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Original Article

Antiviral Microcapsule Design and Characterization Using Bio Muco-Resident Polymer

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ABSTRACT

Zidovudine is used in the treatment of Human immune deficiency Virus (HIV) infection so aim of this study was to develop a sustained release dosage form. Formulations of Zidovudine microcapsule was prepared by using drug with Chitosan and Cellulose Acetate in different ratio and their evaluation study was done by Scanning Electron Microscope, Particle size analysis, FT-IR, DSC and in vitro dissolution.The surface morphology of Zidovudine micro capsules was seen by bifocal microscope and Scanning Electron Microscope. Magnification was done 50 x, 250 x, 2500 x. Surface of Zidovudine microcapsule was found smooth and spherical. The particle size analysis was carried out by Optical Microscope. The maximum particle size range was found to be 60-80 µm in Zidovudine Chitosan for FCH-1, and 80-100 µm in case of Zidovudine Cellulose Acetate for FCA-1 formulation. In formulation with Chitosan, minimum entrapment efficiency was found to be 15.30% and maximum 62.55%. In formulation with Cellulose Acetate, minimum entrapment efficiency was found to be 60.70 % and maximum 99.29 %. The entrapment data show that increase in the polymer concentration increases the entrapment efficiency. FT-IR was done for pure drug and with different formulation for the identification of drug. It indicates no chemical interaction occurred between drug and polymer and also determined the stability of drug during formulation process. Thermal analysis (DSC) was done for the standard and formulations As per standard (pure drug) peak value is between 117.87°C – 257.59°C.So it is concluded that all Formulation peaks are within the range of standard. Cumulative % drug release of FCH-1 and FCA-1 was found 28 % and 44.31% respectively in acidic pH for first two hours and in 6 hours cumulative % drug releasewas52.526% and 83.50% respectively. Various kinetic data studies were done including zero order, first order, Higuchi model and Korsmeyer - Peppas model.

Keywords: Zidovudine, Chitosan, Cellulose Acetate, Microcapsule, Higuchi model

1. INTRODUCTION

Sustained release dosage forms have many advantages in comparison to conventional dosage form. They maintain blood level of the drug for long duration at time and minimize the systemic side effect. Zidovudine

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is used in the treatment of Human immune deficiency Virus (HIV) infection so aim of this study was to develop a sustained release dosage form which is microencapsulated using Chitosan and Cellulose Acetate at specific ratio for different concentration of drug and polymer. Chitosan and Cellulose Acetate are biocompatible, non-toxic, excellent gel and film forming ability. It's widely used in controlled delivery (e.g. gels, membrane and micro spheres). Chitosan has a very good mucoadhesive property and stick to the gut wall and also villi of the intestine, therefore the release of the drug is for a longer period and the material slowly get diffused from the gut wall and maintain a specific concentration in the blood. Chitosan is used as DNA deliveries for enhance delivery and significantly higher and express level. Cellulose Acetate is used for sustained release formulation such as microspheres and transdermal Drug delivery. [1]

2. MATERIALS AND METHODS

Zidovudine is used in the treatment of Human immune deficiency Virus was procured from Cipla Banglore and polymers such as Chitosan and Cellulose acetate was procured from Loba chemi Mumbai.

Standard graph of Zidovudine in 0.1 N HCl pH 1.2, phosphate buffer pH 6.8 and water

One hundred milligram of the drug was accurately weighed and transferred to a 100 ml standard flask. To this small quantity of corresponding solution was added and the drug was made to dissolve. Then the solution was made up to 100 ml. This was considered as stock solution. From the stock solution, 1ml was pipetted out to 100 ml standard flask and it was diluted suitably with HCl buffer to get 10µg/ml. The same procedure was repeated by taking 1.5 ml, 2.0 ml, 2.5 ml, 3.0 ml, 3.5 ml, 4.0 ml, to get 15µg/ ml, 20µg/ml, 25µg/ml, 30µg/ml, 35µg/ml, 40µg/ml, respectively. The absorbance's for the solutions were determined at 266nm by Elico make UV- visible spectrophotometer. After the samples were scanned, the concentration within the range of 10µg/ ml and 40µg/ ml was found to obey the Beer- Lambert's law.

