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Effect of process and formulation variables on release kinetics of cellulosic nanoparticulate formulation of Stavudine

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ABSTRACT

Nanoparticles represent a promising drug delivery system of controlled drug release. Recently, researchers have increasing interest in cellulosic polymers for optimizing the efficiency of existing drugs utilizing better-designed drug delivery systems. In the present research nanoparticles were prepared using cellulose esters to obtain a product with a good oral bioavailability and biological half life. Stavudine was formulated by combining the modified solvent evaporation/extraction technique, sonicator and high pressure homogenization approaches. Various formulation parameters such as effect of sonication-time, concentration of cellulose esters and surfactants on variables were studied. Nanoparticles were obtained with enhanced encapsulation efficiency, negatively charged zeta potential, defined shape, size and with homogeneous size distribution. In vitro release was studied in variable pH using various kinetic models to determine the pattern and mechanism of drug release which showed a sustained Stavudine release upto 12 days. The developed formulation overcome and alleviates the drawbacks and limitations of Stavudine sustained release formulations and could possibility be advantageous in terms of increased bioavailability of Stavudine.

Keywords: *Stavudine, Cellulose esters, Nanoparticles, Modified solvent evaporation/extraction technique, Release kinetic models.*

1. INTRODUCTION

Nanotherapeutics is most promising tool for treatments of various disease, as they solve many problems associated with conventional drug delivery system such as nonspecific biodistribution, lack of targeting, lack of aqueous solubility and low therapeutic indices [1-2]. Drug delivery systems present an opportunity for formulation scientists to overcome the many challenges associate with antiretroviral (ARV) drug therapy. The currently available anti– HIV drugs classified into the nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors and recently fusion, integration inhibitors. These drugs bear some significant drawbacks such as relatively short half-life, low bioavailability, poor permeability and undesirable side effects. So the efforts have been made to design drug delivery systems for antiretroviral therapy as reducing dosing frequency, increase bioavailability, decrease degradation/metabolism in GIT, improve CNS penetration and inhibit CNS efflux, delivery them to target cells and selectively minimal side effects. ARV delivery systems that have been developed by achieving designed drug release kinetics specifically targeting drugs to macrophages, brain, gastric mucosa and for addressing formulation difficulties such as solubility, stability and drug entrapment [3].

The polysaccharide cellulose is considered to be the most abundant and inexhaustible organic polymer known to mankind on earth having applications ranging from packaging and foods to medical devices and pharmaceutics [4-5]. Cellulose derivatives such as cellulose esters have a number of advantages including recyclability, reproducibility, biocompatibility, biodegradability, non toxicity, cost effectiveness and availability in a wide variety of forms [6-7]. Recently, cellulose esters are developed for coatings, films, membranes, building materials, drilling techniques, pharmaceuticals and foodstuffs. These materials also have ability to form micro and nanoparticles thus playing a vital role in the development of modern drug delivery technology [8].

They possess properties that are not only well-suited to the needs of pharmaceutical applications, but that enable construction of drug delivery systems that address critical patient needs. This suite of properties has enabled the creation of a wide range of drug delivery systems employing cellulose esters as key ingredients.

Stavudine (D4T), a pyrimidine nucleoside analog, was approved by the U.S. Food and Drug Administration (FDA) in 1994 and could replace the clinical use of Zidovudine [9]. D4T is activated by thymidine in T-lymph cells to yield stavudine-5'-triphosphate and to compete the association with pyrimidine-5'-triphosphate. Thus, the duplication of HIV is inhibited. However, the residence time of D4T in plasma and the elimination half life of D4T after tissue binding are only 0.5-0.75 hr and 0.9-1.2 hrs, respectively [10].

Taking account of the above information, the principal objective of the present study was to develop nanoparticulate based controlled release formulations of Stavudine to retain the safety and efficacy profiles of Stavudine while greatly improving the bioavailability by encapsulating it in biodegradable polymers, cellulose esters. The nanoparticles were prepared by modified solvent evaporation/extraction technique. Importantly, both the cellulose esters were compared to find out the one producing enhanced entrapment efficiency. The processing parameters, such as the sonication time, effect of surfactant and concentration of polymer were systematically investigated.

