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RELEASE STUDY OF STAVUDINE LOADED TO CHEMICALLY GRAFTED NOVEL POLYMER CHITOSAN-G-HEMA

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ABSTRACT

Current study involves the in-vitro release study of Stavudine from chemically grafted co-polymer Chitosan-g-HEMA. Chemical grafting of 2-Hydroxyethylemethacrylate onto chitosan was carried out to improve its solubility and relevance. The grafting procedure consists of surface activation followed by free radical graft polymerization of HEMA onto chitosan with benzoyl peroxide as an free radical initiator. Whole reaction was carried out at 70-80°C. To the polymeric chain HEMA was grafted terminally and covalently bonded. The grafting was confirmed by FTIR spectroscopy, XRD and the thermal property was studied by DSC. The drug (Stavudine) loading to the grafted co-polymer was performed and the percentage of drug entrapment was determined. The drug polymer interaction and in-vitro release of drug in phosphate buffer 6.8 and 0.1N HCl from drug loaded polymer was studied.

KEYWORDS: graft co-polymer; chitosan; HEMA; free radical graft polymerization stavudine; swelling; in-vitro drug release.

INTRODUCTION

Chitosan is a biopolymer having immense structural possibilities for chemical and mechanical modifications[1] to generate novel properties, functions and applications particularly in biomedical area.[2] Chitosan which is the result of complete or partial alkaline deacetylation of chitin is a polysaccharide composed[3] by copolymers of glucosamine and N-acetyl glucosamine. Chitin is the second most prolific polysaccharide, found in the exoskeleton of crustaceans, cuticles of insects and the cell wall of fungi, is a linear homopolymer composed[4] of β (1-4) – linked N-acetyl glucosamine.

Chitosan due to its crystalline nature is only soluble[5] in few dilute acid solutions, which restricts its extensive application. Graft copolymerization amplifies the importance and interest in chemical modification of chitosan to improve[6-8] its solubility and widen its relevance, because chitosan have both reactive Hydroxyl and Amino groups that can be grafted. Graft copolymerization of chitosan with synthetic monomers can introduce desire properties and extend the field of potential application[9-13] of them by using various types of side chains, using free radical initiation gas been reported.

Presence of 2-Hydroxyethyle Methacrylate (HEMA) in copolymers improve [14-16] the biocompatibility of the materials.

Use of chitosan - based copolymers and the use of HEMA as a grafting monomer onto a range of polymeric substrates have been reported with increasing success as biomaterials.[17-20]

The present investigation looks forward to prepare a graft copolymer of chitosan to HEMA in order to obtain a biocompatible copolymer for sustain release of drugs (Stavidine). Here we report the graft copolymerization of HEMA onto chitosan induced by Benzoyl Peroxide (BPO) as an initiator by free radical emulsifier polymerization reaction process and to deliver stavudine, which is a synthetic antiretroviral agent (dideoxynucleoside reverse transcriptase inhibitor)[22-23] in a controlled manner through the prepared graft co-polymer, chitosan-g-HEMA.

EXPERIMENT

Materials: Chitosan medium molecular weight ($1.9 \times 10^5 - 3.1 \times 10^5$ Da) was acquired from Mercury Lab. LTD, Baroda-16. The chemical initiator BPO was obtained from S.D. Fine-Chem. Ltd, India. Stavudine was a gift sample from Dr. Reddy's laboratories, India. The other chemicals used were of analytical grade.

Synthesis of graft co-polymer chitosan-g-HEMA: Chitosan solution (2.5%) was made with glacial acetic acid and water in same proportion. Chitosan solution and 2-HEMA in ratio 2:1 was taken in reaction vessel maintained in inert atmospheric condition by passing N_2 gas through it. Then the reaction vessel was subjected to stirring while heating by placing it on a magnetic stirrer with heating facility. When the temperature

inside the reaction vessel reaches 70-80^{o}C, BPO 0.5% solution in benzene was injected slowly inside the reaction vessel in order to initiate the polymerization reaction. The addition was continued till completion of the reaction that is for 1.5h. Subsequently the product was collected washed thrice with double distilled water and finally freeze dried for further usage.