Formulation and composition:

For Chitosan microcapsules: Chitosan dissolved in 20 ml 5% Acetic acid solution and drug was dispersed in the solution. This solution was added drop wise in 100 ml light liquid paraffin containing 1 % Tween 80 and continuous stirring at a speed of 2000 rpm for 3 hrs. At the end of stirring 5 ml Glutaralehyde (40 %) was added and kept for 4 hours for cross linking. The resulting product was filtered and washed with Petroleum Ether 4 to 5 times and kept in decicator for 5 days.[2, 3]

For Cellulose Acetate microcapsules: Cellulose acetate dissolved in 20 ml Acetone and drug was dispersed in the solution. This solution was added in 100 ml light liquid paraffin containing 1% Span-60 and continuous stirring for 3 hrs. At the end of stirring 5 ml of Gluteraldehyde (40 %) was added and kept for 4 hours for cross linking. The resulting product was filtered and washed with Petroleum ether 4 to 5 times and kept in decicator for 48 hrs. [4]

Entrapment Efficiency of Zidovudine Microcapsules

Method: 25 mg of the microencapsulated product were crushed in to power and add 25 ml distilled water. The resulting mixture was kept for 24 hours then the solution was filtered through membrane filter and the 1 ml of this solution was diluter using distilled water and analyzed absorbance in UVvisible spectrophotometer at 266nm.The entrapment efficiency of the microencapsulated product was calculated from the absorbance obtained for 14 samples. [5, 6]

Bifocal Microscopy

Bifocal Microscope are used to produce clear morphology of the specimens at various magnifications

light passes through a small pinhole and expands to fill the entrance pupil of microscope objective lens. The objective lens focuses the light to a small spot on the specimen at the local place of the objective lens. Light reflected back from the illuminated spot on the specimen is collected by the objective and is partially reflected by a beam splitter.

Applications: The Surface characteristics of various excipients have also been studied. [7]

Imaging has also been used to study the morphology of bulk drug. To study morphology with higher magnification power and other constituents

Scanning Electron Microscope

Particle size and shape characteristics can be determined by microscopic evaluation using scanning electron microscope. The scanning electron microscope provides magnifications up to 200*1000 xs with a resolution approximately 25A.To determine detailed particle surface morphology as well as individual particles surface characteristics. The scanning electron microscope adds the dimension of depth by tilting the stage to several angles of view during operation. The ability to resolve various shapes helps the investigator determine whether a sample is morphologically homogenous and the presence of other materials can be detected.

Procedure: As the encapsulated sample is non-conductive in nature, the sample was sputtered within the golden coating, Electric current was passed through the sample and was viewed through SEM (Philips 200 FEI) to determine the surface morphology [8, 9].

In Vitro Release Studies of Different Batches of Zidovudine Microcapsules

Design of in vitro dissolution apparatus: In vitro dissolution apparatus used in the present study was specially designed. Dialysis membrane was tied at the bottom of glass tube. 100 mg of sample was weighed and placed in glass tube. Dialysis membrane was soaked in dissolution medium. 250 ml beaker with 187.5 ml of dissolution fluid was kept on a magnetic stirrer. A magnetic bead was placed and stirred at 100 rpm. The temperature of 250 ml beaker was maintained at 37 ± 0.5^{0}c. Tube containing sample was immersed in the dissolution fluid so that it touches the surface of the dissolution fluid. Samples were withdrawn every half an hour for 2 hours in case of 0.1N HCl (pH 1.2) as dissolution medium. After two hours, to the above dissolution media 62.5 ml of 0.2 M Tri-basic sodium phosphate solution was added to change the pH of dissolution media from pH 1.2 to pH 6.8. And samples were withdrawn at one hour interval up to six hours. 5 ml of the sample was withdrawn and replaced with 5 ml of the dissolution medium. The samples were analyzed spectrophotometrically at 266 nm with suitable dilution. The dissolution as carried out for 14 samples using 0.1N HCl, pH 1.2 and phosphate buffer, pH 6.8 as dissolution medium

In vitro release profile of Zidovudine Microcapsule from FCH- 1 formulation in the GI simulated condition: 100 mg of sample contains 20mg of active drug (Entrapment 62.20 %) 115 The release study of Zidovudine Microcapsules was done for the first twohours at acidic pH using 0.1N HCl and then it was continued at alkaline pH using phosphate buffer pH 6.8 .The Percentage Cumulative release found to be 28% in acidic pH for first two hours .In alkaline pH, the Percentage Cumulative release found to be 52.526 % for six hours. The release study of Zidovudine Microcapsules was done for the first twohours at acidic pH using 0.1N HCl and then it was continued at alkaline pH using phosphate buffer pH 6.8 .The Percentage Cumulative release found to be 32.438 % in acidic pH for first two hours .In alkaline pH, the Percentage Cumulative release found to be 46.68 % for six hours.