2. EXPERIMENTAL SECTION

2.1. Materials.

Stavudine was purchased from Balaji Drug Ltd., (Surat, India). Cellulose acetate butyrate (CAB, Mn=12,000) and cellulose acetate propionate (CAP, Mn=15,000) was purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI, USA). Poly vinyl alcohol (Mw=20,000-30,000) and dichloromethane was purchased from S. D. Fine Chemicals (Mumbai, India). Dialysis membrane-110 was purchased from Himedia Laboratories Pvt. Ltd., (Mumbai, India). Double-distilled water was used throughout the work. All other reagents and solvent used were of analytical grade.

2.2. Preparation of Cellulosic nanoparticles.

Nanoparticles were prepared by solvent evaporation/extraction technique [11] with modification. The objective of the present study was to understand the effect of different formulation parameters on particle formation, particle morphology and release behavior leading to lowest effective dose preparation of Stavudine. CAB and CAP were dissolved in the 20 mL organic solvent mixture consisting of dichloromethane/acetone (9:1, v/v). Stavudine was dissolved in 5 mL water. The organic phase was emulsified with the aqueous phase by sonication using a microtip probe sonicator (XL 2002 Sonicator® ultrasonic liquid processor) in ice bath for 1-10 min to produce the water-in-oil emulsion. The resulted solution was slowly dropped into 50 mL PVA (0.5 – 1 % w/v) as an external phase. During this process, the mixture was homogenized using a high speed homogenizer (CHG-15A, Daihan Scientific Co. Ltd., Korea), at agitation speed of 20,000 rpm and kept on iced-water bath. The formed emulsion was gently stirred at room temperature for 3 h to evaporate the organic solvents. The nanosuspensions were then centrifuged at 14,000 rpm (Remi Elecktrotechnic Ltd, Vasai, Maharashtra, India) 20 °C for 20 min. Supernatants from previous step were undergone for a further centrifugation to ensure for obtaining all the dispersed nanoparticles. The collected nanoparticles were washed (3 times) with 5 ml distilled water. The purified nanoparticles were further freeze-dried.

2.3. Measurement of the drug content and encapsulation efficiency.

To determine entrapment efficiency, 5 mg of nanoparticles were dissolved in deionized water (10 mL) and stirred magnetically for 6 hrs[12]. Then 1ml of solution was taken, filtered through membrane filter and absorbance of aqueous phase was measured at 265 nm using UV-spectrophotometer (UV-spectrophotometer 1601, Shimadzu Co. Ltd., Japan). Drug contents and loading efficiency were calculated as follows:

%Drug loading= ((Weight of drug in nanoparticles) / (weight of nanoparticles)) ×100 (1)

%Encapsulation Efficiency= ((Drug loading) / (Theoretical loading)) ×100 (2)

2.4. Transmission electron microscopy.

The surface morphology of nanoparticles was studied by transmission electron microscopy (TEM, JEOL, Japan). The surface morphology & surface characteristics of samples were studied by transmission electron microscopy (TEM). A drop of nanoparticle suspension containing 0.03% (w/v) of phosphotungstic acid was placed on a TEM copper grid coated with carbon film and dried at room temperature. Observation was performed at 200 kV with Tecnai 20, Philips (JEOL, Japan).

2.5. Particle size studies and zeta potential.

Particle size, zeta potential (ZP) and polydispersity index (PDI) were determined by dynamic light scattering technique using Zetasizer Nano ZS90 (Malvern Instruments Ltd., Malvern, UK).

2.6. Fourier transform infrared spectroscopy (FTIR).

Interaction between the drug and polymer were studied by employing Fourier transform infrared spectroscopy (FTIR; Spectrum GX, Perkin Elmer, USA). The spectrum was recorded in the wavelength region of 400–4000 cm-1 and the resolution of 0.15 cm^{-1} at scan speed 10 scan/s.

