3. EXPERIMENTAL

3.0.PLAN OF THE WORK

3.1.0 EXPERIMENTAL METHODS WITH RESULTS AND DISCUSSION

3.0. PLAN OF THE WORK

1. Literature survey.

2. Drug profile study. (Model drugs: Zidovudine).

3. Experimental work

4. Preparation of standard curve of drug.

5. Preparation of microencapsulation by solvent evaporation, coaccervation and inotropicgelation techniques using different polymer ratio Various combinations of Eudragit RL 100, RS 100, Hydroxy propyl methyl cellulose (HPMC),Ethyl cellulose, sodium alginate and chetochan.

6. Evaluations of particle size analysis, drug content, Entrapment efficiency, flow properties, *in-vitro* drug release profile and optimized the microcapsules.

7. optimized the microcapsules encompass with Prepared gel by using different polymer like carbopol 940, carbopol 934, Hydroxy propyl methyl cellulose, Sodium carboxyl methyl cellulose in different ratio.

8. Evaluation of gel by different parameter like Extrudability, Spreadability, Rheological study, pH and swelling index.

9. *In- vitro* diffusion study by using different membrane

10. *In-vivo* study of final gel formulation

11. Vaginal irritancy test for final formulation.

12. Stability study for final formulation.

13. Comparison study final gel with marketed product of same drug.

14. Comparison study final gel with vaginal films and vaginal tablet of respective drug.

15. Statistical analysis.

3.1.0 EXPERIMENTAL METHODS WITH RESULTS AND DISCUSSION

3.1. Method of Preparation of microcapsules

While choosing for the preparation of microcapsules, following methods were tried.

3.1.1. Preparation of microcapsules by ionic gelation method:-

Vaginal microcapsules containing AZT were prepared employing sodium alginate in combination with Carbopol 940, methyl cellulose (MC), hydroxypropyl methyl cellulose (HPMC) and sodium CMC as coat material. Sodium alginate (200mg) and the Mucoadhesive polymers (200mg) were dissolved in purified water (12.8ml) to form a homogeneous polymer solution. Core material, AZT (200mg) was added to the polymer solution and mixed thoroughly to form a smooth viscous dispersion.The resultant solution was extruded drop wise with the help of syringe and needle (gage 18) into 100ml of (10%) aqueous calcium chloride solution and stirred 100 rpm[1]. After stirring for 15 min microcapsules were separated, washed with water and dried at 70^0 C for 6hr in an oven table 3.1.

Table 3.1. Formulation design of microcapsule by ionic gelation method

Drug(mg)	Sodium alginate(mg)	HPMC(mg) (F1)	Carbopol(mg) (F2)	Methyl cellulose (F3)	Sodium CMC(mg) (F4)
200	200	200	----	---	---
200	200	----	200	---	---
200	200	-----	---	200	---
200	200	----	---	---	200

Drug content for all microencapsulated formulations (F1-F4) was observed .The observations given in the column 2 in Table 3.2. The drug content of all microcapsules was obtained between the ranges of **1.005±0.007 to 1.306± 0.054** which was too low. All the microcapsules formulation with entrapment efficacy are observed (three times for each batch) for the loading efficiency. The observed data given column 3 in Table 3.2,

the percent drug entrapment efficiency of all microcapsules were observed between the 5.36± 0.02 to 7.5±0.020 which was too low again. The particle size analysis of microcapsules was studied by using optical microscopy and SEM with magnification power 32X and 198X. SEM photograph was shown microcapsules were not formed figure 3.1.

Table 3.2 Percent Yield, drug content, and encapsulation efficiency of microcapsule formulation F1, F2, F3, F4

Formulation code	% yield	Drugcontent/50(mg) (mg)	Encapsulation efficiency (%)
F1	94± 1.02	1.089± 0.046	5.69± 0.03
F2	88± 0.75	1.049± 0.003	5.7± 0.01
F3	89± 1.15	1.005± 0.007	5.36± 0.02
F4	96.3± 1.2	1.306± 0.054	07.5±0.02

All values are expressed in mean ± standard deviation (n=3)

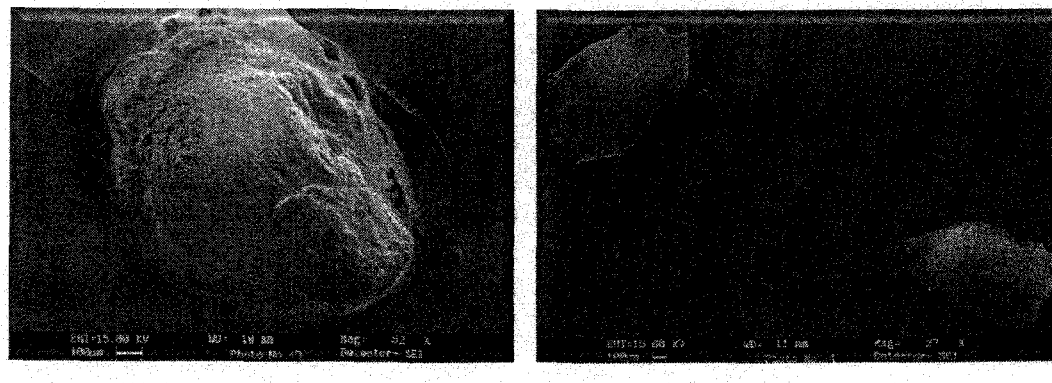


Figure 3.1: SEM photographs of microcapsules by ionic gelation method

This method was tried for the preparation of microcapsules but for further study, it was not used because drug was diffused out resulting in low drug content.

3.1.2. Preparation of microcapsules by thermal change method:-

An aqueous internal phase (30ml) of an emulsion (w/o) containing 400mg bovine serum albumin, 100 mg AZT was emulsified with 250ml of pure olive oil under constant stirring at 1200rpm while the temperature was maintained at 4^0C. This emulsion was

added gradually (10-15drops/min) to preheated (120^0c) pure olive oil under constant stirring at 750 rpm for 15minutes and mixture was allowed to cool down to room temperature. The heat stabilized albumin microcapsules were separated by centrifugation at 3000rpm for 5 minutes. The separated microcapsules were washed with 60ml of anhydrous ether [2].

This method was tried for the preparation of microcapsules but for further study, it was not used because difficulty in removing oil phase. SEM photograph was shown microcapsules were not formed figure 3.2.

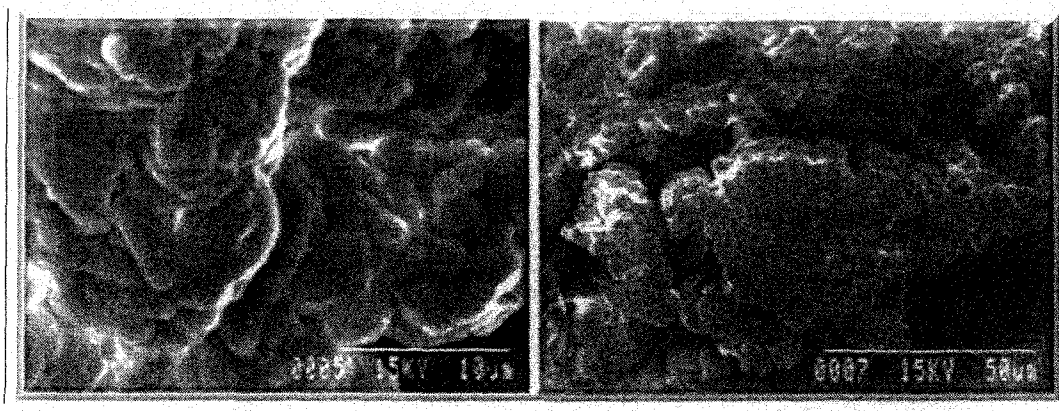


Figure 3.2: SEM photographs of microcapsules by thermal change method

3.1.3. Preparation of microcapsules by AZT microcapsule by coacervation phase separation technique

About 1 gm of ethyl cellulose was added to 50ml of cyclohexane and heated to 80^0c. After this stage 100mg of AZT was added to above polymeric solution. Then the mixture was stirred continuously with the help mechanical stirrer, reduced to room temperature than filter the solution. The collected microcapsule was dried in room temperature. The same procedure was adopted for preparing microcapsule with hydroxyl propyl methylcellulose[3].

This method was tried for the preparation of microcapsules but for further study, it was not used because aggulramation was form duering preparation. SEM photograph was shown microcapsules were not formed figure 3.3.

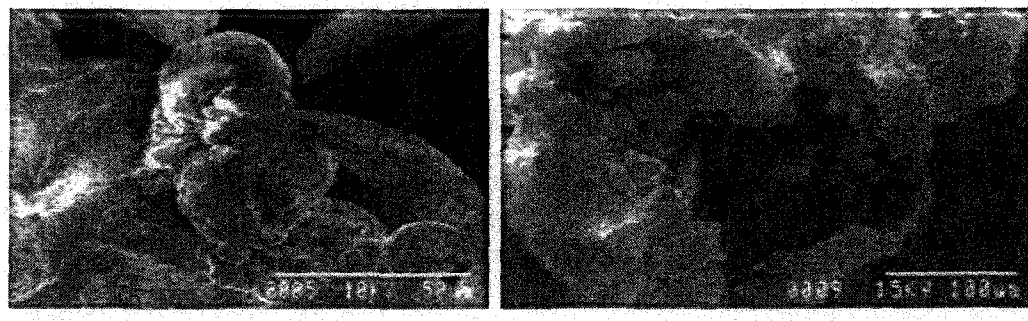


Figure 3.3: SEM photographs of microcapsules by coacervation phase separation technique

3.1.4. Preparation of microcapsules by the solvent evaporation method (I)

The drug and polymer (in different ratios) were dissolved in acetone with the help of mechanical stirring at about 500 rpm for 30 minutes and this viscous solution was added to havey liquid paraffin with uniform stirring with the help of mechanical stirring at about 500 rpm for 30min. Microcapsules (MF) were washed with petroleum ether recovered by filtering with vacuum filter. Then the microcapsules were dried in room temperature for 12 hrs to get rigid microcapsules.[4-7] The codes for the microcapsules were given as MF1, MF2, MF3, MF4, MF5, MF6, and MF7 table 3.3.

Table 3.3: Formulation design microcapsules by the solvent evaporation method (I)

Formulation code	Drug/polymer ratio	Amount of drug(mg)	Amount of polymer(mg)
MF1	1:1	200	200
MF2	1:2	200	400
MF3	1:3	100	300
MF4	1:4	100	400
MF5	1:5	100	500
MF6	1:6	100	600
MF7	1:7	100	700

Percent yield of microcapsules was found 85.00±1.31 to 97.50±0.15% table 3.4. Drug content for all microencapsulated formulations was observed good.

Table 3.4: Percent Yield of Microcapsules

Formulation code	Yield (mg)	Yield % (X±SD)
MF1	390	97.50±0.15
MF2	553	92.16±1.52
MF3	340	85.00±1.31
MF4	485	97.00±0.06
MF5	412	97.36±0.29
MF6	490	99.70±0.03
MF7	597	90.64±0.84

*All values are expressed in mean ± standard deviation (n=3)

The observations given in the column 2 in table 3.5. The drug content of all microcapsules was obtained between the ranges of 10.15±0.72 to 37.73±0.86 and the highest for MF2 3 and lowest for MF7. All the microcapsules formulation with high percent entrapment efficacy are observed (three times for each batch) for the loading efficiency. The observed data given column 3 in table 3.5, the percent drug entrapment efficiency of all microcapsules were observed between the 67.12±0.93 to 99.70±0.15 and the highest for MF6 and lowest for MF1. The particle size analysis of microcapsules was studies by using optical microscopy and SEM with magnification power 10X, 32X and 198X. The average diameter of all different microencapsulated formulations has shown column 5 in table 3.5 Polymer concentrations have a positive effect on mean particle size.

Table 3.5: Drug content, encapsulation efficiency, % CR (cumulative drug release), and average particle size of various MFs formulations

Formulation code	Drug content* (mg)(%)	*Encapsulation efficiency (%)	% CR*	*Average particle size (µm)
MF1	34.42±0.65	67.12±0.93	64.54±1.27	247.36±0.095
MF2	37.73±0.86	99.0±0.61	65.07±3.49	334.23±0.012
MF3	22.9±0.91	77.89±0.75	67.59±4.56	357.89±0.124
MF4	23.08±0.76	99.31±0.83	71.53±5.89	389.46±0.153
MF5	23.63±0.84	97.36±0.45	76.17±6.37	482.98±0.154
MF6	20.34±0.69	99.70±0.15	85.48±4.21	545.87±0.023
MF7	10.15±0.72	95.64±0.38	98.25±8.54	578.54±0.128

*All values are expressed in mean ± standard deviation (n=3)

Micromeritic studies:

The flow properties of the MFs were shown in column 3-6 of table 3.6.Form the Carr's Index, most of the MFs were having excellent to good flow properties as represented in column 3 of table 3.6.Form the Hausner's ratio, all MFs were found to have good flow properties as represented in column 4 and of table 3.6. From the angle of repose data as in column 6 of table 3.6 all MFs possessed the free flowing properties. As a general guide, values of Hausner ratio 1.25 indicate good flow (=20.0% Carr's index), while greater than 1.25 indicates poor flow (=33.0 % Carr's index). Between 1.25 and 1.5, added glidant normally improves flow. While for angle of repose $\leq 30^{\circ}$ usually indicating the free flowing material and angle $\geq 40^{\circ}$ suggested the poorly flowing material. Powder with angle of repose $>50^{\circ}$ have unsatisfactory flow properties, where as minimum angle close to 25° corresponds to very good flow property.

Table 3.6: Bulk density, angle of repose, hausner's ratio & car's index of various MFs

Formulation Code	*Bulk density (g/cc)	Carr's index	Haunser's ratio	*Angle of Repose (°)	Comment
MF1	0.571±0.31	14.26	1.16	24.7±0.07°	Excellent
MF2	0.502±0.49	17.86	1.21	23.2±0.06°	Good
MF3	0.441±0.71	11.20	1.12	20.1±0.04°	Excellent
MF4	0.667±0.63	16.75	1.19	26.4±0.07°	Good
MF5	0.526±0.81	15.84	1.18	26.4±0.02°	Good
MF6	0.400±0.23	9.90	1.11	27.1±0.05°	Excellent
MF7	0.510±0.49	12.43	1.14	21.7±0.10°	Excellent

*Each value represents as mean ± standard deviation, n=3.

Fourier transforms infrared analysis (FTIR):

FTIR is used to study the presence of drug – polymer (Chemical) interaction in the formulation. In FT-IR studies, -C-O stretching at around for Carbohydrates groups 1066.67 cm^{-1}, O-H deformation (for 1°alcohol) at around 1095.60 cm^{-1}, C-N stretching at around for 3°amine 1278.85 cm^{-1},C=O stretching at around for six member ketones 1697.41 $cm^{-1}$and $-N_3$ (for azide group) stretching at around 2085.12 cm^{-1} was clearly

distinguished in all the drug MFs formulations. All the IR peaks present in different formulations were matched with the drug peaks, were observed suggesting no drug-polymer chemical interaction figure 3.4.

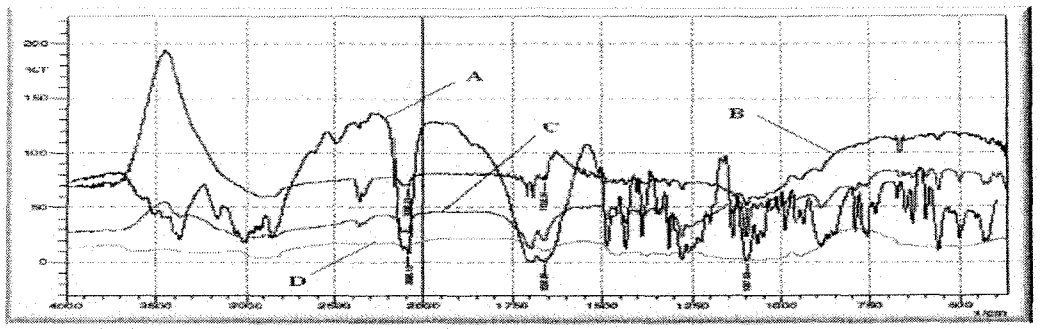


Figure 3.4:FT-IR spectra of pure drug (A); EC containing MF (B) ; MF containing Carbopol (C); MF containing HPMC(D)

The microcapsules of MFs prepared by the solvent evaporation methods were found to be discrete, spherical, free flowing. The microcapsules were size range of 247.36-578.54 µm. The SEM photographs indicated that microcapsules were nearly spherical, discreet and completely cover the coat polymer Figure 3.5.

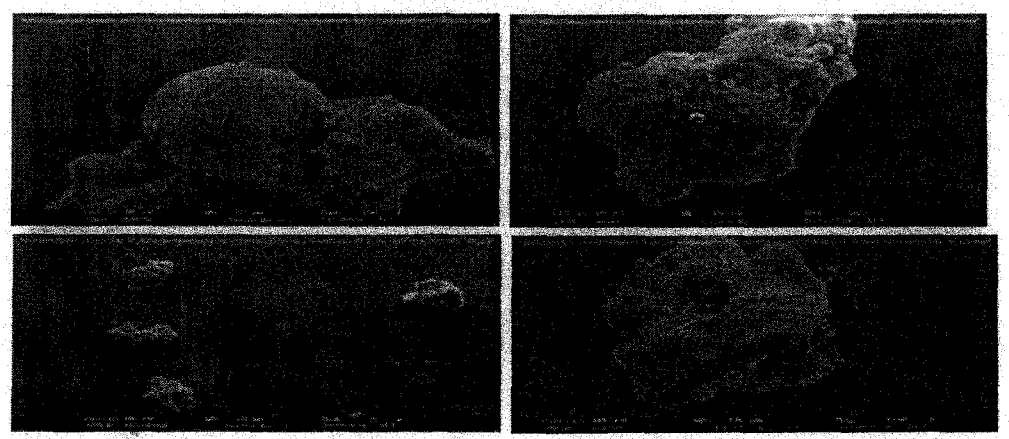


Figure 3.5: SEM study of MFs A using magnification X 500 Scanning electron microscopy of B using magnification 1500

Table 3.7: In-Vitro Drug Dissolution Profile of Vaginal Microcapsules Formulations MF1-MF7

	% Cumulative drug release(CR)						
Time (hrs)	**MF1**	**MF2**	**MF3**	**MF4**	**MF5**	**MF6**	MF7
1	**53.39±1.96**	**53.45±8.92**	**57.29±1.92**	**65.77±2.64**	**40.37±1.57**	**63.61±1.74**	**43.28±2.48**
2	53.63±2.93	54.32±4.65	61.61±6.82	66.95±7.31	53.54±2.9	70.79±2.1	54.77±5.72
3	58.79±4.35	57.95±2.9	62.66±3.57	67.13±4.64	59.37±3.72	74.77±3.65	62.90±2.19
4	60.28±3.90	59.07±1.73	65.37±1.73	67.70±5.19	65.07±1.83	77.75±3.5	69.86±1.57
5	62.82±0.89	60.86±1.37	64.45±9.57	69.82±0.92	68.32±0.84	78.35±2.16	77.35±0.93
6	61.79±1.45	62.67±6.81	53.10±1.54	68.71±1.79	70.24±3.66	81.90±3.73	84.13±1.73
7	63.64±3.71	62.73±2.73	66.72±2.9	69.22±6.83	74.23±4.62	83.10±5.35	92.77±9.21
8	65.11±0.39	64.05±0.91	69.73±3.64	72.67±7.81	73.87±0.71	87.31±8.64	92.37±0.83
9	64.21±1.96	65.90±3.39	66.24±1.63	69.94±0.93	76.24±0.85	87.31±3.64	92.37±1.73
10	64.54±1.27	65.07±3.49	67.59±4.56	71.53±5.89	76.17±6.37	85.48±4.21	98.25±8.54

The *in vitro* release profiles were applied on various kinetic models in order to find out the mechanism of drug release figure 3.6 and table 3.7. The best fit with the highest correlation coefficient was shown in Higuchi, first order and followed by zero-order equations as given in table 3.8. The rate constants were calculated from the slope of the respective plots. High correlation was observed in the Higuchi plot rather than first-order and zero-order models. The drug release was proportional to square root of time, indicating that the drug release from ethyl cellulose microcapsules was diffusion controlled. The data obtained were also put in Korsmeyer-Peppas model in order to find out n value, which describes the drug release mechanism. The n value of microcapsules of different drug to polymer ratio was ranged 0.036 to 0.370, indicating that the mechanism of the drug release was diffusion controlled. The release also showed higher correlation with the Korsmeyer- Peppas model, as shown in table 3.8.

Table 3.8: Drug release kinetic study of various MF formulations

Formulation code	Zero order release model	First order release model	Higuchi square root model	Korsmeyer and Peppas model	
	regression co-efficient(r^2)				n
MF1	0.71	0.79	0.96	0.95	0.096
MF2	0.69	0.77	0.95	0.96	0.097
MF3	0.59	0.61	0.89	0.33	0.067
MF4	0.59	0.64	0.96	0.77	0.036
MF5	0.90	0.97	0.99	0.97	0.270
MF6	0.76	0.90	0.97	0.98	0.136
MF7	0.95	0.96	0.99	0.99	0.370

n- Diffusion exponent related to mechanism of drug release, according to Korsmeyer and Peppas equation, $\mathbf{m_t / m_\alpha = kt^n}$.

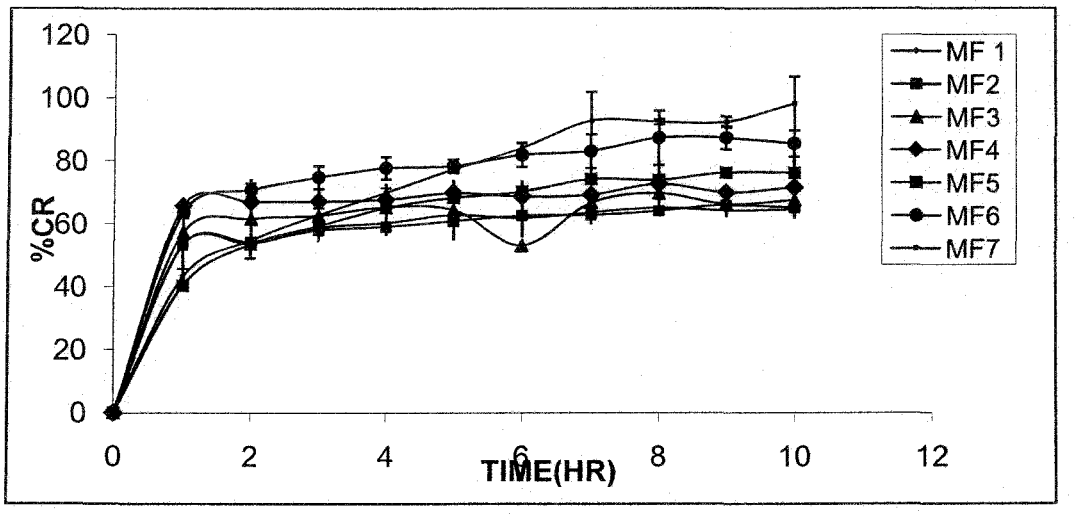


Figure 3.6: ***In-vitro*** **drug release of prepared MFs** each point represents as mean ± S.D, n=3

Initial drug release from all MFs **methods (I)** was **50%** thus selected methods was **methods (II)**.

3.1.5. Preparation of microcapsules by the solvent evaporation O/O emulsion method (II)

AZT, due to its hydrophilicity is likely to preferentially partition out into the aqueous medium, leading to low entrapment efficiency, when encapsulated using aqueous phase as the processing medium. Depending on the processing conditions as much as 80% of the AZT can partition out into the outer processing medium. In our study, attempt was made to encapsulate AZT with sufficiently high entrapment efficiency by O/O emulsion solvent diffusion method using a non-aqueous processing medium. The primary requirement of this method to obtain microcapsules is that the selected solvent system for polymer should be immiscible with non-aqueous processing medium. The optimal proportion of acetone and light liquid paraffin was found to be, which enabled emulsion formation and yielded good microcapsules. Span 60 as a surfactant was used for stabilizing primary emulsion, since ethylcellulose (EC) has the additional property of stabilizing O/O emulsion. It has the HLB value of 4.3 and is expected to have a high disparity for the present emulsion system by reducing the surface tension at the interface.

AZT microcapsules (AZMC) were prepared by solvent evaporation method using an acetone and light liquid paraffin. Span 60 was used as the droplet stabilizer. Accurately weighed, different quantities of Ethyl cellulose (EC) were dissolved in 20 ml acetone (HPLC Grade) by using a magnetic stirrer. The drug was mixed with the polymer solution followed by stirring with magnetic stirrer for 15 minutes. The resulting dispersion was then poured into 250 ml beaker containing the mixture of 50 ml light liquid paraffin (as continuous phase), while stirring. A mechanical stirrer with a three bladed paddle was used for stirring (at 1000 rpm) with heating (at 40°C) and was continued for 2 hours, until complete evaporation of acetone. After evaporation of

acetone, the AZMC formed were filtered using filter paper [8-10]. The residue was washed for 4-5 times by 10 ml of ether. AZMC were dried at room temperature for 24 hours and kept in desiccators for further evaluations. All batches were prepared in triplicate as shown in table 3.9 and figure 3.7. The microcapsules were prepared by O/O single emulsion solvent evaporation method I using ethyl cellulose as polymer in different ratio with other ingredients and solvents as given in table 3.9. All the prepared microencapsulated formulations contains different drug: polymer ratio and coded as AZMC1 (1:1), AZMC 2 (1:2), AZMC3 (1:3), AZMC4 (1:4), AZMC 5 (1:5), AZMC 6 (1:6) and AZMC 7 (1:7).

