INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES
IJPAES
Volume-6, Issue-1, Jan-Mar-2016 Coden: IJPAJX-CAS-USA, Copyrights@2016 ISSN-2231-4490
Received: 4th Jan-2016 *Revised: 17th Jan--2016* *Accepted: 19th Jan--2016*
Research article

COMPARATIVE STUDY OF CONTROLLED RELEASE MECHANISM OF CURCUMIN FROM DIFFERENT POLYMER MATRICES

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ABSTRACT: In our previous work we have successfully synthesized chitosan nanocomposites and chitosan hybrid nanocomposites by blending chitosan with other polymers like polyvinyl alcohol (PVA), Polycaprolactone (PCL) and chitosan cross linked derivative by using glutaraldehyde (GLU) ascrosslinker. The nanoclay used for the study was Cloisite 30B.In this research programme we have chosen the best among the each individual polymers(previously synthesized formulationsof polymer) to study the release behaviour of a model anticancer drug curcumin from those polymer/ hybrid polymer nanocomposites. Among chitosan nanocomposites films chitosan dissolved in 2% acetic acid was chosen, similarly CS-PVA (80:20)/ C30B (2.5%) and CS-PCL (80:20)/ C 30B (2.5%), CS cross linked with glutaraldehyde and containing C 30B of 2.5 wt % were chosen for studying the drug release mechanism. Same formulations of drug % were loaded in different polymer matrices and the release pattern was studied in three similar dissolution medium i.e. having pH 4.5, pH 7 and pH 7.5. Their release kinetics were also evaluated by applying various mathematical models proposed by different workers from different parts of the world. And it was concluded that Chitosan- PCL hybrid nanocomposites were proved to be the most suitable matrix for releasing the drug in a controlled manner and for longer duration.
Key words: Hybrid polymer nanocomposites, Cloisite 30B, Curcumin, Drug release kinetics

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INTRODUCTION

Chitosan (CS) is a biopolymer that has received great attention in a variety of applications because of their biodegradability and biocompatibility [1]. It is derived from chitin, which is the second most abundant biomass on earth next to cellulose [2]. Chitosan can be dissolved in dilute acid. This solubility is typically reduced with the introduction of hydrophobic groups [3]. Chitosan has both reactive amino and hydroxyl groups, and can be used for modification of its physicochemical properties [4,5].For non toxic and biocompatibility nature of chitosan it has been chosen as the polymer of first interest to be used as a carrier for controlled release of various drugs [5, 6]. Curcumin (diferuloylmethane), a polyphenol, is a low molecular- weight active principle of theperennial herb Curcuma longa (commonly known as turmeric) [7]. Recent evidence suggests thatcurcumin is a highly pleotropic molecule that interacts physically with its diverse range ofmolecular targets including transcription factors, growth factors and their receptors, cytokines,enzymes, and genes regulating cell proliferation and apoptosis [8]. Curcumin possesses antioxidant, anti-inflammatory, anticarcinogenic, and antimicrobial properties, and suppresses proliferation ofa wide variety of tumor cells. Several clinical trials dealing with cancer have addressed thepharmacokinetics, safety, and efficacy of curcumin in humans [9]. Despite extensive research anddevelopment, poor solubility of curcumin in aqueous solution remains a major barrier in itsbioavailability and clinical efficacy [7-9]. Being hydrophobic in nature, it is insoluble in water butsoluble in ethanol, dimethylsulfoxide, and acetone [8]. To increase its solubility and bioavailability, attempts have been made through encapsulation in liposomes, polymeric and lipo-NPs, biodegradable microspheres, cyclodextrin, and hydrogels [10]. In this research programme we aim to study the release behaviour of curcumin from chitosan and chitosan blended with other polymers in order to find out the most suitable polymer for drug carrier.

EXPERIMENTAL

MATERIALS

Chitosan was purchased from India Sea Foods, Kerala. Nano clay Cloisite 30B (C 30B), was purchased from Southern Clay Co.(USA). Polycaprolactone (PCL) was purchased from Solvay Interox, USA. Poly vinylalcohol, acetic acid, formic acid, citric acid and other chemicals were used as analytical grade and purchased from Sigma–Aldrich Company. Formaldehyde, glutaraldehyde and furfuraldehyde are reagent grade chemicals (BDH, India). Curcumin was a generous gift from VINS Bioproducts, Medak, Andhra Pradesh.