2.7. XRD.

X-ray diffraction (XRD) patterns were obtained using Philips X-ray diffractometer (X pert) to analyze degree of sample crystallinity (voltage - 40 kV, current - 40 mA and Cu α-radiation source - range of 3° to 50° of 2θ, scan time - 1 s)

2.8. Drug release study.

To study drug release, the nanoparticles (all containing 10 mg Stavudine nanoparticles) were placed in the vessels containing 100 mL of phosphate buffer solution (PBS pH 1.2 and 6.8) as the dissolution medium. The vessels were incubated at 37°C with continuous orbital mixing on a shaker (Lab-term, sic/incu shaker/01 Kuhner, Switzerland) at 50 rpm. The release medium was exchanged everyday to maintain the sink condition. At predetermined time schedule, release medium was taken and concentration of released drug was measured with (UV-spectrophotometer 1601, Shimadzu Co. Ltd., Japan) at 265 nm. The volume withdrawn was replenished with an equal volume of fresh and pre-warmed PBS 37°C to maintain the constant volume. All experiments were done in triplicate. Percentage drug release at different time intervals were calculated using the calibration curves constructed from the reference standards. Various release models such as zero order equation, first order equation, Higuchi model and Korsmeyer-Peppas were employed to determine the mechanism and kinetics of drug release from nanoparticles [13].

2.9. Release kinetics.

Data obtained from *in vitro* analysis were fitted to various kinetic equations to find out the mechanism of drug release from microspheres. The kinetic equations used were zero order equation, first order equation and Higuchi model.

$$C = k_0 t \quad (3)$$

Where, k_0 is zero-order rate constant expressed in units of concentration/time and t is the time.

$$\text{Log}C = \text{Log}C_0 - k_1 t / 2.303 \quad (4)$$

Where, C_0 is the initial concentration of drug and k1 is first order constant.

$$Q = kH\, t_{1/2} \quad (5)$$

Where, kH is the higuchi constant reflecting the design variables of the system.

The following plots were made; Qt vs t (zero order kinetic model), log(Q0-Qt) vs t (first order kinetic model) and Qt vs square root of t (Higuchi model) [14] where Qt is the total amount of drug released at time t and Q0 is the initial amount of the drug present in the microspheres. The rate constants were calculated for the respective models. Further, to find out the mechanism of drug release, the first 60% drug release was fitted in Korsmeyer-Peppas model [15].

$$Qt = k\, tn \quad (6)$$

Where, k is the kinetic constant and n is the diffusion exponent, a measure of the primary mechanism of drug release.

3. RESULTS SECTION

3.1. Effect of polymeric concentration.

Variable concentrations of CAB and CAP from 1%-3% were used in this study where the drug concentration was kept constant. The results (Table 1) showed that, an increase in initial amount of CAB and CAP increases in entrapment efficiency and mean particle size. The formulation A3 renders Stavudine entrapment efficiency (72.58%) compared to all the formulations whereas formulation A6 renders entrapment efficiency (69.25%).

Table 1. Compositions, encapsulation efficiency and mean particle size of different formulations

Formul-ation	CAB (%, w/w)	CAP (%, w/w)	Stavudine (Wt %)	E.E (%)	Particle size (*M*±SD) (nm)	PDI±SD	ZP (mV)
A1	1		20	64.79	126± 0.57	0.083±0.024	-17.8±0.5
A2	2		20	69.85	146± 1.27	0.042±0.032	-21.0±1.2
A3	3		20	72.58	174.1±0.15	0.070±0.072	-22.2±1.9
A4		1	20	67.34	140± 1.37	0.137±0.043	-19.3±1.7
A5		2	20	68.48	188± 0.75	0.213±0.065	-24.6±2.3
A6		3	20	69.25	194.4±0.72	0.112±0.030	-29.1±2.0

3.2. Effect of sonication time.

In order to obtain emulsified systems, the addition of energy is a fundamental step. The effect of sonication time (1–10 min) on entrapment efficiency and particle size was studied and the results for Stavudine and formulations were tabulated in (Table 2) respectively. In the case of formulation A3 and A6, is increase in entrapment efficiency with lowered mean particle sizes were observed when the time of sonication was increased. The emulsification can be considered one of the most important steps of the process, because an insufficient dispersion of phases results in large particles [16]. Drug loaded CAB and CAP Nanoparticles prepared with 10 min of sonication demonstrated best entrapment efficiency and with lowest particle size.