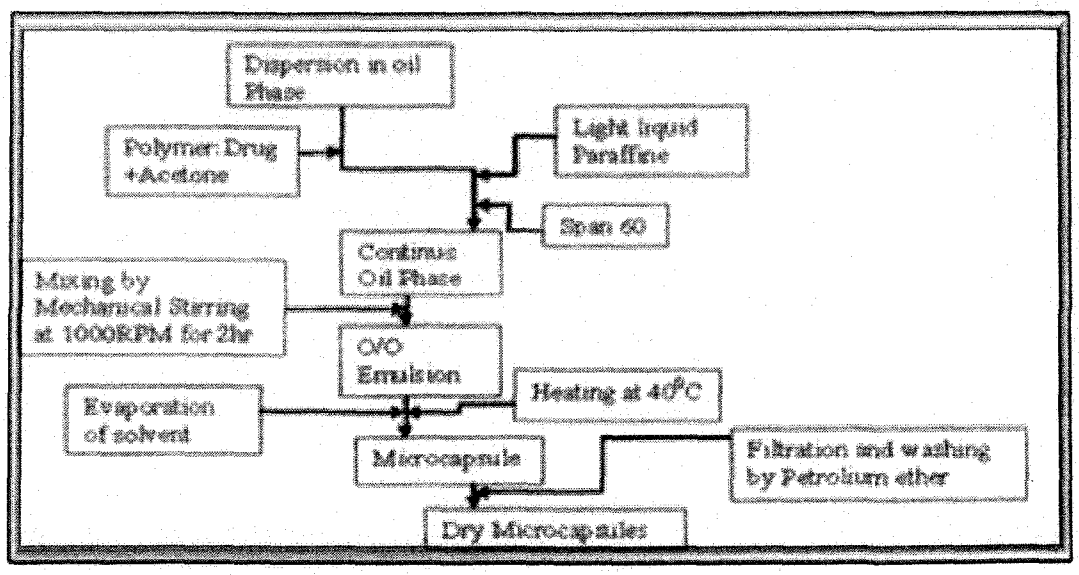


Figure 3.7 Schematic representation of method II

Table 3.9: Formulation design of AZMC

Formulation Code	Drug/polymer ratio	Drug (in mg)	Polymer (in mg)	Span 60 (in mg)	Light liquid paraffin (ml)
AZMC 1	1:1	100	100	10	50
AZMC 2	1:2	100	200	10	50
AZMC 3	1:3	100	300	10	50
AZMC 4	1:4	100	400	10	50
AZMC 5	1:5	100	500	10	50
AZMC 6	1:6	100	600	10	50
AZMC 7	1:7	100	700	10	50

3.2.1. Scanning of Pure AZT for λ_{max} and Preparation of standard curve of AZT in acetate buffer pH 4.7

Method: 50 mg Standard sample of AZT was dissolved in 100 ml of acetate buffer I.P. of pH 4.7 (as a Simulated Vaginal Fluid SVF)[11,12] and from that 1ml was further diluted in 100 ml of acetate buffer pH 4.7. This final dilution with the concentration of 5.0µg/ml was scaned for λmax using UV spectrophotometer (UV – 1700, Shimadzu, Japan). Accurately 100 mg of AZT was taken and dissolved in 100 ml of SVF[12] (Solution – A). 1 ml solution was pipette out from solution – A, and then diluted it in a 10 ml volumetric flask (Solution - B). From solution – B different volumes of 0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml were taken and diluted up to 10 ml with acetate buffer. The absorbance was scanned against blank here used as SVF. A graph was plotted by taking concentration versus absorbance. The slope and regression value was calculated from the graph table 3.10 and figure 3.8.[13]

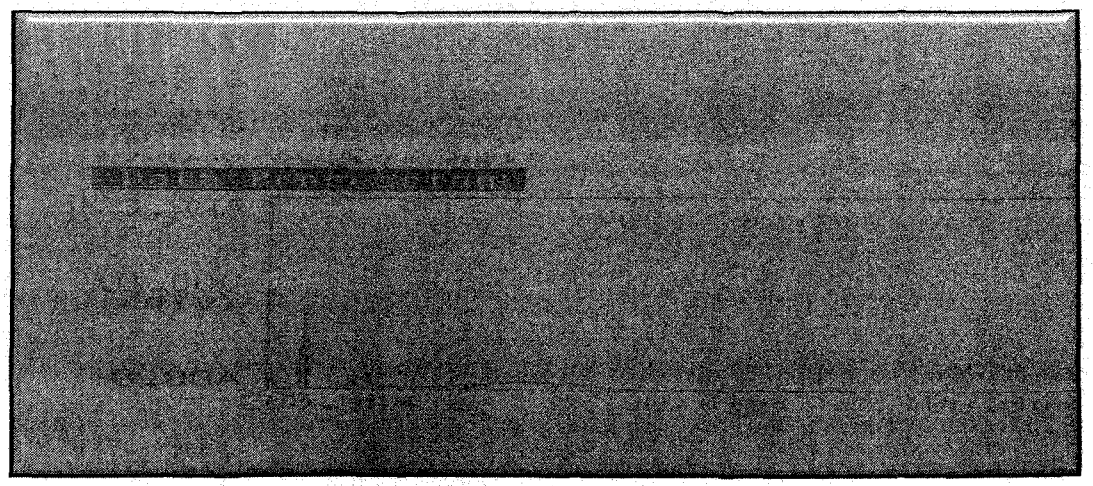


Figure 3.8 Scanning of Pure AZT

From the Figure 3.8, the λ_{max} of AZT in SVF (Simulated Vaginal Fluid) was found to be 267 nm.

Table 3.10: Standard curve data of AZT using acetate buffer pH 4.7:

Serial No.	Conc. (µg/ml)	Absorbance
1.	0.000	0.000
2.	2.000	0.076
3.	4.000	0.154
4.	6.000	0.235
5.	8.000	0.309
6.	10.000	0.384
7.	12.000	0.460

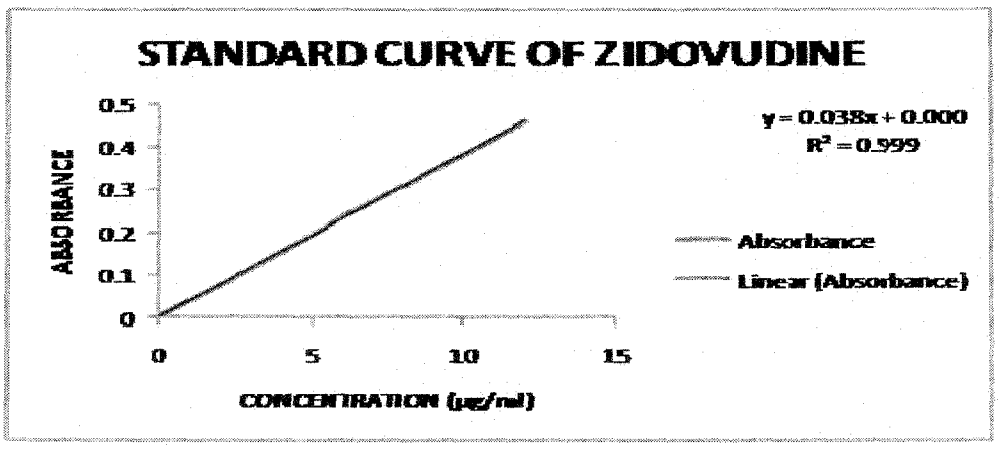


Figure 3.9: Standard curve of AZT using acetate buffer pH4.7

The standard curve of AZT in acetate buffer pH 4.7 was found to be given in Figure 3.9. The regression co – efficient (r^2) was found to be 0.999 and equation was Y = 0.0384X + 0.000. The data of standard curve was represented in table 3.10 and figure 3.9.

3.2.2 EVALUATION OF PREPARED MICROCAPSULES:

3.2.2.1 Percentage yield:

Method: Microcapsules dried at room temperature were then weighed and the yield of microcapsules was calculated using the following formula[14]

% Yield= (Weight of the AZMCs recovered from each batch / Total weight of drug and polymer used to prepare that batch) x 100

The percent yields of microcapsules were calculated and found to be into the range of 65.16 ± 5.89 to 83.15 ± 6.46. The percent yield of AZMC4 was found to be 72.93 ± 0.73 in column 2 in table 3.11.

3.2.2.2 Determination of drug content:

Method: Drug content means the actual amount of drug present in the formulation. About 50 mg of accurately weighed drug – loaded microcapsules were dissolved in 2 ml of acetone and than make up its volume was made up to 50 ml in volumetric flask with SVF. The resulting mixture was agitated on a mechanical shaker and then kept aside for 24 hours.[15] The solution was then filtered and the absorbance was measured at 267 nm using UV – Visible spectrophotometer and drug content was determined by using the following formula:

$$\mathbf{D_C = (C_C \times D_F \times V) / C_F}$$

Where as D_C =Drug Content,C_C = Concentration,D_F Dilution Factor, V =Volume taken, C_F =Conversion Factor

Drug content for all microencapsulated formulations was observed good. The drug content of all microcapsules was obtained between the ranges of 9.13 ± 0.62 to 36.97 ± 5.35 and the highest for AZMC3 and lowest for AZMC7shown in column 3 in table 3.11. Drug content for AZMC4 was found 24.58 ±0.87mg/100mg of microcapsules.

3.2.2.3 Determination of drug entrapment efficiency:

Method: The entrapment efficiency of the microcapsules or the percent entrapment can be determined by allowing washed microcapsules to lyses. The lysate was then subjected

to the determination of active constituents. The percent encapsulation efficiency is calculated using following equation[15]

$$\% E_f = (A_{dc} / T_{dc}) \times 100$$

Where as E_f =Entrapment Efficiency, A_{dc} = (Actual Drug content, T_{dc} =Theoretical Drug Content)

The drug encapsulation efficiency was found to be within the range of 15.37 ± 0.45 to 93.92 ± 4.59% and the highest for AZMC3 and lowest for AZMC1 was shown in table 3.11. AZMC4 was shown 89.75 ± 3.52 % in column 4 of table 3.11.The increased encapsulation efficiency may be attributed to the hydrophobic nature of ethyl cellulose and sparingly soluble AZT. The encapsulation efficiency was found to be nearly increased with increase in polymer content but after some limit, the concentration of polymer did not affect encapsulation efficiency, due to the complete encapsulation of drug. As usual, it was not affected even if the concentration of polymer increased. The highest 93.92 ± 4.59% entrapment efficiency was achieved by increasing polymer-drug ratio from 1:1 to 1:4 and further increased in polymer-drug ratio from 1:5 to 1:7, a significant decrease was observed. Among the different polymer-drug ratios investigated, 1:4 polymer- drug ratios had the optimum capacity for drug encapsulation.

Table 3.11: Percent yield, drug content, %drug entrapment and average particles diameter of AZMCs formulation

Formulation code	Percent yield (Mean ± SEM)	Drug content (mg/100mg of microcapsules)	Percent drug entrapment efficiency (Mean ± SEM)	Average particles diameter (µm) (Mean ± SEM)
AZMC 1	76.33 ± 1.45	10.07 ± 0.23	15.37 ± 0.45	84.50 ± 2.00
AZMC 2	70.99 ± 8.18	23.96 ± 4.81	50.62 ± 9.77	87.80 ± 5.71
AZMC 3	65.16 ± 5.89	36.97 ± 5.35	93.92 ± 4.59	94.64 ± 3.21
AZMC 4	**72.93 ± 0.73**	**24.58 ± 0.87**	**89.75 ± 3.52**	**84.33 ± 4.35**
AZMC 5	83.15 ± 6.46	16.99 ± 1.42	83.60 ± 4.63	92.34 ± 4.18
AZMC 6	74.33 ± 6.98	14.44 ± 1.24	67.32 ± 8.86	84.49 ± 2.03
AZMC 7	68.31 ± 5.54	09.13± 0.62	49.85 ± 5.28	70.12 ± 9.17

All values are expressed in mean ± SEM (n=3)

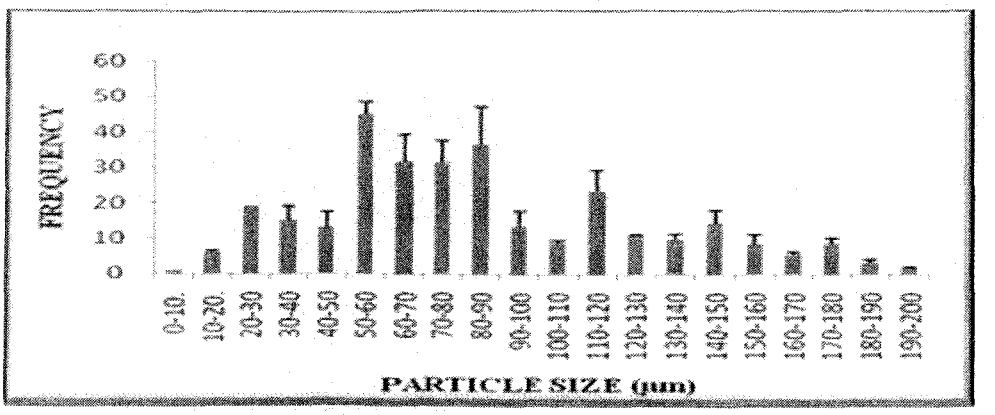
3.2.2.4 Particle size analysis of microcapsules:

Method: About 200 particles of each batch of preparations were transferred on a slide and their size and shape were investigated by a scaled optical microscope with magnification ratio of 10x.[17,18]

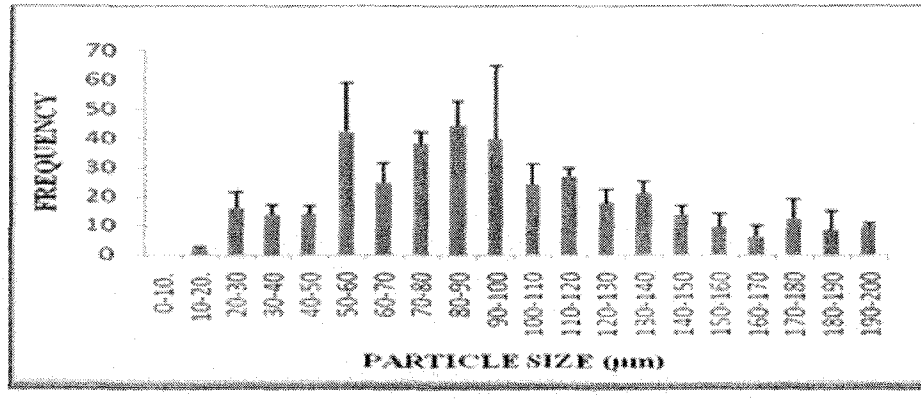
$$\mathbf{D = \sum nd / \sum n}$$

Where, D=Average particle size, $\sum$nd = Sum of the product of mean size range and no. of particles each size range, $\sum$n = Total no. of particlesFrom the weight distribution the average mean diameter.

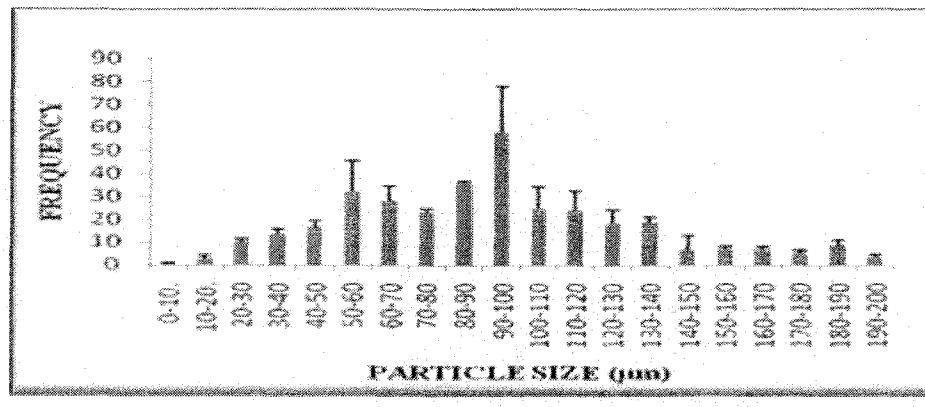
The particle size analysis of microcapsules was studies by using optical microscopy 10X. The average diameter of all different microencapsulated formulations has shown column 5 in table 3.11 and the highest average particles diameter for AZMC3 (94.64 ± 3.21 μm), lowest for AZMC7 (70.12 ± 9.17 μm) and AZMC4 (84.33 ± 4.35) were found figure 3.10-3.16.



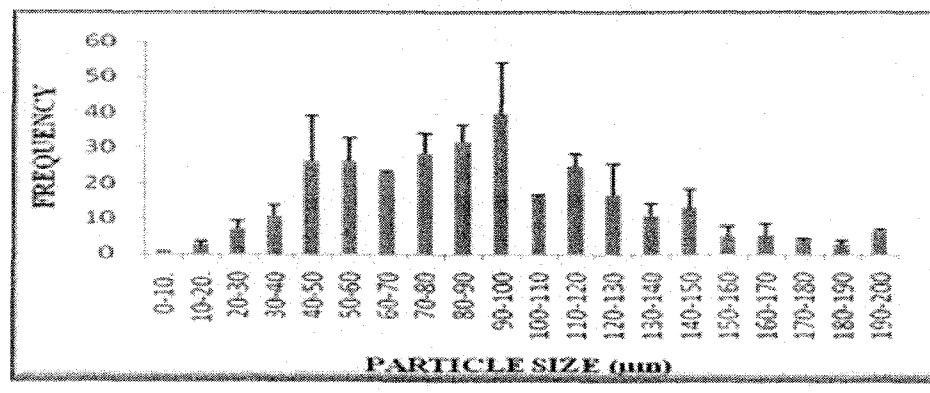
Figurer 3.10 Particle size analysis of AZMC1



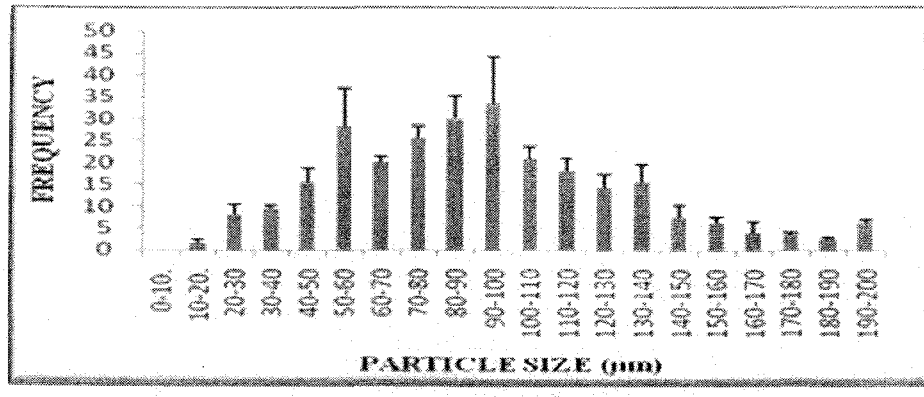
Figurer 3.11 Particle size analysis of AZMC2



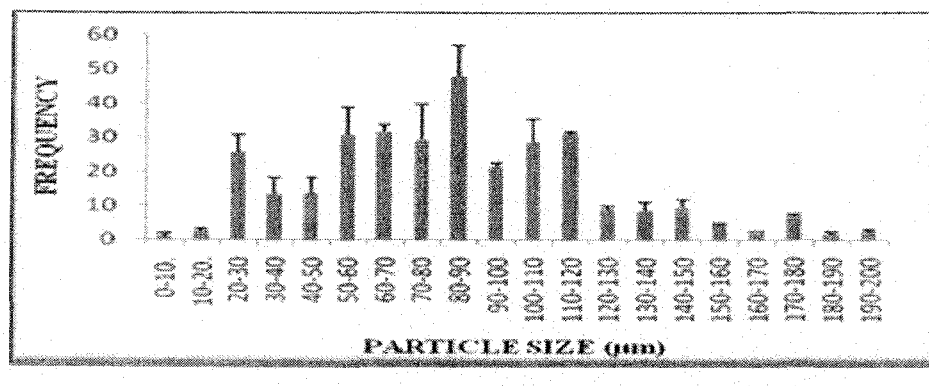
Figurer 3.12 Particle size analysis of AZMC3



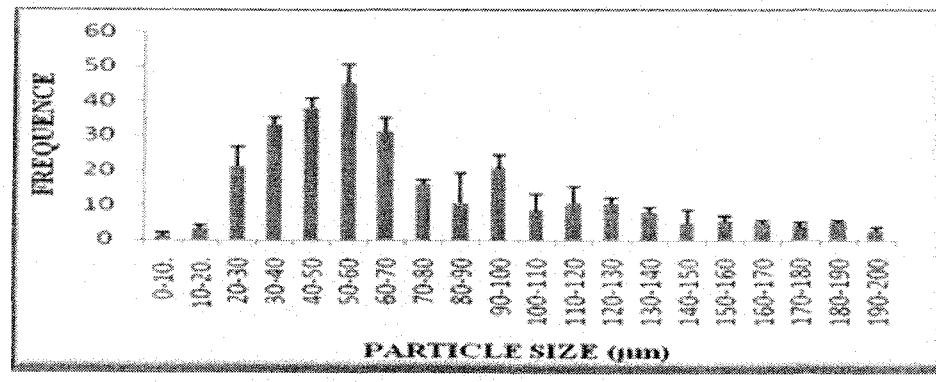
Figurer 3.13 Particle size analysis of AZMC4



Figurer 3.14 Particle size analysis of AZMC5



Figurer 3.15 Particle size analysis of AZMC6



Figurer 3.16 Particle size analysis of AZMC7

3.2.2.5 Determination of percentage of moisture loss:

Method: The AZT loaded microcapsules was evaluated for % of moisture loss which sharing an idea about its hydrophilic nature. The microcapsules weighed initially kept in

desiccators containing calcium chloride at 37^0C for 24 hours. The final weight was noted and moisture loss was calculated using following equation[19]

$$\mathbf{M_L=[(I_0-F_0)/F_W]x100}$$

Where as M_L =moisture loss, I_0=Initial weight, F_0 =Final weight, F_W =Final weight

Table 3.12: Determination of percentage of moisture loss

Formulation code	Percent moisture loss (Mean ± SEM)
AZMC 1	28.03 ± 2.87
AZMC 2	26.40 ± 7.25
AZMC 3	25.40 ± 6.44
AZMC 4	**23.53 ± 2.76**
AZMC 5	20.87 ± 3.78
AZMC 6	20.23 ± 0.07
AZMC 7	18.17 ± 1.57

The observation table 3.12 shows the moisture loss content for all microcapsules of moisture loss contents highst (AZMC1) 28.03 ± 2.87 and lowest (AZMC7) 18.17 ± 1.57. In case of AZMC4 was found 23.53± 2.76.

3.2.2.6 Scanning Electron Microscopy (SEM):

Method: The morphology and surface characteristics of prepared microcapsules were observed by (LEO, 435 VP, U.K.). Microcapsules were coated by putting these in stub with gold in an argon atmosphere, and then put these for 20 minutes in a stub (or plate). The surface morphology of the microcapsules was then studied by scanning electron microscopy (SEM).The samples were imaged using the magnification 1500 and 600.[14,20] The microcapsules of AZT prepared by the solvent evaporation methods were found to be discrete, spherical, free flowing. The microcapsules were size range of 70-94 μm. The SEM photographs indicated that microcapsules were spherical and completely cover the coat polymer figure 3.17

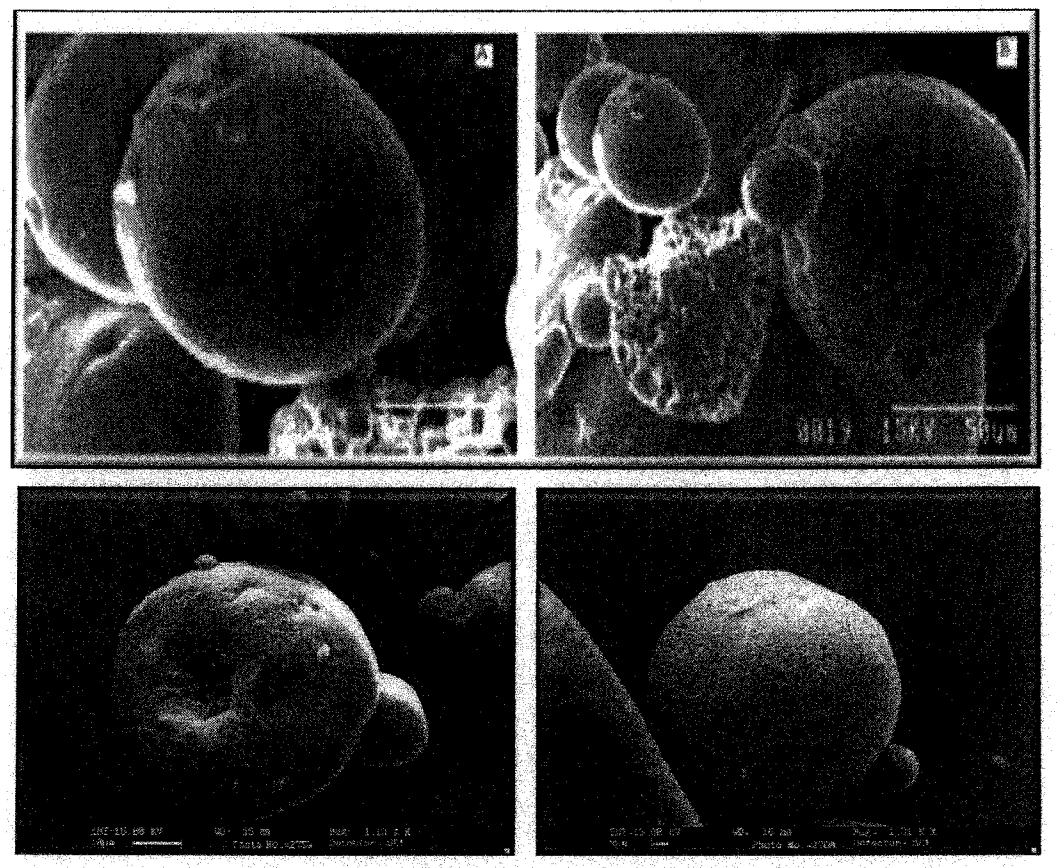


Figure 3.17: SEM study of AZMCs A using magnification X 600 Scanning electron microscopy of B using magnification X 1500

3.2.2.7 Fourier Transforms Infrared Analysis (FTIR):

FTIR (8400S, Shimadzu, Japan) is used to study the presence of drug – polymer (Chemical) interaction in the formulation.

Method: FTIR spectra of AZT, black microcapsules and drug loaded microcapsules were obtained[14,15] in KBr pellets using a FTIR the ranges 400 to 4000 cm^{-1}.

FTIR is used to study the presence of drug – polymer (Chemical) interaction in the formulation. In FT-IR studies, -C-O stretching at around for Carbohydrates groups 1066.67 cm^{-1}, O-H deformation (for 1°alcohol) at around 1095.60 cm^{-1}, C-N stretching at

around for 3°amine 1278.85 cm^{-1},C=O stretching at around for six member ketones 1697.41 $cm^{-1}$and $-N_3$ (for azide group) stretching at around 2085.12 cm^{-1} was clearly distinguished in all the drug AZMCs formulations. All the IR peaks present in different formulations were matched with the drug peaks, were observed suggesting no drug-polymer chemical interaction figure 3.19a, 3.19b and table 3.13.

