CHAPTER-3
(METHOD)

PREFORMULATION

To develop the final dosage forms to the highest quality in the shortest time. It is essential that certain fundamental physical and chemical properties of the drug molecule and other derived properties of the drug powder are determined, understood, and effectively utilized. This information dictates many of the subsequent events and approaches in formulation development. This first learning phase is known as Preformulation.

- A stage of development during which the physicochemical properties of drug substance are characterized.
- A case of learning before doing.
- Reducing time to market, increasing efficacy and enhancing solubility for improved drug delivery.

Preformulation Drug Characterization

- Assay development
- Melting point
- Hygroscopicity
- Loss on drying
- Partition coefficient
- Microscopy
- Micrometrics
- Ionization behaviour
- Solubility studies
- pH profile
- Dissolution studies
- Thin layer chromatography

2.1 DRUG IDENTIFICATION TEST

Analytical method for Tizanidine HCl[69]

The technique of ultra-violet spectrophotometry is one of the most frequently employed in pharmaceutical analysis. It involves the measurement of the amount of the ultra violet (190-380nm) or visible (380-800 nm) radiation absorbed by a substance in solution

Tizanidine HCl can be estimated by UV spectrophotometrically in pharmaceutical formulation. A solution of Tizanidine HCl in methanol gives maximum absorbance at λ_{max} of 318.5 nm.

Preparation of calibration curve

100µg/ml (1mg/ml) stock solution of drug was prepared in methanol and suitable dilutions were made i.e.5 µg/ml, 10 µg/ml, 15 µg/ml, 20 µg/ml, 25 µg/ml respectively. The sample was scanned using UV spectrophotometer (schimadzu, uv-1700), and λmax was 318.5nm was obtained. The absorbance of samples of different concentration at λmax (at 318.5 nm) were measured. The graph was plotted between the absorbance and concentration. The graph obeyed the Beer-Lambert’s law in the selected concentration range. A linear relationship was observed over the range of 5-25µg/ml.

TABLE 2.1 Calibration curve of Tizanidine HCl

S.NO	CONC. (µg/ml)	Absorbance at ג max nm
1	5	0.025
2	10	0.04
3	15	0.072
4	20	0.137
5	25	0.101

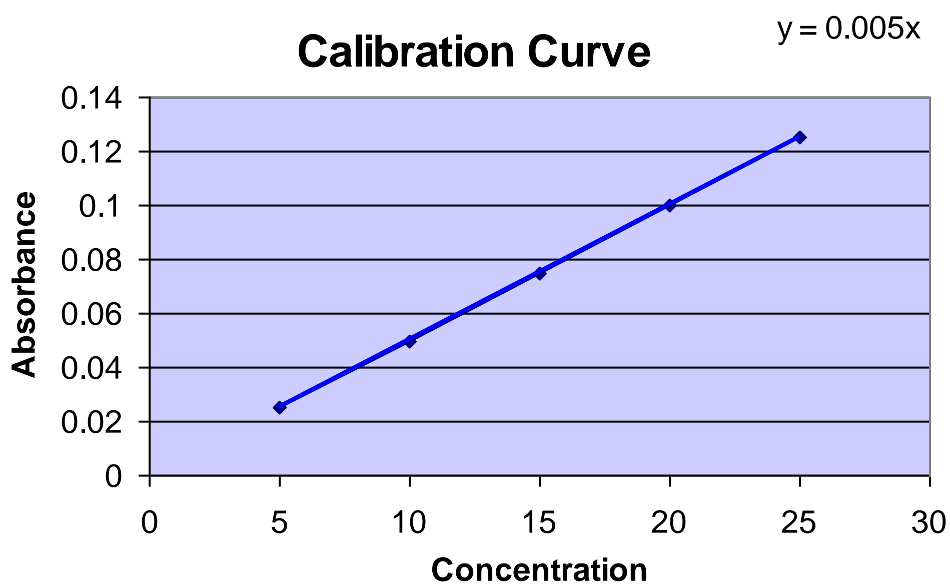

Figure. 1.6: Calibration Curve of Tizanidine HCl

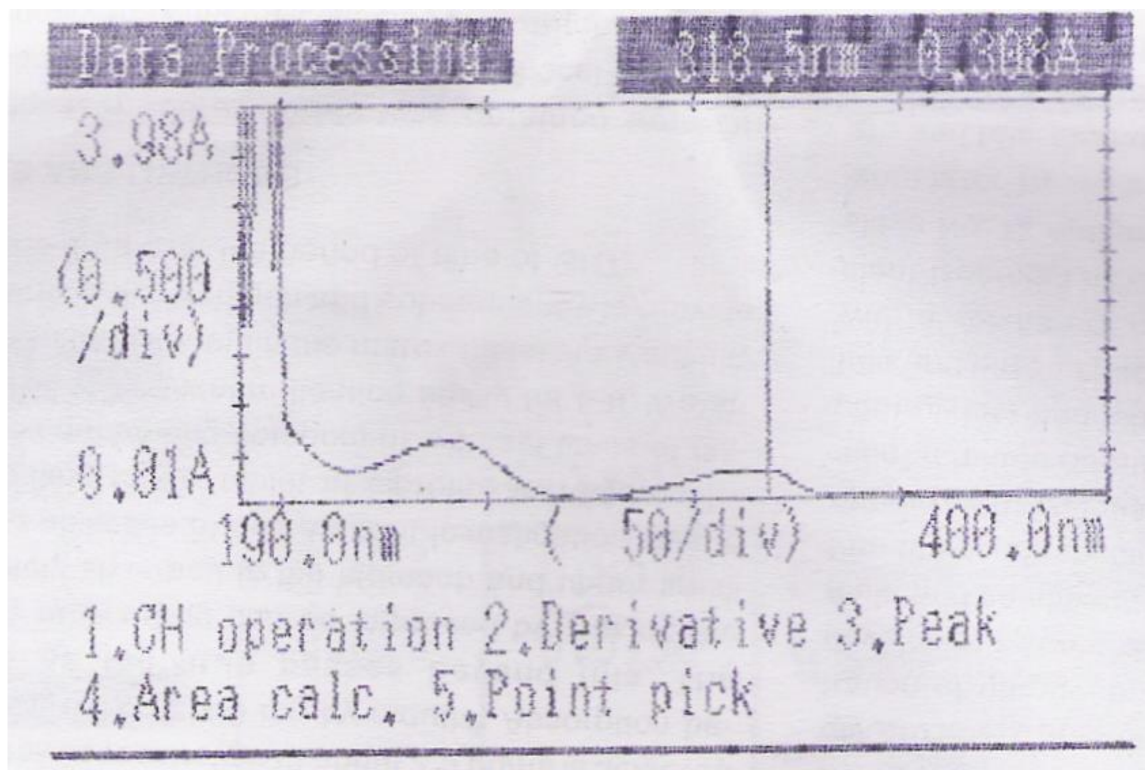

Figure. 1.7: UV Spectrum of the drug

2.2 THIN LAYER CHROMATOGRAPHY[70]:

Thin layer chromatography is an important analytical tool in the separation, identification and estimation of different drugs. In this technique the different compounds are separated by the difference in migration of solute between two phases, a stationary phase and a mobile phase. The principle of separation is adsorption and the stationary phases act as an adsorbent.

Application of the sample: -

The solution of Tizanidine HCl(0.1%w/v) was made in methanol. About 5µl sample was spotted by capillary tube on the pre coated silica gel G-TLC plates by keeping a distance of about 2 cm above the base of the plate. Allow it to dry in air.

- **Development of the chromatogram: -**

The plate was then placed in TLC chamber previously saturated with appropriate solvent system (Toluene; Acetone, 1:1 v/v) for the development of chromatogram, the plate was removed and the spot was observed under the UV chamber at the short wavelength 254nm, the principal spot in the chromatogram obtained. The Rf value was calculated and tabulated.