3.3. Concentration of surfactants.

The surfactant used in the present study was PVA with variable concentrations (0.1 - 1 % w/v). PVA was incorporated in external aqueous phase to further optimize the formulation to study the effect of surfactant on entrapment efficiency and particle size. Formulation prepared with 1 % w/v PVA concentration showed a remarkable increase in entrapment efficiency as compared to other formulations. Also, the results shown in (Table 3) indicate that there was a decrease in particle size, when the PVA concentration in the external aqueous phase was increased from 0.1 to 1.0 % (w/v). Also, the increase in amount of PVA leads to narrower granulometric distribution.

In case of Stavudine, A3 where as for A6 formulations with CAB as a polymeric matrix, 10 min of sonication and 1% w/v PVA concentration had the highest encapsulation efficiency. Further the narrow particle size distribution was observed with higher concentration of PVA. This could be explained by the fact that high concentration of PVA stabilizes the Nanoparticles surface effectively and hence leads to a reduced size of the Nanoparticles [17]. The theory is that an insufficient amount of surfactant would not succeed in stabilizing all the Nanoparticles, leading to aggregation of the particles thus resulting in larger size Nanoparticles. The emulsification and stabilization of the particles plays a vital role in the solvent evaporation/extraction technique. Along with these factors, the amount of surfactant also plays an important role in the emulsification process as it protects the droplets avoiding the coalescence of globules. Thus the observations revealed that PVA with 1%, w/v concentration is a better surfactant in terms of entrapment efficiency, and particle size.

3.4. Particle size.

The mean diameter of the entire nanoparticles was determined and listed in (Table 1). The Stavudine and loaded nanoparticles prepared by using CAB and CAP as an excipients, 3% w/v polymer concentration, sonication time of 10 min and 1% PVA as stabilizer, have 174.1±0.15 nm and 194.4±0.72 nm sizes respectively with optimum entrapment efficiency. The results showed that sonication time, polymer and PVA concentration, along with the type of cellulosic polymers have a significant influence on encapsulation efficiency, particulate size and size distribution of the Nanoparticles.

3.5. Transmission electron microscopy.

The prepared Stavudine loaded nanoparticles and according to the optimized formulation and preparation conditions were rigid and almost spherical (Figure 2). The Nanoparticles obtained with greater surfactant concentrations showed spherical shape and absence of agglomerates.

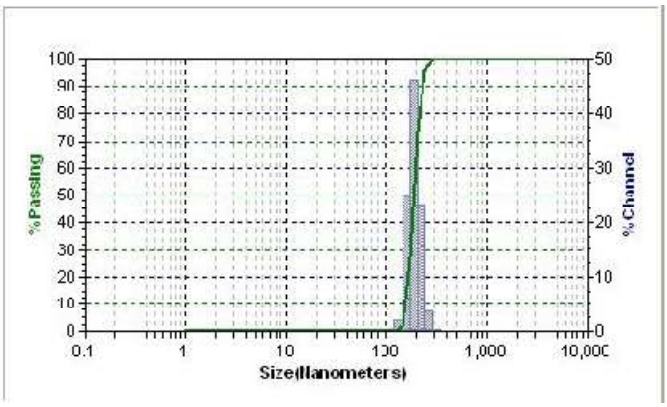

Figure 1. Particle Size formulation A1

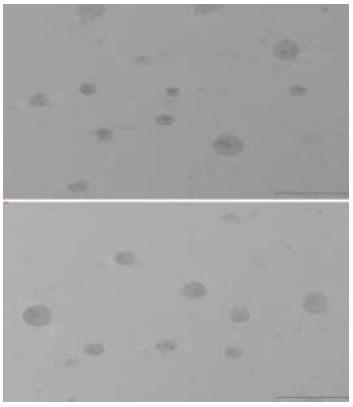

Figure 2. TEM image of Stavudine nanoparticles

Table 2. Effect of Sonication time

Sonication Time (min)	1	5	10
A3			
Encapsulation Efficiency (%)	45.11	59.33	72.58
Particle Size (*M*±SD) (nm)	249± 1.86	199± 1.23	174.1± 0.15
PDI±SD	0.140±0.052	0.125±0.082	0.070±0.072
ZP (mV)	-30.8±2.9	-33.5±1.5	-22.2±1.9
A6			
Encapsulation Efficiency (%)	40.32	56.46	69.25
Particle Size (*M*±SD) (nm)	230±1.54	201±1.38	194.4±0.72
PDI±SD	0.142±0.092	0.128±0.046	0.112±0.030
ZP (mV)	-32.2±0.8	-30.4±1.6	-29.1±2.0