Grafting Parameters

$$\text{Yield of grafting} = \frac{\text{Wt. of graft polymer} - \text{Wt. of chitosan}}{\text{Wt. of chitosan}} \times 100$$

$$\text{Efficiency of grafting (\%)} = \frac{\text{Wt. of graft polymer - Wt. of chitosan}}{\text{Wt of initial monomer}} \times 100$$

INFRARED SPECTROSCOPY

IR spectra of 2-HEMA, chitosan and the obtained copolymeric material were studied by FTIR. IR spectra were recorded in KBr disks on a **FT/IR-4100typeA** series FTIR spectrometer with serial number **B131661016.** All spectra were recorded at ambient temperature at the resolution of **4 cm^{-1}**, apertures of **7.1mm,** scanning speed of **2mm/sec** and 4 times scanning.

XRD ANALYSIS

X-ray diffraction of the copolymeric material was studied by XRD technique. The scanning was done at a speed of 5.0 degree/min, with sampling pitch of 0.020 degree to a range of 10-80 degree.

DSC ANALYSIS

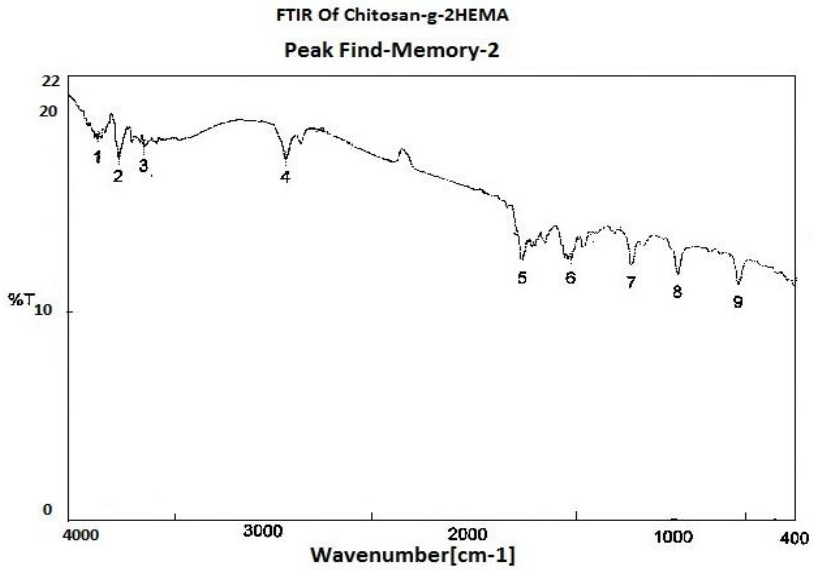
The thermal property of the copolymer was evaluated by DSC. DSC was carried out by Cell constant calibration method by the instrument **DSC Q20 V24.4 Build 116.** The analysis was done over a temperature range of 0 – 300 ˚C.

RESULT AND DISCUSSIONS

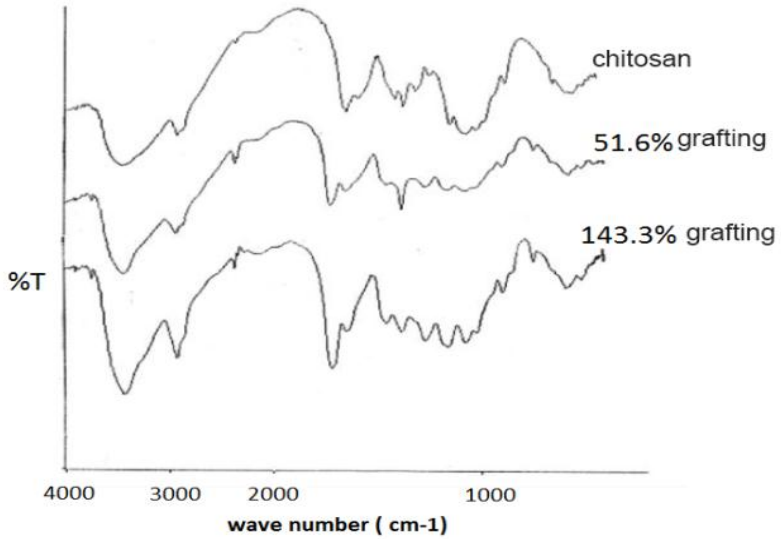
The infrared spectra of chitosan and two copolymers with different grafting yields are shown in fig-2 & an infrared spectrum of one of the above two graft copolymer is shown in fig-1. Near 701cm^{-1} (9 in fig-1), the continuously increasing peak, characteristic of the methacrylic polymers that exhibits here a strong CH_2 rocking peak, can be observed in spectrum of copolymers with increasing grafting. The peaks at 1752cm^{-1}