- **Stationary phase** = pre coated silica gel- G plate
- **Mobile phase** = Toluene: Acetone, 1:1v/v

TABLE 2.2: Rf values of Tizanidine Hydrochloride

Drug	Rf Value	Rf value Standard
Tizanidine HCl	0.44	0.46

- **Interpreting the data**

The R_f value for each spot should be calculated. R_f stands for "ratio of fronts" and is characteristic for any given compound on the same stationary phase using the same mobile phase for development of the plates. Hence, known Rf values can be compared to those of unknown substances to aid in their identifications.

$$R_f = \frac{\text{Distance from start to center of substance spot}}{\text{Distance from start to solvent front}}$$

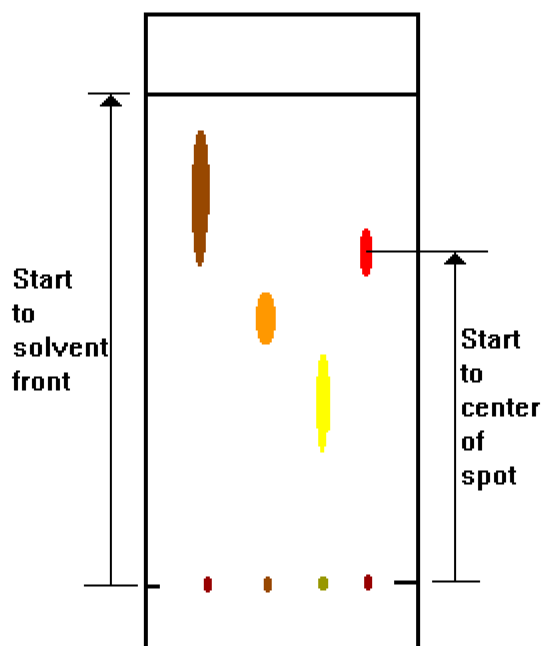

FIGURE 1.8 Development of chromatogram

R_f Value of Tizanidine HCl= 0.80

2.3 MELTING POINT DETERMINATION[71]

The measurement of melting point is a measure concern in pharmaceutical studies. The most important reason to determine melting point during preformulation is crystalline solubility such studies are particularly important because the scarcity of available drug powder often precludes accurate solubility determinations. Melting point and solubility are related via the latent heat of fusion, which is the amount of heat generated during melting or fusion.

Auto melting point apparatus

Melting point was determined by digital auto melting point apparatus (decibel). Similar procedure was repeated here in this case. Automatically the temperature at which drug starts to melt was shown by digital auto melting point apparatus, noted and compared with literature value.

It was found that melting point of drug is 286 ^{o}C.

2.4 HYGROSCOPICITY[72, 73]

Hygroscopicity is an important characteristic of a powder. It can be shown, roughly, for a fairly soluble compound that the hygroscopicity is related to its solubility. A substance that absorbs sufficient moisture from the atmosphere to dissolve itself is deliquescent. Materials unaffected by relative humidity are termed non-hygroscopic, whereas those in dynamic equilibrium with water in the atmosphere are hygroscopic.

Drug salts should have limited hygroscopicity to ensure good chemical and physical stability under all reasonable climatic conditions. As a working limit it should be < 0.5% H_2O at <95% RH.Hygroscopicity experiment is carried out most easily by exposing drug to an atmosphere of an known relative humidity.

A weighed amount of drug was exposed at atmosphere condition and to a RH of 60%, 70% in Humidity chamber for 48 hrs the weighing of petridishes was done and hygroscopicity was calculated.

TABLE 2.3 Hygroscopicity of drug at room and accelerated condition

Temperature/ relative humidity	Hygroscopicity
25◦ C and 60 % RH	1.3 %
40◦ C and 75 % RH	0.54 %
At room temperature	0.75 %

2.5 LOSS ON DRYING:

In pharmacy, the term loss on drying is an expression of moisture content on a wet weight basis, which is calculated as follows:-

%LOD = WT OF WATER IN SAMPLE /TOTAL WT OF WET SAMPLE X 100

LOD is loss in wt in percentage weight/ weight resulting from water and volatile matter of any kind that can be driven off under specified condition(if substances is in form large crystal size reduced by rapid crushing to powder).

One gram of drug was kept in petridish, it was then covered and sample was distributed as evenly as possible by gentle shaking.

Pertidish was placed in tray dryer (Hicon, New Delhi) at105 $^{-0}$C. The sample was dried to constant weight After 4 hr it was found that loss on dry was 0.3%, which was, comply with specification as per limit (NMT 0.5%)

Loss on drying was determined by keeping drug powder of known weight at 60◦ C and 0.7 K Pasc. for 4 hrs. and was found to be **0.65 %**[74, 75].

2.6 SOLUBILITY STUDY

Solubility is defined in quantitative terms as the concentration of solute in a saturated solution at a certain temperature and in qualitative way; it may be defined as the spontaneous interaction of two or more substances to form a homogenous molecular dispersion.

The solubility of drug is an important physicochemical property because it affects the bioavailability of the drug, the rate of drug release into the dissolution medium, and consequently, the therapeutic efficacy of the pharmaceutical product. Preformulation solubility studies focus on drug-solvent system that could occur during the delivery of a drug candidate.

Common solvents used for solubility are:

- Water
- Chloroform
- Methanol
- Acetone
- Cyclohexane
- 2% Acetic acid solution
- Liquid paraffin light
- Liquid paraffin heavy

Preparation of saturated solution:

The solubility of a material is usually determined by the equilibrium solubility method, which employs a saturated solution of the material, obtained by stirring an excess of material in the solvent for a prolonged period (24 hr) until equilibrium is achieved.

Analysis of saturated solutions:

The solution were filtered through sintered filter and absorbance were measured at 318.5 nm using UV spectrophotometer the result are shown in the table[76].

TABLE 2.4 Solubility in different solvents

S No.	Solvent	Conc.(mg/ml)	Volume of solvent in ml/gm of solute	Solubility
1.	Cyclohexane	27777	More than 10,000 part	Practically insoluble
2.	Methanol	1.785	1-10	Freely soluble
3.	Chloroform	9.46	1-10	Freely soluble
4.	Water	0.9	1-10	Freely soluble
5.	Liq Paraffin Heavy	14001.68	More than 10,000	Practically insoluble
6.	Liq Paraffin Light	70028.01	More than 10,000	Practically insoluble
7.	5% Acetic acid	3.94	1-10	Freely soluble

2.7 PARTITION COEFFICIENT

Partition coefficient is the solvent water quotient of drug distribution. The partition coefficient of a compound that exists as a monomer in two solvents is given by

$$K = C_1/C_2$$

If it exist in as an n-mer in one of the phases, the equation becomes

$$K = C_1 / C_2$$

$$\text{Or } \log K = n \log C_1 - \log C_2$$

It is defined as the ratio of un-ionized drug distributed between the organic and aqueous phases at equilibrium

$$P_{o/w} = (C_{oil}/C_{water})_{equilibrium}$$

Partition coefficient (oil/water) is a measure of a drug's lipophilicity and an indication of its ability to cross cell membranes.

Drugs having values of log P much greater than 1 are classified as lipophilic, whereas those with partition coefficients much less than 1 are indicative of a hydrophilic drug[77, 78].

$$\text{Partition coefficient} = \frac{\text{concentration of drug in organic phase}}{\text{Concentration of drug in aqueous phase}}$$

log P=O0.653.

2.8 PARTICLE SIZE DETERMINATION:

A dispersion of drug (1mg) in glycerine was taken on glass slide and mounted under optical microscope initially at 10X magnification. After the adjustment for view, magnification was increased to 50X. Particle size analyses were carried out by Photomicroscope and USB digital scale computer program (software).

Table 2.5: Particle size determination of Tizanidine HCl

S.No.	Particle size range (µm)	No. of particles
1.	0-5	18
2.	5-10	22
3.	10-15	16
4.	15-20	15
5.	20-25	12
6.	25-30	10
7.	30-35	8
8.	35-40	5
9.	40-45	2
10.	45-50	2
11.	50-55	1
12.	55-60	1