Table 3. Effect of PVA Concentration

PVA (%, w/v)	0.1	0.5	1.0
A3			
Encapsulation Efficiency (%)	42.20	56.34	72.58
Particle Size (*M*±SD) (nm)	299± 1.82	298± 1.38	174.1± 0.15
PDI±SD	0.172±0.048	0.155±0.095	0.070±0.072
ZP (mV)	-30.8±1.9	-24.5±2.2	-22.2±1.9
A6			
Encapsulation Efficiency (%)	50.32	66.46	69.25
Particle Size (*M*±SD) (nm)	379±1.85	288±1.34	194.4±0.72
PDI±SD	0.179±0.065	0.148±0.058	0.112±0.030
ZP (mV)	-23.1±1.2	-21.5±1.9	-29.1±2.0

3.6. Zeta potential.

The zeta potential is an important particle characteristic as it influences stability of nanoparticles. Zeta potential measurements provide information about the particle surface charge. Zeta Potential is the charge at the electrical double layer, created by ions of the liquid, which exists around each particle.

More pronounced zeta potential values, irrespective of the charge type, tend to stabilize nanoparticle suspension. The same electric charge leads to electrostatic repulsion between particles which prevent the aggregation of the spheres. All the formulations of nanoparticles exhibited zeta potentials ranging from -17.8 to -29.1 mV (negatively charged). These values are adequate to from a stable cellulose esters nanoparticles suspension. Only a small difference in zeta potential was obtained between the different kinds of nanoparticles prepared. However, it was observed that the formulations with lower surfactant concentration exhibited high negative charge compared to that of formulation with higher surfactant concentration. This can be explained by the fact that the residual surfactant may be present on the surface of nanoparticle which can create a shield between the surface and surrounding medium thus masking the possible charged groups existing on the particle surface [18].

Furthermore, values of PDI were smaller than 0.3, indicating a narrow and homogenous nanoparticles size distribution [19].

3.7. FT-IR.

There is no significant difference in characteristic peaks of pure drug loaded nanoparticles suggesting drug stability during encapsulation process. This show the absence of any chemical interaction between drug & polymer.

3.8. *In vitro* drug release studies.

Sustained release formulations were developed to release the required quantity of drug with predetermined kinetics in order to maintain an effective drug plasma concentration. To achieve this for Stavudine, delivery systems were formulated with CAB and CAP to release the drug in a predetermined and reproducible manner. *In vitro* release experiments of Stavudine from the

cellulose ester nanoparticles were performed in simulated gastric (pH 1.2) conditions for first 4 h and continued in intestinal (pH 7.2) conditions.