(5 in fig-1) & 1515cm^{-1} (6 in fig-1) indicate C=O & C=C ring stretching due to resonance of ester group respectively. The other peaks at 1225cm^{1} (7 in fig-1) & 998cm^{-1} (8 in fig-1) indicate -CH_3 stretching & -CH_2 stretching group respectively. The appearance of the new peaks in the spectrum clearly demonstrates that HEMA is successfully grafted onto chitosan.

FTIR Analysis



(Fig 1- FTIR of chitosan-g- 2HEMA)



(Fig 2- FTIR of chitosan and grafted copolymeric materials)

XRD Analysis

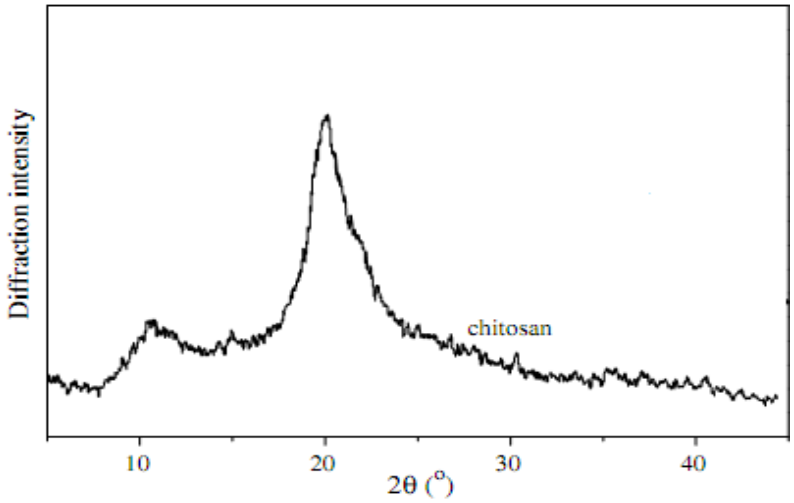


Figure-3: XRD pattern of pure chitosan

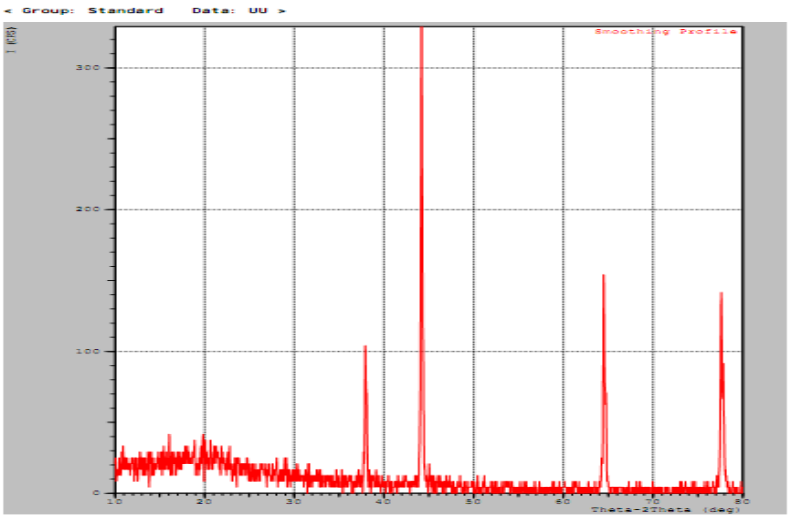


Fig-4: XRD pattern of chitosan-g-2HEMA

From fig-3 at 2Θ = 10.4° and 20.1°, the XRD pattern of chitosan shows two reflections. 2Θ = 10.4 is attributed to the hydrated crystals of low crystallinity and corresponded to the form I [21], while the reflection appeared at 2Θ = 20.1° was identified as representative of the crystallinity of the form II (Wu et al., 2005). For the grafted polymer, the peak at 2Θ = 10.4° and 2Θ = 20.1° disappeared leads to decrease in crystallinity. This suggests that the hydrogen bonding ability of chitosan was reduced after the grafting of 2-HEMA onto chitosan. On comparison with the generally stated formula the figure states that initially the crystallinity decreases but at the end with the 2Θ value (44.16^0) informs the pure crystallinity of the sample.