In vitro release profile of Zidovudine Microcapsule from FCH- 2

The release study of Zidovudine Microcapsules was done for the first twohours at acidic pH using 0.1N HCl and then it was continued at alkaline pH using phosphate buffer pH 6.8 .The Percentage Cumulative release found to be 21.447% in acidic pH for first two hours .In alkaline pH, the Percentage Cumulative release found to be 33.811 % for six hours.

In vitro release profile of Zidovudine Microcapsule from FCH- 3

The release study of Zidovudine Microcapsules was done for the first twohours at acidic pH using 0.1N HCl and then it was continued at alkaline pH using phosphate buffer pH 6.8 .The Percentage Cumulative release found to be 13.441% in acidic pH for first two hours .In alkaline pH, the Percentage Cumulative release found to be 25.398 % for six hours.

In vitro release profile of Zidovudine Microcapsule from FCH- 4

The release study of Zidovudine Microcapsules was done for the first twohours at acidic pH using 0.1N HCl and then it was continued at alkaline pH using phosphate buffer pH 6.8 .The Percentage Cumulative release found to be 10.227% in acidic pH for first two hours .In alkaline pH, the Percentage Cumulative release found to be 15.126 % for six hours.

In vitro release profile of Zidovudine Microcapsule from FCH- 5

The release study of Zidovudine Microcapsules was done for the first twohours at acidic pH using 0.1N HCl and then it was continued at alkaline pH using phosphate buffer pH 6.8 .The Percentage Cumulative release found to be 16.87% in acidic pH for first two hours .In alkaline pH, the Percentage Cumulative release found to be 21.94 % for six hours.

In vitro release profile of Zidovudine Microcapsule from FCH- 6

The release study of Zidovudine Microcapsules was done for the first twohours at acidic pH using 0.1N HCl and then it was continued at alkaline pH using phosphate buffer pH 6.8 .The Percentage Cumulative release found to be 20.796% in acidic pH for first two hours .In alkaline pH, the Percentage Cumulative release found to be 25.832 % for six hours.

In vitro release profile of Zidovudine Microcapsule from FCH- 7

The release study of Zidovudine Microcapsules was done for the first twohours at acidic pH using 0.1N HCl and then it was continued at alkaline pH using phosphate buffer pH 6.8 .The Percentage Cumulative release found to be 44.31 % in acidic pH for first two hours .In alkaline pH, the Percentage Cumulative release found to be 83.50 % for six hours.

In vitro release profile of Zidovudine Microcapsule from FCA- 1

The release study of Zidovudine Microcapsules was done for the first twohours at acidic pH using 0.1N HCl and then it was continued at alkaline pH using phosphate buffer pH 6.8 .The Percentage Cumulative release found to be 53.04% in acidic pH for first two hours .In alkaline pH, the Percentage Cumulative release found to be 75.00 % for six hours.

In vitro release profile of Zidovudine Microcapsule from FCA- 2

The release study of Zidovudine Microcapsules was done for the first twohours at acidic pH using 0.1N HCl and then it was continued at alkaline pH using phosphate buffer pH 6.8 .The Percentage Cumulative release found to be 59.20% in acidic pH for first two hours .In alkaline pH, the Percentage Cumulative release found to be 78.806 % for six hours.

In vitro release profile of Zidovudine Microcapsule from FCA- 3

The release study of Zidovudine Microcapsules was done for the first twohours at acidic pH using 0.1N

HCl and then it was continued at alkaline pH using phosphate buffer pH 6.8 .The Percentage Cumulative release found to be 52.694% in acidic pH for first two hours .In alkaline pH, the Percentage Cumulative release found to be 72.074 % for six hours.

In vitro release profile of Zidovudine Microcapsule from FCA- 4

The release study of Zidovudine Microcapsules was done for the first twohours at acidic pH using 0.1N HCl and then it was continued at alkaline pH using phosphate buffer pH 6.8 .The Percentage Cumulative release found to be 38.900% in acidic pH for first two hours .In alkaline pH, the Percentage Cumulative release found to be 59.507 % for six hours.

In vitro release profile of Zidovudine Microcapsule from FCA- 5

The release study of Zidovudine Microcapsules was done for the first twohours at acidic pH using 0.1N HCl and then it was continued at alkaline pH using phosphate buffer pH 6.8 .The Percentage Cumulative release found to be 47.999% in acidic pH for first two hours .In alkaline pH, the Percentage Cumulative release found to be 68.324 % for six hours.