METHODS

Drug Loading

Curcumin of different loadings, i.e., 5wt%, 10wt%, 15wt%, 20wt% and 25wt% were then added to each polymer matrix separately. In case of chitosan nanocomposites films different % of drugs were added to chitosan solution having 2.5% of Cloisite 30B and stirred for 5hr and then the composites were kept at room temperature for drying. Similar procedure was adoptedin case of CS-PVA(75:25)/ C 30B (2.5%), and CS-PCL (80:20)/ C30B (2.5%) where the formed solution along with the drug was poured into a labeled Petri dish and allowed to evaporate the solvent overnight at room temperature. In case of cross linked chitosan product Curcumin of different loadingswere added to the chitosan solution having 2.5% of Cloisite 30B and stirred for 5hrs and then it was cross linked by glutaraldehyde (GLU) (2.5%) and the composites were kept at room temperature for drying.

Dissolution Experiments

Dissolution experiments were performed at 37^{o} C using the dissolution tester (Disso test, Lab India, Mumbai, India) equipped with six paddles at a paddle speed of 100 rpm. About 900 ml of phosphate buffer solution (pH 4.5, pH 7 and pH 7.4) was used as the dissolution media to stimulate gastrointestinal tract (GIT) conditions. A 5 ml aliquot was used each time for analyzing the curcumin content at a fixed time interval. The dissolution media was replenished with a fresh stock solution. The amount of curcumin released was analyzed using a UV spectrophotometer (Systronics, India) at the λ_{max} value of 490 nm.

Swelling Studies

Water absorption of the polymer-drug conjugates was measured following ASTM D 570-81. The samples were preconditioned at 50^{o}C for 24h and then cooled in a desiccator before being weighed. The preconditioned samples were submerged in distilled water at 25 ^{o}C for 24h.The samples were removed and dried with a paper towel before weighing. Water absorption was calculated as a percentage of initial weight. The soluble material loss was checked by weighting the specimens after drying them in an oven at 50^{o}C for another 24h. The total water absorption for 24h was calculated including the soluble material loss

$$\% \text{ Swelling} = \frac{W_1 - W_2}{W_2} \times 100$$

RESULTS AND DISCUSSION

Equilibrium Swelling Studies

Swelling studies are important to understand the drug release characteristics of the polymer drug conjugate. It depends upon the nature and extent of interaction between solvent molecules and polymer chains in addition to porosity of the polymer and the nature of hydrophilic groups present on the polymer.The swelling behaviours of the CS-PVA nanocomposites (2.5% of C30B) and with different % of drug loadings are presented in Figure 1. It is known that the swelling behaviour of the polymer network depends upon the nature of the polymer, polymer solvent compatibility and degree of cross-linking. However, in the case of ionic networks, swelling behaviour depends upon mass transfer limitations, ion exchange and ionic interaction.

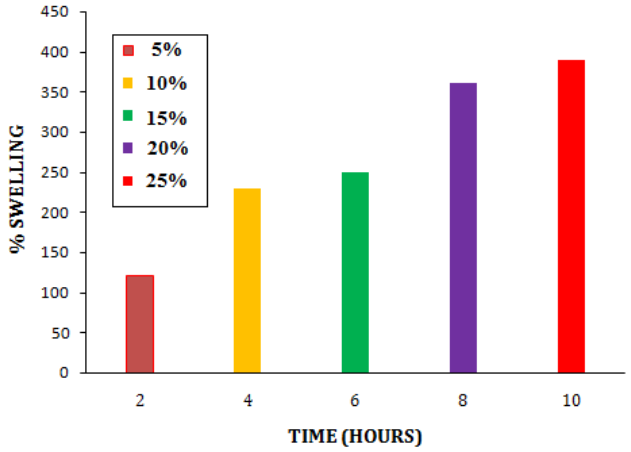

Figure 1. % Swelling of CS-PVA nanocomposites (2.5% of C30B) and with different % of drug loadings

The CS-PVA/ C30 B nanocomposite matrix with different drug loadings were directly immersed in pH 7.5 at room temperature and it showed that the swelling increases with time up to a certain level, and then levels off (Figure 1). All the polymer drug nanocomposites have shown the maximum swelling equilibrium within 10hours hydrophilicity of different drug loading becomes greater with an increase of drug loading, so the swelling of CS-PVA/ C30 B nanocomposites increases with increasing amount of drug loading.

In case of CS/PCL nanocomposites and CS/GLUnanocomposites the percentage of swelling increases with increase in the percentage of drug loading in CS/PCL nanocompositesFigure.2 and Figure 3.