DSC Analysis

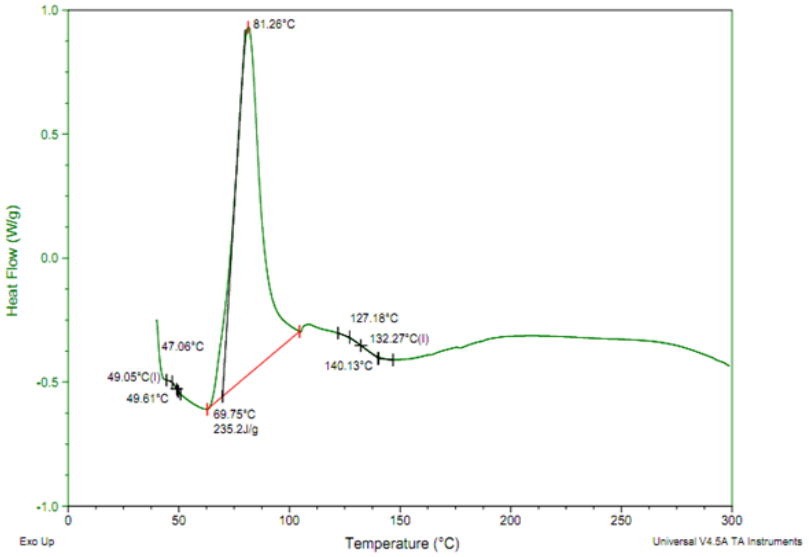


Figure-5: DSC spectrum of chitosan-g-2HEMA

The DSC spectrum of the graft copolymer had carried out at a heating rate of 10^0 C /min. from O^0C - $3OO^0C$ under nitrogen atmosphere. As shown in figure the glass transition temperature of 2-HEMA and chitosan were 49.61 °C and 132.27 °C respectively. The polymer had Tg 81.26^0C & energy consumption for transition was 235.2 Joules/gm.

Swelling behavior

As the prepared polymer is made up of P(chitosan-g-2HEMA) the hydroxyl group bound to their polymer chains from where the H^+ ion comes off and combines with OH^- ion to form water . The charge is compensated by cations that enter the polymer together with another OH^- ion, thus charges neutrality is maintained. The increased cation concentration gives rise an osmotic pressure that causes to swell the polymer. It has been marked that the polymer swells faster in the presence of buffered solution. The swelling behavior of the graft polymer was computed by calculating the percentage of swelling (%S).

$$\%S = \frac{(Mt - Mo)}{Mo} \times 100$$

Where:

Mt: mass of the swollen sample at time 't'

Mo: mass of the dry sample

The water intake or the swelling responses of P(chitosan-g-2HEMA) at intervals is shown in the table in distilled water and buffer solution of PH = 6.8. It has been marked that the polymer swells more in buffer.

Percentage swelling of graft polymer chitosan-g-HEMA(initial weight = 1 gm .) at various time period in distilled water and 6.8 buffer solution.

Time	Distilled water	6.8 Buffer solution
1 Hr.	788	884
2 Hr.	845	907
3 Hr.	1232	942
24 Hr.	3390	3527
48 Hr.	5568	5930
1 Week	6312	6816
1 Month	7864	8545

Drug loading

1000mg stavudine was accurately weighed and dissolved in 10ml of phosphate buffer of pH 6.8. To the above drug solution 1gm of the prepared polymer was added and allowed to swell for 24hours. The swollen drug loaded polymer was placed in a vacuum oven and dried under vacuum at 37°.

Drug entrapment efficiency

$$\% \text{ Drug entrapment efficiency} = \frac{\text{Practical drug content}}{\text{Theoretical drug content}} \times 100$$

The drug content was found to be 67.85% in the formulation. The entrapment efficiency was found to be 84.81%.