In vitro release profile of Zidovudine Microcapsule from FCA- 7

The release study of Zidovudine Microcapsules was done for the first twohours at acidic pH using 0.1N Hill and then it was continued at alkaline pH using phosphate buffer pH 6.8 .The Percentage Cumulative release found to be 51.309% in acidic pH for first two hours. In alkaline pH, the % Cumulative release found to be 73.430 % for six hours.

3. RESULTS AND DISCUSSION

Table 1: Materials used for preparation of Zidovudine Microcapsule by using Chitosan

	FCH-1	FCH-2	FCH-3	FCH-4	FCH-5	FCH-6	FCH-7
Zidovudine(mg)	75	150	225	300	300	300	300
Chitosan(mg)	300	300	300	300	75	150	225
Cellulose acetate(mg)	---	---	----	----	----	----	-----
Tween 80(ml)	1	1		1	1	1	1
Span 60(ml)	---	---	----	----	----	----	-----
Acetone (ml)	---	---	----	----	----	----	-----
Liquid paraffin(ml)	100	100	100	100	100	100	100
Gluteraldehyde (ml)	5	5	5	5	5	5	5
Speed (R.p.m)	2000	2000	2000	2000	2000	2000	2000

Table 2: Materials used for preparation of Zidovudine Microcapsule by using Cellulose acetate

	FCA-1	FCA-2	FCA-3	FCA-4	FCA-5	FCA-6	FCA-7
Zidovudine(mg)	75	150	225	300	300	300	300
Chitosan(mg)	---	---	----	----	----	----	-----
Cellulose acetate(mg)	300	300	300	300	300	300	300
Tween 80(ml)	1	1		1	1	1	1
Span 60(ml)	1	1		1	1	1	1
Acetone (ml)	20	20	20	20	20	20	20
Liquid paraffin(ml)	100	100	100	100	100	100	100
Gluteraldehyde (ml)	5	5	5	5	5	5	5
Speed (R.p.m)	2000	2000	2000	2000	2000	2000	2000

Table 3: Percentage yield and Entrapement efficacy of Zidovudine microcapsule after formulation with Chitosan and Cellulose Acetate

S.no	Formulations	% yield	Entrapement efficacy	Formulations	Entrapement efficacy	% yield
1	FCH-1	94.60	62.20	FCA-1	99.29	98.60
2	FCH-2	91.66	51.81	FCA-2	85.65	98.66
3	FCH-3	83.33	35.60	FCA-3	81.65	97.33
4	FCH-4	78.83	25.65	FCA-4	76.00	96.83
5	FCH-5	57.33	15.30	FCA-5	60.70	93.33
6	FCH-6	73.33	22.10	FCA-6	70.10	91.33
7	FCH-7	86.19	26.10	FCA-7	75.45	91.19

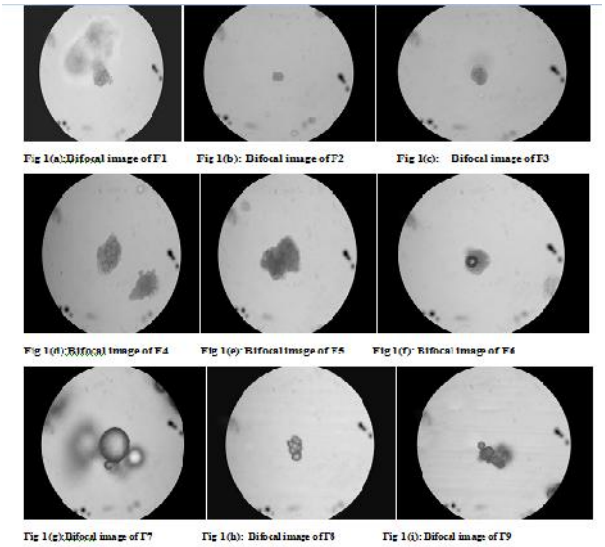
Table 4: Comparative study of in vitro release of Zidovudine-Chitosan Microcapsules

