus. Samples were withdrawn at regular time intervals from the dissolution apparatus and the percentage of released ddI and 5'-O-succinyldidanosine were quantified using HPLC. The HPLC conditions were developed and validated. Each experiment was carried out in triplicate and expressed as the mean value ± S.D. The results are reported in figures 3 and 4.

In Fig. 3, it can be seen that at pH 1.2 both ddI (**3**) and 5'-O-succinyldidanosine (**6**) were delivered but the rates of release of **3** and **6** were slow. In the first hour the amounts of **3** and **6** released were only 3.21 % and 1.72 % respectively. The maximum amounts of **3** and **6** released over a period of 24 h were 25.65 % and 15.74 % respectively.

In Fig. 4, the amounts of **3** and **6** released from the polymeric conjugate at pH 7.4 were significantly higher than at pH 1.2. The percentage amounts of **3** and **6** released in the initial hour at pH 7.4 were 10.52 and 6.13 respectively and over a period of 24 h the amounts were found to be 64.74 and 20.62 respectively.

The higher release pattern in case of ddI compared to 5'-O-succinyldidanosine can be attributed to successive hydrolysis of ester linkages of succinoylated didanosine to release free ddI. The hydrolysis of ester linkages to release **3** and **6** took place predominantly at higher pH. This can be well justified on the basis of the well known fact that the hydrolysis pattern of the ester in acid medium is reversible and in alkaline medium it is non-reversible [13].

The free succinate (spacer) released on hydrolysis will be metabolized to fumarate by succinate dehydrogenase in the biological system. In AIDS compromised patients, the free spacer is metabolized by the same process as in healthy humans [14]. Moreover the polymeric backbone Poly(HEMA) after releasing 5'-O-succinyldidanosine and ddI will be excreted as such as it will not be absorbed by the mucosal surfaces. Hence the free spacer and the polymeric drug carrier would not accumulate and would thus be safe for long term use of the Poly(HEMA-Suc-ddI) conjugate.

CONCLUSION

The *in-vitro* drug release studies of the macromolecular prodrug obtained by linking didanosine to Poly(HEMA) through succinic spacer showed a pH dependent drug release behavior. The prodrug exhibited a sustained drug release behavior over a period of 24 hour and the amount of drug released was more at pH 7.4 when compared to the drug release at pH 1.2. In other words, the drug release takes place predominantly at the alkaline environment of the lower GI tract in a site-specific and sustained manner as hypothesized. This drug release behavior of the prodrug reveals its potential to overcome the limitations of poor bioavailability, short $t_{1/2}$ and dose limiting toxicities associated with didanosine and on proper dose adjustment the prodrug can be a good candidate for AIDS compromised patients.

ACKNOWLEDGEMENT

The authors acknowledge the facilities provided at J.S.S College of Pharmacy, Ooty in carrying out the work. The authors would like to thank Hetero Labs, Hyderabad for providing gift sample of the drug.

REFERENCES

1. Suwanpidokkul N., Thongnopnua, P. and Umprayn K. 2004. Transdermal delivery of zidovudine (AZT): the effects of vehicles, enhancers, and polymer membranes on permeation across cadaver pig skin. AAPS PharmSciTech. 5(3): 1-11.
2. http://en.wikipedia.org/wiki/Didanosine
3. Brittain H. G. Analytical profiles of drug substances and excipients. Vol. 22, Academic press, Elsevier, 219.
4. Manouilov K. K., Xu Z. S., Boudinot F. D., Schinazi R. F. and Chu C. K. 1997. Lymphatic targeting of anti-HIV nucleosides: distribution of 2',3'-dideoxyinosine after intravenous and oral administration of dipalmitoylphosphatidyl prodrug in mice. Antiviral research. 34(3): 91-99.

5. Høyem S., Bruheim S., Gunhild M., Standal M., Olberg D. E., Brudeli B., Åsberg A., Klaveness J and Rongved P. 2009. Didanosine ester prodrugs: Synthesis, albumin binding properties and pharmacokinetic studies in rats. European Journal of Medicinal Chemistry. 44(10): 3874-3879.
6. Bourgeois J., Pierson L. A., Nicolas J., Lalanne M., Couvreur P. and Andrieux K. 2009. Application of thermal analysis to the study of lipidic prodrug incorporation into nanocarriers. Journal of Thermal Analysis and Calorimetry. 98(1): 65-71.
7. Sriram D., Yogeeswari P., Nagell R. B. and Kurre P. N. 2007. Synthesis and *in vitro* anti-HIV activities of didanosine prodrugs. Journal of Enzyme Inhibition and Medicinal Chemistry. 22(1): 51-55.
8. Schacht E., Soyez H. and Vanderkerken S. 1996. The crucial role of spacer groups in macromolecular prodrug design. Advanced Drug Delivery Reviews. 21: 81-106.
9. Chappard D., Filmon R., Grizon F. and Basle M. F. 2002. Effects of negatively charged groups (carboxymethyl) on the calcification of poly(2-hydroxyethyl methacrylate). Bio materials, 23(14): 3053-3059.
10. Montheard J. P., Chatzopoulos M. and Chappard D. 1992. 2-hydroxyethyl methacrylate (HEMA) ; chemical properties and applications in biomedical fields. J. Macromol. Sci. Macromol. Rev, 32: 16-21.
11. Zalipsky S., Gilon C. and Zilkha A. 1983. Attachment of drugs to polyethylene
12. glycols. Eur. Polym. J. 19: 1177–1183.
13. Mabilleau G., Cincu C., Basle M.F. and Chappard D. 2008. Polymerization of 2- (hydroxyethyl) methacrylate by two different initiator / accelerator systems: a Raman spectroscopic monitoring. J. Raman Spectroscopy. 39: 767–771.
14. Bhosale A.V., Agrawal G. P. and Mishra P. 2004. Hydrolysis Kinetics Studies of Mutual Prodrugs of Ibuprofen. Indian J. Pharm. Sci. 66(5): 626-630.
15. Nimmannit U., Wannachaiyasit S. and Chanvorachote P. 2008. A Novel Anti-HIV Dextrin–Zidovudine Conjugate Improving the Pharmacokinetics of Zidovudine in Rats. AAPS PharmSciTech. 9(3): 840-850.