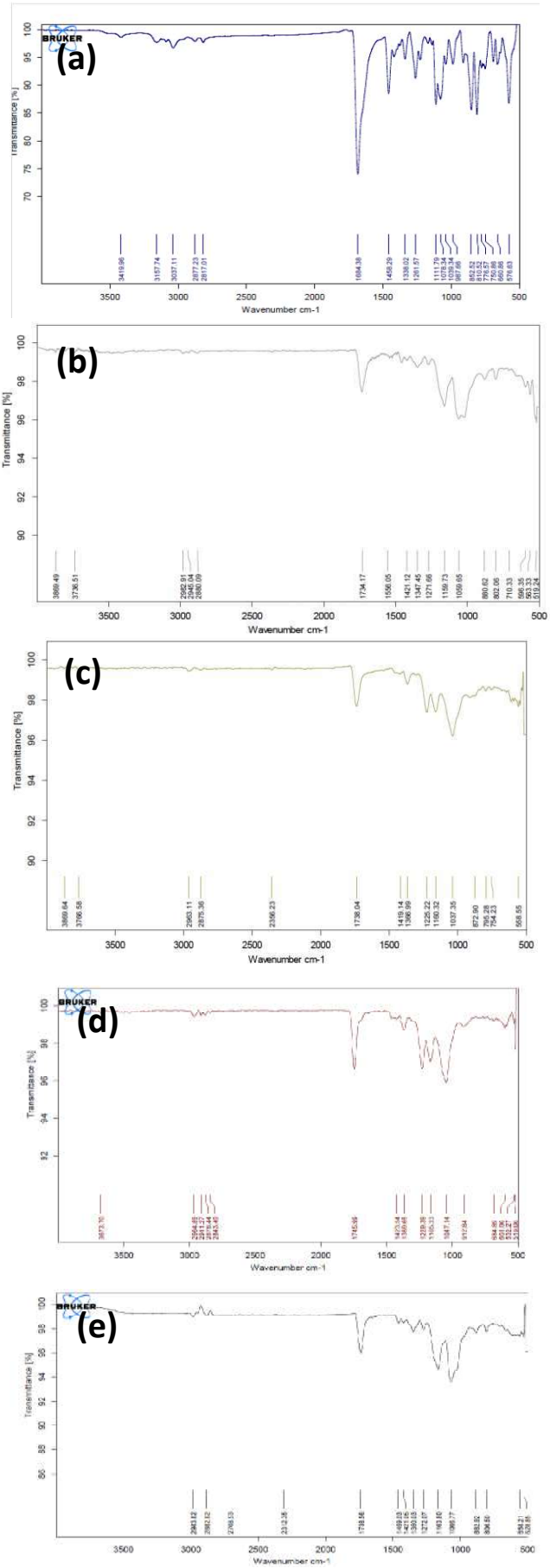

Figure 3. FTIR spectra of (a) pristine Stavudine, (b) CAP, (c) CAB, (d) Formulation A1 and (e) Formulation A6

To avoid erroneous results due to sudden temperature changes, dissolution media (phosphate buffers) was maintained to 37^0C, throughout the experimental conditions. Here, *in vitro* release data has been discussed in terms of effect of type and concentration of polymer, concentration of surfactant. Average % cumulative release vs. time plots for Stavudine -loaded nanoparticles of all the formulations have been displayed in (Figure 4).

Stavudine release profiles from nanoparticles appeared to have a biphasic release pattern. First an initial slight exponential phase releasing 11-19% of the drug in first 4 hour followed by second phase of slow sustained release. The initial release phase was probably due to small amount of drug which was adsorbed or close to the surface of the nanoparticles [20]. Another reason for initial burst effect in case of Stavudine might be the high solubility of drug in acidic pH. The second phase for Stavudine was a relatively slow release up to more than 12 days, which could be caused due to the diffusion of the dissolved Stavudine within the cellulose matrix core of the nanoparticles and the polymer degradation in to the release medium.

The release rate of Stavudine from the CAP nanoparticles was higher than that of CAB, which could explain that the CAB nanoparticles exhibited stronger sustained release effect than that of CAP nanoparticles, and the release rate was higher in case of the formulation containing lower polymer concentration. One reason could be the increased particle size with increase in polymer concentration. With the increase in particle size, surface area/volume ratio decreases leading to decreased buffer penetration and slower release of the drug. Moreover another reason could be that when the viscosity of the solution is low (viz. decreased concentration of polymer), causes decreased density of the polymer matrix, resulting in decreased diffusional path length and so the nanoparticles show a fast controlled release phase, and as the viscosity of the increases, the opposite situation occurs resulting in slow controlled drug release. This may be the reason for slower release of the drug from CAB nanoparticles prepared with higher concentration.

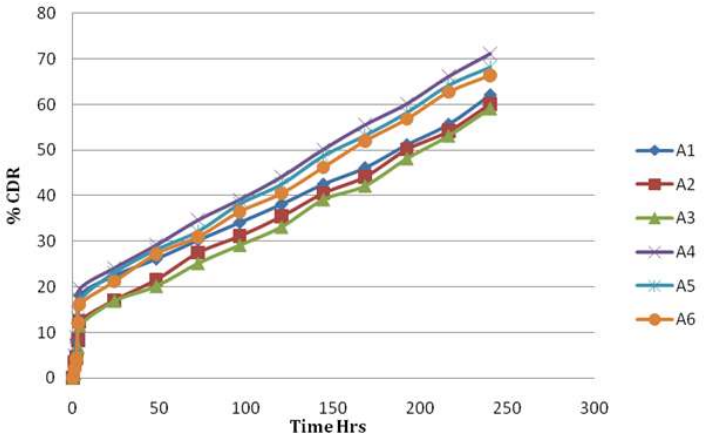

Figure 4. The cumulative release profiles of Stavudine Nanoparticles.