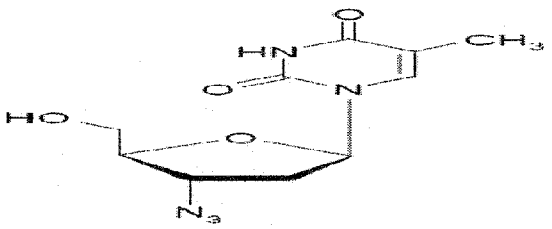


Figure 3.18: Chemical Structure of AZT

Table 3.13: Peaks table for AZT and AZMCs formulations

Formulation Code	C-O str (for Carbohydrates) cm^{-1}	O-H def (for 1°alcohol) cm^{-1}	C-N str (for 3°amine) (cm^{-1})	C=O str (for six membered ketones) (cm^{-1})	$-N_3$ (for azide group) (cm^{-1})
Zidovudine	1066.67	1095.60	1278.85	1697.41	2085.12
AZMC1	1066.67	1095.60	1278.85	1697.41	2085.12
AZMC2	1066.67	1097.53	1278.85	1697.41	2087.05
AZMC3	1066.67	1097.53	1278.85	1697.41	2088.98
AZMC4	**1066.67**	**1095.60**	**1278.85**	**1697.41**	**2085.12**
AZMC5	1066.67	1095.60	1278.85	1697.41	2085.12
AZMC6	1066.67	1097.53	1278.85	1697.41	2087.05
AZMC7	1066.67	1097.53	1278.85	1697.41	2087.05

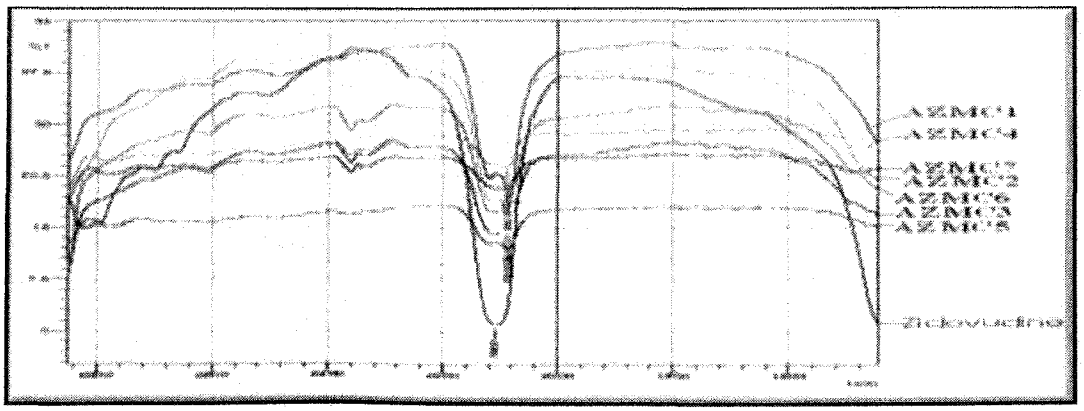


Figure 3.19a: Combined FTIR graph of AZT and all formulations

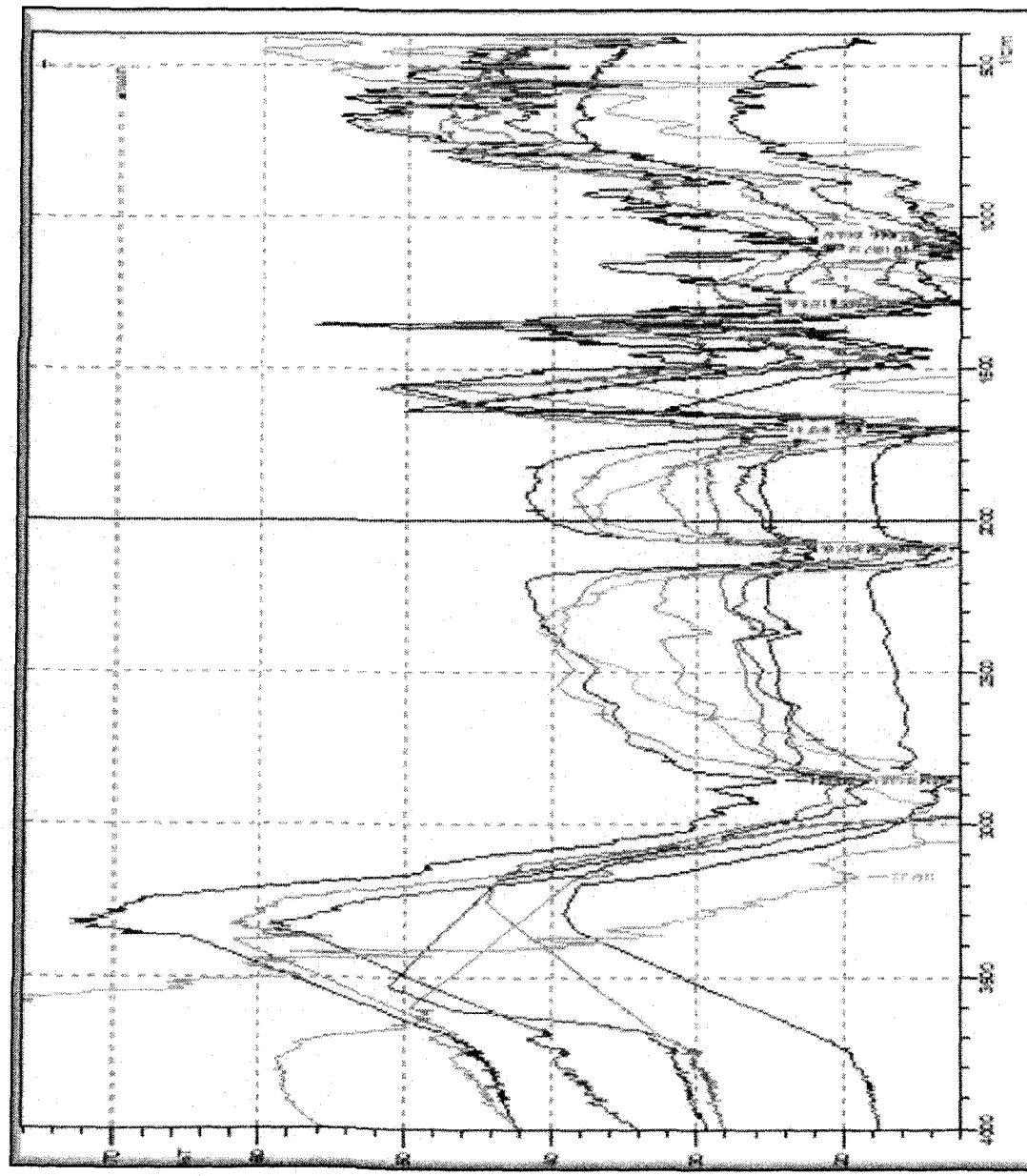


Figure 3.19b: Combined FTIR graph of AZT and all AZMCs formulations

3.2.2.8 Micromeritic studies:

The flow properties of the microcapsules were investigated by measuring the bulk density, tapped density, Carr's index, Hausner ratio and angle of repose.

3.2.2.8.1 Angle of repose:

Method: The angle of repose was determined by fixed funnel method.The angle of repose was measured from a heap carefully built up by dropping the 100 gm of microcapsules through a glass funnel to the horizontal plate of a powder characteristic tester.[21,22] The angle of repose was calculated using the following equation:

$$\theta = \tan^{-1}(h/r)$$

Where, θ is the angle of repose, h is the height of the pile of microcapsules, and r is the radius of the base.

3.2.2.8.2 Carr's index:

Method: The AZMCs were placed in a measuring cylinder and tapped using bulk density apparatus (Excel Enterprises, Kolkata) for 100 taps and the change in volume was measured. [21,22]

$$CI = [(T^d - B^d) / T^d] \times 100$$

CI=Carr's index (%), T^d =Tapped density, B^d= Bulk density

3.2.2.8.3 Hausner ratio:

Method: Hausner ratio is a measure of flow ability of microcapsules or drug and is calculated using equation given below.[23]

$$H^0 = B^d / T^d$$

Where as H^0 =Hausner ratio, B^d = Bulk density, T^d =Tapped density.

The flow properties of the AZMCs were shown in column 2-6 of table 3.14. Form the Carr's Index, most of the AZMCs were having excellent to good flow properties as represented in column 6 of table 3.14.Form the Hausner's ratio, all AZMCs were found to have good flow properties as represented in column 4 and column 5 of table 3.14. From the angle of repose data as in column 6 of table 3.14all AZMCs possessed the free flowing properties. AZMC4 has the good angle of repose (28.15 ° ±0.001), Carr's index (14.24%± 0.080) and Hausner ratio (1.17± 0.001) values and having the good flow properties. As a general guide, values of Hausner ratio 1.25 indicate good flow (=20.0% Carr's index), while greater than 1.25 indicates poor flow (=33.0 % Carr's index). Between 1.25 and 1.5, added glidant normally improves flow. While for angle of repose ≤ 30° usually indicating the free flowing material and angle ≥40° suggested the poorly flowing material. Powder with angle of repose >50° have unsatisfactory flow properties, where as minimum angle close to 25° corresponds to very good flow property.

Table 3.14: Observation table for bulk density, tapped density, Carr's index, Hausner ratio and angle of repose

Formulations Code	Bulk Density	Tapped Density	Carr's Index	Hausner Ratio	Angle of Repose
AZMC **1**	0.31 ± 0.001	0.37 ± 0.001	17.27 ± 0.496	1.21 ± 0.007	28.38 ± 0.118
AZMC **2**	0.31 ± 0.002	0.37 ± 0.001	16.99 ± 0.258	1.21 ± 0.004	28.673 ± 0.526
AZMC **3**	0.31 ± 0.002	0.37 ± 0.000	17.30 ± 0.468	1.21 ± 0.007	28.676 ± 0.366
AZMC 4	**0.31 ± 0.002**	**0.36 ± 0.002**	**14.24 ± 0.080**	**1.17 ± 0.001**	**28.15 ± 0.001**
AZMC **5**	0.31 ± 0.002	0.36 ± 0.002	16.28 ± 0.936	1.19 ± 0.001	29.084 ± 0.479
AZMC **6**	0.31 ± 0.001	0.36 ± 0.004	16.27 ± 0.990	1.19 ± 0.014	29.372 ± 0.63
AZMC **7**	0.31 ± 0.001	0.37 ± 0.000	17.57 ± 0.270	1.21 ± 0.004	29.89 ± 0.172

3.3. *In-vitro* drug release study:

Method: *In-vitro* drug release study of drug from the AZMCs was studied by using USP type I dissolution test apparatus (TDT-08L USP, Electrolab, Kolkata, India). The receptor compartment (a cylindrical vessel) was contained 900 ml SVF that was within the vaginal pH and maintained at a temperature of 37±1°C. The AZMCs, equivalent to 100 mg of AZT were placed in a basket covered with muslin cloth. The *in-vitro* drug release studies were carried out for 24 hr and the dissolution medium was stirred at 50 rpm. At predetermind time intervals 5 ml aliquots were withdrawn and replaced by an equal volume of fresh pre-warmed dissolution medium maintaining sink condition, the samples were analyzed for drug quantification at 267 nm using UV – Visible Spectrophotometer, (UV – 1700, Shimadzu, Japan). The concentrations of AZT in samples were calculated .[24] The *in vitro* drug releases of acquired AZMCs were shown in figure 3.20, table 3.15 and table 3.16. The *in vitro* drug release profile of all the AZMC1-AZMC7 was in the range of 63.41± 5.36 to 85.46± 7.14% respectively upto 24hrs only. *In vitro* drug release of all AZMCs were shown significantly drug released ($p<0.01$) in single factor ANOVA. It means null hypothesis was nullified and alternative hypothesis is accepted i.e. conclude that the drug release among the AZMCs (AZMC1-AZMC7) differ significantly table 3.17-3.23. The microcapsules AZMC4 was found to release the drug of about 63.41 ± 5.36% only, even after 24hrs, thus concluded to have sustained drug release profile for longer period of time when compared to other AZMCs. The *in vitro* release of AZT from ethylcellulose microcapsules exhibited initial burst effect, which was due to the presence of drug particles on the surface of the microcapsules. The initial burst effect may be attributed as a desired effect to ensure initial therapeutic plasma concentrations of drug.

Factors such as polymer drug ratio and presences of surfactant for emulsification the drug release from microcapsules. In order to keep the total surface area of the microcapsules constant and thus to get comparable results, the release studies were carried out using the same size fractions of microcapsules containing equivalent amount of AZT from different batches. Drug release rates increased with increasing amounts of AZT in the formulation. Higher level of AZT corresponding to lower level of the polymer in the formulation resulted in an increase in the drug release rate. In addition, higher drug levels in the microcapsules formulation produced a higher drug concentration gradient between the microcapsules and dissolution medium, thus drug release rate was increased. This may be attributed to the presence of more free drug on the surface of the microcapsules with Span 60 for emulsification process. The faster drug release was observed from microcapsules prepared using large volume of processing medium. It may be due to the higher migration of drug due to free movement of emulsion droplets with increasing volume of processing medium. The release rate was generally decreases with increased proportion of polymer up to AZMC4.

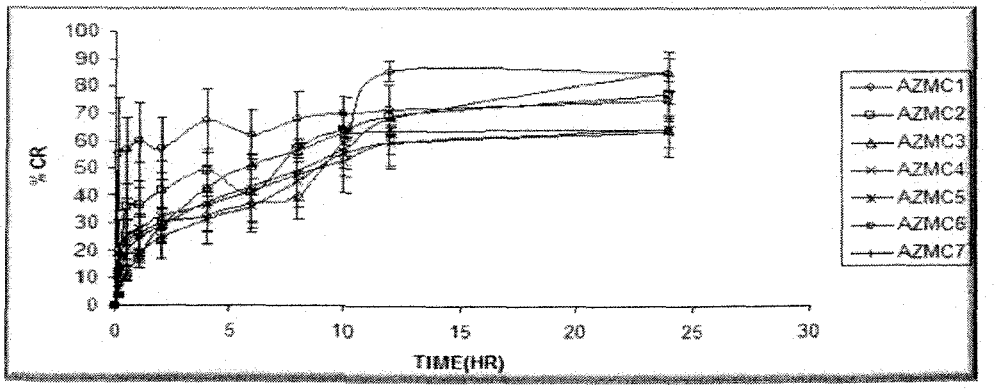


Figure 3.20: *In-Vitro* Drug Release Profile of AZMCS Formulations

Table 3.15: Percent cumulative drug release study for AZMC1 – AZMC4:

Time (hrs.)	% Cumulative Drug Release (%CR)			
	AZMC1	AZMC2	AZMC3	AZMC4
0.17	11.53 ± 6.52	11.58 ± 1.75	4.15 ± 0.17	**8.39 ± 4.79**
0.50	23.68 ± 7.21	35.58 ± 20.85	10.54 ± 1.97	**17.79 ± 3.78**
1.00	26.00 ± 5.78	36.12 ± 16.15	18.05 ± 0.65	**19.23 ± 1.28**
2.00	29.82 ± 5.64	41.57 ± 11.27	28.51 ± 0.43	**24.87 ± 2.56**
4.00	33.04 ± 6.66	49.14 ± 8.31	43.06 ± 0.36	**31.48 ± 4.59**
6.00	37.05 ± 7.13	41.14 ± 14.64	51.06 ± 0.15	**36.39 ± 5.86**
8.00	39.96 ± 8.45	57.48 ± 3.55	56.67 ± 1.27	**45.35 ± 7.31**
10.00	60.58 ± 10.13	64.42 ± 0.93	62.89 ± 1.18	**55.45 ± 8.04**
12.00	85.59 ± 3.67	68.88 ± 2.57	63.89 ± 1.14	**59.59 ± 8.22**
24.00	84.42 ± 5.77	76.81 ± 7.37	64.18 ± 1.34	**63.41 ± 5.36**

All values are expressed in mean ± SEM (n = 3).

Table 3.16: Percent cumulative drug release study for AZMC 5 – AZMC 7:

Time (hrs.)	%Cumulative Drug Release (%CR)		
	AZMC5	AZMC6	AZMC7
0.17	19.09 ± 11.93	54.96 ± 20.76	13.33 ± 4.88
0.50	24.58 ± 14.16	56.48 ± 12.23	18.81 ± 7.29
1.00	28.04 ± 14.42	59.73 ± 14.34	24.16 ± 8.83
2.00	32.49 ± 15.63	57.18 ± 11.29	28.77 ± 5.88
4.00	36.63 ± 14.63	67.71 ± 11.59	37.75 ± 5.66
6.00	41.53 ± 13.38	62.54 ± 8.89	43.44 ± 3.12
8.00	47.61 ± 11.63	68.14 ± 10.14	49.38 ± 3.18
10.00	52.88 ± 11.88	69.89 ± 6.38	56.87 ± 1.92
12.00	59.41 ± 9.25	71.29 ± 9.32	68.15 ± 12.42
24.00	64.24 ± 9.82	74.85 ± 7.05	85.46 ± 7.14

All values are expressed in mean ± SEM (n = 3)

Table 3.17 Analysis of variance of *in-vitro* drug release of all AZMCs:

Source	SS	df	Mean Squares	*F*-ratio	Tabulated value
Treatment between column	524.555	7	74.936	521.967	$F_{0.05}$=8.89 $F_{0.01}$ =27.67
Residual	0.431	3	0.144		
Total		10			

Since the observed value *F* is larger than the 1% tabulated value corresponding to *d.f* (7,3), we reject the null hypothesis and conclude that the drug release among the AZMCs differ significantly (P<0.01).

Table 3.18 Analysis of Variance of *in-vitro* drug release AZMC 4 and AZMC1

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	421.535	2	210.768	16.299	$F_{0.05}$=4.46 $F_{0.01}$=8.65
Residual	103.450	8	12.931		
Total		10			

Since the observed value *F* is larger than the 1% tabulated value corresponding to *d.f* (2,8), we reject the null hypothesis and conclude that the drug release among the AZMC4 and AZMC1 differ significantly (P<0.01).

Table 3.19 Analysis of Variance of *in-vitro* drug release AZMC 4 and AZMC2

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	361.166	2	180.583	8.819	$F_{0.05}$=4.46 $F_{0.01}$=8.65
Residual	163.820	8	20.477		
Total		10			

Since the observed value *F* is larger than the 1% tabulated value corresponding to *d.f* (2,8), we reject the null hypothesis and conclude that the drug release among the AZMC4 and AZMC2 differ significantly (P<0.01).

Table 3.20 Analysis of Variance of *in-vitro* drug release AZMC 4 and AZMC3

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	427.001	2	213.500	17.431	$F_{0.05}$=4.46 $F_{0.01}$=8.65
Residual	97.985	8	12.248		
Total		10			

Since the observed value *F* is larger than the 1% tabulated value corresponding to *d.f* (2,8), we reject the null hypothesis and conclude that the drug release among the AZMC4 and AZMC3 differ significantly (P<0.01).

Table 3.21 Analysis of Variance of *in-vitro* drug release AZMC 4 and AZMC5

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	381.686	2	190.843	10.654	$F_{0.05}$=4.46 $F_{0.01}$=8.65
Residual	143.299	8	17.912		
Total		10			

Since the observed value *F* is larger than the 1% tabulated value corresponding to *d.f* (2,8), we reject the null hypothesis and conclude that the drug release among the AZMC4 and AZMC5 differ significantly (P<0.01).

Table 3.22 Analysis of Variance of *in-vitro* drug release AZMC 4 and AZMC6

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	343.014	2	171.507	7.540	$F_{0.05}$=4.46 $F_{0.01}$=8.65
Residual	181.971	8	22.746		
Total		10			

Since the observed value *F* is larger than the 5% tabulated value corresponding to *d.f* (2,8), we reject the null hypothesis and conclude that the drug release among the AZMC4 and AZMC6 differ significantly (P<0.05).

Table 3.23 Analysis of Variance of *in-vitro* drug release AZMC 4 and AZMC7

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	486.527	2	243.264	50.604	$F_{0.05}$=4.46 $F_{0.01}$=8.65
Residual	38.458	8	4.807		
Total		10			

Since the observed value *F* is larger than the 1% tabulated value corresponding to *d.f* (2,8), we reject the null hypothesis and conclude that the drug release among the AZMC4 and AZMC7 differ significantly (P<0.01).

3.3.1 *In-vitro* drug release kinetics:

Method: In order to investigate the mechanism of AZT release from microcapsules of different Ethyl cellulose: AZT ratios, the release data were analyzed with the following mathematical models: Zero-order kinetic (equation a), first – order kinetic (equation b), and Higuchi kinetic (equation c).

$$Q_t = K_0 t \quad \text{---------- (a)}$$

$$\ln Q_t = \ln Q_o - K_1 t \quad \text{---------- (b)}$$

$$Q_t = K_h t^{1/2} \quad \text{---------- (c)}$$

The following plots were made: Q_t verses t (zero – order kinetic model), ln (Q_0 – Q_t) verses t (first – order kinetic model) and Q_t verses $t^{1/2}$ (Higuchi model).[24,26]

Where, Q_t is the percent of drug released at time t, Q_0 is the percent of drug present in the microcapsuless, K_0, K_1 and K_h are the constants of the equations.

Further, to confirm the mechanism of drug release, the first 60 % of drug release was fitted in Korsmeyer – Peppas model (equation d):

$$M_t/M_\alpha = K_p t^n \quad \text{---------- (d)}$$

Where, M_t/M_α is the fraction of the drug release at time t, K_P is the rate constant, n value is used to characterize different release mechanisms, and is calculated from the slope of log of fraction of drug released (M_t/M_α) verses of time (t).[24,27]

The following plots were made: cumulative % drug release vs time (zero order kinetic models); log cumulative of % drug remaining vs time (first order kinetic model); cumulative % drug release vs square root of time (Higuchi model); and log cumulative % of drug released vs log time (Korsmeyer Peppas model).[25]

The *in vitro* release profiles were applied on various kinetic models in order to find out the mechanism of drug release. Inc case of AZMC4 shown the best fit with the highest correlation coefficient was shown in, zero-order, Higuchi and followed first order by equations as given in table 3.24. The rate constants were calculated from the slope of the respective plots. High correlation was observed in the zero-order and Higuchi plot models rather than first-order models. The drug release was proportional to square root of time, indicating that the drug release from ethyl cellulose microcapsules was diffusion controlled. The data obtained were also put in Korsmeyer-Peppas model in order to find out n value, which describes the drug release mechanism. The n value of microcapsules of different drug to polymer ratio was ranged from 0.071 to 0.564. Indicating that the mechanism of the drug release was diffusion controlled. In case of Fickian release mechanism, the rate of drug release is much lesser than that of polymer relaxation (swelling/erosion). So the drug release was chiefly dependent on the diffusion through the matrix.The release also showed higher correlation with the Korsmeyer- Peppas model, as shown in table 3.24.

Table 3.24: Drug release and kinetic study of AZMCs

Formulations	**Cumulative % Drug release (24 h) study**	**Zero order release**		**First order release**		**Higuchi square root**		**Korsmeyer and Peppas equation**	
	%CR	K_0	r^2	K_1	r^2	K_h	r^2	r^2	n
AZMC1	84.42± 5.77	3.13	0.81	1.93	0.78	0.18	0.91	0.82	0.349
AZMC2	76.81± 7.37	2.79	0.74	1.83	0.87	0.14	0.89	0.85	0.213
AZMC3	64.18± 1.34	2.79	0.65	1.88	0.71	0.24	0.91	0.98	0.564
AZMC4	**63.41± 5.36**	**3.96**	**0.97**	**1.91**	**0.86**	**0.18**	**0.90**	**0.97**	**0.432**
AZMC5	64.24± 9.82	1.89	0.84	1.88	0.84	0.12	0.92	0.96	0.264
AZMC6	74.85± 7.05	0.84	0.77	1.62	0.82	0.03	0.90	0.89	0.071
AZMC7	85.46± 7.14	3.27	0.88	1.91	0.94	0.16	0.94	0.99	0.394

All values are expressed in mean ± standard deviation (n=3)

3.4. Stability studies:

Method: Stability studies of AZMCs were conducted to find out stability of product under storage. The microcapsules were stored in amber colored glass bottles at elevated temperature i.e. 4 ± 1°C (FT), 25 ± 1 °C (RT) and 50 ± 1 °C (HT) for a period of 2 – month and observed for change in drug content.[28,29,30]

Table 3.25 shows the concentration, potency and log percent concentration of AZMC4 for 60 days stability study, and table 3.26 shows the parameters determined for the stability of AZMC4. Shelf life in year in different temperatures like 4± 1°C, 25± 1°C and 50± 1°C result were found 1.468 years and 2.207 years respectively. Degradation half life also calculated in year of AZMC4 in different temperatures like 4± 1°C, 25± 1°C and 50± 1°C results were found 9.608 years, and 14.438 years for respective temperatures.

Table 3.25: Stability studies (concentration, potency and log % concentration) of AZMC 4

Temperatures	4± 1°C			25± 1°C			50± 1°C		
Time(in Days)	**Conc. (in mg /100mg)**	**Poten-cy (%)**	**Log % Conc.**	**Conc. (in mg/ 100mg)**	**Poten-cy (%)**	**Log % Conc.**	**Conc. (in mg/ 100mg)**	**Poten-cy (%)**	**Log % Conc.**
0	33.99	100.00	2.000	33.99	100.00	2.000	33.99	100.00	2.000
7	33.86	99.63	1.998	33.99	100.00	2.000	33.86	99.63	1.998
14	33.86	99.63	1.998	33.99	100.00	2.000	33.86	99.63	1.998
21	33.86	99.63	1.998	33.99	100.00	2.000	33.99	100.00	2.000
30	33.99	100.00	2.000	33.99	100.00	2.000	33.99	100.00	2.000
38	33.73	99.23	1.997	33.86	99.63	1.998	33.86	99.63	1.998
45	33.86	99.63	1.998	33.86	99.63	1.998	33.86	99.63	1.998
52	33.59	98.84	1.995	33.86	99.63	1.998	33.86	99.63	1.998
60	33.59	98.84	1.995	33.73	99.23	1.997	33.73	99.23	1.997

Table 3.26: parameters determined for stability studies of AZMC4

Parameters	**Temp. 4± 1°C**	**Temp. 25± 1°C**	**Temp. 50± 1°C**
Zero – order (R^2)	0.554	0.776	0.210
First – order (R^2)	0.594	0.783	0.382
First order rate constant (k_1) in day^{-1}	1.976×10^{-4}	1.315×10^{-4}	1.315×10^{-4}
Degradation Half life (in year)	9.608	14.438	14.438
Shelf life (in year)	1.468	2.207	2.207

3.5. Optimization of microcapsules:

Method: Selection of optimized formulation was done by considering the various parameters like percentage yield, drug release study, particles size and shape, flow properties and drug content, FTIR study, stability studies, etc. Then this optimized formulation was incorporated into the bioadhesive intra-vaginal gel for further study.