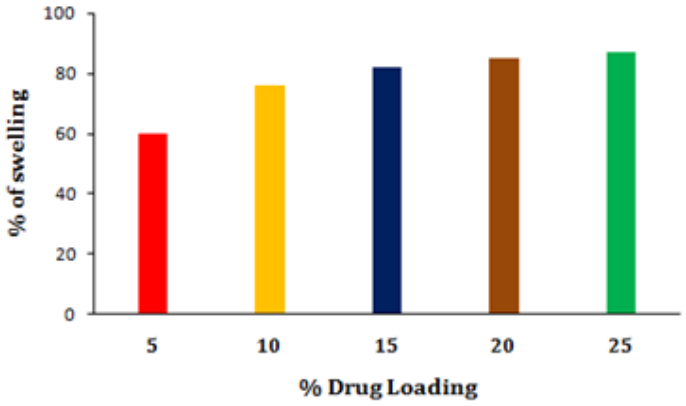

Figure 2. Water Absorption of the CS–PCL / C30B nanocomposites with different % drug loadings

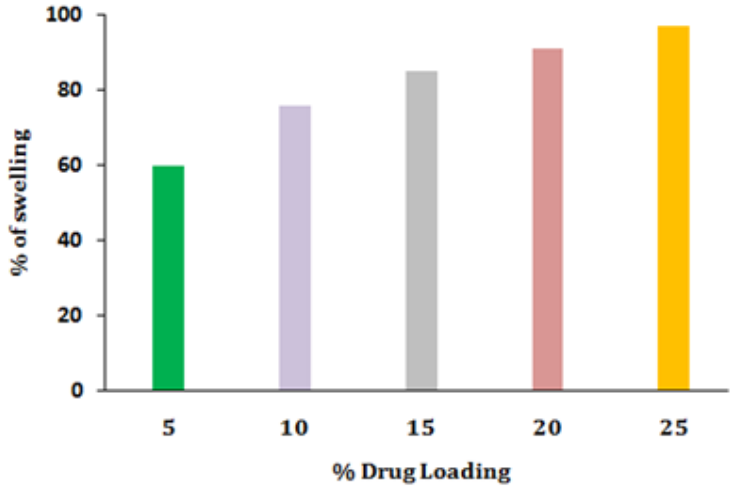

Figure3. % swelling of the chitosan / GLU nanocomposites with different % drug loadings

In-Vitro Drug Release

Effect of pH

The effect of pH on the swelling of chitosan-acetate nanocomposites films (2.5%), the % cumulative release in pH4.5, pH 7 and pH 7.5 media was measured. Cumulative release data presented in Figure 4 indicate that by increasing the pH from 4.5 to 7.5, a considerable increase in the cumulative release was observed for all composites. From Figure 4(a), (b) and (c)it can be seen that the 5 % drug loaded in polymer composites have shown slower and longer drug release rates than the other composites. Interestingly, more than 80 wt% curcumin is released from composites at pH 7.5 within 48hrs, whereas less than 60wt% of the drug is released at pH 4.5 within the same time period.