Thus, the proportionate increase in the polymer concentration reflected in sustaining the release to a greater extent. So the CAB and higher concentration of CAB could control the release rate of both the drugs Stavudine from the nanoparticles for

longer period of time. Furthermore, the surfactant, PVA appeared to be the most suitable emulsifier in reducing aggregation between nanoparticles which suspends immediately after formation. Nanoparticles were prepared with 1% surfactant concentrations gave longer drug release profile for 12 days for Stavudine.

3.9. Release kinetics.

The aim of this section was to utilize the already existing mathematical models in literature such as zero-order, first-order, Higuchi's square root of time and Korsmeyer-Peppas model to determine the pattern and mechanism of drug release from the nanoparticles. The zero order rate describes, the systems, where the drug release rate is independent of its concentration. The first order rate, which describes the rate of drug release, is dependent of its concentration. Higuchi model describes the release of drugs from an insoluble matrix as a square root of time dependant process, based on Fickian diffusion. Korsmeyer-Peppas model describes the release of drug when the prevailing mechanism is a combination of Fickian and non-Fickian mechanisms. The release constants were obtained from the slope of the appropriate plots, and regression coefficient (R2) was determined by linear regression analysis.

The correlation coefficient of different kinetic models for Stavudine nanoparticles containing various CAB and CAP have been tabulated in (Table 4). The *in vitro* releases of Stavudine from CAB nanoparticles were best explained by Higuchi plots as it indicated highest linearity. Thus the Stavudine release from the CAB nanoparticles was proportional to square root of time. Moreover, drug release from CAP nanoparticles were best explained by first order equation, as the plots showed the highest regression coefficient compared to that of zero order and Higuchi's model.

Korsmeyer-Peppas used n-value to characterize different release mechanisms. When n takes a value of 0.5, the drug diffuses through and is released from the polymer following Fickian diffusion mechanism. For n>0.5, an anomalous, non-Fickian solute diffusion mechanism is observed. When n=1, purely relaxation-controlled delivery which is referred to as Case II transport is observed.

In case of drug, the calculated n-values for all examined formulations prepared by using CAB were found to be less than 0.5, indicating Fickian diffusion drug release while CAP formulations were found to have n-values more than 0.5 thus following non-Fickian diffusion mechanism. In release kinetic A1, A2 and A3, follows Higuchi model while A4, A5 and A6 follows First order model.

Table 4. Release Kinetics of Stavudine Nanoparticles

Batch code	Zero order		First order		Higuchi		Korsmeyer-Peppas	
	R^2	K_0	R^2	K_0	R^2	K_h	R^2	N
A1	0.8177	0.2634	0.9164	0.0065	0.9929	2.9013	0.8103	0.4021
A2	0.8587	0.2697	0.9231	0.0081	0.9839	2.9104	0.8674	0.4527
A3	0.8502	0.2600	0.9155	0.0098	0.9822	2.8322	0.7643	0.4422
A4	0.8408	0.3066	0.9864	0.0067	0.9277	3.3498	0.8091	0.9941
A5	0.8399	0.3074	0.9928	0.0074	0.9342	3.3602	0.8061	0.7653
A6	0.8298	0.3115	0.9893	0.0089	0.9195	3.4120	0.7530	0.8477

4. CONCLUSIONS

The present investigation suggests that drug release can be tailored significantly by varying the polymer, concentration of polymer and surfactant. Furthermore, cellulosic nanoparticles have substantial role in enhancing the delivery aspects of potent drugs such as Stavudine. The release profile of the cellulosic nanoparticles consists of two idiosyncratic phases, an initial little fast release phase followed by a slow controlled release phase. Also the release kinetics depended upon the type of polymer such as CAB nanoparticles followed Higuchi kinetics while CAP nanoparticles followed first order release kinetics. The present study has concluded that the prepared cellulosic nanoparticles with enhanced encapsulation efficiency could explore a promising carrier system for controlled and sustained release preparation for effective management of drug therapy with improved oral bioavailability, decreased dose frequency, side effects and maximizing the patients' compliance.

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