Table 3.27 optimization of microcapsules for further Study:

S.No	Parameters		Results
1.	Percent yield (%)		72.93 ± 0.73
2.	Percent moisture loss (%)		23.53 ± 2.76
3.	Drug content (mg/100mg of microcapsules)		24.58 ± 0.87
4.	Percent Drug Entrapment (%)		89.75 ± 3.52
5.	Particle size (μm)		84.33 ± 4.35
6.	Optical microscopy		Spherical shape
7.	SEM Photography		Sphere and porous
8.	FTIR		No chemical interaction
9.	In-vitro drug release study (% CR/24 hrs.)		63.41 ± 5.36
10.	In- vitro drug release kinetic study		
	Korsmeyer – Peppas	(r^2)	0.956
		n	0.432
11.	Micromeritic Analysis		Good flow properties
12.	Stability Studies		Stable (room & above temperature)

Selection of optimized formulation was done by considering the various parameters like percent yield, drug release study, particles size and shape, flow properties and drug content, FTIR study, stability studies, etc. Then this optimized formulation was incorporated into the bioadhesive intra-vaginal gel for further study. On the basis of above study table 3.27 it was concluded that AZMC4 formulation best for the preparation of microencapsulated vaginal gel.

3.6 PREPARATION OF MICROENCAPSULATED VAGINAL GEL:

Method: Microencapsulated vaginal gels were prepared by cold mechanical method described by *Schmolka et al.*[31, 32] Required quantity of polymer (Carbopol 940 and Hydroxyethyl cellulose) was weighed and it was sprinkled slowly on surface of purified water for 2 hrs. After which it was continuously stirred by mechanical stirrer, till the polymer soaked in the water. With continuous stirring, triethanolamine was added to neutralize the gel and it maintains the pH of the gel. Now the appropriate quantity of DMSO (Dimethyl sulfoxide) was added to the gel, which behaves as the penetration enhancer, followed by the required quantity of methyl paraben as a preservative. Finally the best microencapsulated formulation was added to the gel with continuous stirring till the microcapsules get dispersed in gel completely. Eight formulations of microencapsulated vaginal gel were prepared by using Carbopol 940 and Hydroxyethyl cellulose in different ratio. The prepared gel were packed in wide mouth glass jar covered with screw capped plastic lid after covering the mouth with an aluminum foil and were kept in dark and cool place.[33,34] The formulations were preserved for further study figure 3.21.

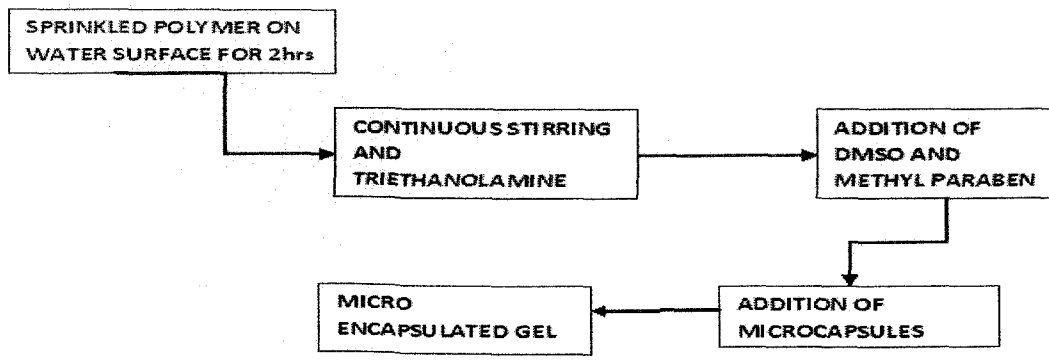


Figure 3.21 Preparation of microencapsulated vaginal gel

3.6.1 Formulation design of Microencapsulated vaginal gel:

Table 3.28: Formulation design for the preparation of microencapsulated bioadhesive vaginal gel:

Ingredients	AZMB VG 1	AZMB VG 2	AZMB VG 3	AZMB VG 4	AZMB VG 5	AZMB VG 6	AZMB VG 7	AZMB VG 8	BV G1
Microcapsules(AZMC4) (mg)	407.00	407.00	407.00	407.00	407.00	407.00	407.00	407.00	----
Pure drug(mg)	----	----	----	-----	-----	----	-----	------	100
Carbopol P940 (mg)	100.00	200.00	300.00	400.00	100.00	100.00	100.00	100.00	400
Hydroxy propyl methyl cellulose (mg)	-----	-----	------	-----	100.00	200.00	300.00	400.00	----
Triethanolamine (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Dimethyl Sulfoxide(ml)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Methyl Paraben (%)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Distilled Water (gm)	up to 100	up to 100	up to 100	up to 100	up to 100	up to 100	up to 100	up to 100	upto 100

Method: The intra-vaginal gels were prepared by using cold mechanical method using Carbopol and HPMC as polymer in different ratio with other ingredients. All the prepared microencapsulated vaginal gel formulations contain different microcapsules: polymer ratio and without microcapsuleted gel coded as AZMBVG 1, AZMBVG 2, AZMBVG 3, AZMBVG 4, AZMBVG 5, AZMBVG 6, AZMBVG 7 , AZMBVG 8 and BVG1 shown in table 3.28 and figure 3.21.

3.7 EVALUATION OF MICROENCAPSULATED VAGINAL GEL:

3.7.1 Percent Yield of Microencapsulated Vaginal Gel:

Method: The yield was calculated as the weight of the microencapsulated gel recovered from each batch divided by total weight of drug containing microcapsules and all other ingredients used to prepare microencapsulated gel multiplied by 100. The percentage yield

of each formulation was replicated three times[14]. The yield of microencapsulated vaginal gel was calculated using the following formula:

$$\mathbf{Y= \{P_m(Z_G)/T_m[P+M+I_g]\} \times 100}$$

Where as Y=Yield,P_m=Practical mass, Z_G =Microencapsulated vaginal Gel,T_m =Theoretical mass , P =Polymer, M =Microcapsules, I_g= Ingredients

The percentage yields of AZMBVGs were calculated and found to be 99.10 ± 0.06 to 99.63 ± 0.32%. The percent yields of AZMBVG4 was found 99.40 ± 0.31% and BVG1 was found 94.35±0.01% shown in column 2 of table 3.31. .

3.7.2 Drug Content Evaluation:

Method: Drug content was determined by dissolving accurately weighed quantity of gels in acetate buffer pH 4.7. After suitable dilution absorbance was recorded by using UV-visible spectrophotometer (UV – 1700, Shimadzu, Japan) at 267 nm.[35] The drug content was determined by using following equation:

$$\mathbf{D_C= (C_C \times D_F \times V)/ C_F}$$

Where as D_C =Drug Content,C_C = Concentration,D_F Dilution Factor, V =Volume taken, C_F =Conversion Factor

The drug content of AZMBVGs was found to be in the range of 1.07 ± 0.01 to 1.21 ± 0.12 mg/1gm of gel. AZMBVG4 was shown 1.20± 0.08 mg/1gm and BVG1 was shown drug content 98.68±0.19 mg/1gm in column 3 tables 3.31.

3.7.3 pH and Color Evaluation of Microencapsulated Vaginal Gel:

Method: 2.5 gm of gel was accurately weighed and dissolved in 25 ml of distilled water and equlibated for two hours. The pH of dispersions was determined using digital pH meter (Digital pH meter MK VI, Systronics, Naroda). The measurement of pH of each

formulation was in triplicate and the average values are presented.[35,36] The color of gel was also visualized by naked eyes.

Table 3.29: pH and color evaluation study of AZMBVG1- AZMBVG 8:

Formulation code	pH	Color of Vaginal Gel
AZMBVG1	6.94±0.05	Transparent
AZMBVG2	7.13±0.09	Transparent
AZMBVG 3	7.47±0.12	Transparent
AZMBVG 4	7.20±0.28	Transparent
AZMBVG 5	7.13±0.09	Light Brownish-White
AZMBVG 6	7.30±0.14	Light Brownish-White
AZMBVG 7	7.43±0.12	Light Brownish-White
AZMBVG 8	7.50±0.06	Light Brownish-White
BVG1	7.10±0.08	Transparent

Table 3.29 shows the pH and color of microencapsulated vaginal gel. From the pH study of microencapsulated vaginal gel concluded that all formulations having the neutral pH and visual color conclude the gel having good appearance. The pH of BVG1 also found 7.10±0.08 with transparent color where as AZMBVG4 was shown 7.20±0.28 with transparent appreance.

3.7.4 Spreadability of Microencapsulated Vaginal Gel:

Method: Spreadability was determined by apparatus suggested by *Mutimer et al.,*[37] which was suitably modified in the laboratory and used for the study. It consists of a wooden block, which was provided by a pulley at one end. By this method, spreadability was measured on the basis of 'Slip' and 'Drag' characteristics of gels. A ground glass slide was fixed on this block. An excess of gel (about 2 gm) under study was placed on this ground slide. The gel was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. A one Kg weight was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the gel between the slides. Excess of the gel was scrapped off from the

edges figure 3.22. The top plate was then subjected to pull of 80 gm. With the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance (cm) be noted. A shorter interval indicates better Spreadability.[38]

Spreadability was then calculated using the following formula:

$$S = M \times L / T$$

Where, S is the spreadability, M is the weight in the pan (tied to the upper slide), L is the length moved by the glass slide and T represents the time taken to separate the slide completely from each other.

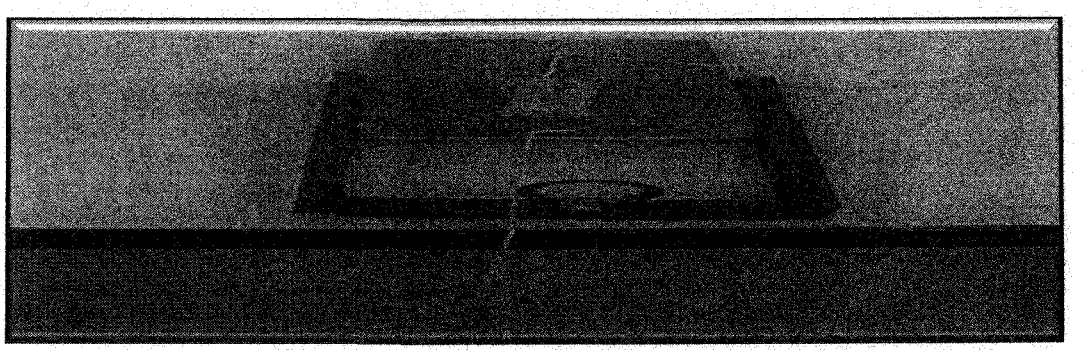


Figure 3.22: Photograph during spreadability study preformed

Spreadability of all microencapsulated vaginal gels was in between range of 12.86 ± 0.09 to 15.00 ± 0.22 gm.cm/sec. AZMBVG4 was shown 14.38 ± 0.12 gm.cm/sec and BVG1 was shown 13.74 ±0.10 gm.cm/sec in column 4 tables 3.31. As the concentration of polymer increases the spreadability of AZMBVGs decreases the spreadability.

3.7.5 Extrudability:

Method: It is a usual empirical test to measure the force required to extrude the material from tube. The method applied for determination of applied shear in the region of the

rheogram corresponding to a shear rate exceeding the yield value and exhibiting consequent plug flow one such apparatus is described by *wood et al.*[38]

In the present study, the method adopted for evaluating gel formulation for extrudability was based upon the quantity in percentage of gel and gel extruded from lacquered aluminum collapsible tube on application of weight in grams required to extrude at least 0.5 cm ribbon of gel in 10 seconds. More quantity extruded better was extrudability. The measurement of extrudability of each formulation was in triplicate and the average values are presented. The extrudability was than calculated by using the following formula:

$$\mathbf{E_P = G_m / A}$$

Where as E_P =Extrudability, G_m = Applied weight to extrude gel from tube (in gm) / A =Area (in cm^2)

Extrudability of all AZMBVGs was in between range of 16.17 ± 0.08 to 18.08 ± 0.08 gm/cm^2. AZMBVG4 and BVG1 were shown extrudability 16.67 ± 0.08 gm/cm^2 column 5 table 3.31. As the concentration of polymer increases the extrudability of AZMBVGs also increases, because as the concentration of polymer increases weight required to extrude gel from tube also increases.

3.7.6 Swelling index:

Method: Swelling of the polymer depends on the concentration of the polymer, ionic strength and the presence of water. To determine the swelling index of prepared microencapsulated vaginal gel, 1 gm of gel was taken on porous aluminum foil, and then placed separately in a 50 ml beaker containing 10 ml acetate buffer pH 4.7. The beakers were removed at different time intervals, put it on dry place for some time and then reweighed. Swelling index was calculated as follows[39]

Swelling Index (S_W) % = $[(W_t - W_o) / W_o] \times 100$

Where, (S_W) is equilibrium percent swelling, W_t is weight of swollen gel after time t, W_o is original weight of gel at zero time.

Table 3.30: Percent swelling index of AZMBVG 1- AZMBVG8 and BVG1

Formulation code	Swelling Index (%) (X± SD)				
Time(hrs)	0.5	1	2	4	6
AZMBVG1	8.40 ± 0.15	14.80 ± 0.12	25.30 ± 0.40	38.67 ± 0.29	46.07 ±0.93
AZMBVG 2	10.70 ± 0.29	19.33 ± 0.26	32.03 ± 0.67	49.07 ± 0.49	55.43 ±0.45
AZMBVG 3	12.17 ± 0.37	20.60 ± 0.46	37.23 ± 0.67	52.90 ± 0.78	60.40 ±1.28
AZMBVG 4	**16.34 ± 0.67**	**21.70 ± 0.59**	**36.17 ± 0.61**	**54.23± 1.28**	**64.70±0.95**
AZMBVG 5	9.67 ± 0.29	17.40 ± 0.61	28.43 ± 0.79	42.80 ± 0.55	57.57 ±1.25
AZMBVG 6	9.17 ± 0.23	17.10 ± 0.12	29.10 ± 0.12	44.43 ± 1.69	57.73 ±0.75
AZMBVG 7	13.33 ± 0.47	23.33 ± 0.72	39.03 ± 0.09	56.60 ± 0.61	73.37 ±0.38
AZMBVG 8	16.77 ± 0.09	22.93 ± 0.46	36.90 ± 0.55	58.10 ± 0.45	78.03 ±0.83
BVG1	**15.14±0.03**	**22.30±0.16**	**35.70±0.16**	**54.64±0.25**	**67.83±0.24**

All values are expressed in mean ± SEM ($n = 3$).

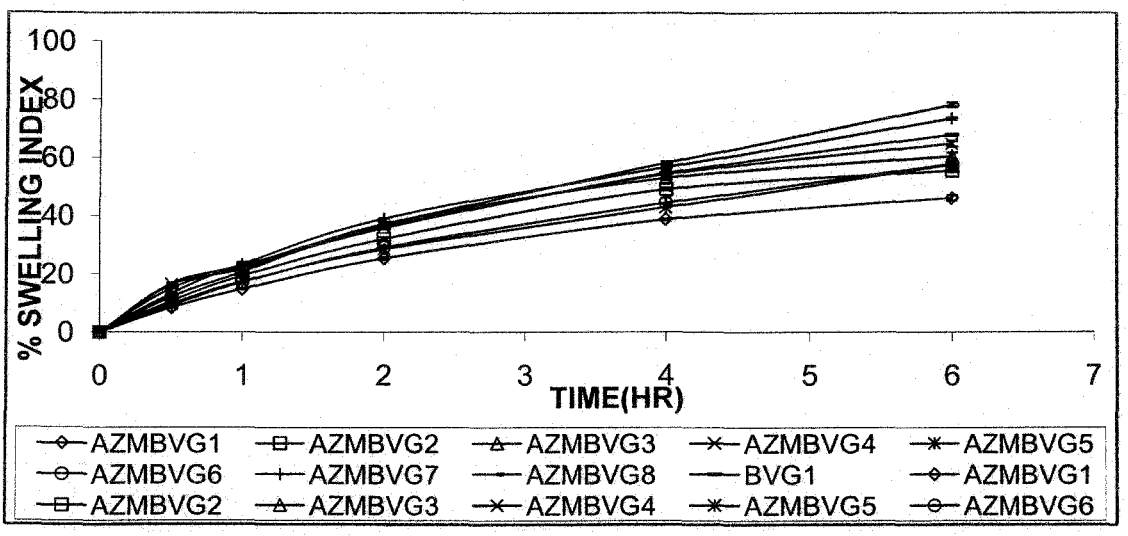


Figure 3.23: Percent swelling index of AZMBVG 1- AZMBVG8 and BVG1

In order to understand the influence of the polymer system on drug release and swelling study on gel matrices containing the polymers (HPMC and Carbopol P940) was evaluated. It is clear that the gel matrices underwent swelling at the same time as it was placed in the dissolution media. The pH of the media influenced swelling. On the other hand, the percentage of matrix swelling as a function of pH ranged from 78.03% at pH

7.5 to 46.07% at pH 6.9 (table 3.29 and 3.30 and figurer 3.23).AZMBVG4 was shown 67.70% at ph 7.20. Other hand in case of BVG1 showed 67.83% at pH 7.1 this results demonstrates that gel matrix swelling depends on the pH of the media. As the pH of the media increases, swelling of the matrix increases. The table 3.30 and figure 3.23 showed swelling indexes of different formulations. Swelling index increased in the following order of formulations AZMBVG1<AZMBVG2<AZMBVG5 <AZMBVG6 < AZMBVG3 <AZMBVG4 <AZMBVG7 <AZMBVG8. Formulation AZMBVG8 (1:4) was contain highest proportion of HPMC and lowest proportion of carbopol showed highest swelling index. Other hand formulation AZMBVG1 was contain lowest proportion of carbopol, showed lowest swelling index respectively. It was indicated that as the proportion of HPMC and carbopol increased, swelling index increased.

3.7.7 Bioadhesive strength measurement:

Method: Isolated goat vaginal tissue (*Capra hircus*, local breed, obtained immediately after sacrifice of animals at a slaughter house) was cleaned, separated from the supporting muscular and connective tissues taking care to maintain integrity of mucosa, and kept at 0°C till further use. Before experiments, goat vaginal tissue was thawed in SVF medium. The bio adhesion measurement was performed by using a modified balance method intact with goat vaginal tissue. The two pans of physical balance were removed. Right side pan was replaced with a 100 ml beaker and on left side, a glass slide was hanged. For balancing the assembly a weight of 20g was hanged on left side. Another glass slide was placed below the hanged slide. Portions of vaginal membranes were attached with both slides. The height of this set up was so adjusted, leaving a space of about 0.2 cm between two vaginal membrane faces. One gm of gel was placed between two vaginal membrane

faces. Little pressure was applied to form bio adhesion bond, and then slowly drop of water was added on right side beaker, till the vaginal gel / microencapsulated vaginal gel was separated from one face of vaginal membranes attached. Volume of water added was converted to mass Figure 3.24a and 3.24b. This gave the bioadhesive strength of film in gm. An initial investigation examined the reproducibility of the system using five same formulations. Then the study was carried out for all formulations.[40,41]

The bioadhesive strength was calculated by using following formula:

$\mathbf{B_s=W_g/Q_b}$, Where as B_s=Bioadhesive Strength, W_g =Weight required (in gms), Q_b =Area (cm^2)

Figure 3.24a and 3.24b: Photograph during bioadhesive strength measurement

Vaginal bioadhesive strengths of all AZMBVGs, using goat vagina, were found in the following order AZMBVG4>AZMBVG8>AZMBVG3> AZMBVG7 > AZMBVG6 > AZMBVG5 > AZMBVG2 >AZMBVG1 accordingly. Thus it was concluded that AZMBVG4 and BVG1 were showed the highest bioadhesive strength 1.69 ± 0.02 gm/cm^2 in column 6 of table 3.31.The bioadhesive property of Carbopal was reported due to carboxyl groups present on its acrylic acid backbone, which possess an ability to interact with sialic acid molecules present in the vaginal mucus layer.

Table 3.31: Percent Yield, Drug Content, Spreadability, Extrudability and Bioadhesive Strength and Of Microencapsulated Vaginal Gel

Formulation code	% Yield (Mean ± SEM)	Drug Content (mg/1gm of gel)	Spreadability (gm.cm/sec)	Extrudability (gm/cm^2)	Bioadhesive strength (gm/cm^2) (Mean ± SEM)
AZMBVG 1	99.63 ± 0.32	1.16 ± 0.08	15.00 ± 0.22	16.17 ± 0.08	1.20 ± 0.02
AZMBVG 2	99.33 ± 0.33	1.21 ± 0.12	14.74 ± 0.10	17.58 ± 0.08	1.31 ± 0.02
AZMBVG 3	99.40 ± 0.31	1.18 ± 0.03	14.66 ± 0.10	17.42 ± 0.08	1.61 ± 0.03
AZMBVG 4	**99.40 ± 0.31**	**1.20 ± 0.08**	**14.38 ± 0.12**	**16.67 ± 0.08**	**1.69 ± 0.02**
AZMBVG 5	99.40 ± 0.31	1.11 ± 0.05	13.54 ± 0.10	17.33 ± 0.08	1.41 ± 0.02
AZMBVG 6	99.10 ± 0.06	1.15 ± 0.03	13.54 ± 0.21	17.66 ± 0.08	1.52 ± 0.02
AZMBVG 7	99.13 ± 0.03	1.07 ± 0.01	13.16 ± 0.12	17.92 ± 0.08	1.59 ± 0.02
AZMBVG 8	99.40 ± 0.15	1.02 ± 0.09	12.86 ± 0.09	18.08 ± 0.08	1.67 ± 0.03
BVG1	**94.35±0.01**	**98.68±0.19**	**13.74 ± 0.10**	**16.67 ± 0.08**	**1.69 ± 0.02**

All values are expressed in mean ± SEM (n=3)

3.8 *In-Vitro* Drug Diffusion and kinetic Study:

Method: Cellophane membrane obtained from sigma chemicals was used for this study. In Kiescary Chien (KC) diffusion cell, 1.0 gm of gel was kept in donor compartment. The entire surface of membrane was in contact with the receptor compartment containing 85 ml of acetate buffer pH 4.7. The receptor compartment was continuously stirred (100 rpm) using a magnetic stirrer. The temperature maintained was 37 ± 1°C. The study was carried out for 28 hrs with the interval of 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, and 28 hrs. The sample was withdrawn at predetermined period of time and same volume was replaced with fresh acetate buffer. The absorbance of withdrawn sample was measured at 267 nm to estimate AZT. The experiment was carried out in triplicate and average values were reported table 3.32 and 3.33.[19,21,42]

Table 3.32: *In-vitro* drug diffusion study of AZMBVG1-AZMBVG4:

Time (hrs.)	% Cumulative Drug Release (%CR)			
	AZMBVG 1	**AZMBVG 2**	**AZMBVG 3**	**AZMBVG 4**
0.50	11.48 ± 1.94	16.14 ± 7.61	9.61 ± 4.36	**9.75 ± 2.32**
1.00	28.41 ± 6.46	22.73 ± 10.78	11.80 ± 2.07	**10.92 ± 2.02**
2.00	36.12 ± 3.31	35.14 ± 14.64	18.65 ± 0.42	**16.16 ± 2.03**
3.00	43.51 ± 1.56	40.92 ± 17.77	25.85 ± 2.14	**20.03 ± 2.27**
4.00	48.95 ± 0.49	46.01 ± 20.62	29.60 ± 1.41	**23.66 ± 2.48**
5.00	53.31 ± 1.27	57.91 ± 16.98	32.29 ± 1.04	**26.45 ± 2.41**
6.00	62.17 ± 1.83	67.71 ± 16.16	33.90 ± 1.95	**29.44 ± 3.26**

8.00	73.03 ± 4.06	75.59 ± 14.66	39.99 ± 1.22	**32.94 ± 2.91**
10.00	79.86 ± 6.12	86.32 ± 15.94	44.03 ± 1.75	**33.65 ± 4.25**
24.00	89.19 ± 10.85	86.40 ± 6.15	47.67 ± 3.43	**41.18 ± 2.53**
28.00	90.72 ± 11.84	90.57 ± 12.79	65.89 ± 1.59	**48.58 ± 8.91**

Table 3.33: *In-vitro* drug diffusion study of AZMBVG5- AZMBVG8:

Time (hrs.)	% Cumulative Drug Release (%CR)			
	AZMBVG 5	**AZMBVG 6**	**AZMBVG 7**	**AZMBVG 8**
0.50	10.60 ± 4.12	19.20 ± 4.06	33.42 ± 2.25	12.19 ± 2.94
1.00	12.11 ± 4.81	29.31 ± 11.26	44.89 ± 3.54	16.13 ± 4.12
2.00	28.85 ± 2.45	34.78 ± 15.34	49.38 ± 6.00	17.64 ± 4.00
3.00	37.52 ± 5.07	40.79 ± 20.08	58.59 ± 2.37	22.42 ± 2.29
4.00	57.67 ± 9.98	48.98 ± 22.74	65.82 ± 6.27	26.49 ± 0.86
5.00	67.64 ± 11.73	58.56 ± 22.54	67. 30 ± 6.08	31.06 ± 1.65
6.00	74.39 ± 9.47	59.46 ± 16.83	72.15 ± 5.25	34.25 ± 2.36
8.00	79.11 ± 7.06	71.73 ± 17.07	75.51 ± 6.26	40.43 ± 7.24
10.00	84.23 ± 4.78	80.87 ± 12 .66	73.18 ± 9.06	59.49 ± 12.59
24.00	88.16 ± 3.46	83.65 ± 11.16	84.39 ± 1.47	55.24 ± 7.68
28.00	92.82 ± 3.79	90.40 ± 7.68	92.88 ± 4.76	67.81 ± 13.78

All values are expressed in mean ± standard deviation (n=3)

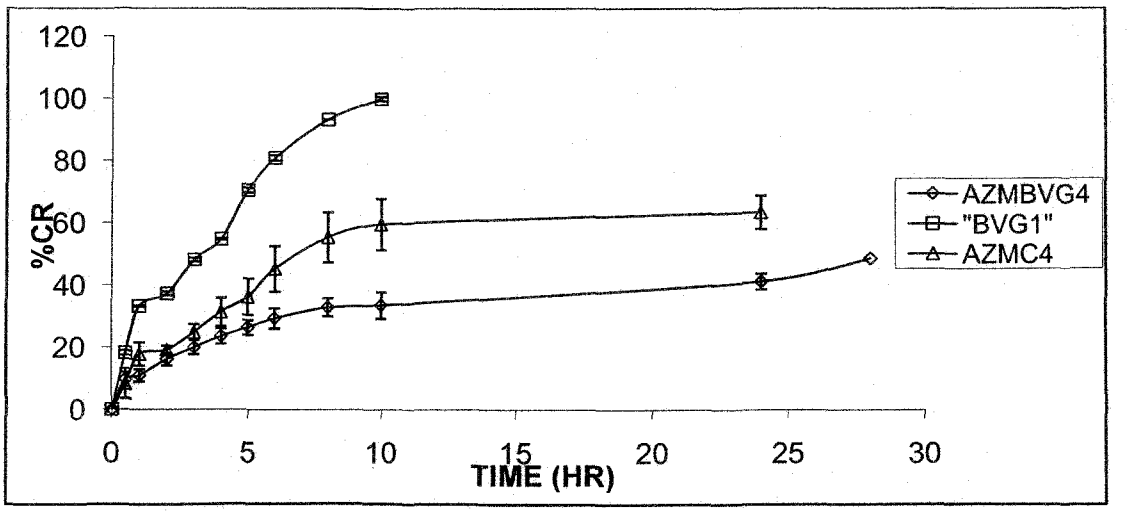


Figure 3.25: *In-vitro* drug release study of AZMBVG4 and AZMC4 with vaginal gel

Figure 3.25,3.32 and table 3.34,3.33 shown AZMBVG4 %cr drug release upto 28hr in comparision with BVG1 10hrs and AZMC4 24hrs.