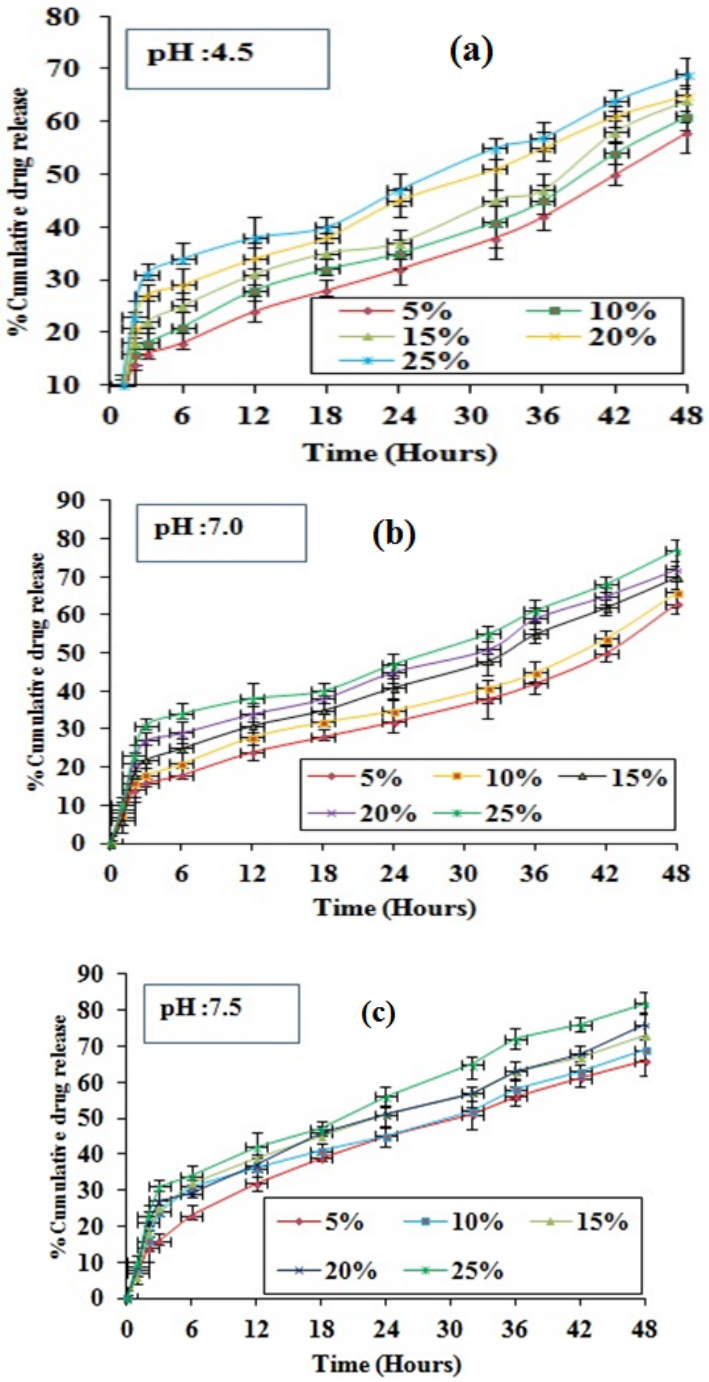

Figure 4. % Cumulative release Vs. Time for different formulation of drug loaded in CS-acetate nanocompositesat (a) pH 4.5, (b) pH 7 and (c) pH 7.5media

Figure 5 shows the cumulative release curves of Curcumin from CS-PVAnanocomposite films at various pH at 4.5, pH 7 and pH 7.5 with constant time gap. It can be seen that curcumin released from CS-PVAnanocomposite matrixes are 54.2%, 58.3%, 62.1%, 65.0%, 68% at 5, 10, 15, 20, 25 % drug loadings respectively at pH 4.5, and the % cumulative release data varies from 59 to 73 % in case of pH 7.0 and 65% to 77 % in case of pH 7.5 respectively. Within 48 hours. The release profile was characterized by an initial burst effect in two media at first two hours, followed by a continuous and controlled release phase within 3 hours.