Drug polymer interaction study

Drug polymer interactions were studied by FTIR spectroscopy. As shown in following fig. There was no significant difference in the IR-spectra of pure stavudine and stavudine loaded polymer. The characteristic OH stretching, NH stretching of secondary amine, C-H stretching and C=O stretching of pure stavudine was unchanged in case of stavudine loaded polymer. The results suggest drug stability during the encapsulation process.

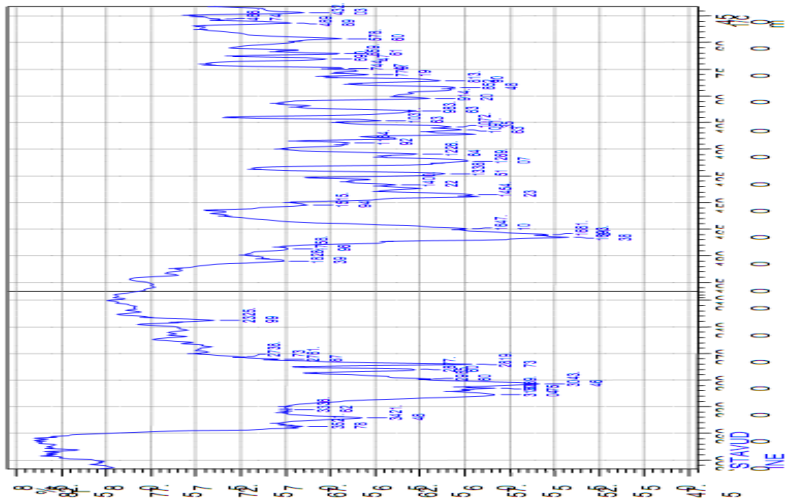


Fig 6: FTIR spectra of pure stavudine

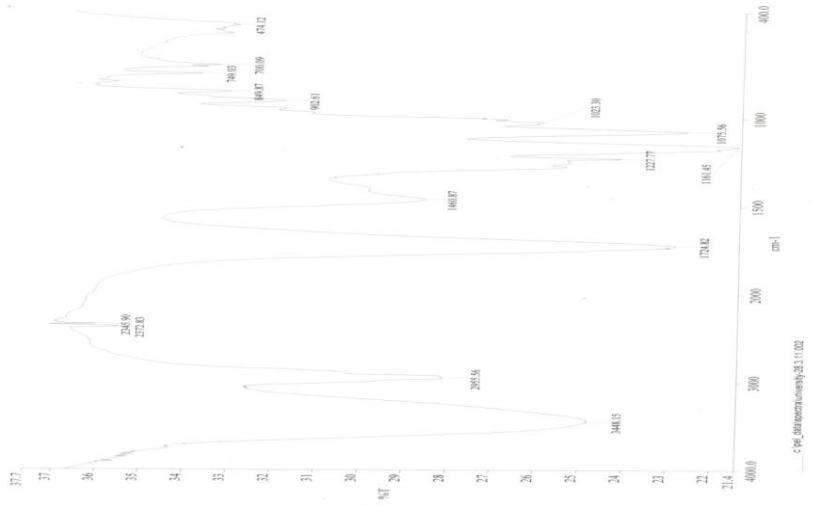


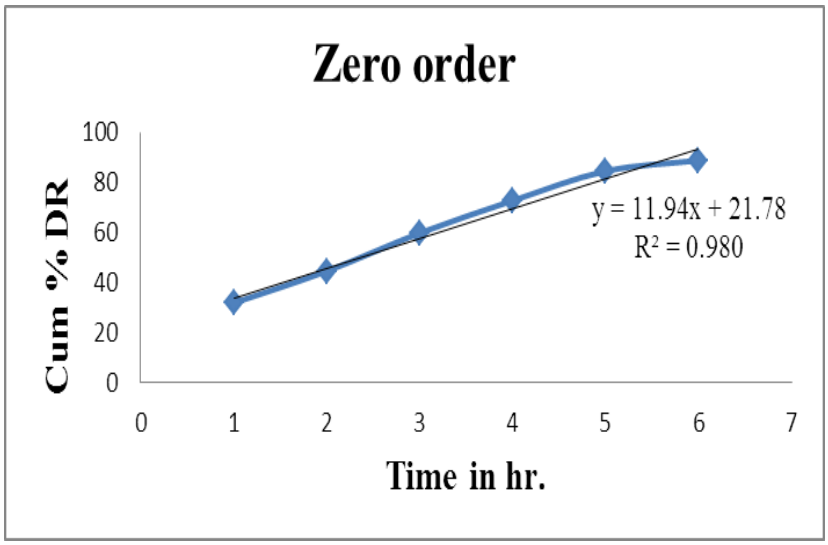
Fig-7: FTIR spectra of stavudine loaded chitosan-g-HEMA