Table 3.34: *In-vitro* drug release study of AZMBVG4 and AZMC4 with BVG1

AZMBVG4	BVG1	AZMC4	TIME(HR)
00	00	00	00
9.75 ± 2.32	18.26±0.73	8.39 ± 4.79	0.50
10.92 ± 2.02	33.27±0.23	17.79 ± 3.78	1.00
16.16 ± 2.03	37.35±0.61	19.23 ± 1.28	2.00
20.03 ± 2.27	48.25±0.64	24.87 ± 2.56	3.00
23.66 ± 2.48	54.92±0.24	31.48 ± 4.59	4.00
26.45 ± 2.41	70.67±0.77	36.39 ± 5.86	5.00
29.44 ± 3.26	81.05±0.88	45.35 ± 7.31	6.00
32.94 ± 2.91	93.34±0.68	55.45 ± 8.04	8.00
33.65 ± 4.25	99.91±0.08	59.59 ± 8.22	10.00
41.18 ± 2.53	-------	63.41 ± 5.36	24.00
48.58 ± 8.91	-------		28.00

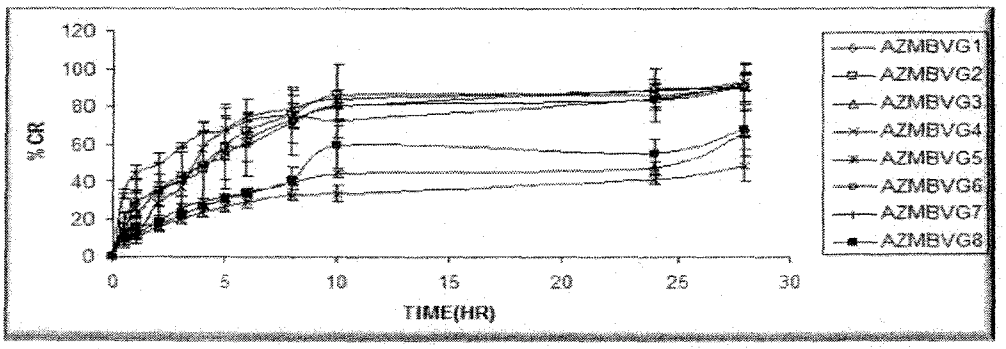


Figure 3.26: *In-vitro* drug release profile of AZMBVGs formulations

In vitro drug release profile of all the AZMBVGs (AZMBVG1-AZMBVG8) was in the range of 48.58 ± 8.91 to 92.88 ± 4.76% respectively. *In vitro* drug release of all AZMBVGs were shown significantly drug released different ($p<0.01$) table 3.35 to 3.42. It means null hypothesis was nullified and alternative hypothesis is accepted i.e. the variation in formulations in polymeric type and content (AZMBVG1-AZMBVG8) has significant effect on drug release profile. The AZMBVG4 was found to release the drug of about 48.58 ± 8.91% only, up to 28hrs, thus concluded to have sustained drug release profile for longer period of time when compared to other AZMBVGs. BVG1 was showed 99.91±0.08% cumulative drug release up to 10^{th} hr followed by diffusion controlled (Higuchi square root equation, $r^2 = 0.98$), non Fickian Case II anomalous release

(n=0.682). While AZMBVG4 released showing only 63.41± 5.36% drug up to 28 hrs in constant manner in comparison to other formulations followed by zero order (r^2 = 0.95), Fickian case I (n=0.433) drug release mechanism. The 'n' values for all the formulations ranged from 0.227 to 0.529 table 3.43, indicated release patterns was Case I Fickian release ($n \leq 0.5$). The designed AZMBVG4 which release 10.92 ± 2.02% in 1st hour and extend the release up to 48.58 ± 8.91% in 28th hours can overcome the disadvantages associated with conventional gel (BVG1). The mechanism of drug release from hydrophilic polymeric matrices involves solvent penetration, hydration and swelling of the polymer, diffusion of the dissolved drug in the matrix and erosion of the gel layer. In case of Fickian release mechanism, the rate of drug release is much lesser than that of polymer relaxation (swelling/erosion). So the drug release was chiefly dependent on the diffusion through the matrix. The *in vitro* drug releases of acquired AZMBVGs were shown in Figure 3.26 and Table 3.32 and 3.33. The *in vitro* drug release profile of all the AZMBVGs (AZMBVG1-AZMBVG8) was in the range of 48.58 ± 8.91 to 92.82 ± 3.79% respectively. The AZMBVG4 was found to release the drug of about 48.58 ± 8.91% only, even after 28hrs, thus concluded to have sustained drug release profile for longer period of time when compared to other AZMBVGs.

Table 3.35: Analysis of Variance of all AZMBVGs:

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	913.228	8	114.153	79.006	$F_{0.05}$=8.85 $F_{0.01}$=27.49
Residual	4.335	3	1.445		
Total		11			

Since the observed value F is larger than the 5% tabulated value corresponding to $d.f$ (8,3), we reject the null hypothesis and conclude that the drug release among the AZMBVGs differ significantly (P<0.01).

Table 3.36: Analysis of Variance of AZMBVG4 and AZMBVG1:

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	719.583	2	359.792	16.356	$F_{0.05}$=4.26 $F_{0.01}$=8.02
Residual	197.979	9	21.998		
Total		11			

Since the observed value F is larger than the 1% tabulated value corresponding to $d.f$ (2,9), we reject the null hypothesis and conclude that the drug release among the AZMBVG4 and AZMBVG1 differ significantly (P<0.01).

Table 3.37: Analysis of Variance of AZMBVG4 and AZMBVG2:

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	739.895	2	369.948	18.740	$F_{0.05}$=4.26 $F_{0.01}$=8.02
Residual	177.667	9	19.741		
Total		11			

Since the observed value F is larger than the 1% tabulated value corresponding to $d.f$ (2,9), we reject the null hypothesis and conclude that the drug release among the AZMBVG4 and AZMBVG2 differ significantly (P<0.01).

Table 3.38: Analysis of Variance of AZMBVG4 and AZMBVG3

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	712.167	2	356.084	15.603	$F_{0.05}$=4.26 $F_{0.01}$=8.02
Residual	205.395	9	22.822		
Total		11			

Since the observed value *F* is larger than the 1% tabulated value corresponding to *d.f* (2,9), we reject the null hypothesis and conclude that the drug release among the AZMBVG4 and AZMBVG3 differ significantly (P<0.01).

Table 3.39: Analysis of Variance of AZMBVG4 and AZMBVG5

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	756.776	2	378.388	21.180	$F_{0.05}$=4.26 $F_{0.01}$=8.02
Residual	160.786	9	17.865		
Total		11			

Since the observed value *F* is larger than the 1% tabulated value corresponding to *d.f* (2,9), we reject the null hypothesis and conclude that the drug release among the AZMBVG4 and AZMBVG5 differ significantly (P<0.01).

Table 3.40: Analysis of Variance of AZMBVG4 and AZMBVG6

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	735.657	2	367.828	18.199	$F_{0.05}$=4.26 $F_{0.01}$=8.02
Residual	181.906	9	20.212		
Total		11			

Since the observed value *F* is larger than the 1% tabulated value corresponding to *d.f* (2,9), we reject the null hypothesis and conclude that the drug release among the AZMBVG4 and AZMBVG6 differ significantly (P<0.01).

Table 3.41: Analysis of Variance of AZMBVG4 and AZMBVG7

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	767.420	2	383.710	23.001	$F_{0.05}$=4.26 $F_{0.01}$=8.02
Residual	150.142	9	16.682		
Total		11			

Since the observed value *F* is larger than the 1% tabulated value corresponding to *d.f* (2,9), we reject the null hypothesis and conclude that the drug release among the AZMBVG4 and AZMBVG7 differ significantly (P<0.01).

Table 3.42: Analysis of Variance of AZMBVG4 and AZMBVG8

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	719.703	2	359.852	16.369	$F_{0.05}$=4.26 $F_{0.01}$=8.02
Residual	197.859	9	21.984		
Total		11			

Since the observed value *F* is larger than the 1% tabulated value corresponding to *d.f* (2,9), we reject the null hypothesis and conclude that the drug release among the AZMBVG4 and AZMBVG8 differ significantly (P<0.01).

Table 3.43: Drug release and kinetic study of AZMBVG

Formulations	Cumulative % Drug release (28 h) study	Zero order release		First order release		Higuchi square root equation		Korsmeyer and Peppas equation	
	%CR	K_0	r^2	K_1	r^2	K_h	r^2	r^2	n
AZMBVG1	90.72 ± 1.84	2.08	0.81	1.89	0.36	0.13	0.82	0.91	0.440
AZMBVG2	90.57 ±12.79	2.01	0.75	1.89	0.77	0.12	0.90	0.94	0.422
AZMBVG3	65.89 ± 1.59	1.74	0.91	1.93	0.91	0.15	0.89	0.97	0.483
AZMBVG4	**48.58 ± 8.91**	**1.19**	**0.95**	**1.92**	**0.90**	**0.13**	**0.91**	**0.99**	**0.433**
AZMBVG5	92.82 ± 3.79	2.03	0.66	1.89	0.88	0.15	0.93	0.85	0.529
AZMBVG6	90.40 ± 7.68	1.86	0.80	1.85	0.91	0.11	0.91	0.95	0.369
AZMBVG7	92.88 ± 4.76	1.31	0.77	1.70	0.91	0.07	0.90	0.95	0.227
AZMBVG8	67.81 ± 3.78	1.62	0.88	1.92	0.91	0.14	0.90	0.96	0.446
BVG1	99.91±0.08 (10hrs)	1.23	**0.96**	1.90	**0.94**	0.13	**0.98**	**0.98**	**0.682**

All values are expressed in mean ± standard deviation (n=3)

3.8.1 Effect of Swelling Index on AZT Release:

In all the cases, the release rate was increased with increased proportion of hydrophilic polymer carbopol and HPMC due to more swelling. Initially, the diffusion coefficient of drug in the dehydrated polymer will be less and increases significantly as the polymer imbibes more and more water, and forms a gel, as the time progresses. The hydration rate of the polymer and thereby the gel formation significantly depended on polymer proportion. The overall effect of polymer was observed as follows. Formulation AZMBVG4 (Carbopol) with swelling index 64.70±0.95% table 3.30, showed lowest % cumulative drug release 48.58±8.91% up to 28^{th}hr table 3.32 and BVG1 swelling index of 67.83±0.24% table 3.30 showed 99.91±0.08% cumulative drug release up to 10^{th} hr table 3.34. Formulation AZMBVG1 (Carbopal minimum) with lowest swelling index of 46.07 ±0.93% table 3.30 showed 90.72 ± 11.84% cumulative drug release up to 28^{th} hr table 3.32. Thus concluded that carbopol contend EC microcapsules has a effect on drug release and it was as a batter rate controlling polymer which gave better drug release as comparer to BVG1.

AZMBVG8 was (Carbopal:HPMC=1:4) highest swelling index 78.03 ±0.83% showed 67.81 ± 13.78% drug release up to 28^{th} hrs table 3.30 and table 3.33. while formulation AZMBVG5(Carbopal:HPMC=1:1) with swelling index 57.57 ±1.25%, was found to release the drug only about 92.82 ± 3.79% up to 28th hrs table 3.30 and table 3.33. This finding can be attributed to the higher water retentions property of carbopol and HPMC, thus concluded that in present of EC microcapsule drug release was significantly influence of both the polymers. Carbopol contained EC microcapsules was a better rate controlling polymer to sustain the release of drug for longer period of time when compared to formulations containing HPMC and carbopol with EC microcapsules. The

overall *in vitro* drug release form AZMBVG4 formulation and other AZMBVGs formulations were differ significantly ($P<0.01$) table 3.35-3.42.

3.8.2 *In-Vitro* Drug Diffusion Kinetic Study:

Method: In order to investigate the mechanism of AZT release from AZMBVGs of different polymer ratio was discuss were in **3.3.1.** [25-27]

3.9. Selection of optimum microencapsulated vaginal gel:

Method: Optimum formulation was selected on the basis of various parameters of evaluation like *in-vitro* drug release diffusion study, bioadhesive strength measurement, vaginal irritation study, extrudability, spreadability, etc.

Table 3.44: optimization of vaginal gel:

S.No	Parameters		Results
1.	Percent yield (%)		99.40 ± 0.31
2.	Drug content (mg/gm of gel)		1.20 ± 0.08
3.	Ph		7.0
4.	Color		Transparent
5.	Spreadability (gm.cm/sec.)		13.74 ± 0.10
6.	Extrudability (gm/cm^2)		17.58 ± 0.08
7.	Percent Swelling Index (%)		100.40 ± 0.84
8.	Bioadhesive Strength (gm.cm^2)		1.69 ± 0.02
9.	In-vitro drug release study (% CR/28 hrs.)		48.58 ± 8.91
10.	In- vitro drug release kinetic study		
	Zero – order (r^2)		0.913
	First – order (r^2)		0.903
	Higuchi model (r^2)		0.914
	Korsmeyer – Peppas	(r^2)	0.989
		n	0.433

Optimum formulation was selected on the basis of various parameters of evaluation like *in-vitro* drug release diffusion study, bioadhesive strength measurement, vaginal irritation study, extrudability, spreadability, etc. On the basis of above study table 3.44 it was concluded that AZMBVG4 formulation best for the further study of microencapsulated vaginal gel.

3.10. Preparation of standard curve of AZT in phosphate buffer pH 7.4(blood pH):

3.10.1. Scanning of AZT for λ max and Preparation of Standard Curve in Phosphate Buffer pH 7.4:

Method: 50 mg Standard sample of AZT was dissolved in 100 ml of phosphate buffer I.P. of pH 7.4 and from that 1ml was further diluted in 100 ml of acetate phosphate buffer pH 7.4. This final dilution has the concentration of 5.0µg/ml and than scan this final concentration using UV – visual spectrophotometer (UV – 1700, Shimadzu, Japan).

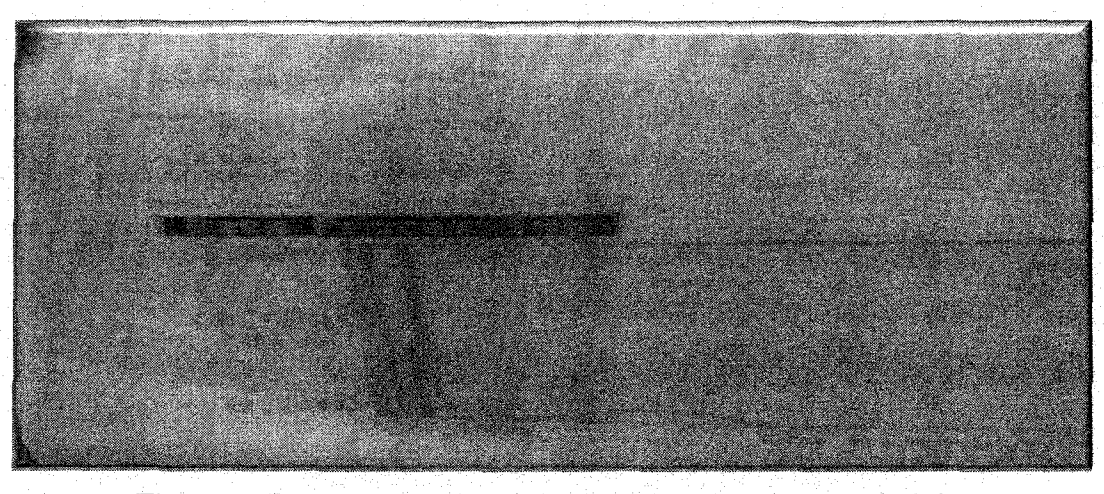


Figure 3.27 Scanning report of AZT in phosphate buffer pH 7.4

From the Figure 3.27, the λ_{max} of AZT in phosphate buffer was found to be 267 nm.

Method: Accurately 100 mg of AZT was taken and dissolved in 100 ml of phosphate buffer pH 7.4(SBF) (Solution – A). 1 ml solution was pipette out from solution – A, and then diluted it in a 100 ml volumetric flask (Solution - B). From solution – B different volumes of 1, 3, 4, 5 and 6 ml were taken and diluted up to 10 ml with phosphate buffer. The absorbance was scanned and its result found was 267 nm in UV –Visible spectrophotometer (UV – 1700, Shimadzu, Japan) against blank here used as phosphate

buffer pH 7.4. A graph was plotted by taking concentration versus absorbance. The slope and regression value was calculated from the graph.[11,12]

Table 3.45: Standard curve data of AZT using phosphate buffer pH 7.4:

Serial No.	Conc. (µg/ml)	Absorbance
1.	0.00	0.000
2.	1.00	0.044
3.	3.00	0.113
4.	4.00	0.147
5.	5.00	0.188
6.	6.00	0.229

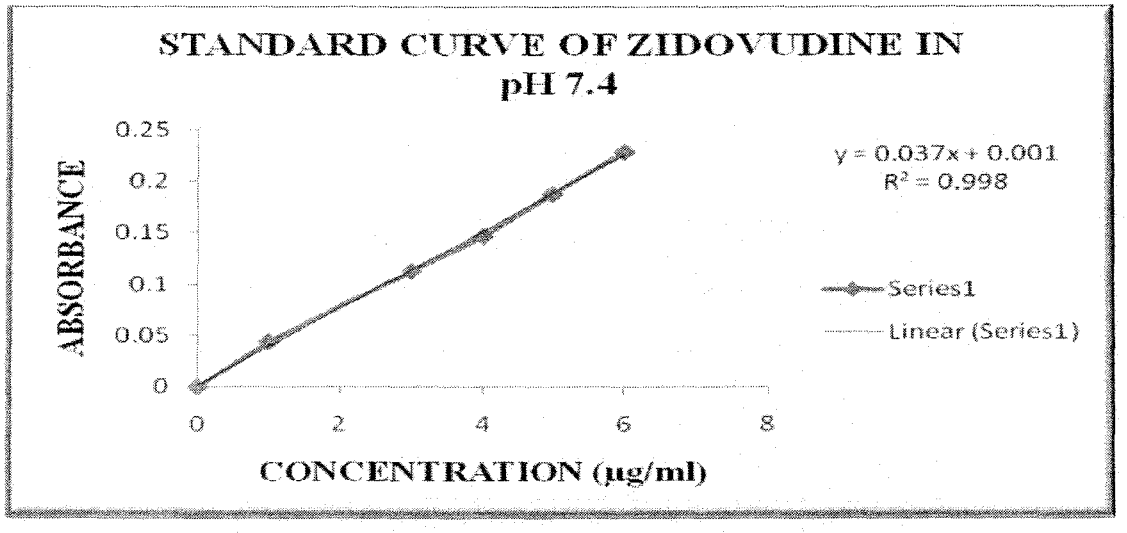


Figure 3.28: Standard curve of AZT using phosphate buffer pH7.4

The standard curve of AZT in phosphate buffer pH 7.4 was found to be given in Figure 3.28. The regression co-efficient (r^2) was found to be 0.999 and equation was Y = 0.037X + 0.001. The data of standard curve was represented in Table 3.45 and figure 3.28.

3.11 *In-Vitro* Permeation Studies:

Method: Keshery-Chien (KC) diffusion cell mounted with goat vaginal membrane was used for the permeation study of microencapsulated vaginal gel. 1.0 gm of microencapsulated vaginal gel was taken into the donor compartment and phosphate buffer pH 7.4 was taken into receptor compartment which is agitated using magnetic

stirrer (100 rpm) and temperature maintained to 37 ± 1°C. The sample was withdrawn at predetermined intervals of time and same volume was replaced with fresh buffer medium. Absorbance was measured at 267 nm to estimate AZT.[35]

Observations for percent *in-vitro* drug permeation study of AZMBVG4 in table 3.46 and graphical representations in figure 3.29 are given. *In-vitro* drug permeation of AZMBVG4 is 81.47 ± 8.19% in 28 hrs only.

The best fit with the highest correlation coefficient was shown in zero-order and first order followed by Higuchi equations as given in table 3.47. The drug release was proportional to square root of time, indicating that the drug release from AZMBVG4 was diffusion controlled. The *n* values for AZMBVG4 was found 0.363 indicated release patterns viz. Case I Fickian release ($n \leq 0.5$).

Table 3.46: Observation table for *In-vitro* drug permeation study of AZMBVG4:

Time (hrs.)	X	Y	Z	Percent Cumulative Drug Release (%CR)
0.50	23.96	18.79	2.48	15.07 ± 6.47
1.00	39.41	26.63	7.71	24.59 ± 9.21
2.00	40.65	34.78	15.33	30.26 ± 7.65
3.00	46.83	36.96	19.43	34.41 ± 8.01
4.00	51.24	38.12	21.71	37.02 ± 8.54
5.00	57.73	40.68	24.29	40.90 ± 9.65
6.00	67.31	52.87	33.33	51.17 ± 9.85
8.00	69.63	63.20	32.57	55.13 ± 11.43
10.00	72.18	67.00	35.33	58.17 ± 11.52
24.00	79.75	72.98	51.81	68.18 ± 8.42
28.00	83.93	77.64	56.95	72.84 ± 8.15

All values are expressed in mean ± SEM (n = 3) and X, Y, Z are the repetition of formulations.

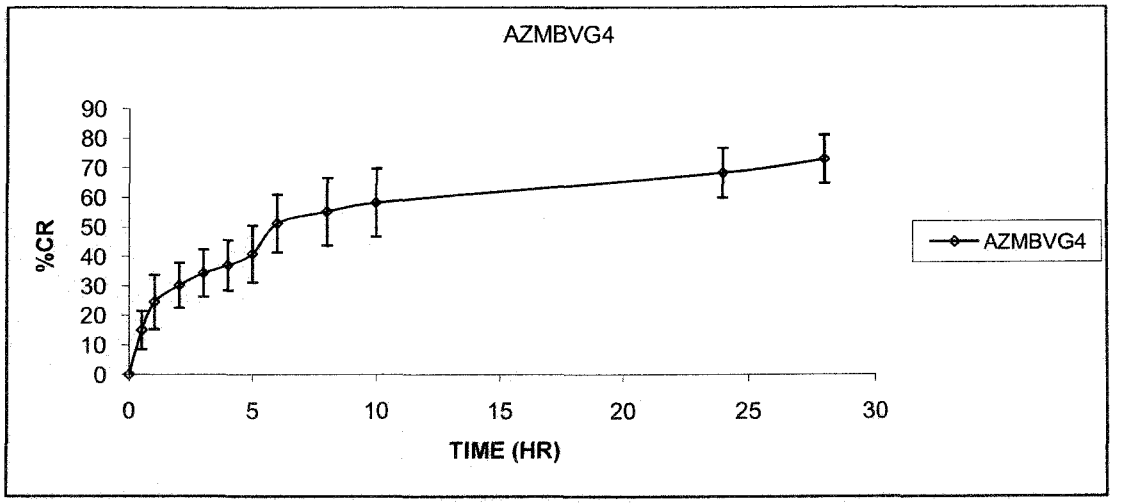


Figure 3.29: *In-vitro* drug permeation kinetic study:

3.11.1. *In-Vitro* Drug Permeation Kinetic Study:

Method: In order to investigate the mechanism of AZT release from AZMBVG4, the release data were analyzed with the following mathematical model which was explained in 3.11. [25-27]

Table 3.47: *In-vitro* drug permeation kinetic study of AZMBVG4:

Formulation Code	Kinetic models							
	Zero-order release		First-order release		Higuchi model		Korsmeyer-Peppas model	
	r^2	K_0	r^2	K_1	r^2	K_h	r^2	n
AZMBVG 4	0.94	1.527	0.90	1.855	0.85	0.107	0.968	0.363

The correlation between in vitro drug release rate and in vitro drug permeation across the goat vaginal mucosa was found to be positive, with a correlation coefficient (R^2) of 0.99 figures 3.30.

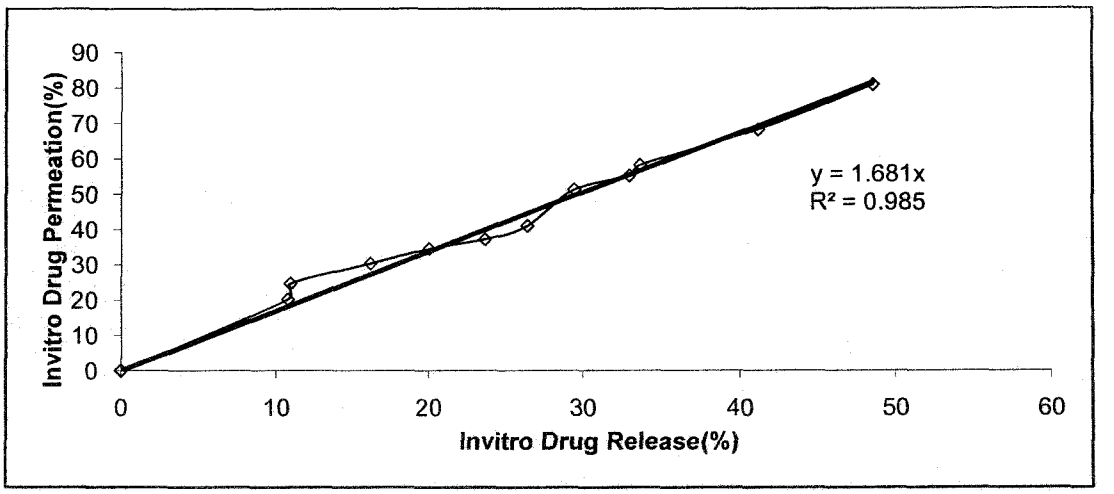


Figure 3.30: Correlation between in vitro drug release and in vitro drug permeation study

3.11. Preparation of Microencapsulated Bioadhesive Vaginal Tablet (MBVT)

Opimized batches of AZT microcapsule (ZMC4) were incorporated in tablet by direct compression method using various grades of bioadhesive polymer, such as Guar gum, Hydroxyl Propyl Methyl Cellulose, Carbopol 934, Carbopol 940 with other formulation excipients. For all batches, the microcapsules were mixed with bioadhesive polymer and other formulation additives[63-65] of tablet as designed in table 3.48.