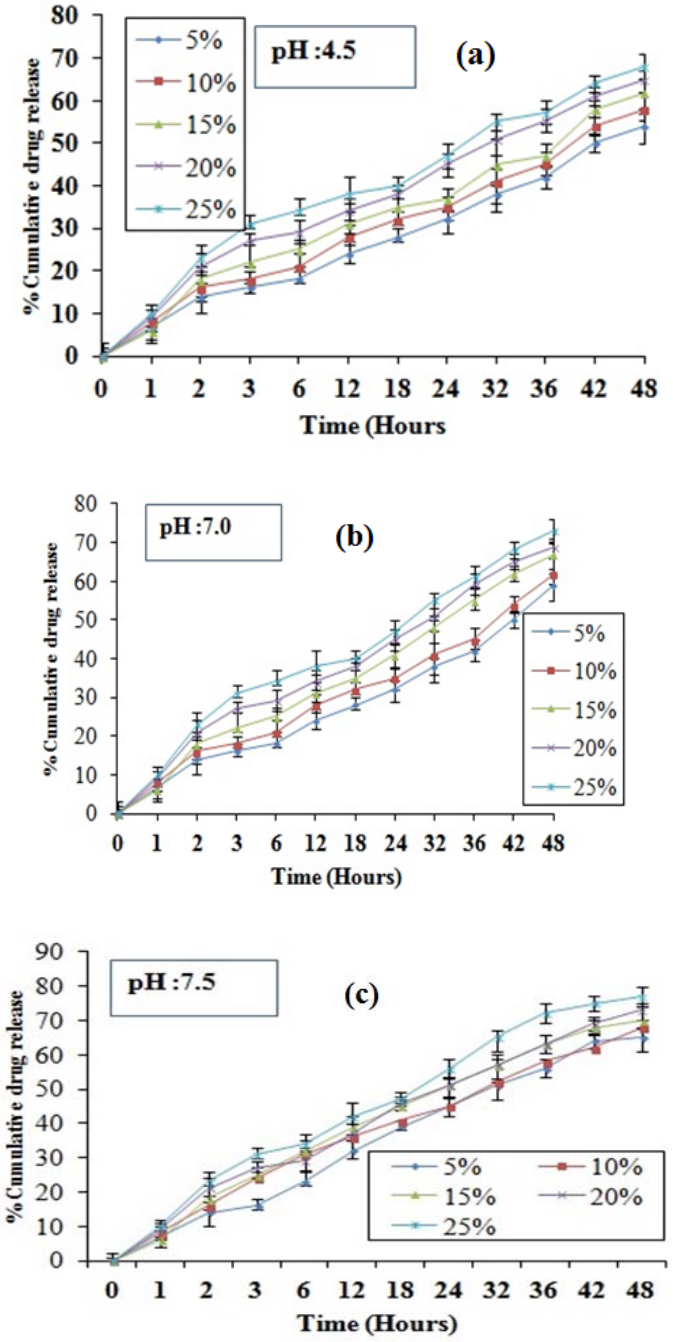

Figure 5. % Cumulative release of curcumin from CS-PVA nanocomposite (80:20) with different drug loadings in (a) pH 4.5, (b) pH 7 and (c) pH 7.5

In order to investigate the effect of pH on the swelling of composite CS-PCL (75:25) / 2.5 %C 30B, the % cumulative release in pH4.5, pH 7.0 and pH 7.5 media was measured. The cumulative release data presented in Figure.6 indicates that by increasing the pH from 4.5 to 7.5, a considerable increase in the cumulative release was observed for all composites.From Figure.6.(A), (B) and (C) it can be seen that the 50 % drug- polymer composites have shown longer drug release rates than the other composites.

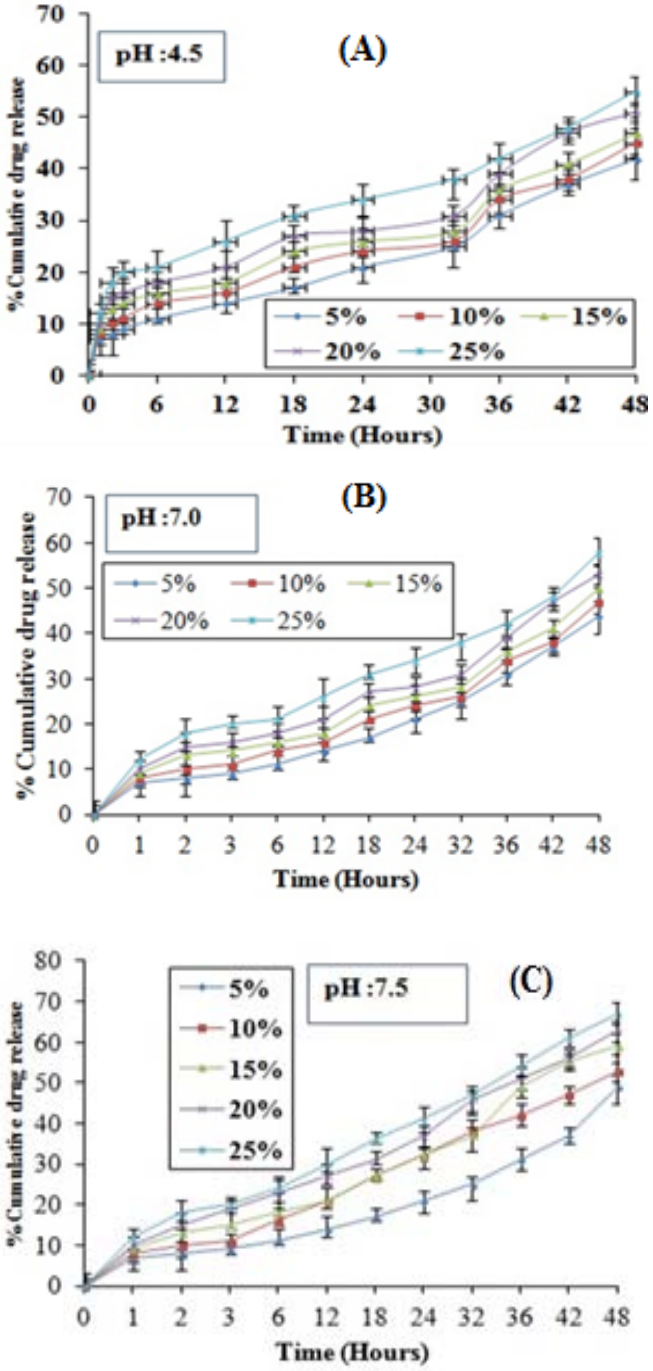

Figure 6.% Cumulative release Vs. Time for different formulation loaded with CS-PCL(75-25)/ C 30B (2.5%) in (A)pH4.5, (B)pH 7 and (C) pH 7.5 media

In order to investigate the effect of pH on the swelling of composite CS-GLU /C 30B 2.5 %, the % cumulative release in pH 4.5, pH 7.0 and pH 7.5 media was measured. The cumulative release data presented in Figure 7indicates that by increasing the pH from 4.5 to 7.5, a considerable increase in the cumulative release was observed for all composites.