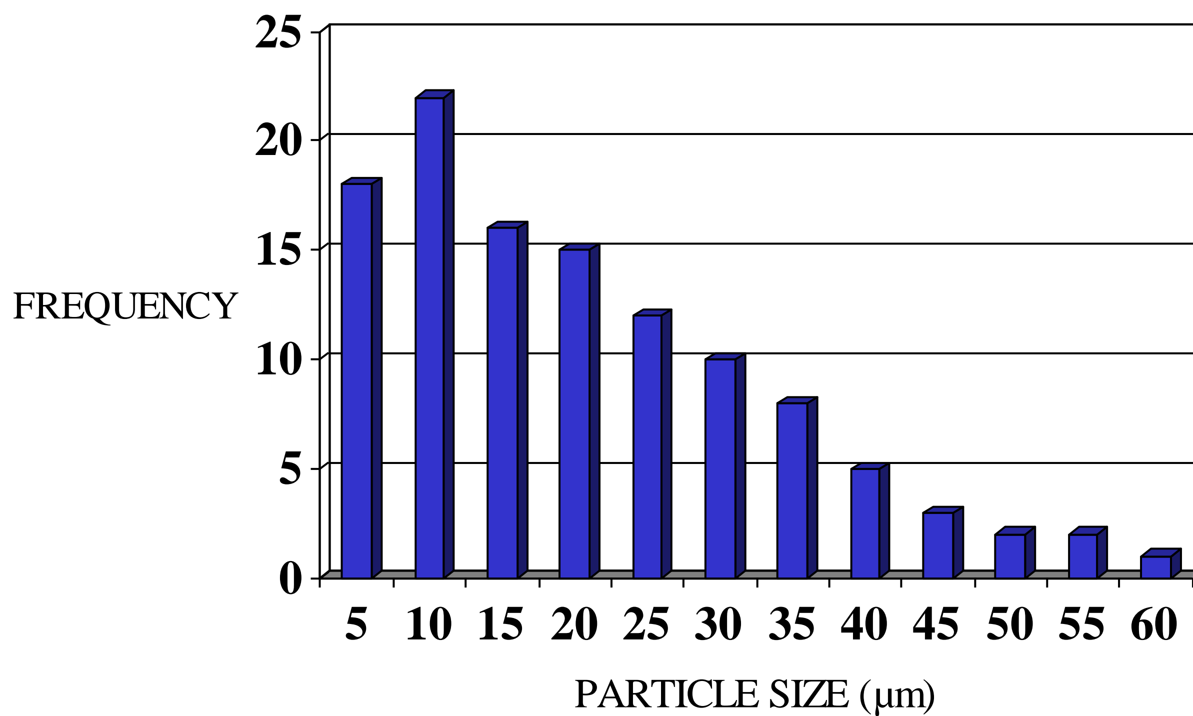

FIGURE. 1.9 Particle size distribution of Tizanidine HCl

2.9 MICROMETRICS STUDY

- **Bulk density:**

1 GM of pure drug was taken and fill in 10 ml capacity measuring cylinder and note the volume/height of drug filled.

Bulk density = mass of powder \ bulk volume of powder

TABLE 2.6 BULK DENSITY OF DRUG POWDER

S.no	Mass of drug Gm	Height of drug	Untapped density	Average density
1.	1	2.8	0.36	
2.	1	2.7	0.37	0.36
3.	1	2.9	0.35	

Result: The Untapped density of powder is = 0.36gm\ml.

- **Tapped density**

1 GM of pure drug was taken and fills in 10 ml capacity measuring cylinder and tapped the measuring cylinder (100 times) until the constant height obtained note the volume/height of drug filled.

Tapped density = mass of powder \ volume of powder after tapping

TABLE 2.7 Tapped density of drug powder

S.no	Mass of pure drug	Height of drug	Tapped density	Average
1.	1	1.9	0.52	
2.	1	2.0	0.50	0.51
3.	1	1.95	0.51	

Result: the Tapped density of drug is 0 .51gm\ml

- **Carr index:**

Carr index = (Tapped density – Untapped density) / Tapped density

Carr's index = 29.3 %(poor flow)

- **Angle of repose**:

Angle of repose was determined in preformulation to find out the flow properties of drug.

Placed a glass funnel on a ring supported by a stand, 4 gm of drug was taken and passed through funnel; maintain the gap between the bottom of the funnel and the top of powder from pile. After emptying the powder from the funnel measure the height of the pile and diameter[79, 80].

Angle of repose = tan Ø 2h/D

Where d=diameter

h= height

Angle of repose= 53.33(very poor flow)

2.10 DETERMINATION OF pH VALUE:

The pH value conventionally represents the acidity or alkalinity of an aqueous solution. the determination was carried out at a temperature of $25^0 \pm 2^0$.

The pH value of solution is determined potentiometrically means of glass electrode (Hicon, New Delhi) and a digital pH meter (Systrnics-Digital pH meter 802). Potentiometric titration of 1% Tizanidine HCL solution is done.

It was obtained that pH of prepared solution of Tizanidine HCl in 1% suspension in water is in between 3-5, which comply with the pH value as given in certificate of analysis[81] (ENNDOC PHARMA, GUJRAT).

2.11 IONIZATION CONSTANT (pKa)

Determination of the dissociation constant for a drug capable of ionization within a pH range of 1 to 10 is important since solubility, and consequently absorption, can be altered by orders of magnitude with changing pH. The Henderson-Hasselbalch equation provides an estimate of the ionized and un-ionized drug concentration at a particular pH.

Ionization constant was determined by using potentiometeric titration[81]

Potentiometric (Hicon, New Delhi) titration of 1% drug solution was done and pH determined by digital pH meter (Systrnics-Digital pH meter 802)

2.12 DRUG POLYMER INTERACTION STUDY

While designing any drug delivery system, it is imperative to give consideration to the compatibility of drug and polymer used within the system. Therefore it is necessary to confirm that the drug is not interacting with polymer under experimental conditions and shelf life. The interaction studies can be done on the basis of Assay, U.V, Infra red and TLC analysis.

For the present study, the drug-polymer interaction was studied by comparing it with the pure drug (Tizanidine HCl) and physical mixture of drug-polymer by TLC analysis.

(i) Thin Layer Chromatographic Studies

The compatibility of drug and polymer is an important prerequisite to formulation. So a thin layer chromatographic method was used as follows for the drug carrier interaction.

- **Stationary Phase**: Recoated silica gel GF 254 plate
- **Mobile Phase**: mixture of 95 volume of toluene and 5 volume of acetic acid.
- **Sample preparation:** Apply 20 µl of mixture of chloroform containing 0.1%w/v of drug

The plate was spotted with standard solution at about 2 cm from the bottom. The sample solution was spotted adjacent to the pure drug spot at a distance of 2 cm. The plate was kept in a chamber saturated previously with the solvent system. The solvent was allowed to rise on the plate to a sufficient level. The distance traveled by the solvent front was noted. The distance traveled by the solute was determined by measuring the sample spot. The R_f value was calculated.

It was observed that the R_f values for the pure drug (Tizanidine HCl) and the mixture were proximal[81].

TABLE 2.8 a Thin layer chromatography of drug polymer interaction

S.NO	PHYSICAL MIXTURE	Rf values	
		ROOM TEMP	ACCELERATED
1	Drug + Chitosan	0.81	0.79
2	Drug + Sodium Alginate	0.81	0.82
3	Drug + Ethyl Cellulose	0.81	0.78
4	Drug + Dextran	0.82	0.82
5	Drug+ Carbopol 934P	0.79	0.81

R_f Value of Tizanidine HCl= 0.80

IR ANALYSIS

Table 2.8 b : DRUG-Tizanidine HCl

TYPE	NATURE	WAVE NUMBER
C-H str	Variable	3100 cm^{-1}
C-H bend	Sharp, Strong	700 cm^{-1}
C-C str	Weak	1490 cm^{-1}
C-Cl	Medium	650 cm^{-1}
C=N	Sharp, Strong	1650 cm^{-1}
N-H str	Medium	3650 cm^{-1}
N-H bend	Sharp, Strong	1600 cm^{-1}
Ar-NH-R	Medium	3250 cm^{-1}
	Sharp, Strong	1600 cm^{-1}
	Medium	1300 cm^{-1}
CN str	Medium	950 cm^{-1}
	Medium	1200 cm^{-1}

Table 2.8 c: CHITOSAN

TYPE	NATURE	WAVE NUMBER
C-O str	Broad	1100 cm^{-1}
C-H str	Broad	2900 cm^{-1}
C-H bend	Medium	1400 cm^{-1}
C-N bend	Medium	1150 cm^{-1}
N-H str	Medium	3600 cm^{-1}
N-H bend	Medium	1650 cm^{-1}
O-H str (polymeric association)	Broad	3400 cm^{-1}

From the IR spectra of Drug(Tizanidine HCl) and Polymer(Chitosan) it can be concluded that there is no interaction between drug polymer mixture.