Table 3.48: Formulation Design of Microencapsulated Vaginal Bioadhesive Tablet:

FC	ZMC4* (mg)	GG	HPMC	Carbopol 934 (mg)	Carbopol-940 (mg)	Starch (mg)	Mg. stearate (mg)
MBVT1	101.68	101.68			-	186.64	10
MBVT2	101.68		101.68		-	186.64	10
MBVT3	**101.68**			**101.68**	-	**186.64**	**10**
MBVT4	101.68			-	101.68	186.64	10

FC= Formulation code, ZMC4= Microcapsule, CP= Carbopol, Mg.stearate= Magnesium stearate, HPMC= Hydroxyl propyl methyl cellulose and GG= Guar gum
*Weight of the microcapsule is equivalent to the 25 mg of drug.

3.12. Evaluation of Microencapsulated Bioadhesive Vaginal Tablet

3.12.1. Weight Variation of Tablet

Twenty tablets were randomly selected from each batch and weighed individually. The average weight was calculated. Then the deviation of individual weight from the average weight and the standard deviation were calculated.[63]

The total weight of MBVT was 400mg. Weight variation of the formulated tablets (20 in number) was tested according to the official monograph in Indian Pharmacopoeia. Average weight of different formulation was found in the range of 398.50±1.48to 400.58±1.03mg column 2 of table 3.49.

3.12.2 Disintegration of Tablet

As the vaginal tablets, prepared using the polymers stated above are swelling matrices, disintegration tests of the prepared tablets were performed to observe, whether tablets were disintegrated or softened within a prescribed time when placed in a liquid medium under the prescribed experimental conditions. The modified disintegration apparatus resembling to B.P was designed in a vessel of suitable diameter containing water at 36^0 to 37^0 C. The level of the liquid was adjusted by the gradual addition of water at 36^0 to 37^0 C until the perforations in the metal disc were just covered by a uniform layer of water. One vaginal tablet on the upper perforated disc was placed and the apparatus was covered with a glass to maintain appropriate conditions of humidity. The operation was repeated with two more vaginal tablets .[66]

Disintegration of MBVT was determined similarly, as per monograph of B.P. Disintegration time of different MBVT was varied between 0.20-1.35hr columns 4 of table 3.49.

3.12.3. Hardness of Tablet

The hardness of the tablet was calculated with the help of a Monsanto hardness tester. Five tablets from each batch of formulations were tested. Then average hardness and standard deviation were calculated.[63]

Hardness of the formulated tablets (10 in number) of each batch, were evaluated using the Monsanto hardness tester. The hardness of different formulation was found 04.9±0.01to 6.10±0.02kg/cm^2 column 3 of table 3.49.

3.12.4. Friability of Tablet

The friability test was done using Roche's Friabilator. Twenty tablets were selected and weighed individually. Then the friability test was carried out at 25 rpm for 4 minutes. These tablets were then de-dusted, again weighed and percentage loss in weight was calculated.[63]

Friability of different MBVT was determined according to USP, in the range of 0.002±0.001to 0.005±0.006% column 5 of table 3.49.

The thickness and diameter of different formulation were found 4.76 ±0.05to 5.74±0.05mm and 10.12±0.02to 11.06±0.04mm respectively column 6 & column 7 of table 3.49.

Table 3.49: Weight variation, Hardness, Friability, Disintegration time, Thickness and Diameter Parameter of Tablet:

FC	Weight uniformity (mg)	Hardness (Kg/cm^2)	Friability (%)	Disintegration Time (hr:min)	Thickness (mm)	Diameter (mm)	% drug content
MBVT 1	399.37±1.95	5.78±0.083	0.003±0.007	0.20	5.74±0.05	10.36±0.04	88.25±0.71
MBVT 2	398.50±1.48	6.10±0.02	0.005±0.006	0.35	5.14±0.04	10.68±0.02	93.58±0.73
MBVT 3	**399.56±0.94**	**04.9±0.01**	**0.002±0.001**	**1.2**	**5.08±0.04**	**10.12±0.02**	**94.65±0.80**
MBVT 4	400.58±1.03	5.25±0.05	0.003±0.005	1.35	4.76±0.05	11.06±0.04	98.49±0.44

*All values are expressed in mean ± SD (n=3).

3.12.5. Drug Content of the Tablet

Tablets of each formulation were ground in a mortar to form powder. An accurately weighed amount of the powder, equivalent to 100 mg of AZT, was transferred to a 100 ml volumetric flask. The powder was kept in methanol overnight. After filtration, the solution was assayed spectrophotometrically for AZT at 267 nm against methanol as blank. The content was calculated using a pre constructed calibration curve for the drug.[67] Drug content of vaginal tablet formulations was found to be in the range of 88.25± 0.71to 98.49± 0.44% column 8 of table 3.49, indicating the suitability of the present method for the preparation of novel MBVT.

Three tablets were chosen randomly from each of the formulation and taken separately into three 100 ml volumetric flask. In each flask 100 ml of acetate buffer pH 4.7 was kept for 24 hr. After filtration the solution, the absorbance of the filtrate was measured in UV visible spectrophotometer at 267 nm.

3.12.6. *In Vitro* Dissolution and Kinetic Study of Tablet

In vitro drug release study from bioadhesive vaginal tablet was carried out in USP Type II dissolution test apparatus (TDT-08L USP, Electrolab, Kolkata, India) using SVF as dissolution medium. Volume of dissolution medium was 900 ml and bath temperature was maintained at 37±1°C throughout study. Paddle speed was adjusted to 50 rpm. At an interval of 1 hr, five ml of sample was withdrawn with replacement of five ml fresh medium and analyzed for AZT content by UV-Visible spectrophotometer at 267nm. The entire release tests were performed in triplicate.[67]

The *in vitro* drug releases of acquired MBVTs were shown in figure 3.31 and table 3.50. The *in vitro* drug release profile of all the MBVTs (MBVT1-MBVT4) was in the range of 82.93±8.01- 98.76±0.7% respectively. The MBVT3 was found to release the drug of

about 82.93±8.01% only, even after 24hrs, thus concluded to have sustained drug release profile for longer period of time when compared to other MBVTs. Also, *in vitro* release of zidovudine form EC ZMC4 exhibited initial burst effect, which was due to presence of drug particle on the surface of the ZMC4. The initial burst effect was attributed as a desired effect to ensure initial therapeutic plasma concentrations of drugs. Although, drug release from all the MBVT formulations (MBVT1-MBVT4) was observed up to 24 hrs, the drug release mechanism of all MBVT was found to be predominately influenced by the different bioadhesive polymer added. The mechanism of drug release from hydrophilic polymeric matrices involves solvent penetration, hydration and swelling of the polymer, diffusion of the dissolved drug in the matrix and erosion of the gel layer. Form table 3.51 the *n* values for all the formulations ranged from 0.25 to 0.55 indicating different release patterns viz. Fickian (n = 0.5), diffusion control studies, tablets underwent case I Fickian diffusion control, during the dissolution study.

Table 3.50: *In Vitro* Drug Release Study of AZT Loaded Microencapsulated Bioadhesive Tablet Formulation in Acetate Buffer pH 4.7:

Time (hr)	√t	% of cumulative Release±SD			
		MBVT1	MBVT2	**MBVT3**	MBVT4
0.25	0.5	23.7±1.5	29.1±1.48	**7.63±0.58**	10.0±2.02
0.5	0.7	31.7±1.8	38.6±1.58	**10.3±0.55**	12.8±2.48
1	1.0	39.3±2.2	48.3±0.97	**11.6±0.89**	18.4±2.07
2	1.4	49.8±0.5	65.5±1.26	**18.6±0.34**	29.4±2.52
4	2.0	66.4±2.2	74.6±2.89	**26.7±0.56**	36.8±3.38
8	2.8	83.9±1.6	82.2±1.99	**55.5±0.83**	66.4±2.59
16	4	95.2±1.0	90.3±1.55	**72.42±7.1**	81.7±4.29
24	4.89	98.76±0.7	98.14±0.64	**82.93±8.01**	92.01±2.11

***All the results are expressed Mean ± SD (n=3).**

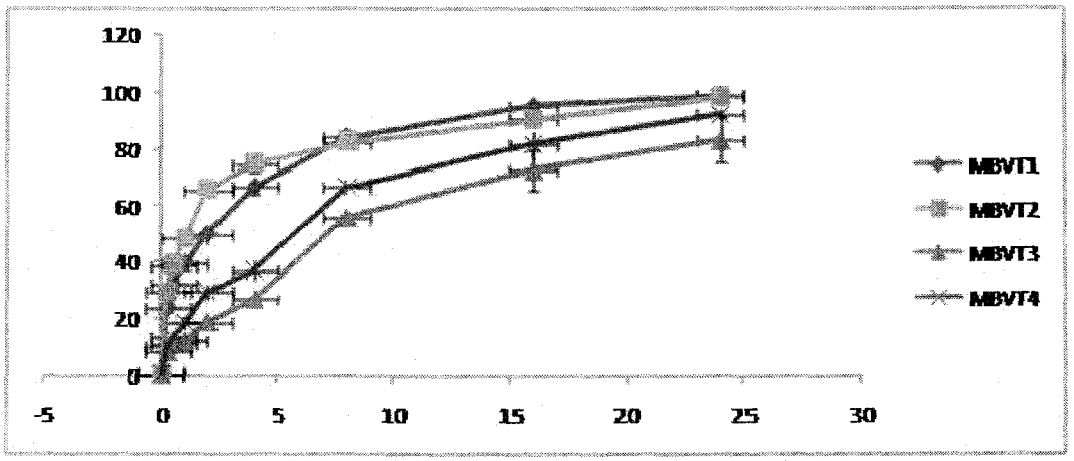


Figure 3.31: *In Vitro* Drug Release Study of AZT Loaded Microencapsulated Bioadhesive Tablet Formulation

Table 3.51: *In Vitro* Drug Release Kinetic Studies of AZT Loaded Microencapsulated Bioadhesive Tablet Formulation:

FC	Zero order		First order		Higuchi model		Korsmeyer-Peppas model		
	r^2	Ko (µg/sec)	r^2	K_1	r^2	K_H (µg/√sec)	r^2	n	Comment
MBVT1	0.72	3.43	0.99	0.077	0.92	19.56	0.986	0.32	Fickian diffusion
MBVT2	0.62	2.98	0.94	0.059	0.84	17.61	0.958	0.25	Fickian diffusion
MBVT3	**0.92**	**2.92**	**0.96**	**0.034**	**0.98**	**17.61**	**0.990**	**0.55**	Fickian diffusion
MBVT4	0.84	2.74	0.93	0.066	0.97	17.91	0.986	0.49	Fickian diffusion

3.12.7 Drug Release Kinetic Studies of Tablet

Method: In order to investigate the mechanism of AZT release from MBVTs, the release data were analyzed with the following mathematical model which was explained in **3.12.6**.

3.12.8. Bioadhesive Strength of Tablet

Method: One MBVT tablet was placed between two vaginal membrane faces. Little pressure was applied to form bio adhesion bond, and then slowly drop of water was added on right side beaker, till the tablet was separated from one face of vaginal membranes attached. Bioadhesive strength of tablet was discussed in **3.7.7** sections.

Table 3.52 represent the vaginal bioadhesive strength of the prepared microencapsulated bioadhesive tablets (MBVT1-MBVT4) in goat vagina and the result shows that all vaginal bioadhesive strengths were found in the following order MBVT1> MBVT3> MBVT2> MBVT4. In above data MBVT 4 shows the highest bioadhesive strength (17.21gm).

Table 3.52: Bioadhesive Strength Measurement Study of Microencapsulated Bioadhesive Tablets:

Formulation code	Bioadhesive strength (gm)
MBVT1	11.67± 0.02
MBVT 2	14.85±0.05
MBVT 3	13.15± 0.04
MBVT 4	17.21±0.04

3.12.9. Swelling Index of Tablet

To determine the swelling index of prepared bioadhesive tablets, MBVTs was taken on porous aluminum foil, and then placed separately in a 50 ml beaker containing 10 ml acetate buffer pH 4.7[41] were discussed in **3.7.6** sections. Swelling index plays an important role in the drug release pattern. The swelling index lied in the range of 0.431 to 3.36 as given in table 3.53. The highest swelling index was achieved by the formulation MBVT 4.

Table 3.53: Swelling Index Study of Microencapsulated Bioadhesive Tablets:

Formulation code	Swelling index				
	20 (min)	40 (min)	1 (hr)	2 (hr)	4 (hr)
MBVT1	1.16	*	*	*	*
MBVT 2	0.431	1.15	1.5	*	*
MBVT 3	0.636	1.163	1.82	2.22	*
MBVT 4	1.11	1.31	1.48	2.87	3.36

*Swelling could not be possible to measure.

3.13. Preparation of Bioadhesive Vaginal Film of AZT

VF of AZT was prepared by solvent casting method containing different ratios of Acrycoat S-100 (AC) or EC and HPMC in di-butyl phthalate or glycerol or sorbitol or PEG 400 as a plasticizer. 40 % w/w of polymeric solution was allowed to stir for 1 h. After that, drug and plasticizer were added with constant stirring and this solution was allowed to stir until we got clear solution. The solution was allowed to stand overnight to remove all the air bubbles. The solution was then casted onto a petri dish and dried in the oven at 60°C until complete drying. The film was carefully removed from the petridish, checked for any imperfections and cut according to the size required for testing. The films thus prepared, were wrapped in a aluminum foil and kept in a desiccators for further study. Each formulation was replicated three times table 3.54.[68,69]

Table 3.54: Composition and plasticizer of Bio-adhesive vaginal films

Formulation	Polymer	Drug : polymer	Plastisizeer (% w/w)
VF 5	EC : HPMC (4: 1)	1: 5	DBP
VF 8	EC : HPMC (1 : 4)	1: 5	DBP
VF 10	AC : HPMC (4: 1)	1: 5	DBP
VF 13	AC : HPMC (1: 4)	1: 5	DBP

4.13. Physical characteristics of films:

Physical Characteristics of different VF were optimized plasticizers. Films containing PEG 400 and sorbitol as plasticizers could not be removed from glass plate after drying. Film containing Glycerol as a plasticizer, was appeared transparent and easily removed from plate but was brittle. Films containing DBP as a plasticizer appeared transparent,

easily removed from plate and were soft. So for such composition of Film, DBP was selected as plasticizer of choice.

3.13.1. Morphological Characterization

Films were analyzed in Scanning Electron Microscopy (LEO, 435 VP, U.K.) to reveal the surface morphology of the films[24] were discuss in **3.2.2.6.**

SEM photographs of blank and drug loaded films were shown in figures 3.32a and 3.32b accordingly. Films appeared to be homogenous and continuous. Drug was distributed on the surface, over the drug loaded film.

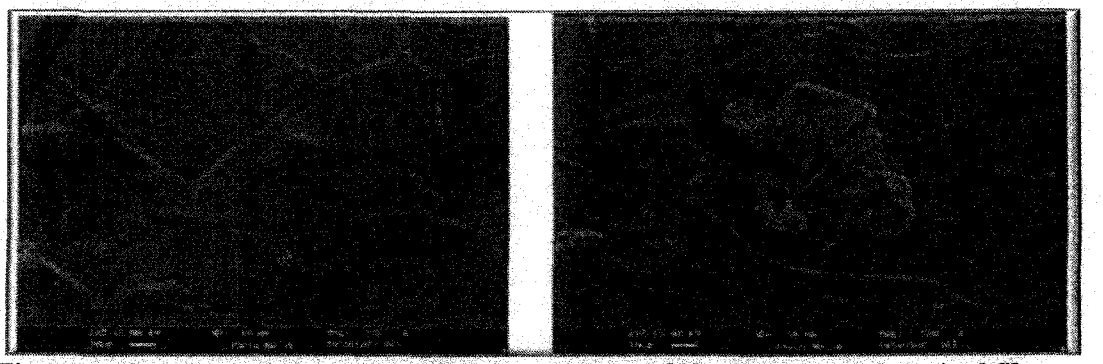


Figure 3.32a: SEM photograph of Blank **Figure 3.32b: Drug loaded vaginal film**

3.13.2. Measurement of Mechanical Properties (EVALUATION)

Mechanical properties of the films were evaluated using a modified instrument based on the similar working principle as reported by *Kok Khiang Peh et al.* Film strip in dimension of 50x10 mm and free from air bubbles or physical imperfections, were held between two clamps positioned at a distance of 3 cm. A cardboard was attached on the surface of the clamp via a double-sided tape to prevent the film from being cut by the grooves of the clamp. One clamp remains fixed and another one is movable. During measurement, the strips were pulled by the movable clamp at a rate of 2.0 mm/s to a distance of 5 mm before returning to the starting point. The force and elongation were measured when the films broke. Results from film samples, which did not broke at

between the clamps, were not included in calculations. Measurements were triplicated for each film. The following equations[70] were used to calculate the mechanical properties of the films:

$$\text{Tensile strength } \left(\frac{\text{kg}}{\text{mm}^2}\right) = \frac{\text{Force at break(kg1)}}{\text{Initial cross-sectional area of the sample(mm}^2\text{)}} \quad (1)$$

$$\text{Elastic modulus} \left(\frac{\text{kg}}{\text{mm}^2}\right) = \frac{\text{Force at corresponding strain(kg)}}{\text{Cross - sectional area of the sample(mm}^2\text{)}} \text{x} \frac{1}{\text{Corresponding strain}} \quad (2)$$

$$\text{Elongation break}(\%\text{mm}^{-2}) = \frac{\text{Increase in length(mm)}}{\text{Original length(mm)}} \text{x} \frac{100}{\text{Cross sectional area(mm}^2\text{)}} \quad (3)$$

$$\text{Strain} = \frac{\text{Tensile strength}}{\text{Elastic mdulus of the sample}} \quad -(4)$$

The reproducibility of the system was examined in the initial investigations using three same formulations of VFs. Then the study was carried out for different formulations.

The tensile testing is an indication of the strength and elasticity of the film, reflected by the parameters, tensile strength (TS), elastic modulus (EM) and elongation at break (E/B). A soft and weak film is distinguished by a low TS, EM and E/B; a hard and brittle film is defined by a moderate TS, high EM and low E/B; a soft and tough polymer is characterized by a moderate TS, low EM and high E/B; whereas a hard and tough polymer is characterized by a high TS, EM and E/B. Another parameter, Strain has been used as an indicator of the overall mechanical quality of the film. A high strain value indicates that the film is strong and elastic. Hence, it is suggested that a suitable vaginal film should have a relatively high TS, E/B and Strain but a low EM table 3.55 showed mechanical properties of different formulations. For EC film, decrease HPMC content TS and EM decreases, E/B increases but no significant difference in case of strain. For AC film, decrease TS, EM and strain, E/B increases. These results indicated that HPMC

generally increase the strength while decreased the softness, elasticity and flexibility of both EC as well as AC films. The greater elasticity exhibited by films containing lower HPMC content. From table 3.55, VF 8 (EC:HPMC=1:4) with moderate TS and EM with low B/Band low strain indicated that film were soft & weak nature, while formulation VF10(AC: HPMC=4:1) with low TS, low EM, high E/B with high strain was found indicated film were soft, strong and elastic; while Formulation VF 5 (EC: HPMC=4:1) with low TS ,low EM and high E/B with low strain indicated soft & weak film ,whereas formulation VF13(AC:HPMC=1:4) high TS and high EM, with low E/Band low strain indicated that film was hard , brittle elastic nature. These results indicated that AC generally reduced the strength while increased the softness, elasticity and flexibility of both EC as well as AC films. The greater elasticity exhibited by films containing higher AC content could be related to its conformation and configuration, which is highly cross linked. In comparison, the mean TS values of both EC and AC films were closely comparable for similar compositions. Increase in AC content rendered the HPMC films more elastic than EC films.

Table 3.55: Mechanical properties of different vaginal formulations

Formulation	*Tensile strength ($kgmm^{-2}$)	*Elastic modulus ($kgmm^{-2}$)	*Elongation at break (% mm^{-2})	*Strain
VF 5	1.23±0.17	3.41±0.23	12.57±0.20	0.36±0.09
VF 8	2.45± 0.65	5.83 ±0.32	10.99±0.09	0.42±0.09
VF 10	1.24±0.18	2.88±0.21	12.54±0.08	0.43±0.05
VF 13	2.71±0.32	6.15 ±0.42	11.67±0.14	0.44±0.12

Each value represents as mean ± standard deviation (n=3).

1.13.3. Folding Endurance

The folding endurance is expressed as the number of folds (no. of times the film is folded at the same place) either to break the film or to develop visible cracks. This test is important to check the ability of sample to withstand folding during handling and transport. The measurements of folding endurance of each formulation were replicated in three times.[69]

Evaluation of folding endurance involves determining the folding capacity of the films subjected to frequent extreme conditions of folding. The column 4 of table 3.56 showed folding endurance of different formulations. The folding endurance the prepared VFs were found to be 296-324 numbers of times for all formulation indicating that all formulations were flexible and soft. This also gives an indication of brittleness; less folding endurance indicates more brittleness.

3.13.4. Estimation of Drug Content

AZT content in film was estimated by UV spectrophotometric method in simulated vaginal fluid (SVF). The accurately weighed film strip in dimension of 50x10 mm, were dissolved first in solvent (2ml methanol) so that polymer get dissolved to release drug into the solution. Then volume was made up to 25 ml with SVF and kept for 1 hr under stirring. Similarly, a blank was carried out using drug free film. The solution was filtered and absorbance was measured at 267nm (λmax) using UV-Visible spectrophotometer (UV-1700, Shimadzu, Japan).[69]

The drug content of all the prepared VFs was found to be satisfactory and each formulation demonstrated high drug contents, as summarized in column 2 of table 3.56. The drug contents of the prepared VFs were found to be in the range of 77.87(VF 5) - 97.65% (VF 13). The formulation VF 13 showed highest drug contents among all the

formulations. Further, as shown in table 3.56, the drug content analysis of the prepared films showed that the process used to prepare the films in this investigation is capable of giving optimum drug content and minimum batch variability.

Table 3.56: % Drug content, moisture content, folding endurance, bioadhesive strength and swelling index of different formulations

Formulation	% w/w Drug content*	%Moisture content*	Folding endurance* (no of times)	*Bioadhesive strength (gm)	*Swelling index (up to 25 min)
VF 5	77.87±0.74	2.94±0.65	308±21	5.1±3.2	19.72±0.83
VF 8	87.75±0.75	4.13±0.95	324±15	17.5±29	56.56±0.77
VF 10	**87.44±0.45**	**1.23±1.11**	**321±23**	**4.4±1.8**	**17.08±0.67**
VF 13	97.65±1.32	3.43±0.84	296±76	14.6±26	51.32±1.56

*Each value represents as mean ± standard deviation (n=3).

3.13.5. Estimation of moisture content:

The prepared films were cut into 50 ×10 mm strips. The films were weighed individually and kept in a desiccator containing Calcium Chloride as desiccant at 37° c for 24hr. The films were reweighed individually until a constant weight was obtained. Percentage of moisture content was then calculated based on the change in the weight with respect to the initial weight of the film.[51,52]

The column 3 of table 3.56 showed % moisture content of different formulation. The moisture content in the formulations was found to increase with the increasing concentration of drug and hydrophilic polymer HPMC. Formulation containing EC and HPMC showed higher % moisture content than formulation containing AC and HPMC. Formulation VF 8 showed highest (4.13 %) moisture content and Formulation VF 10 showed lowest (1.231%) moisture content indicating that as ratio of HPMC increases % moisture content increases and vice-versa. The low moisture content is likely to protect the formulation from microbial contamination and bulkiness of the Films.

3.13.6. Determination of Swelling Index

To determine the swelling index of prepared bioadhesive vaginal film, VFs was taken on porous aluminum foil, and then placed separately in a 50 ml beaker containing 10 ml acetate buffer pH 4.7.[70] were discussed in **3.7.6** sections.

The column 6 of table 3.56 showed swelling indexes of different formulations. Swelling index increased in the following order of formulations VF 10 < VF 5 < VF 13 < VF 8. Formulation VF 8 and VF 13, containing highest proportion of HPMC, showed highest swelling index. Formulation VF 10 and VF 5, containing highest proportion of AC and EC, showed lowest swelling index respectively. It indicated that as the proportion of HPMC increased, swelling index increased.

3.13.7. *In Vitro* Drug Release and Release Kinetics of VFs

In vitro drug diffusion studies were carried out by using K.C. cell with a semi permeable barrier. Cellophane membrane was soaked in SVF. Film of specified diameter was placed on the surface of processed cellophane membrane and was fixed to one end of the cylindrical donor compartment by cyanoacrylate adhesives, such that the lower end just touched the surface of SVF medium. Also 0.5 ml of SVF was placed and maintained at same level throughout the study in donor compartment. Temperature was maintained at 37 ± 2°C with constant stirring at 50 ± 10 rpm. A quantity of 5 ml sample was withdrawn from the receptor compartment at definite time interval and replaced with 5 ml of SVF to maintain sink condition. The drug was estimated by using UV-Visible spectrophotometer at 267 nm (λ_{max}).[50,53]

Method: In order to investigate the mechanism of AZT release from VFs, the release data were analyzed with the following mathematical model which was explained in **3.3.1**.

Table 3.57: Drug release profile and kinetic of different formulations

Formulation	%cumulative Drug release (11h study)*	Zero order equation		First order equation		Higuchi Square root eq.		Korsmeyer and Peppas equation	
		r^2	K_0	r^2	K_1	r^2	K_H	r^2	n
VF 5	21.30±0.96	0.991	2.055	0.99	0.010	0.926	7.300	0.988	0.979
VF 8	59.30±0.84	0.873	5.185	0.91	0.033	0.952	19.89	0.924	0.766
VF 10	**13.54±1.12**	**0.981**	**1.229**	**0.97**	**0.005**	**0.889**	**4.298**	**0.939**	**0.785**
VF 13	44.86± 1.11	0.823	3.015	0.99	0.014	0.960	11.96	0.952	0.304

Each value represents as mean ± SD. n=3.