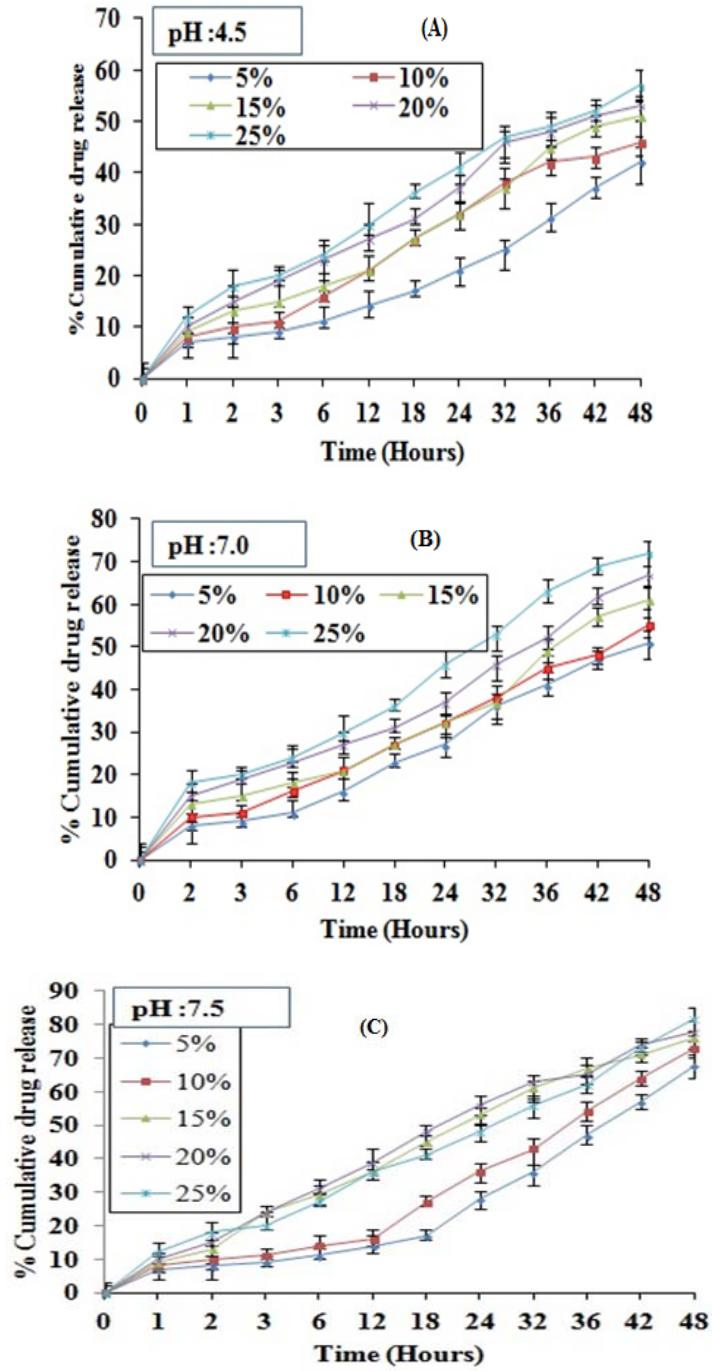

Figure 7.Cumulative release Vs. Time for different formulation of Curcuminloaded in CS-GLU/ C 30B (2.5%) in (A) pH 4.5, (B) pH 7 and (C) pH 7.5 media

From above all the results obtained (Figure 4 to Figure 7) it can be concluded that the drugs in the nanocomposites can be used to be suitable for the basic environment. Probably because the electrostatic interaction of composites is more easily broken at pH 7.5 than at pH 4.5, leading to curcumin being released more rapidly at pH 7.5 than 4.5.Thus, drug release depends upon the nature of the polymer matrix as well as pH of the media. This suggests that the drugs in the blend can be used to be suitable for the basic environment of the large intestine, colon, and rectal mucosa for which there are different emptying times.

Effect of Drug Loading

Figure 4.displays the release profiles of drug from composites at different amounts of drug loadings. Release data show that formulations containing highest amount of drug (25 %) displayed fast and higher release rates than those formulations containing a small amount of drug loading. The release rate becomes quite slower at the lower amount of drug in the matrix, due to the availability of more free void spaces through which a lesser number of drug molecules could transport.