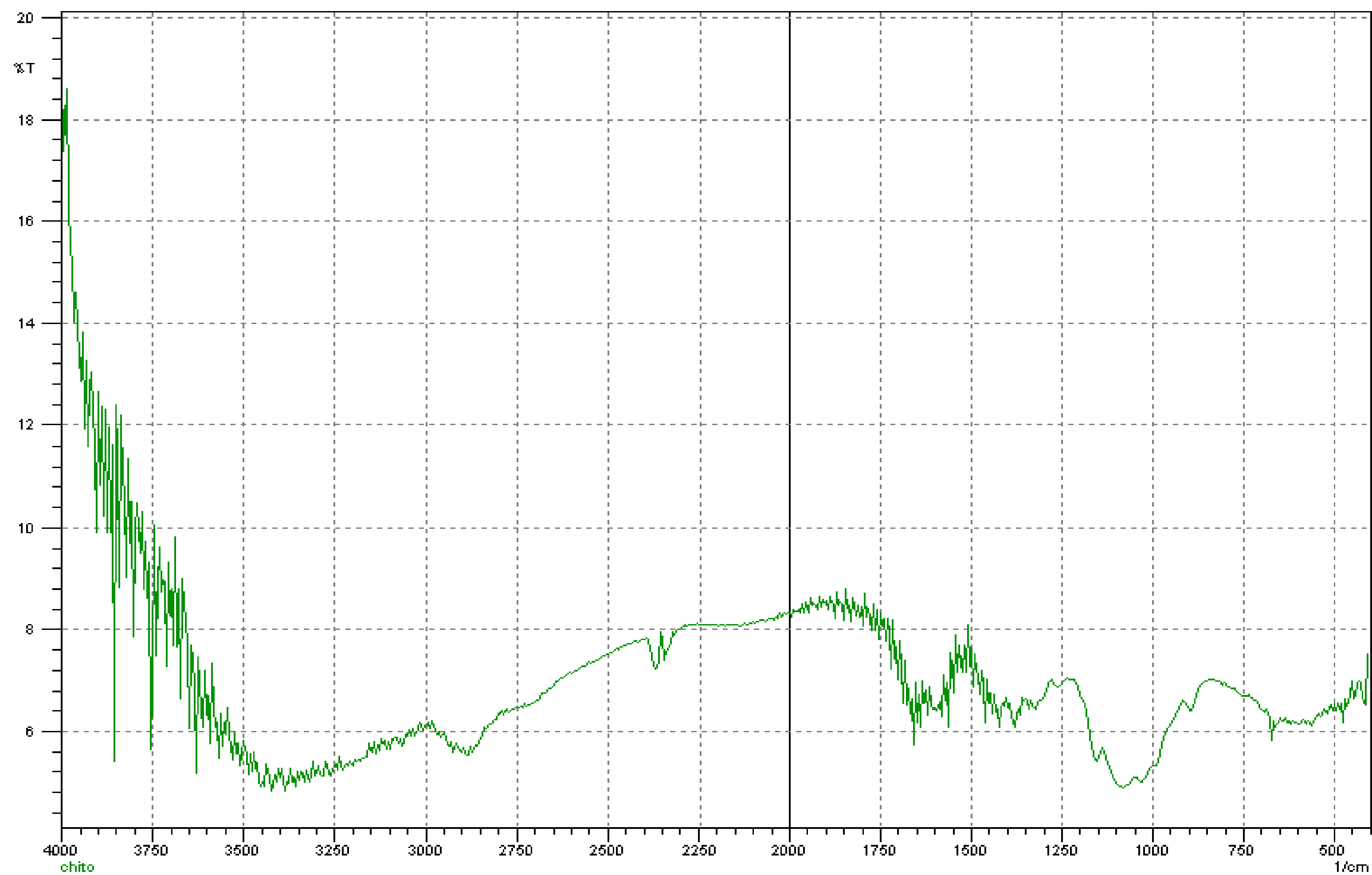

Figure 1.10:IR Spectrum of Chitosan

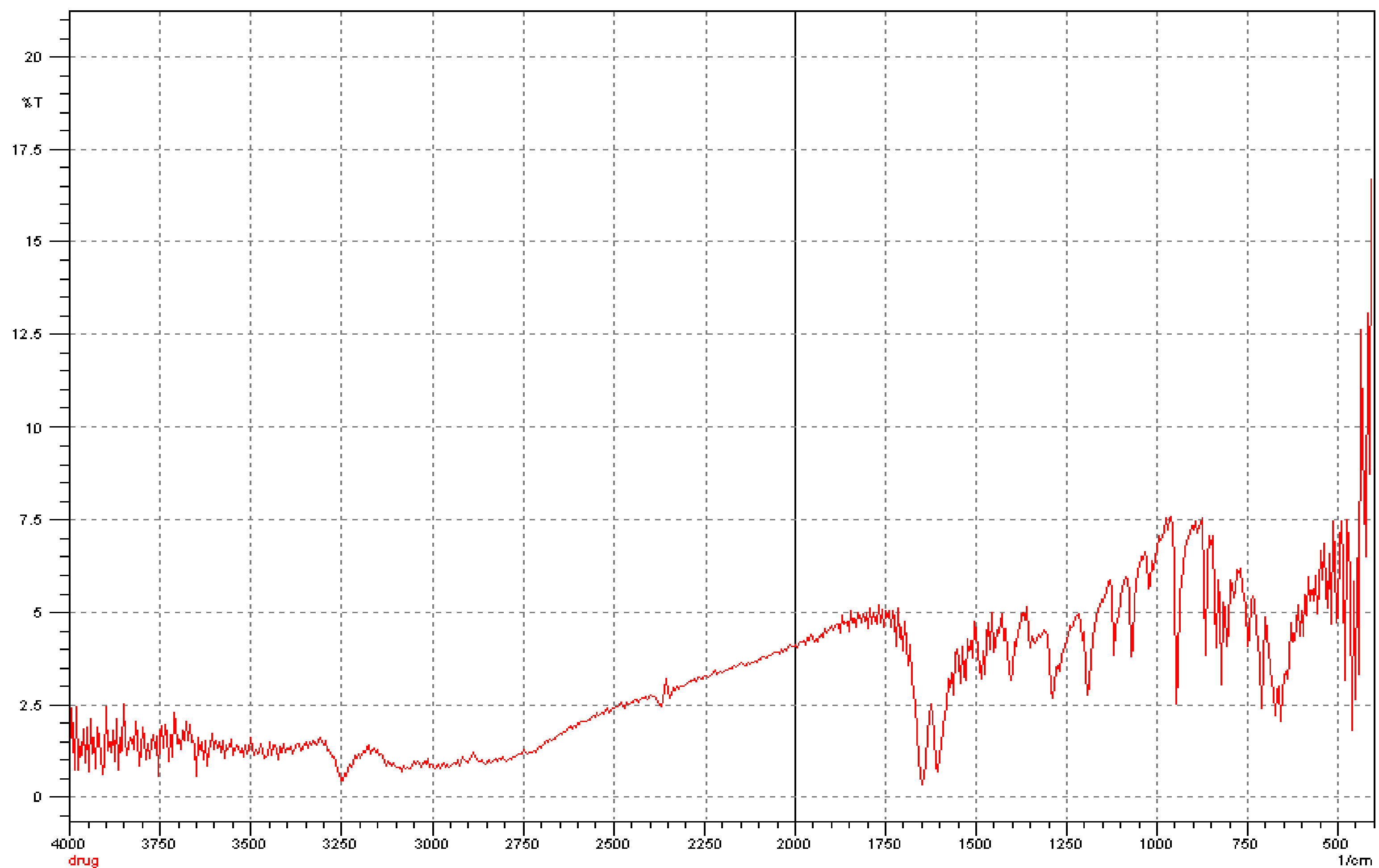

Figure 1.11: IR Spectrum of Pure Drug

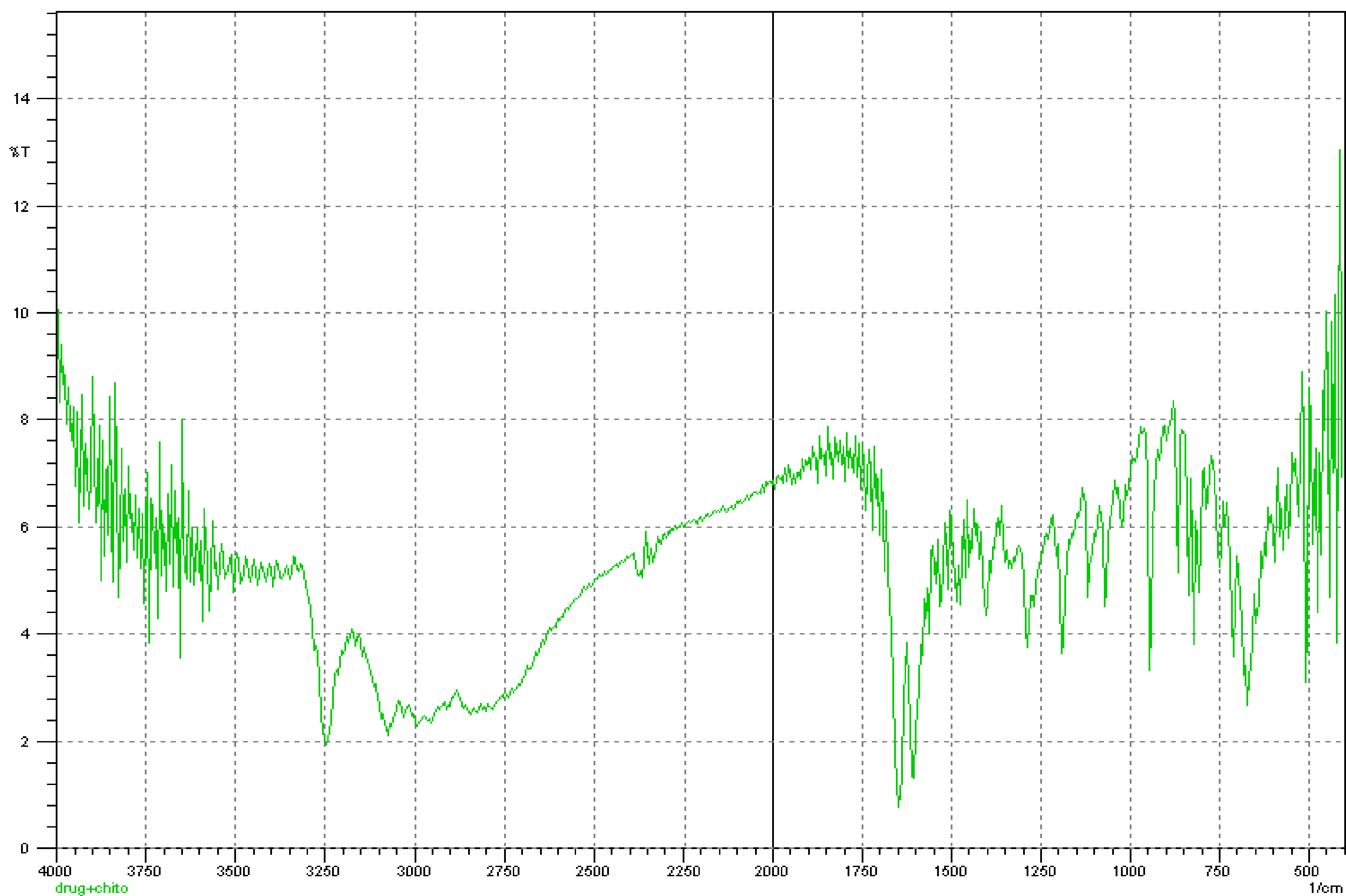

Figure. 1.12: IR Spectrum of drug polymer mixture

FORMULATION

Microspheres were prepared by Emulsification phase separation technique.

Sixteen batches were prepared with following process variables[82]:

- Polymer concentration
- Drug concentration
- Amount of cross linking agent
- Type of external phase
- Stirring speed
- Concentration of surfactant

EVALUATION

Prepared microspheres were evaluated for the following parameters[83, 84, 85, 86, 87]:

- Surface morphology and shape
- Optical photomicrography
- Incorporation efficiency
- Swelling ability
- Bulk density & Angle of repose
- In vitro bioadhesion test
- In vitro diffusion study

3.1 FORMULATION OF BIOADHESIVE MICROSPHERES

Mucoadhesive microspheres of chitosan were prepared by simple emulsification phase separation technique.100mL of paraffin oil mixture of 50mL heavy liquid paraffin and 50mL light liquid paraffin oil was placed in 500mL plastic beaker. Chitosan 200mg was dissolved in 2% acetic acid solution. The drug 100mg was added in it and the suspension was extruded through syringe in 100ml of liquid paraffin containing 0.2% DOSS(dioctyl sulfosuccinate). This addition was accompanied with stirring of paraffin oil with the help of high-speed stirrer (Remi stirrer) After 20 minutes of stirring, 1ml of glutaraldehyde (25%, solution as cross linking agent) was added and stirring was continued for 3 hours after the complete addition of chitosan solution into oil.

Suspension of chitosan microspheres in paraffin oil thus obtained were allowed to stand to let the microspheres settle down under gravity. Clear supernatant liquid was decanted and microspheres obtained as residue were washed 3-4 times with the solvent cyclohexane to remove oil and finally washed with water to remove excess of glutaraldehyde. After the final wash, microspheres were allowed to dry in air. Dry powder thus obtained was collected and stored in desiccator at room temperature[88].