Different kinetic models (zero-order, first-order and Higuchi's) were applied to interpret the release profile from VFs. The best fit with higher correlation (r^2>0.9) was found with the Higuchi's equation. The rate constants were calculated from the slope of the respective plots. The best fit with the highest correlation coefficient was shown in Higuchi followed by first order and zero-order equations as given in table 3.57. The drug release was proportional to square root of time, indicating that the drug release from VFs was diffusion controlled. However, two factors diminish the applicability of Higuchi's equation to matrix systems as this model fails to allow the influence of swelling of the matrix (upon hydration) and gradual erosion of the matrix. Therefore, the dissolution data were also fitted according to the well-known power law equation (Korsmeyer Peppas' equation).The drug release mechanism of all VFs was found to be predominately influenced by the different bioadhesive polymer added. The mechanism of drug release from hydrophilic-hydrophobic polymeric films involves solvent penetration, hydration and swelling of the polymers, diffusion of the dissolved drug in the matrix and erosion of

the gel layer. Form table 3.57, the *n* values for all the formulations ranged from 0.304 to 0.979 indicating different release patterns viz. Case I Fickian release (n = 0.5), Case II non-Fickian (anomalous) release ($0.5 \leq n \leq 0.89$), super case II type of release (≥ 0.89). It was observed that the VF13 (n= 0.304) films underwent Case I Fickian diffusion control, during the diffusion study. In case of Case I Fickian release mechanism, the rate of drug release is much lesser than that of polymer relaxation (swelling/erosion). So the drug release was chiefly dependent on the diffusion through the films. Also it was observed that the formulations VF8(n= 0.766) and VF10 (n=0.785), underwent Case II non-Fickian (anomalous) diffusion control, indicating the rate of drug release is due to the combined effect of drug diffusion and polymer relaxation. Further VF5 (n= 0.979) endured super case II release, denoting polymer relaxation had a significant role in the drug release mechanism. Super Case II release generally refers to the polymer relaxation.

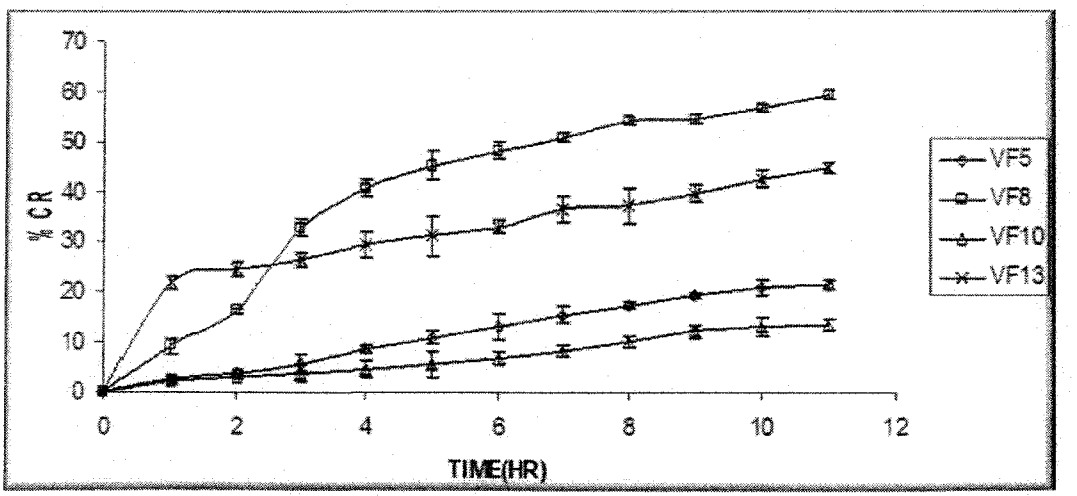


Figure 4.33: *In vitro* drug release profile of different formulations. Each value represents as mean ± standard deviation. n=3.

3.13.7.1 Effect of swelling index on AZT release:

In all the cases, the release rate was increased with increased proportion of hydrophilic polymer (HPMC) due to more swelling. Initially, the diffusion coefficient of drug in the dehydrated polymer will be less and increases significantly as the polymer imbibes more and more water, and forms a gel, as the time progresses. The hydration rate of the polymer and thereby the gel formation significantly depended on polymer proportion. The overall effect of polymer was observed as follows. Formulation VF 8 (EC: HPMC=1:4) with highest swelling index (56.56±0.77), showed highest % cumulative drug release (59.30 ± 0.84% up to 11^{th} hr) while formulation VF10 (AC: HPMC=4:1) with lowest swelling index (17.08±0.67), table 3.56 and 3.57 was found to release the drug only about 13.54± 1.12% upto11hrs. Formulation VF 5 (EC: HPMC=4:1) with swelling index of 19.72±0.77,showed 21.30±0.96% % cumulative drug release up to 11^{th} hr whereas formulation VF13 (AC:HPMC=1:4) with swelling index of 51.32±1.56, was found to release the drug only about 44.86± 1.11% upto11hrs table 3.56 and 3.57. This finding can be attributed to the higher water repelling property of AC, thus concluded that AC was a better rate controlling polymer to sustain the release of drug for longer period of time when compared to formulations containing EC as a rate controlling polymer.

3.13.8. Bio adhesion Strength of vaginal films using Goat Vaginal Mucosa

Method: One film was placed between two vaginal membrane faces. Little pressure was applied to form bio adhesion bond, and then slowly drop of water was added on right side beaker, till the film was separated from one face of vaginal membranes attached. Bio adhesion Strength of vaginal films was found in methods **3.7.7** discusses in that section.

Column 5 of table 3.56, indicates the vaginal bioadhesive properties of the prepared VF (VF4-F13) in goat vagina and the result showed that all vaginal bioadhesive strengths were found in the following order VF8>VF13>VF5>VF10. It was concluded that Bioadhesive strength proportional to the proportion of HPMC in formulation VF 8 (EC: HPMC 1: 4) showed the highest bioadhesive property.

Selected formulation was VF10.

Table 3.58: Comparision study of AZMBVG4, BVG1, MBVT3 and VF10

Formulation	*In vitro* drug release	swelling index	Bioadhesive strength
AZMBVG4	48.58±8.91% upto 24hr	64.70±0.95%(up to 6hr)	1.69±0.02 gm/cm^2
BVG1	99.01±0.08% upto 10hr	67.83±0.24%(up to 6hr)	1.69±0.02 gm/cm^2
MBVT3	82.93±8.01% upto 24hr	2.22% (up to 2hr)	13.15±0.04gm
VF10	13.54±1.12% upto 11hr	17.08±0.67% (up to 25 min)	4.4±1.8gm

On the basis of *invitro* dug release, swelling index and bioadhesive strength table 4.58 selected formulations AZMBVG4 and BVG1 were shown *in vitro* drug release 48.58±8.91% upto 24hr and 99.01±0.08% upto 10hr.While in case of swelling index shown were found 64.70±0.95% (up to 6hr) and 67.83±0.24% (up to 6hr). Bioadhesive strength of AZMBVG4 and BVG1 were found 1.69±0.02 gm/cm^2.

MBVT3 and VF10 were shown *in vitro* drug release 82.93±8.01% upto 24hr and 13.54±1.12% upto 11hrs.While in case of swelling index shown were found 2.22% (up to 2hr) and 17.08±0.67% (up to 25 min). Bioadhesive strength of MBVT3 and VF10 were found 13.15±0.04gm and 4.4±1.8gm.

Reserch protocol was not supported for the vaginal tablet and vaginal films thus **AZMBVG4** and **BVG1** selected for futher stydy.

3.13.9. Vaginal Irritation Study:

Method The vaginal irritation study was done by treating intra-vaginally a group of 3 white female Wister albino rats with 0.2 gm of AZMBVG4, another group of 3 for standard irritant (benzalkonium Chloride, BZK 1%v/v) and one rat, as control (without any formulation). Formulation was applied every day upto10 days. All animals were killed on 11^{th} day. The reproductive tract was examined grossly. The vaginal tissues were rapidly removed and parts of the upper (cervico - vagina), middle and lower (uro - vagina) regions of each vagina were fixed in 10% neutral – buffered formalin for microscopic evaluation. Fixed vaginal tissues were trimmed, embedded in paraffin, sectioned at a thickness of 4 – 6 μm and stained with hematoxylin and eosin and examined under X200 and X400 magnification using a olympus microscope CX21 Leica light microscope (Milton Keynes, Buckinghamshire, United Kingdom) interfaced with an image analysis system (Media Cybernetics, Silver Spring, MD) in conjunction with a 3-CCD camera (DAGE-MTI Inc, Michigan City, IN) for observation and analysis.[9,44] The procedures employed in this study were approved by Institutional Ethical Committee (HPI/ 07/ 60/ IAEC/ 0013) and (HPI/ 07/ 60/ IAEC/ 0002). Histological evaluation of 3 different regions of the vaginal tissues of 6 rats given daily intra vaginal application of AZMBVG4 for 10 consecutive days showed lack of significant vaginal irritation .(Table 3.59 and figurer 3.34) six numbered rats of the treated with AZMBVG4 revealed very mild epithelial ulceration with absence of edema, leukocyte influx, and vascular congestion characteristic of inflammation as quantities by histological scoring according to the method of Eckstein et al indicated AZMBVG4 formulation safe for vaginal application.

Table 3.59: Histological scoring for vaginal irritation study using rat vagina

	Histopathological parameters				
S. No	Batch	Epithelial ulceration	Neutrophil infiltration	Leukocyte infiltration	Vascular congestion
1	Normal(control)	0	--	--	--
2	Standard	+++	+++	++	--
3	Test	--+-	+- -	-- - -	--

+= positive, --= negative (n= 7)

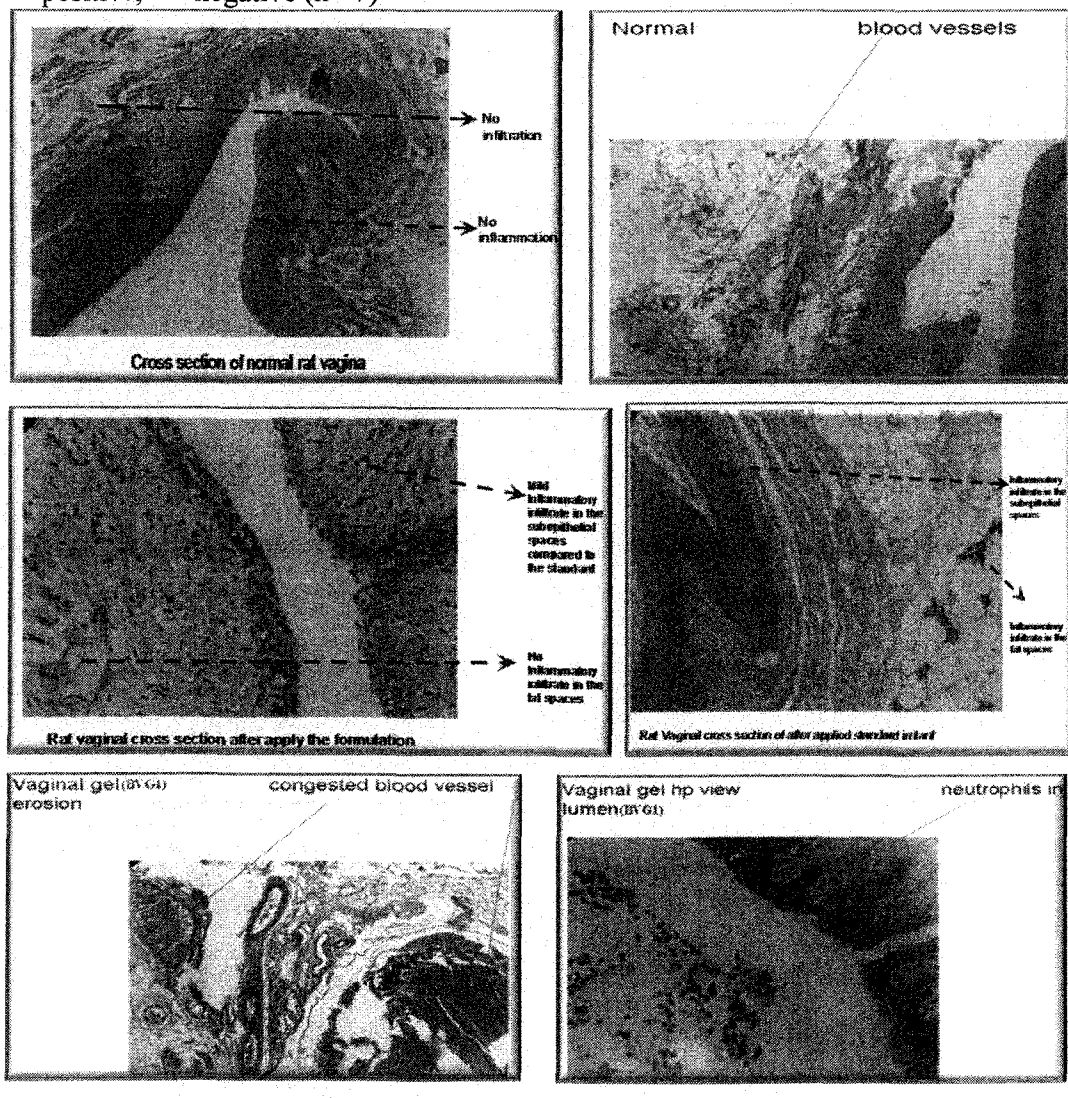


Figure 3.34: Vaginal irritation study on rat vagina

3.14. Retention in simulated vaginal fluid:-

Method: Retention time of microencapsulated vaginal gel in simulated vaginal fluid (SVF) and environment was studied by an *in-vitro* method based on the principle of measuring weight of gel falling down (or retained) as a function of time, from an isolated intact tubular portion of vagina (suspended in vertical position). Dispersion of vaginal formulation (one gram of gel dispersed in 2 ml of SVF) placed inside a vertically suspended excised goat vaginal tube, was allowed to fall under the influence of gravity. The weight of formulation falling down was recorded as percentage leaked out and percentage retained was plotted against time. Also this test was repeated into inclined position at angle of 45^0C.[45]

The percent gel remained in vagina was calculated by using formula:

$$\mathbf{P_{IV} = \{(I_w - F_w) / I_w\} \times 100.}$$

Where as P_{IV} =Percent intra-vaginal gel remained in vagina, I_w =Initial weight, F_w =Final

Table 3.60: vaginal retention study of AZMBVG4:

Time (min.)	**Vertical Position**	**Inclined Position**
	% Gel remained into vagina	% Gel remained into vagina
0	100	100
10	87.48	97.83
20	82.44	96.52
30	77.67	94.11
40	61.54	92.52
50	45.59	90.04
60	33.65	86.87
70	22.87	84.28
80	4.64	81.65
90	3.21	79.52

In-vitro vaginal retention study of selected AZMBVG4formulation was done to determine that how many time the gel retained into the vaginal environment so that it can be absorb from this side. The test was performed in two positions, vertical and inclined position. From table 3.60, it was observed that in inclined position, it retained 79.52 % in 1.5 hrs only, but in vertical position it retained 3.21 % in 1.5 hrs. So it was sufficient to absorb.

3.16. *In – vivo* study of AZMBVG4 in rabbit model:

3.16.1. Preparation of standard curve of AZT in HPLC:

Method: Accurately 50 mg of AZT was taken and dissolved in 50 ml of mobile phase (Solution – A). 5 ml solution was pipette out from solution – A, and then diluted it in a 50 ml volumetric flask (Solution - B) with mobile phase (methanol: water 60:40 V/V). From solution – B different volumes of 1, 2, 3, 4, 5 and 6 ml were taken and diluted up to 10 ml with mobile phase. The 20µl sample was run at 1.2ml/min. flow rate by using λ_{max} 267nm in HPLC (LC-20AT, Shimadzu, Japan) and peak area was noted. A graph was plotted by between concentrations versus peak area. The slope and regression value was calculated from the graph.[11]

Table 3.61: Preparation of standard curve of AZT in HPLC

Serial No.	Conc. (µg/ml)	Absorbance
1.	0.00	0.000
2.	10.00	166.084
3.	20.00	327.614
4.	30.00	489.167
5.	40.00	663.052
6.	50.00	897.550
7.	60.00	974.857

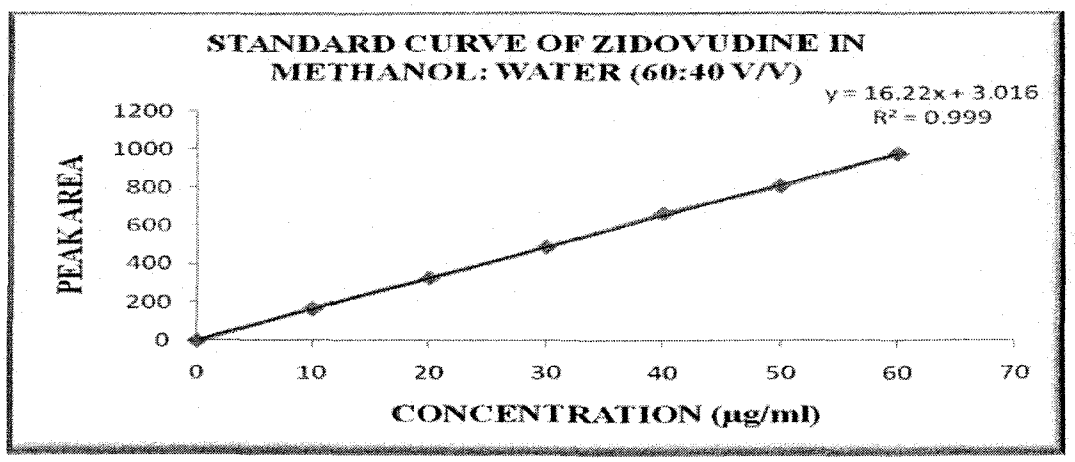


Figure 3.35: Standard curve of AZT in HPLC

The standard curve of AZT in HPLC using mobile phase Methanol: Water (60: 40V/V) was found to be given in figure 3.35. The regression co -efficient (r^2) was found to be 0.999 and equation was Y = 16.22X + 3.016. The data of standard curve was represented in Table 3.61 and figure 3.35.

3.16.2. Experimental design:

Method: Seven adult, female, New Zealand white species rabbits, weighing 1.5 to 1.7 kg were used for the *in vivo* study. The animals were divided into two groups containing four animals each and one animal was used as control.[49] The animals were kept fasted for overnight. Water was given *ad libitum* during fasting and throughout experiment. The rabbits were not anesthetized during or prior to the experiment. The formulation was applied with the help of vaginal applicator and standard oral dose (1mg/ml) with the help of oral cannula. The procedures employed in this study were approved by Institutional Ethical Committee (HPI/ 07/ 60/ IAEC/ 0013/0002). Blood samples (2ml) were collected from marginal ear vein at an interval of 1 hr, 2, 4, 6 and 24 hr during study. The same method was followed for each group (both standard and test). The blood samples withdrawn as above were transferred to a series of graduated centrifuge tube containing 1ml of 10 % w/v EDTA solution and 1 ml of 15 % w/v trichloro acetic acid (TCA).[50] The samples were centrifuged immediately at 3000 rpm for 15 min in cooling centrifuge machine to collect plasma.[51]The plasma was separated and transferred into other set of sample tubes and stored in -20°C until assayed. The plasma samples were analyzed for AZT by firstly passing the samples through silica gel column (for solid phase extraction) and analyzed by HPLC (LC–20 AT, Shimadzu, Japan) using mobile phase methanol:water (60 : 40)[47] at flow rate 1.2 ml/min. Twenty micro liters (20 µl) of

injection volume was eluted in RP C(18) column (4.6 × 150 mm) at room temperature and was monitored at wavelength (λ_{max}) 267 nm using diode array UV detector. [53] The concentration was calculated from standard curve. A standard curve was first prepared using known concentrations of standard AZT against the HPLC peak area and was used throughout for analysis. Standard curve equation was Y = 16.22X +3.016, r^2 = 0.999 (Y = HPLC peak area, X = concentration in µg/ml, r^2 = correlation coefficient).

3.16.3. Calculation of initial and maintenance doses [55]:

One of the most important aims of preformulation study is the initial and Maintenance dose calculation for any sustained release formulation. The amount of AZT to be taken as loading as maintenance doses can be calculated from its pharmacokinetic data. Knowing its biological half life and sustaining its desired blood level for 24 hours. The loading and maintenance doses can be calculated by the following equation-

$$\mathbf{D_t = D_I\ (corrected) + D_m} \quad \text{----------1}$$

Where as, D_t = total dose, D_I (corrected) = Corrected form of non-sustaining loading dose required to achieve initial blood level and D_m = Maintenance dose

Again, $\mathbf{D_I\ (corrected) = D_I - K_1 x W_0\ x\ t_p}$ -------2

$$\mathbf{D_m = K_1 x W_0 x\ h} \quad \text{----------3}$$

Where, D_I = Non-sustaining loading dose, K_1 = Elimination rate constant, W_0 = Initial dose required to produce desired biological half life, t_p = Time required for the onset of action, h = Duration (hours) for which sustaining action is desired

3.16.4. Experimental procedure:

Method: One group was fed with standard AZT at a dose of 2 mg. Other one group was applied the 2gm microencapsulated vaginal gel with the help of vaginal applicator and marked as "Test formulation". One animal was kept as control. Blood samples (2ml) were collected from marginal ear vein at an interval of 1st hr, 2nd , 4th , 6th and 24th hour during study. The same method was followed for each group (both standard and test). The blood samples withdrawn as above were transferred to a series of graduated centrifuged tube containing 1 ml of 10 % w/v EDTA solution.[50] The samples were centrifuge immediately at 3000 rpm for 15 min in cooling centrifuge machine to collect plasma.[44] The plasma was separated and transferred into other set of sample tubes and stored in - 20°C until assayed.[44] About 1 ml plasma was mixed with 1 ml of 15 % w/v Trichloro acidic acid (TCA), shaken well for 3 min and centrifuge at 3000 rpm for 15 min.[44,56]

3.16.5. HPLC analysis of AZT in plasma:

Method: The plasma samples were analyzed for AZT by firstly passing the plasma samples through Silica gel column (for solid phase extraction)[56] analyzed by HPLC (LC – 20 AT, Shimadzu, Japan) using mobile phase methanol : water (60 : 40)[51] at flow rate 1.2 ml/min. Twenty micro liters (20 µl) of injection volume was eluted in RP – C18 column (4.6 × 150 mm) at room temperature. The column eluted was monitored at wavelength (λ_{max}) 267 nm using diode array UV detector.[53] The concentration was calculated from peak area.

After a single oral dose, AZT is rapidly and almost completely absorbed from the gastrointestinal tract. However, the drug undergoes extensive and rapid first-pass

metabolism. Following 1mg oral AZT administration, the plasma level increases rapidly and peaks at about 2hr. However, the plasma level declines rapidly by 6hr after intake and remains low thereafter figure 3.38. In contrast to the oral route, vaginal administration results in plasma concentrations that increase gradually, reaching a maximum level after 4hr and slowly declining, with detectable levels of AZT remaining up to 24 hr after administration figure 3.37 and figure 3.36. Observations of plasma peak area of AZMBVG4, oral standerd suspension and BVG1 given in table 3.63 & 3.36 and figure 3.39. The overall release form BVG1 formulation and pure drug suspension were not significantly different (p<0.05) statistically, indicating that drug amount between the formulations did not vary. The overall release form AZMBVG4 formulation and pure drug suspension and BVG1 were not significantly different (p<0.05) statistically, indicating that drug amount between the formulations did not vary table 3.64-3.67.

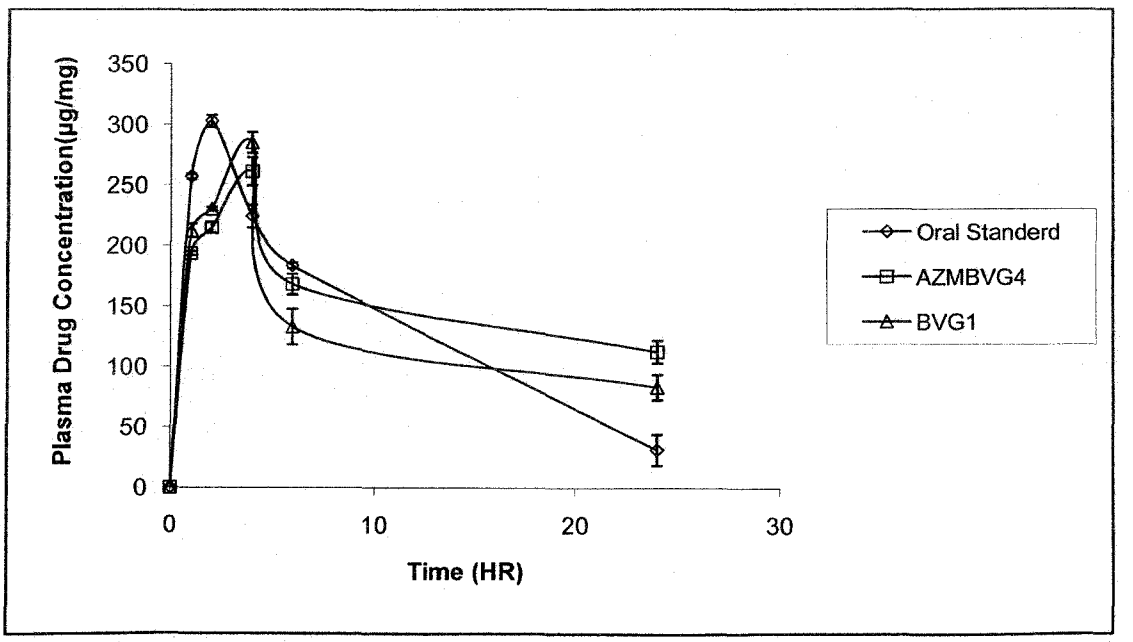
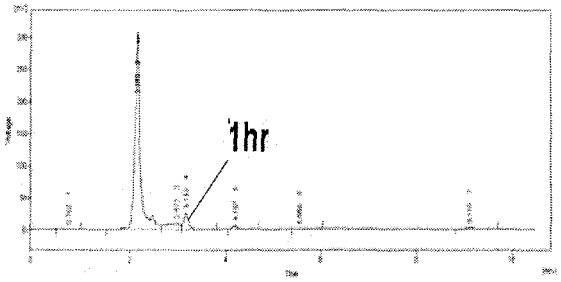
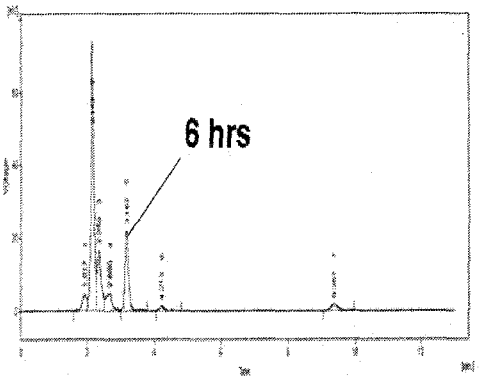


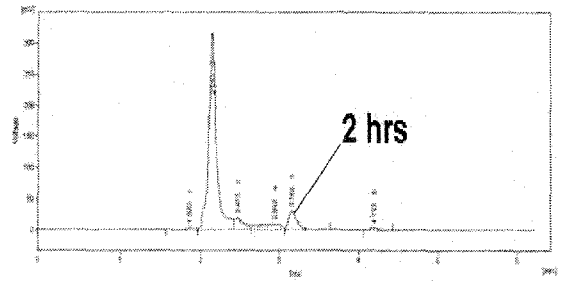
Figure 3.36: Comparision study of oral standard with AZMBVG4