In order to investigate the effect of drug loadingson CS-PVA nanocomposites (2.5%), the % cumulative release in all three dissolution media i.e. pH4.5, pH 7 and 7.5 media was measured. From Figure 5 (a), (b) and (c) it can be seen that the 5 % drug loaded in polymer composites have shown slower and longer drug release rates than the other composites.

The drug release mechanism from the CS-PCL hybrid nanocomposites are shown in Figure.6.Here also we found the same result i.e. a fast and higher release rates are observed in case of higher drug loadings (i.e.25 %) than those containing a small amount of drug loading. Figure7 displays the release profiles of drug from composites at different amounts of drug loadings. From all the above release data it can be concluded that formulations containing highest amount of drug (25 %) displayed fast and higher release rates than those formulations containing a small amount of drug loading. The release rate becomes quite slower at the lower amount of drug in the matrix, due to the availability of more free void spaces through which a lesser number of drug molecules could transport.

DRUG RELEASE KINETICS

Drug Release Mechanism from Matrices

The followings are the most acceptable kinetics that can explain the drug release mechanism.

(i) Zero - Order Kinetics [12,13].

$$W = k_1 t \quad \text{……. (1)}$$

(ii) First - Order Kinetics [14].

$$\ln (100- W) = \ln 100 - k_2 t \quad \text{…… (2)}$$

(iii)Hixon-Crowel's Cube- Root Equation (Erosin Model) [14, 15].

$$(100- W)^{1/3} = 100^{1/3} – k_3 t \text{ …….. (3)}$$

(iv) Higuchi's Square Root of Time Equation (Diffusion Model) [16].

$$W = k_4 t \quad \text{……. (4)}$$

(v) Power Law Equation (Diffusion/ Relaxation model) [16, 17].

$$M_t / M_\infty = k_5 t^n \text{ ……. (5)}$$

Where

M_t/M_∞ = fractional drug release into dissolution medium

k_5= constant incorporating the structural and geometric characteristics of the tablet.

n = the diffusional constant that characterizes the drug release transport mechanism.

The release mechanism mainly depends on the value of "n". When the value of n = 0.5 it means the drug diffuses through the polymeric materials and the mechanism is said to be quasi-Fickian diffusion mechanism. Similarly when the value of $n > 0.5$ or $n \geq 1$ an anomalous, non-Fickian or a non-Fickian, case II or Zero- order release kinetics can be concluded respectively. The kinetics of drug release was analyzed by plotting a graph between the cumulative release data and time, fitting to an exponential equation of the type as represented below [12-17].

$$M_t / M_\infty = k_5 t^n$$

The values of n and k for all the five formulations have been estimated and these data are given in the following Table 1. Analyzing the values of k and n it can be concluded that it dependends on three things (i) drug loading percentage (ii) types of polymer (iii) pH of the dissolution media. Values of k for composites prepared by varying the amounts of drug containing and keeping Chitosan-acetate / C 30 B (2.5wt %) constant, ranged from 0.08 to 0.16 in pH 4.5 and 0.09 to 0.17 in pH 7 and 0.1 to 0.17 in pH 7.5 respectively. However, the drug-loaded composites exhibited n values ranging from 0.76 to 0.93 in pH 4.5, 0.8 to 1.2 in pH 7 and 0.88 to 1.1 in pH 7.5.

Table 1. Release kinetics Parameters of different Formulations at pH 4.5, pH 7 and pH 7.5for Chitosan-acetate / C 30 B (2.5wt %)

Curcumin Loaded %)	Values of "K"			Values of "n"		
	pH 4.5	pH 7	pH 7.5	pH 4.5	pH 7	pH 7.5
5	0.09	0.09	0.12	0.76	0.80	0.88
10	0.08	0.09	0.10	0.79	0.80	0.91
15	0.11	0.11	0.13	0.82	0.82	1.0
20	0.10	0.14	0.15	0.88	1.2	1.1
25	0.16	0.17	0.17	0.93	1.1	1.1

The values of n and k for all the five formulations for CS-PVA polymer hybrid nanocomposites have been estimated and these data are given in the following Table 2. Analyzing the values of k and n it can be concluded that it dependends on three things (i) drug loading percentage (ii) types of polymer (iii) pH of the dissolution media. The values of *n*between 0.5 and 1.2 are an indication of both diffusion controlled drug release and swelling controlled drug release (anomalous transport). Values above 1 indicate case-II transport which relate to polymer relaxation during polymer blend swelling. Values around 0.5 indicate that drug release from polymer was due to Fickian diffusion.