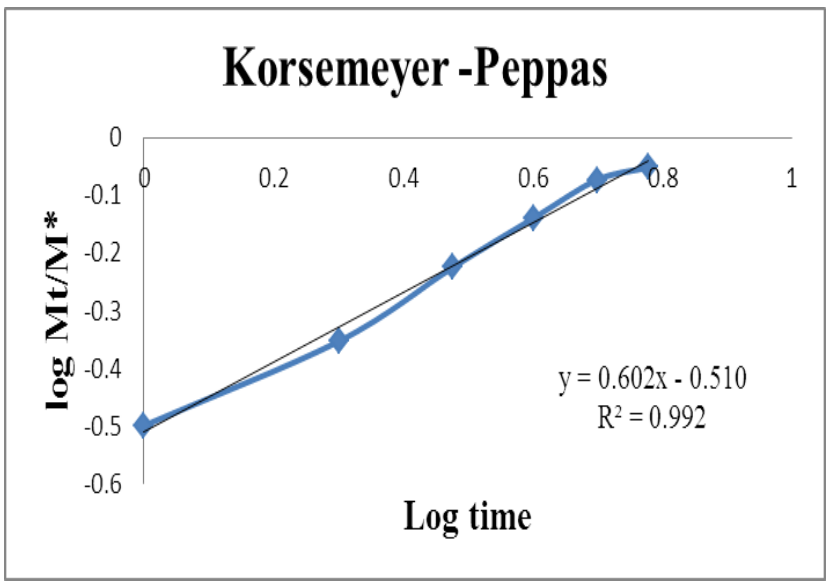
In-vitro dissolution rate study: The *in-vitro* releases of drug were evaluated using dissolution methodology in simulated 6.8 buffer and 0.1N HCL. *In-vitro* releases were carried out in triplicate by incubating 0·1 g of the drug loaded Polymers into a cellophane membrane dialysis bag (D9402, SIGMA-ALDRICH) in 50 ml of 6.8 buffer. At specific time intervals, 1 ml aliquots of sample was withdrawn through a sampling syringe attached with a 0·45 µm Millipore filter and after suitable dilution, the absorption was measured by UV spectrophotometer (UV - VIS spectrophotometer SL 159, Elico India) at 265.1 nm. The concentration of released drug was determined by using the standard curve. Finally the data obtained were fitted to various release kinetics in order to determine the mechanism of drug release from the prepared formulations. The same procedure was duplicated for 0.1N HCl.

Dissolution kinetic data table in 6.8 buffer solution

Time(in hour)	Log time	Absorbance	Concentration	Cum drug release	% Cum drug release 6.8	Mt/M*	log Mt/M*
0	0	0	0	0	0		
1	0	0.254	6.146341463	21.51219512	31.70551971	0.3170552	-0.498865
2	0.30103	0.355	8.609756098	30.13414634	44.4128907	0.4441289	-0.352491
3	0.477121	0.476	11.56097561	40.46341463	59.63657278	0.5963657	-0.224487
4	0.60206	0.579	14.07317073	49.25609756	72.59557489	0.7259557	-0.13909
5	0.69897	0.673	16.36585366	57.2804878	84.422237	0.8442224	-0.073543
6	0.778151	0.707	17.19512195	60.18292683	88.69996585	0.8869997	-0.052077