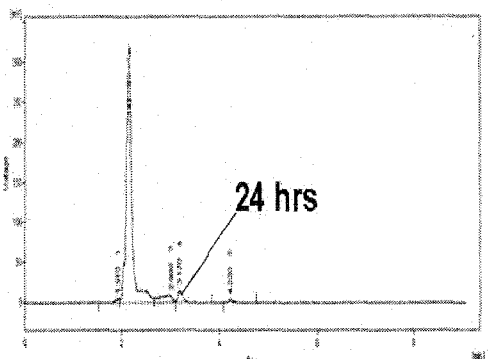
HPLC drug peak of AZMBVG 4 in 1hr



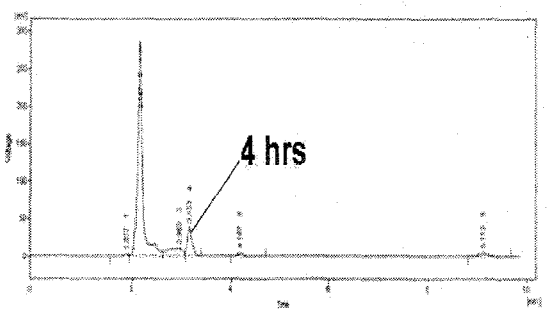
HPLC drug peak of AZMBVG 4 in 6 hrs



HPLC drug peak of AZMBVG 4 in 2 hrs

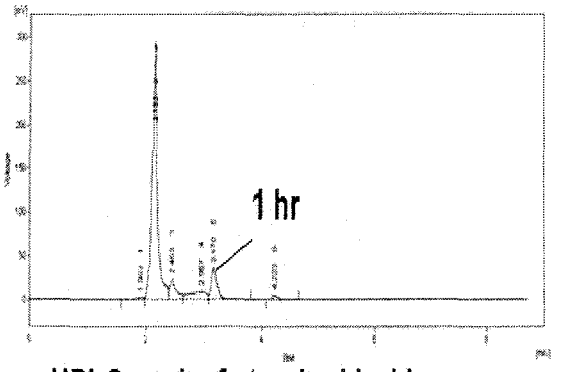


HPLC drug peak of AZMBVG 4 in 24 hrs

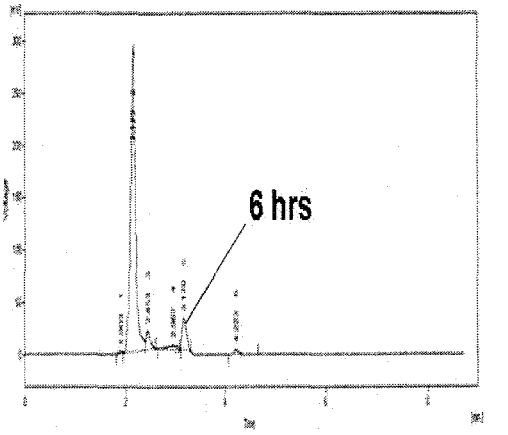


HPLC drug peak of AZMBVG 4 in 4 hrs

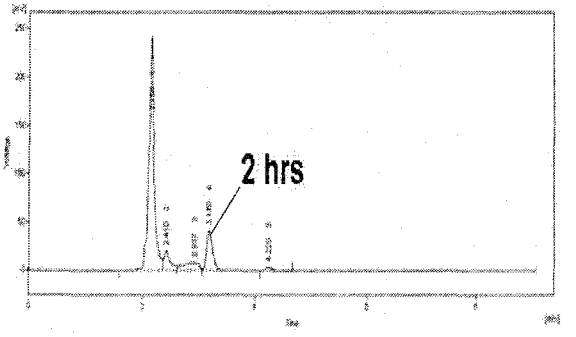
Figure 3.37: HPLC analysis of AZMBVG4



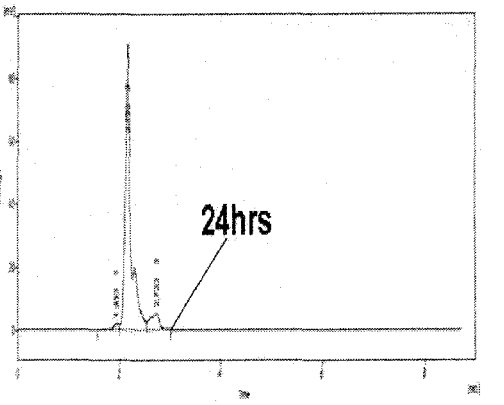
HPLC peak of standard in 1 hr



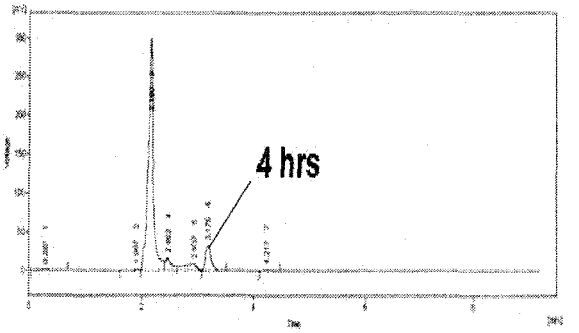
HPLC peak of standard in 6 hrs



HPLC peak of standard in 2 hrs



HPLC peak of standard in 24hrs



HPLC peak of standard in 4 hrs

Figure 3.38: HPLC analysis of oral standerd

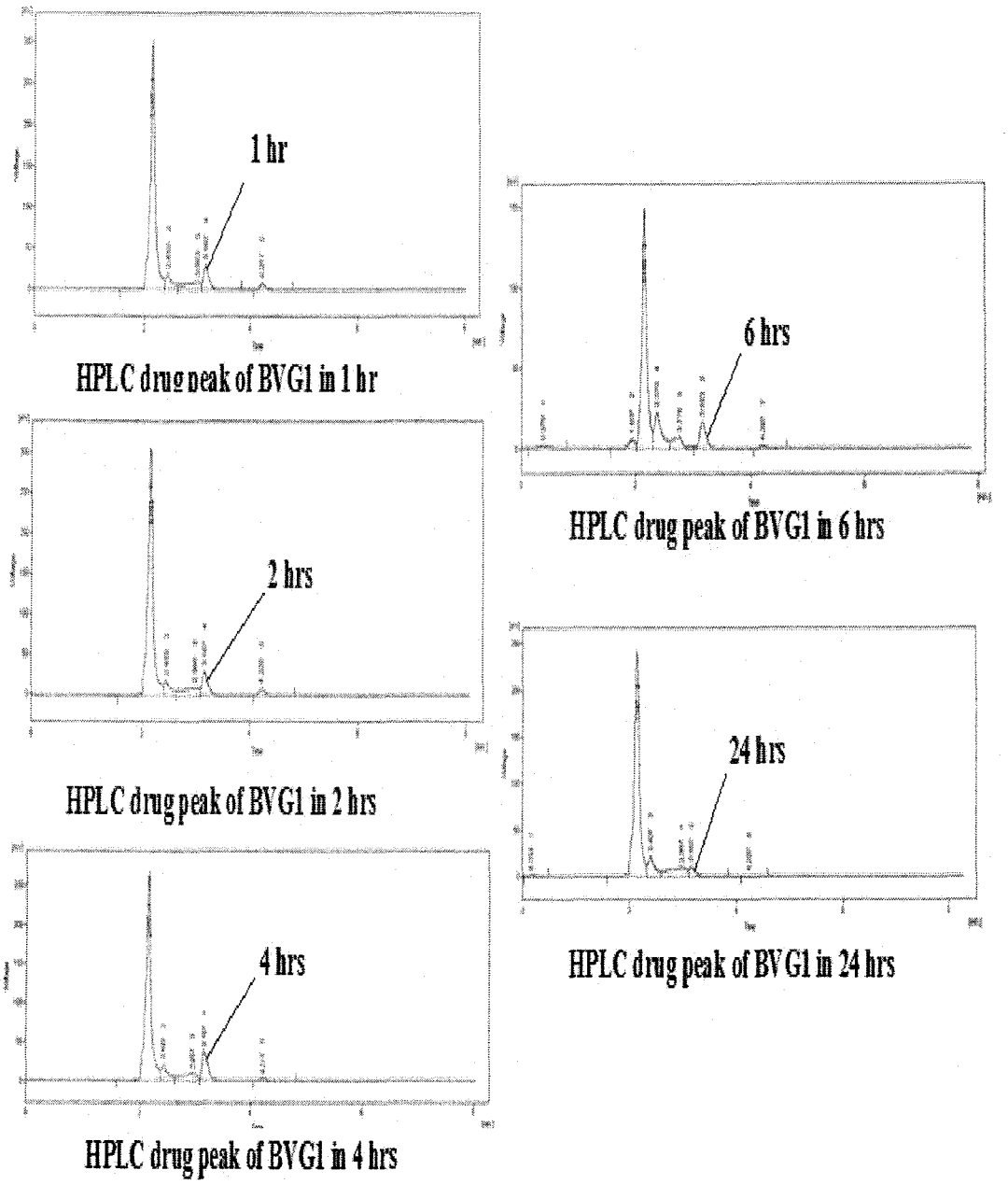
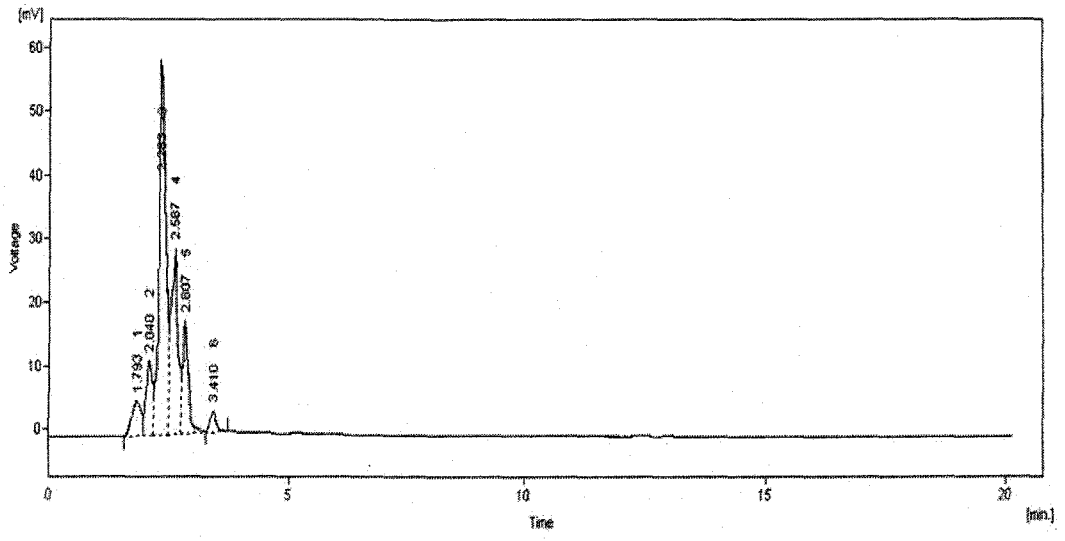


Figure 3.39: HPLC analysis of BVG1



HPLC peak of blank

Figure 3.40: HPLC analysis of blank plasma

Table 3.64: *Invivo* analysis of variance of AZMBVG4, oral standerd and vaginal gel (BVG1):

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	278.682	3	92.894	1.473	$F_{0.05}$=19.16 $F_{0.01}$=99.07
Residual	126.151	2	63.076		
Total		5			

Since the observed value *F* is larger than the 5% tabulated value corresponding to *d.f* (3,2), we accept the null hypothesis and conclude that the drug release among the AZMBVG4, Oral std and Vaginal gel (BVG1) differ not a significantly (P>0.05).

Table 3.65: *Invivo* analysis of variance of AZMBVG4 and oral standard

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	212.275	2	106.138	1.654	$F_{0.05}$=9.55 $F_{0.01}$=30.82
Residual	192.558	3	64.186		
Total		5			

Since the observed value F is larger than the 1% tabulated value corresponding to $d.f$ (2,9), we reject the null hypothesis and conclude that the drug release among the AZMBVG4 and Oral Standard differ not significantly (P<0.05).

Table 3.66: *invivo* analysis of variance of oral standard and BVG1:

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	124.150	2	62.075	0.663	$F_{0.05}$=9.55 $F_{0.01}$=30.82
Residual	280.683	3	93.561		
Total		5			

Since the observed value F is larger than the 1% tabulated value corresponding to $d.f$ (2,9), we reject the null hypothesis and conclude that the drug release among the Oral Standerd and BVG1 differ not significantly (P<0.05).

Table 3.67: *invivo* analysis of variance of AZMBVG4 and BVG1

Source	SS	df	Mean Squares	F-ratio	Tabulated value
Treatment between column	173.852	2	86.926	1.129	$F_{0.05}$=9.55 $F_{0.01}$=30.82
Residual	230.981	3	76.994		
Total		5			

Since the observed value F is larger than the 1% tabulated value corresponding to $d.f$ (2,9), we reject the null hypothesis and conclude that the drug release among the AZMBVG4 and BVG1 differ not significantly (P<0.05).

Table 3.62: Observations of peak area of AZMBVG4 and oral suspension:

S.No.	Time of sampling (hrs.)	Peak area of oral suspension (mV.s)	Concentration (µg/ml)	Peak area of AZMBVG4 (mV.s)	Concentration (µg/ml)
1.	1.0	257.268	11.62464	193.858	8.76
2.	2.0	302.949	13.68606	215.138	9.72
3.	4.0	224.364	10.1398	261.316	11.80
4.	6.0	183.627	8.301489	168.254	7.61
5.	24.0	35.0	0.015072	112.044	5.07

Table 3.63: Observations of peak area of BVG1 and oral suspension:

S.No.	Time of sampling (hrs.)	Peak area of oral suspension (mV.s)	Concentration (µg/ml)	Peak area of BVG1 (mV.s)	Concentration (µg/ml)
1.	1.0	257.268	11.62464	212.643	12.92
2.	2.0	302.949	13.68606	230.549	14.02
3.	4.0	224.364	10.1398	285.163	17.39
4.	6.0	183.627	8.301489	133.055	8.01
5.	24.0	35.0	0.015072	62.982	4.93

3.16.6. Pharmacokinetics analysis:

Method: The primary objectives of the study were to determine AZT plasma concentration, area under the plasma AZT concentration-time curve up to 24hr $[AUC]_0^{24}$ after vaginal and oral administrations of AZT, peak plasma concentration (C_{max}) and time to reach peak plasma concentration (T_{max}). The area under the AZT concentration curve was calculated according to the trapezoidal method. The $[AUC]_0^{24}$ was divided into trapezium segments according to the time intervals of blood sampling. The $[AUC]_0^{24}$ was calculated by summation of the trapezium segments. The highest observed concentration during the study period; C_{max}, and time, at which C_{max} observed, T_{max}, were obtained directly from the plasma concentration – time profiles. The area under the plasma concentration time curve was calculated based on the trapezoidal rule. The volume of distribution (V_d), total body clearance (Cl_T), elimination rate constant (K_E) and half – life ($t_{1/2}$) were also calculated.[58,59]

3.16.6.1. Area under the zero-moment curve (AUC):

The average plasma concentration of AZT obtained each phase of the study was plotted against time and the AUC calculated using the trapezoid rule.[60,61]

3.16.6.2. Area under the first-moment curve (AUMC):

The product of the time and average plasma concentration of AZT obtained each phase of the study was plotted against time and the AUMC calculated using the trapezoid rule.[60,61]

3.16.6.3. Mean Residence Time (MRT): The mean residence time was calculated by using the following relation[61]:

$$\textbf{Mean residence time (MRT)} = {}_0\int^t \textbf{AUMC} / {}_0\int^t \textbf{AUC}$$

3.16.6.4. Area under the zero-moment curve ($_t\int^\infty$AUC): It was calculated using the following relation[56]:

$${}_t\int^\infty \textbf{AUC} = \textbf{Concentration of drug at time (t) in the body} / K_E$$

3.16.6. 5. Area under the first-moment curve ($_t\int^\infty$AUMC): It was calculated using the following relation[56]:

$${}_t\int^\infty \textbf{AUMC} = \textbf{Product of the concentration and time at time (t) in the body} / K_E$$

3.16.6.6. Area under the zero-moment curve ($_0\int^\infty$AUC): It was calculated using the following relation[56]:

$${}_0\int^\infty \textbf{AUC} = {}_0\int^t \textbf{AUC} + {}_t\int^\infty \textbf{AUC}$$

3.16.6.7. Area under the first-moment curve ($_0\int^\infty$AUMC): It was calculated using the following relation[56]:

$${}_0\int^\infty \textbf{AUMC} = {}_0\int^t \textbf{AUMC} + {}_t\int^\infty \textbf{AUMC}$$

3.16.6.8. Peak plasma concentration (C_{max}) and time to peak plasma concentration (T_{max}): These were obtained from the serum concentration versus time curve.[60]

3.16.6.9. Relative bioavailability: The relative bioavailability (F) of tested formulation was finally calculated according to the following formula[59]:

$$F = \{AUC_{0\text{-}24hrs} \text{ (tested formulation)} / AUC_{0-24hrs} \text{ (oral suspension)}\} \times 100$$

3.16.6.10. Half-life of drug in plasma ($t_{1/2}$): The half-life of drug was calculated by using following relation[56]:

$$\text{Half-life } (t_{1/2}) = (0.5\times C_0)/K$$

Where, C_0 = Initial concentration of drug in the body. K = Zero-order rate constant.

3.16.6.11. Elimination rate constant (K_E): The elimination rate constant was obtained from the following relation[56]:

$$K_E = 0.693/t_{1/2}$$

3.16.6.12. Volume of distribution of drug (V_d): This was calculated using the relationship[61]:

$$V_d = \text{Amount of drug in the body (X)/ Plasma drug concentration (C)}$$

3.16.6.13. Plasma clearance rate (Cl_T): The rate of clearance of AZT from the plasma in all the phase of the study was determined using the relation[61]:

$$Cl_T = 0.693\ V_d / t_{1/2}$$

There are only a few reported studies of the pharmacokinetic profiles of AZT through vaginal route. The mean plasma concentration of AZT was determined after oral administration of pure drug suspension (1mg/ml) and vaginal AZMBVG4 formulation containing AZMC4 (407 mg) with the microcapsule: polymer ratio of 1:4. The peak plasma concentration (C_{max}) and the peak time (T_{max}), indications of the rate of absorption, differed between the two groups. The C_{max} (11.807μg ml^{-1}) after vaginal administration was achieved lower, than C_{max} (13.686μg ml^{-1}) after oral administration. After a single oral dose, AZT is rapidly and almost completely absorbed from the gastrointestinal tract, as C_{max} was found higher in 2hr than the C_{max} of vaginal administration of AZMBVG4. However, the drug undergoes extensive and rapid first-pass metabolism as evident from oral AZT administration, the plasma level increases rapidly up to 2hr followed by sharp decline in plasma level up to 24hr thereafter figure 3.37. The area under the plasma concentration time curve $[AUC]_0^{24}$ was calculated by

summation of the trapezium segments according to the time intervals of blood sampling. In contrast to the oral route, vaginal administration results in plasma concentrations that increased gradually, reached a maximum level after 4hr and slowly declining, with detectable levels of AZT remaining up to 24 hr after administration and bioavailability figure 3.37 and 3.36, measured as the area under the plasma level time curve ${}_0\int^{24}$AUC (μg.h/ml) of plasma AZT. Pharmacokinetic studies showed a rapid rise to peak plasma level and a sustained elevation in AZT, resulting in (${}_0\int^{24}$AUC =168.68 μg.h/ml) through vaginal route, which is higher than the oral route (${}_0\int^{24}$AUC=134.58 μg.h/ml). However, vaginal absorption has been shown to be slower and the plasma level of AZT is sustained longer than with oral AZT. The effect of AZT may linger for 24 hr after a single dose administered vaginally. Since, the effects of vaginal and oral AZT are similar and more pronounced than for oral AZT, it seems that it is the sustained plasma levels, rather than the high peak plasma concentration, that are crucial to efficacy. The volume of distribution (V_d) 0.169 L/kg, total body clearance (Cl_T) 0.064 L/kg/hr, elimination rate constant (K_E) 0.379hr^{-1} and half – life ($t_{1/2}$) 1.824 hours was found table 3.68 when compared with oral pure drug suspension.

Table 3.68: Pharmacokinetic study of AZMBVG4 and oral suspension:

S.No.	Parameters	Pharmacokinetics of Oral Suspension	Pharmacokinetics of AZMBVG 4
1.	${}_0\int^{24}$AUC (μg.h/ml)	135.58	168.68
2.	${}_0\int^{24}$AUMC (μg.h^2/ml)	129.72	242.79
3.	${}_0\int^{\infty}$AUC (μg.h/ml)	135.540	189.04
4.	${}_0\int^{\infty}$AUMC (μg.h^2/ml)	130.89	1928.4
5.	MRT at 24 (hrs.)	0.956	1.43
6.	MRT at ∞ (hrs.)	0.965	10.200
7.	C_{max} (μg/ml)	13.686	11.807
8.	T_{max} (hrs.)	2	4
9.	$t_{1/2}$ (hrs.)	1.428	1.824
10.	K_E (hr^{-1})	0.485	0.379
11.	V_d (L/Kg)	0.197	0.169
12.	Cl_T (L/Kg/hr.)	0.070	0.064
13.	F_r (%)	100	71.69

The mean plasma concentrations were found of AZT after oral administration of pure drug suspension (1mg/ml) and vaginal BVG1 formulation containing AZT (100 mg) with the ratio 1:4 but not microcapsules. The peak plasma concentration (C_{max} 17.39μgml ^{1}) achieved is lower than following oral administration but bioavailability, measured as the area under the curve $_0\int^{24}$AUC (μg.h/ml) of plasma AZT, resulting in a ($_0\int^{24}$AUC =193.29 μg.h/ml) that though vaginal route is higher than for the oral route ($_0\int^{24}$AUC =134.58 μg.h/ml) table 3.69. The volume of distribution (V_d) 0.114 L/kg, total body clearance (Cl_T) 0.270 L/kg/hr, elimination rate constant (K_E) 0.188hr^{-1} and half – life ($t_{1/2}$) 3.68 hours was found table 3.69 compare with oral pure drug suspension. Bioavailability (F_r) was found 71.69% for AZMBVG4 and 161% for BVG1 compare with oral pure drug suspension table 3.68 and 3.69.

Table 3.69: Pharmacokinetic study of BVG1 and oral suspension:

S.No.	Parameters	Pharmacokinetics of Oral Suspension	Pharmacokinetics of BVG1
1.	$_0\int^{24}$AUC (μg.h/ml)	135.58	193.29
2.	$_0\int^{24}$AUMC (μg.h^2/ml)	129.72	276.98
3.	$_0\int^{\infty}$AUC (μg.h/ml)	135.540	219.52
4.	$_0\int^{\infty}$AUMC (μg.h^2/ml)	130.89	911.2
5.	MRT at 24 (hrs.)	0.956	1.43
6.	MRT at ∞ (hrs.)	0.965	4.15
7.	C_{max} (μg/ml)	13.686	17.39
8.	T_{max} (hrs.)	2	4
9.	$t_{1/2}$ (hrs.)	1.428	3.68
10.	K_E (hr^{-1})	0.485	0.188
11.	V_d (L/Kg)	0.197	0.114
12.	Cl_T (L/Kg/hr.)	0.070	0.270
13.	F_r (%)	100	161

3.15. Stability studies of gel:

Method: The formulated gel were filled in the sterile lacquered collapsible aluminum tubes and stored at different temperature condition viz. 25 ± 2°C (refrigerator

temperature) and 50 ± 2°C (condition of accelerated stability testing) for a period of three months and studied for color, pH, extrudability and drug content as per International Conference on Harmonization (ICH) guidline on stability testing.[42,46,47]

Table 3.70 and 3.71 shows the concentration, potency and log percent concentration of AZMBVG4 for 90 days stability study, and table 3.71 shows the parameters determined for the stability of AZMBVG4. Shelf life in year in different temperatures like 25± 1°C and 50± 1°C result were found 6.579 and 3.990 years respectively. Degradation Half life was also calculated in year of AZMBVG4 in different temperatures like 25± 1°C and 50± 1°C results were found 1.006 years, and 0.610 years for respective temperatures.

Table 3.70: Stability studies (drug concentration, potency and log % concentration) of AZMBVG 4

Temperatures		25± 2°C			50± 2°C	
Time(in Days)	Conc.(in mg/gm gel)	Potency (%)	Log % Conc.	Concentration (in mg/gm gel)	Potency (%)	Log % Conc.
0	1.20	100.00	2.000	1.20	100.00	2.000
7	1.20	100.00	2.000	1.19	99.17	1.996
14	1.20	100.00	2.000	1.19	99.17	1.996
21	1.19	99.17	1.996	1.18	98.33	1.992
30	1.20	100.00	2.000	1.19	99.17	1.996
38	1.20	100.00	2.000	1.17	97.50	1.989
45	1.19	99.17	1.996	1.18	98.33	1.992
52	1.19	99.17	1.996	1.17	97.50	1.989
60	1.18	98.33	1.992	1.16	96.67	1.985
68	1.19	99.17	1.996	1.16	96.67	1.985
75	1.17	97.50	1.989	1.17	97.50	1.989
82	1.18	98.33	1.992	1.15	95.83	1.982
90	1.17	97.50	1.989	1.15	95.83	1.982

Table 3.71: Parameters determined for stability studies of AZMBVG 4

Parameters	Temp. 25± 2°C	Temp. 50± 2°C
Zero – order (R^2)	0.738	0.828
First – order (R^2)	0.749	0.856
First – order rate constant (k_1) (in day^{-1})	2.886×10^{-4}	4.758×10^{-4}
Degradation Half life (in year)	1.006	0.610
Shelf life (in year)	6.579	3.990

3.17 Viscosity measurement

A Brookfield digital viscometer (Brookfield Engineering Laboratories, Model DV-E, Mumbai) with a suitable sample adaptor and Spindle (S64) was used to measure the viscosities in cps of the microencapsulated gel prepared.[62]

Viscosity is an important parameter for characterizing the gels as it affects the spreadability, extrudability and release of drug. Table 3.72 showed the data of viscosity. The viscosity of gels was increased with the increase in carbopol content which may be due to the increase in formation of three dimensional cross linking structure of gel, as expected.

Table 3.72: Viscosity studies of AZMBVG4

Sample	Spindle	RPM	cP	% (T)
AZMBVG4	64	20	29400	99.2
		50	21200	21.8
		EE	–	–
		50	22953	33.5
		20	19300	24.5

3.18. Statistical Analysis

MYSTAT soft-ware for uni-variables comparison was for statistical analysis used.The Analysis of Variance is presented as mean ±SEM and p=0.01 and p=0.05 was considered significant. Statistical data analyses were performed using the ANOVA one way at 1 % and 5% level of significance $p < 0.01$ and $p<0.05$.

3.19. REFERENCES

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