Table 2. Release kinetics Parameters of different Formulations at pH 4.5, pH 7& pH 7.5formulations for CS-PVA polymer hybrid nanocomposites

Curcumin Loaded (%)	Values of "K"			Values of "n"		
	pH 4.5	pH 7	pH 7.5	pH 4.5	pH 7	pH 7.5
5	**0.05**	**0.06**	**0.06**	**0.5**	**0.66**	**0.83**
10	**0.07**	**0.07**	**0.08**	**0.59**	**0.62**	**0.88**
15	**0.07**	**0.09**	**0.11**	**0.67**	**0.73**	**0.93**
20	**0.13**	**0.15**	**0.17**	**0.75**	**1**	**1**
25	**0.14**	**0.16**	**0.19**	**0.92**	**1**	**1.2**

Similaraly the values of n and k for all the five formulations have been estimated in case of CS-PCL nanocomposites and CS-GLU nanocomposites too (Table 3 and Table 4)Values of n for hybrid nanocomposites prepared by varying the amounts of drug containing and keeping the percentages of Chitosan and PCL constant (i.e.75:25), ranged from 0.23 to 0.89 in case of pH 4.5 and the value equals to 1 in case of pH 7 and pH 7.5 and values of 'k' for composites prepared by varying the amounts of drug containing and keeping CS-GLU/C 30B (2.5 wt %) constant, ranged from 0.05 to 0.12 in pH 4.5, 0.06 to 0.17 in pH 7.0 and 0.08 to 0.16 in pH 7.5 respectively. However, the drug loaded composites exhibited 'n' values ranging from 0.55 to 0.8 in pH 4.5, 0.7 to 1.1 in pH 7.0 and 0.8 to 1 in pH 7.5.

Table 3. Release kinetics Parameters of different Formulations at pH 4.5, pH 7 and pH 7.5for Chitosan and PCL (75:25)

Curcumin Loaded (%)	Values of "K"			Value of "n"		
	pH 4.5	pH 7	pH 7.5	pH 4.5	pH 7	pH 7.5
5	**0.07**	**0.07**	**0.09**	**0.23**	**0.28**	**0.86**
10	**0.07**	**0.08**	**0.12**	**0.34**	**0.36**	**0.85**
15	**0.08**	**0.07**	**0.12**	**0.49**	**0.48**	**1**
20	**0.1**	**0.14**	**0.14**	**0.78**	**0.86**	**0.88**
25	**0.1**	**0.19**	**0.13**	**0.89**	**1**	**0.94**

Table.4. Release kinetics Parameters of different Formulations at pH 4.5, pH 7 and pH 7.5 in CS-GLU/C 30B (2.5 wt %)

Curcumin Loaded (%)	Values of "K"			Values of "n"		
	pH 4.5	pH 7	pH 7.5	pH 4.5	pH 7	pH 7.5
5	**0.06**	**0.06**	**0.08**	**0.55**	**0.7**	**0.8**
10	**0.05**	**0.08**	**0.1**	**0.57**	**0.6**	**0.8**
15	**0.08**	**0.08**	**0.13**	**0.7**	**0.8**	**0.83**
20	**0.1**	**013**	**0.16**	**0.7**	**1.1**	**0.92**
25	**0.12**	**0.17**	**0.16**	**0.8**	**1**	**1**

Hence from all the kinetic parameters studied a common thing was noticed indicating a shift from erosion type release to a swelling controlled, non-Fickian type mechanism. The values of n more than 1 have also been recently reported. Comparing the similar findings reported else where we can conclude that this may happen because of the reduction in the regions of low micro viscosity inside the matrix and closure of microcavities during the swollen state of the polymer.

CONCLUSION

Controlled release of anticancer drugs by usingbiodegradable polymers has a significant advantage over conventional drug delivery. In this research program, a model drug Curcumin which has gained attention because of its anticancer properties worldwide was used to study its release pattern from different polymer matrices prepared. After thoroughly investigating and comparing all the cases of controlled release of curcumin from different polymer nanocomposite matrices we found that by increasing the pH from 4.5 to 7.5, a considerable increase in the cumulative release is observed for all composites.Among all the drug delivery data CS-PCL (75:25) nanocomposites showed the least drug release after 48 hrs from the polymer matrices than the other blended polymers. Also, in all cases we found maximum drug release from nanocomposites at pH 7.5 within 48 hours, whereas least drug release (in some case less than 60% for the maximum drug loading i.e.25% of curcumin loading) was observed within 48 hours at pH 4.5.Hence it suggested that drug release depends upon the nature of the polymer matrix as well as pH of the media. Thekinetic parameters like “k” and “n” have been computed and based on the values of “k” and “n” the fickian or non fickian or anomalous behaviour of drug release have been ascertained.In future this method could be used for in vivo research for commercial applications.

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PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

ISSN 2231-4490

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