TABLE 3.1 Formulation optimization

BATCH CODE	DRUG:POLYMER	STRRING SPEED(rpm)	QTY OF GLUT(ml)	EXTERNAL PHASE	DOSS
A1	1:1	2000	1	LLP: HLP(1:1)	0.1%
A2	1:2	2000	1	-	-
A3	1:3	2000	1	-	--
A4	1:4	2000	1	-	-
P1	1:2	2000	1	-	-
P2	1:3	2000	1	-	-
P3	1:4	2000	1	-	-
A3R1	3:1	1700	1	-	-
A3R2	3:1	1400	1	-	-
A3G1	3:1	2000	2	-	-
A3G2	3:1	2000	4	-	-
A3EP1	3:1	2000	1	Soyaben Oil	-
A3EP2	3:1	2000	1	Cotton Seed Oil	-
A3EP3	3:1	2000	1	Castor Oil	-
A3DOSS1	3:1	2000	1	LPL:LPH(1:1)	0.1%
A3DOSS2	3:1	2000	1	-	0.3%

- **EVALUATION**

3.2. Determination of Tizanidine HCl content of microspheres

Accurately weighed quantities of drug loaded microsphere were suspended in methanol, to extract the drug from microsphere. It was then shaken in mechanical shaker.

After 24 hours, the filtrate was analysed spectrophotometrically at 318.5 nm for drug content against methanol as blank.

Corresponding drug concentration in the sample were calculated from the calibration plot generated by regression of the data.

Preliminary UV scanning showed that the presence of the polymers did not interfere with the absorbance of Tizanidine HCl at 318.5nm.

Drug content was calculated as the detected amount of Tizanidine HCl with respect to theoretical amount of drug used for the preparation of microsphere and expressed as a percentage. Each determination was carried out in triplicate.[89, 90]

TABLE 3.2 Drug incorporation efficiency

BATCH CODE	INCORPORATION EFFICIENCY
A1	72.19
A2	69.14
A3	79.03
A4	60.01
P1	67.78
P2	65.80
P3	63.72
A3R1	52.8
A3R2	49.9
A3G1	72.12
A3G2	63.49
A3EP1	78.5
A3EP2	79.24
A3EP3	79.00
A3DOSS1	77.01
A3DOSS2	78.46

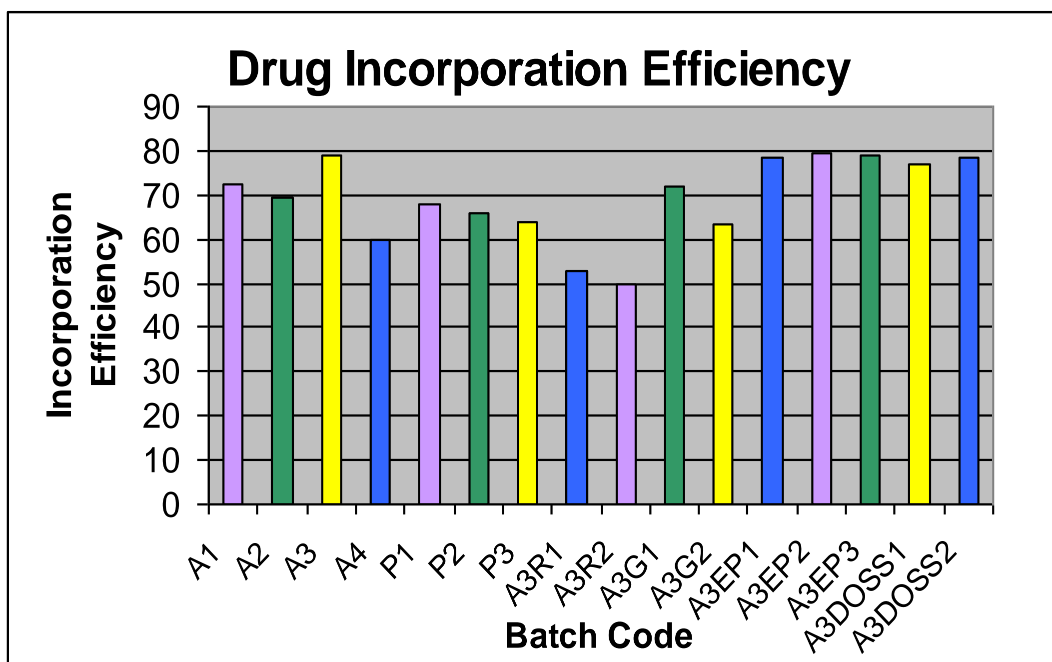

Figure 2.1 Bar graph presentation of Drug Incorporation Efficiency

3.3. Surface morphology and shape

Shape and surface morphology of microsphere formulations was determined using optical microscopy.