Time (Hr)	FCH-1	FCH-2	FCH-3	FCH-4	FCH-5	FCH-6	FCH-7
0.5	19.0	26.4	18.2	7.3	3.7	13.9	17.5
1.0	22.8	28.4	19.2	9.4	5.4	15.1	18.8
1.5	24.8	30.6	20.2	11.2	7.4	16.0	19.8
2.0	28.0	32.4	21.4	13.4	10.2	16.7	20.7
2.5	32.9	34.9	23.7	16.7	12.4	17.1	22.2
3.0	36.5	37.3	25.5	19.1	14.1	19.1	22.3
3.5	40.5	39.6	27.7	22.3	14.4	21.7	24.3
4.0	45.2	42.2	30.0	25.3	14.5	21.5	25.4

5.0	48.2	44.0	32.1	25.3	14.7	21.9	25.7
6.0	52.5	46.6	33.8	25.6	15.1	21.9	25.8

Table 5: Comparative study of in vitro release of Zidovudine-Cellulose acetate

Time (Hr)	FCH-1	FCH-2	FCH-3	FCH-4	FCH-5	FCH-6	FCH-7
0.5	18.0	24.4	18.2	7.3	3.7	13.9	17.5
1.0	21.8	26.4	19.2	9.4	5.4	15.1	18.8
1.5	23.8	32.6	20.2	11.2	7.4	16.0	19.8
2.0	27.0	33.4	21.4	13.4	10.2	16.7	20.7
2.5	31.9	35.9	33.7	26.7	22.4	27.1	32.2
3.0	47.5	48.3	45.5	39.1	34.1	39.1	42.3
3.5	51.5	59.6	47.7	42.3	44.4	41.7	44.3
4.0	54.2	61.2	50.0	55.3	54.5	51.5	55.4
5.0	66.2	66.0	62.1	55.3	64.7	61.9	65.7
6.0	75.0	78.8	72.0	59.5	68.3	73.4	74.9



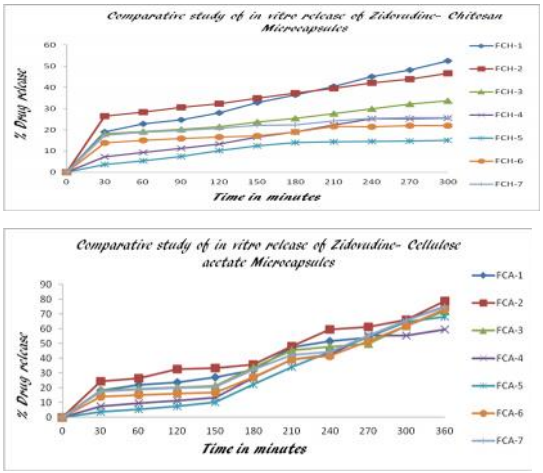
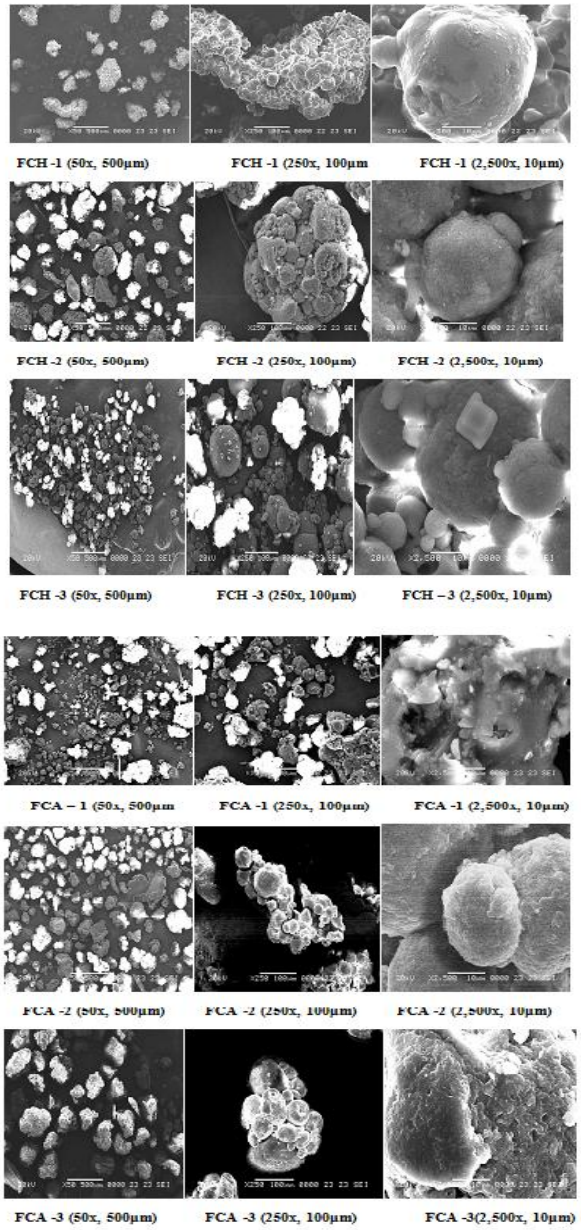