Graphs for drug release in 6.8 buffer

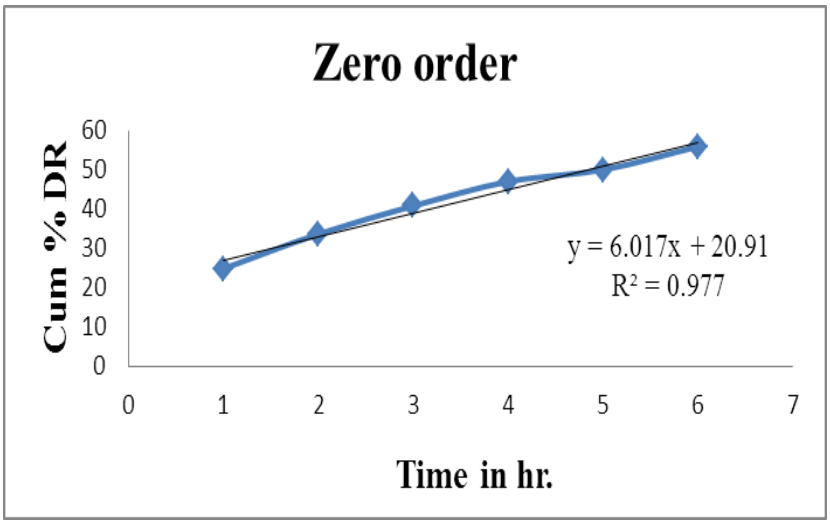


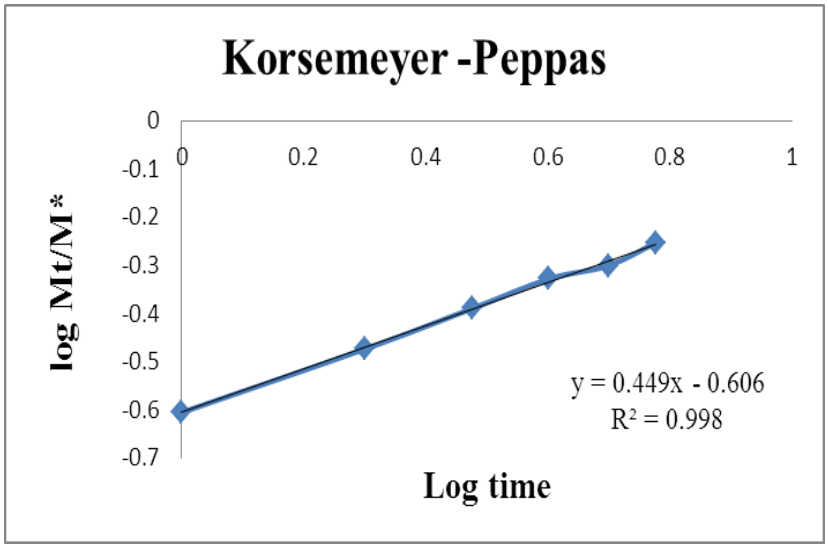


Dissolution kinetic data table in 0.1N HCL

Time(in hour)	Log time	Absorbance	Concentration	Cum drug release	% Cum drug release	Mt/M*	Log Mt/M*
0	0	0	0	0	0		
1	0	0.199	4.804878049	16.81707317	24.78566422	0.2478566	-0.605799
2	0.30103	0.269	6.512195122	22.79268293	33.59275302	0.3359275	-0.473754
3	0.477121	0.326	7.902439024	27.65853659	40.76423962	0.4076424	-0.389721
4	0.60206	0.375	9.097560976	31.84146341	46.92920179	0.469292	-0.328557
5	0.69897	0.399	9.682926829	33.8902439	49.9487751	0.4994878	-0.301475
6	0.778151	0.446	10.82926829	37.90243902	55.86210615	0.5586211	-0.252883