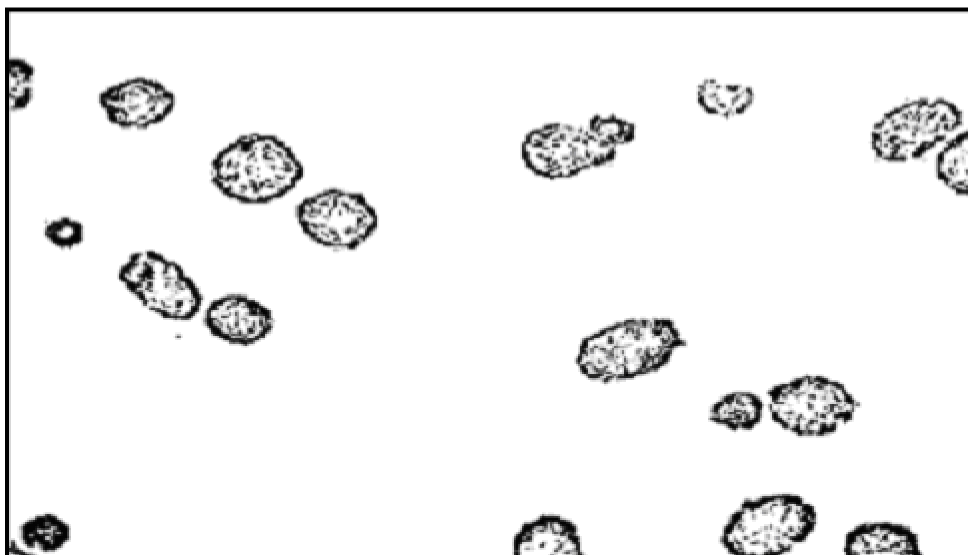

Figure. 2.2 a: Optical Micrograph of batch A3 microspheres.

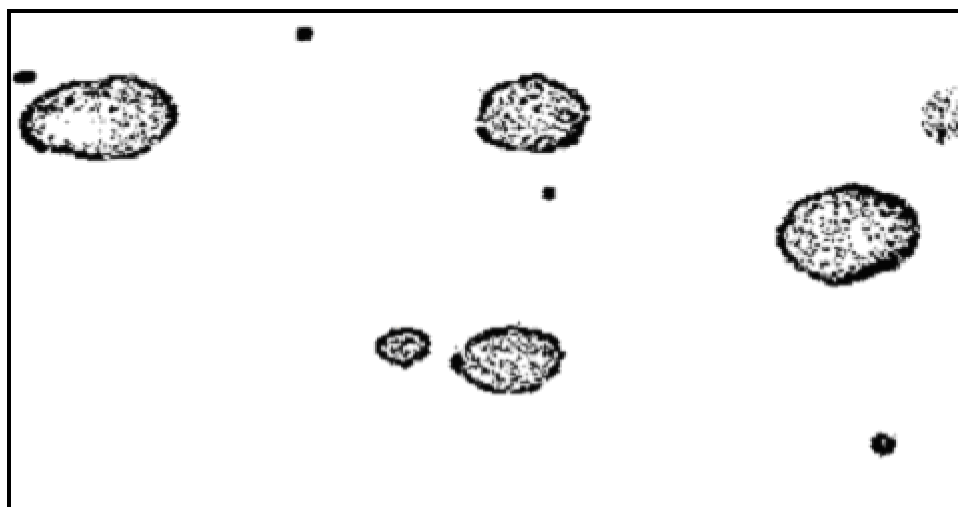

Figure. 2.2 b: Optical Micrograph of batch P3 microspheres.

3.4 SIZE AND SIZE DISTRIBUTION

Microspheres obtained from emulsion technique were studied microscopically for their size and size distribution using a calibrated ocular eyepiece. A small amount of dry microspheres was suspended in glycerine. A small drop of suspension thus obtained was placed on a clean glass slide. The slide containing chitosan microspheres was mounted on the stage of microscope. At least 100 particles was measured using photomicroscope. The process was repeated for each batch prepared. Average of 100 microspheres were used for the study and the mean particle size (arithmetic mean diameter) was considered to be the deciding factor in selecting optimum formulation conditions for each variable parameter studied.

TABLE 3.3 Mean particle size of prepared microspheres

BATCH CODE	MEAN PARTICLE SIZE(µm)
A1	55.3
A2	62.11
A3	65.57
A4	68.72
P1	72.74
P2	79.81
P3	84.47
A3R1	92.5
A3R2	130.4
A3G1	75.67
A3G2	89.5
A3EP1	68.74
A3EP2	66.0
A3EP3	65.75
A3DOSS1	65.5
A3DOSS2	66.6

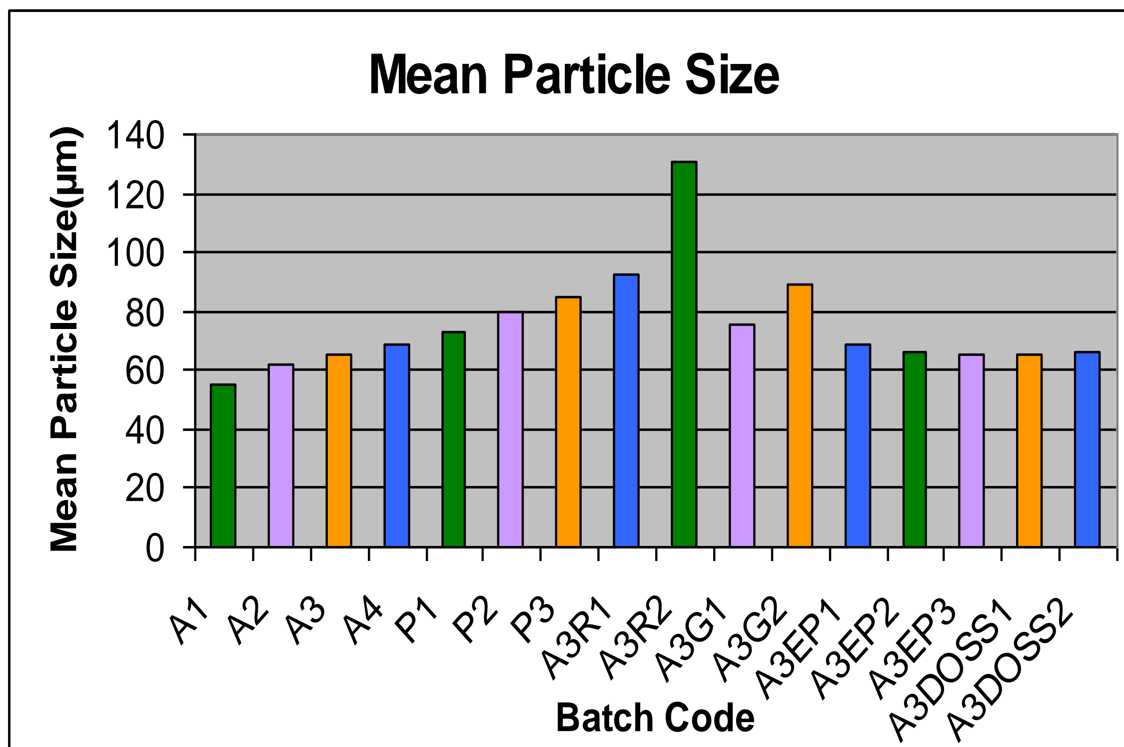

Figure 2.3 Particle size distribution of microspheres

3.5 SWELLING ABILITY

The swelling ability of the microspheres in physiological media was determined by swelling them to their equilibrium. Accurately weighted amounts of microspheres were in little excess of phosphate buffer (pH6.6) and kept for 24 hour. The following formula was used for the calculation of degree of swelling:

DEGREE OF SWELLING = $\alpha = W_S - W_0 / W_S$

Where Wo =Initial Wt of Microspheres

Ws= Wt of microspheres after swelling

TABLE 3.4 Degree of swelling of prepared microspheres

BATCH CODE	DEGREE OF SWELLING
A1	1.23
A2	1.95
A3	1.08
A4	1.03
P1	1.29
P2	1.38
P3	1.45
A3R1	1.1
A3R2	1.19
A3G1	0.81
A3G2	0.65
A3EP1	1.09
A3EP2	1.07
A3EP3	1.00
A3DOSS1	1.09
A3DOSS2	1.07