Fig 2: Comparative study of in vitro release of Zidovudine-Chitosan/cellulose acetate



4. SUMMARY AND CONCLUSION

Sustained release dosage forms have many advantages in comparison to conventional dosage form. They maintain blood level of the drug for long duration at time and minimize the systemic side effect. Zidovudine is used in the treatment of Human immune deficiency Virus (HIV) infection so aim of this study was to develop a sustained release dosage form.

Formulations of Zidovudine microcapsule was prepared by using drug with Chitosan and Cellulose Acetate in different ratio and their evaluation study was

done by Scanning Electron Microscope, Particle size analysis, FT-IR, DSC and in vitro dissolution.

The surface morphology of Zidovudine micro capsules was seen by bifocal microscope and Scanning Electron Microscope. Magnification was done 50 x, 250 x, 2500 x. Surface of Zidovudine microcapsule was found smooth and spherical.

In case of Zidovudine Cellulose Acetate micro capsules surface was wavy. Calibration curve of drug was done in distilled water, 0.1 N HCl, pH 1.2, and phosphate buffer pH 6.8 as per procedure. The linear graph was obtained and R2 value was found in water, 0.1N HCl and phosphate buffer. 0.9998, 0.9995, 09992 respectively.

The particle size analysis was carried out by Optical Microscope. For all formulations 200 microcapsules were selected randomly and their size was determined using Optical Microscope fitted with a standard micro meter scale. The maximum particle size range was found to be 60-80 µm in Zidovudine Chitosan for FCH-1, and 80-100 µm in case of Zidovudine Cellulose Acetate for FCA-1 formulation.

The entrapment efficiency of all formulations was done. In formulation with Chitosan, minimum entrapment efficiency was found to be 15.30% and maximum 62.55%. In formulation with Cellulose Acetate, minimum entrapment efficiency was found to be 60.70 % and maximum 99.29 %. The entrapment data show that increase in the polymer concentration increases the entrapment efficiency.

Dissolution studies of all 14 formulations were done for first two hour in acidic pH (0.1 N HCl) and 6 hours in Phosphate buffer pH 6.8. Cumulative % drug release of FCH-1 and FCA-1 was found 28 % and 44.31% respectively in acidic pH for first two hours and in 6 hours cumulative % drug releasewas52.526% and 83.50% respectively.

Various kinetic data studies were done including zero order, first order, Higuchi model and Korsmeyer - Peppas model. Zero order kinetics was done and R2 value for formulation FCH- 1 and FCA- 1 were found to be 0.9995 and 0.9674 and it confirmed that FCH- 1 followed zero order kinetics. First order kinetics was done and R2 value for formulation FCH- 1 and FCA- 1 were found to be 0.9875 and 0.9975 and it confirmed that FCA- 1 followed first order kinetics.

For Higuchi model, R2 value for FCH- 1 and FCA- 1 were found to be 0.9972 and 0.9791. Its mean formulation FCH-1 follow higuchi model. It indicates that drug release mechanism was diffusion. For Korsmeyer–Peppas model, R2 value for FCH- 1 and FCA- 1 were 0.9973 and 0.9873, And n value for FCA-1 & FCH-1 were found to be 0.4988, 04978 respectively. It proves that both formulations follow Korsmeyer – Peppas model. It indicates that (The exponent n >0.5) drug release mechanism was Fickian diffusion.

It is conclude that drug encapsulated with Chitosan polymer FCH- 1 showed 52.526 % drug released at the end of 8 hours and it followed Zero order kinetics, Higuchi and Korsmeyer- Peppas model. Drug encapsulated with Cellulose Acetate polymer FCA- 1 showed 83.50 % drug released and it followed First order kinetics and Korsmeyer- Peppas model.

Future Plan: The maximum entrapment efficiency of Zidovudine Chitosan formulation was found to be 62.50 %. The entrapment efficiency may be improved by using cross linking agent in higher concentrations. Future studies involve in vivo Studies; scale up studies of the optimized formulation; in vivo – In Vitro Correlation.

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