Graphs for drug release in 0.1N HCL

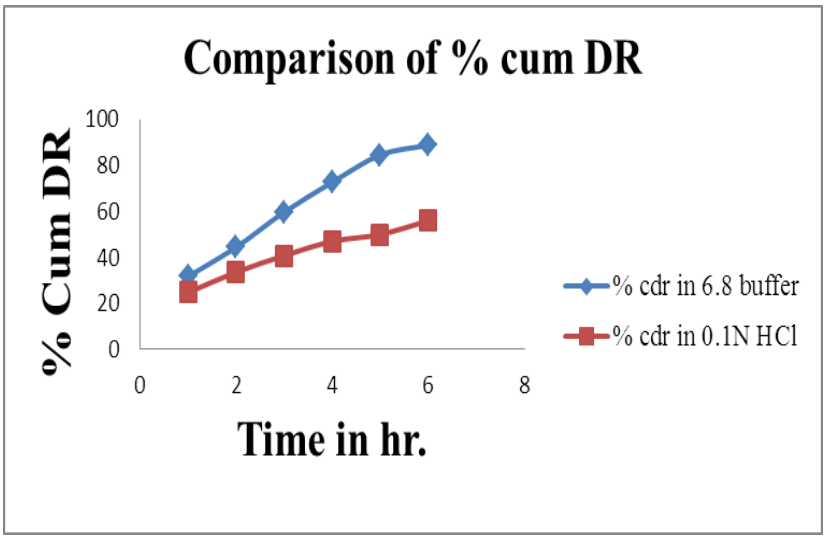


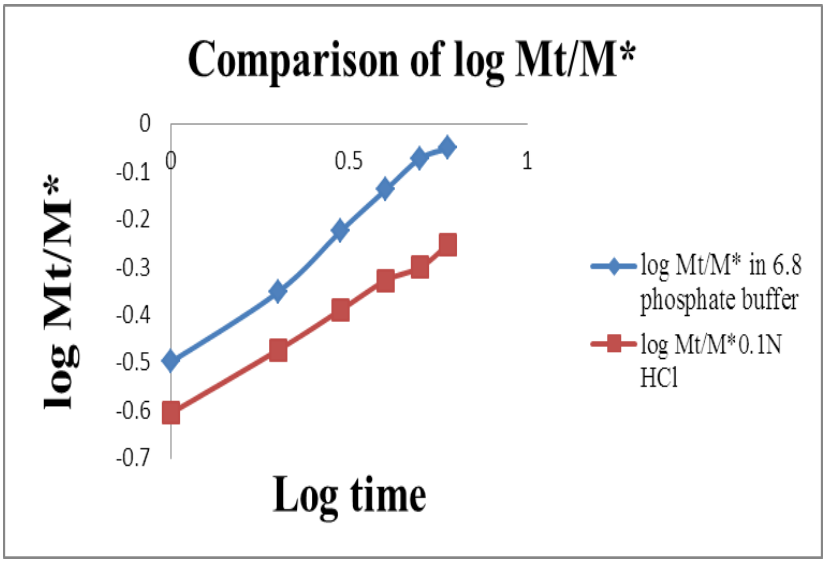


RESULT AND DISCUSSIONS

The result of *in-vitro* drug release study in phosphate buffer of pH 6.8 shows that 88.69% drug was released after 6th indicating the synthesized bulk polymer is capable of releasing the drug in a controlled manner. The release study was also carried out with 0.1N HCl. It was

found that in case of 0.1N HCl the release rate is comparatively lower in comparison to phosphate buffer of pH 6.8 as shown in following figures





In order to found out the mechanism of drug release from the formulation the "n" value of Korsemeyer-Peppas model was used. In both the medium the "n"-value was found to be less than 0.5 indicating the mechanism of drug release to be Fickian type diffusion.

CONCLUSION

From the obtained result it has been confirmed that the grafting of 2-HEMA was successfully done with Chitosan by using Benzoyl peroxide as an free radical initiator. FTIR spectroscopy, XRD study successfully confirmed that the grafting is done. The thermal property of the grafted co-polymer was studied by DSC technique. The synthesized graft polymer shows the swelling behaviour in increasing manner as per the mentioned time

intervals. The higher rate of swelling is observed in case of phosphate buffer of pH 6.8 than the distilled water indicating a good polymer for the study of drug loading and release.

The drug loading and release study of stavudine shows better results. The yield and entrapment efficiency were high for all formulations. The assessment of the release kinetics revealed that drug release followed Korsemeyer-Peppas model. Mechanism of drug release was found to be Fickian type diffusion controlled.

The graft polymer can be used for loading and release study of various drugs. In future the synthesised graft polymer can be implemented to develop nanoparticles for various applications.

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