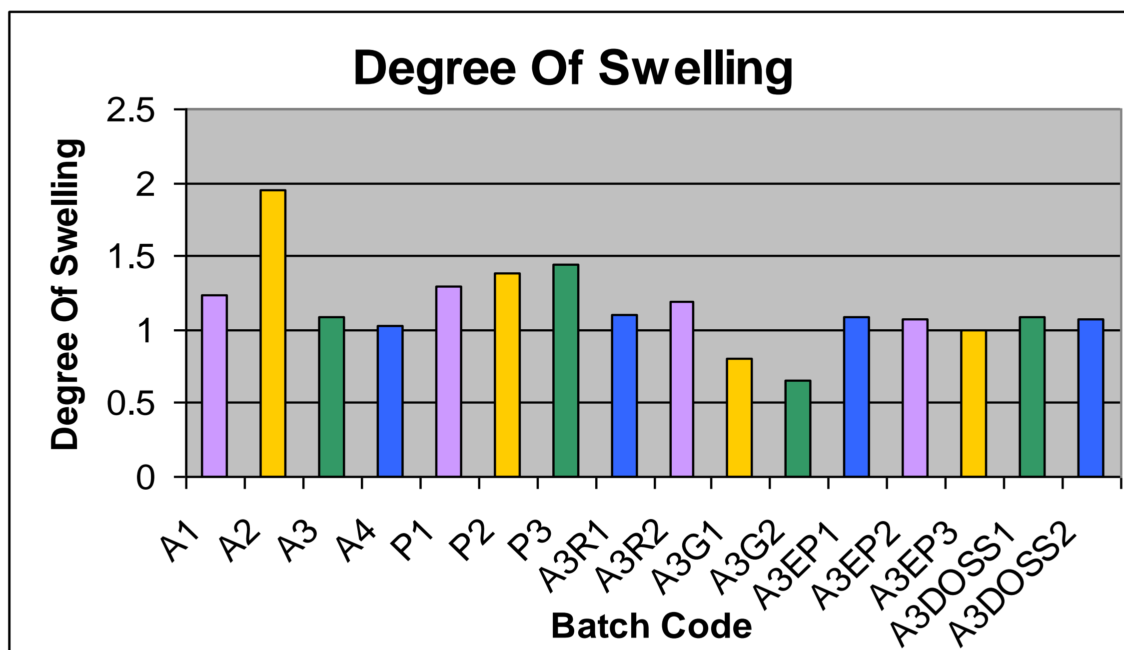

Figure 2.4 Bar Graph Presentation Of Swelling Ability

3.6 IN VITRO BIOADHESION TEST

The invitro mucoadhesion of microspheres was carried out by using intestinal mucosa of mice. The intestinal mucosa obtained from male mice fasted overnight and dissected under chloroform anesthesia was cut longitudinally and rinsed in 10 ml of physiological saline. The experiments to evaluate the adhesive properties were started within two hours after dissection. The microspheres were placed uniformly on the intestinal mucosa which was fixed on the polythene support. The tissue with the microspheres were then placed in the dessicator to maintain at > 80% relative humidity and room temperature to allow the chitosan microspheres to hydrate and to prevent drying of the mucus. Mucosa was washed with phosphate buffer(pH6.6) for 5 minutes.

Tissue was again observed under microscope to see the number of microspheres remaining in the same field area. The adhesion number was found by the following equation

$$Na = N/N_0 \times 100$$

Na = Adhesion number

No = Total number of particles in the particular area

N = Number of particles attached to the mucosa after washing

TABLE 3.5 In vitro bioadhesion test

Batch No. Time (h)	% Bioadhesion				
	1	2	4	6	8
A1	42	36	30	22	8
A2	46	39	33	28	12
A3	60	55	50	42	38
P1	58	51	47	40	32
P2	56	49	43	36	28
P3	50	40	32	23	14

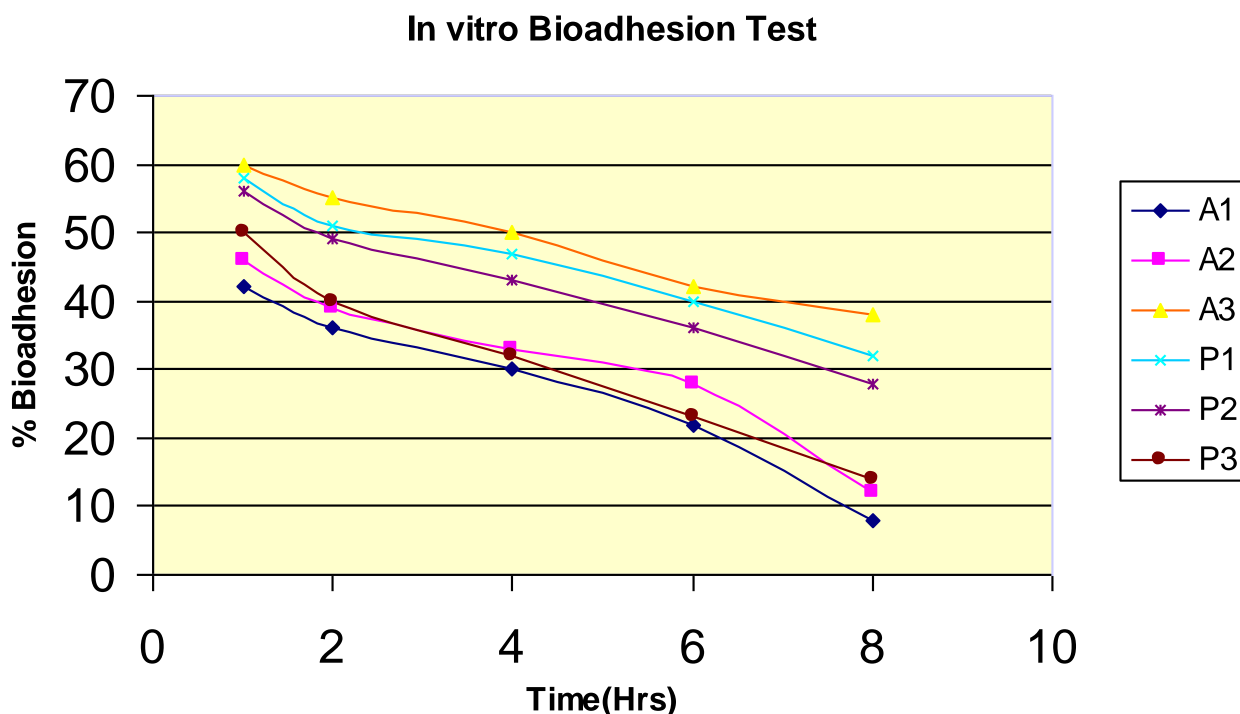

Figure 2.5 Bar Graph Presentation of In vitro Bioadhesion Test

3.7 MICROMETRICS PROPERTIES OF CHITOSAN MICROSPHERES

TABLE 3.6 Micrometrics properties of chitosan microspheres

ROPERTIES	A1	A2	A3	A4	P1	P2	P3	A3R1
ANGLE OF REPOSE	30.5	34.6	32	36.4	33.9	34.36	45.36	42.1
CARR'S INDEX	20.3	19.5	19.84	22.4	24	25.6	26	30.5
HAUSNER RATIO	1.24	1.22	1.22	1.24	1.25	1.25	1.25	1.26

PROPERTIES	A3R2	A3G1	A3G2	A3EP1	A3EP2	A3EP3	A3DOSS1	A3DOSS2
ANGLE OF REPOSE	44.5	43.9	36.7	36.4	33.9	34.36	45.36	42.1
CARR'S INDEX	20.3	19.5	19.84	22.4	24	25.6	26	30.5
HAUSNER RATIO	1.26	1.26	1.27	1.24	1.25	1.25	1.25	1.26

3.8 IN VITRO DIFFUSION STUDIES:

Various approaches used to determine the drug diffusion through nasal mucosa from the formulation. The two important methodologies to study the diffusion profile of the drug are:

Fabrication of apparatus for in-drug permeation studies.

In vitro drug permeation studies were carried out in the Franz diffusion cell.

The assembly consist of 2 chambers

1. The upper cylindrical chamber (donor compartment), which was open from above.

2. The lower flask shaped chamber (receiver chamber), containing a sampling port and had Teflon coated magnetic bead at the base (Figure.). The capacity of the receiver compartment was 15ml and the area of diffusion between donor and receiver compartment of cell was 1.767 cm^2. Hooks were made on the sides of both chambers so that the chambers formed are single unit without any leakage, once the springs were secured over these hooks. The receptor compartment was maintained at 37 ± 2^0C by a water bath.

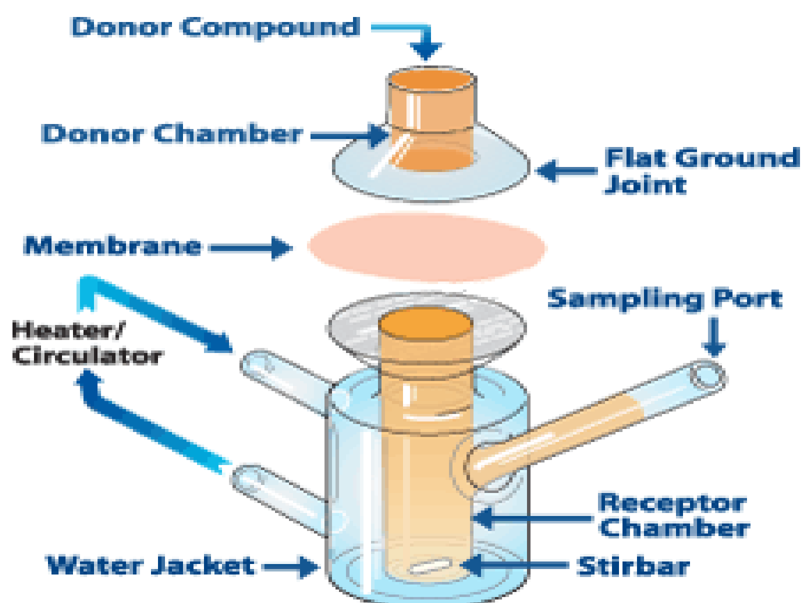

Figure 2.6 Franz Diffusion Cell

vitro drug permeation across the treated cellophane membrane was conducted using Franz diffusion cell. The mucosal membrane was placed in between the donor and receptor compartment of the Franz diffusion cell.

The area of the diffusion cell used for all in vitro diffusion studies was 1.767 $cm^2$and the capacity of receiver compartment was 15.0 ml.The membrane was equilibrated for one hour with the receiver medium. A blank sample (1.0ml) was withdrawn from the receptor compartment and analyzed to ensure any residual absorbance. The receptor medium (phosphate buffer pH 6.8) was replaced with the fresh medium. Weighed quantities of microspheres suspended in phosphate buffer pH 6.8(3ml) were kept in donor compartment, the receptor compartment is also filled with same medium. The receptor chamber was thermo stated at 37 ± 2^0C and magnetic stirrer stirred the solution in the receptor chamber continuously.

Samples (1.0 ml) were withdrawn from the receptor compartment for 8 hrs at the interval of one hour and drug content was analyzed by UV spectrophotometry method at λ_{max} 318.5 nm using phosphate buffer pH 6.8 as blank. The receptor volume was immediately replaced with equal receptor medium. Sampling port and donor chamber were covered by aluminium foil to prevent the evaporation of receptor medium.

TABLE 3.7- In vitro release profile of batch A1

Sl. no	Time (mins)	Conc. (mg/ml)	Conc. (In 5ml)	Conc. (In 15ml)	Cumulative Amount	Cumulative %Released
1.	15	0.39	1.95	5.85	5.85	19.5
2.	30	0.28	1.42	4.26	6.21	20.7
3.	45	0.48	2.41	5.09	7.23	24.1
4.	60	0.53	2.67	5.6	8.01	26.7
5.	120	0.54	2.7	8.1	10.77	35.9
6.	180	0.73	3.67	11.01	13.71	45.7
7.	240	0.87	4.38	13.16	16.83	56.1
8.	300	0.99	4.99	14.97	19.35	64.5
9.	360	1.04	5.22	15.67	20.67	68.9
10.	420	1.12	5.6	16.8	22.02	73.4
11.	480	1.13	5.7	16.99	22.59	75.3

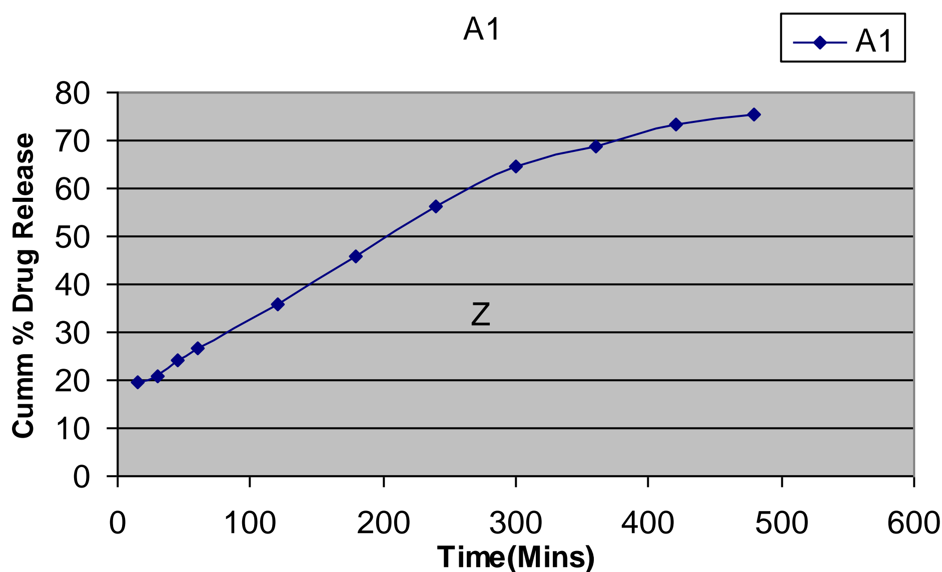

FIGURE 2.7: In vitro release profile of batch A1

TABLE 3.8 In vitro release profile of batch A2

Sl. no	Time (mins)	Conc. (mg/ml)	Conc. (In 5ml)	Conc. (In 15ml)	Cumulative Amount	Cumulative %Released
1.	15	0.36	1.81	5.43	5.43	18.1
2.	30	0.30	1.50	4.52	6.33	21.1
3.	45	0.39	1.96	5.88	7.38	24.6
4.	60	0.44	2.2	6.61	8.61	28.7
5.	120	0.57	2.86	8.6	10.98	36.6
6.	180	0.81	4.07	12.23	15.09	43.4
7.	240	0.86	4.32	12.94	17.01	50.3
8.	300	0.93	4.6	14.06	18.36	56.7
9.	360	0.98	4.9	14.75	19.35	61.2
10.	420	1.02	5.2	15.34	20.34	64.5
11.	480	1.04	6.1	16.12	22.54	67.8

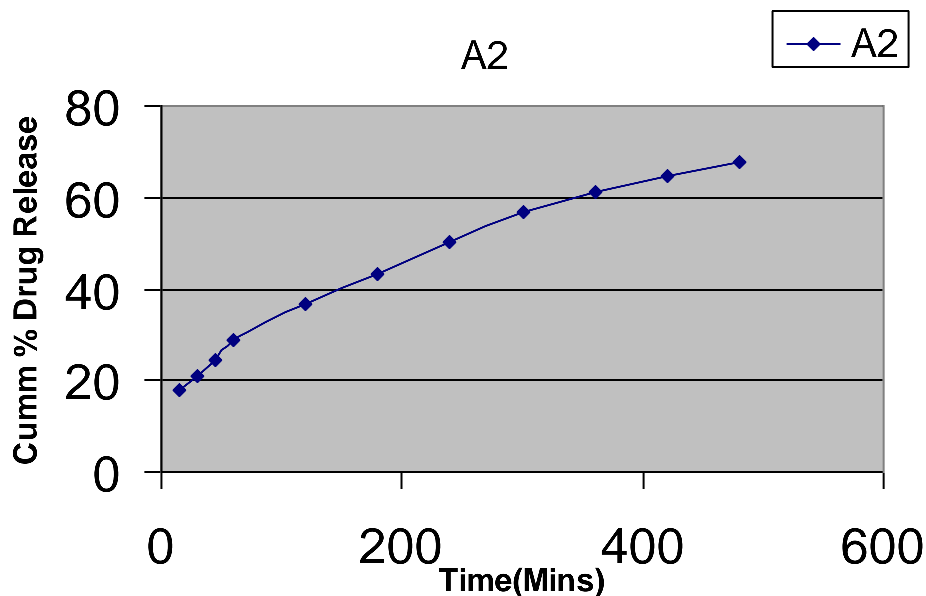

FIGURE 2.8 :In vitro release profile of batch A2

TABLE 3.9 In vitro release profile of batch A3

Sl. no	Time (mins)	Conc. (mg/ml)	Conc. (In 5ml)	Conc. (In 15mll)	Cumulative Amount	Cumulative %Released
1.	15	0.39	1.96	5.88	5.88	19.66
2.	30	0.35	1.77	5.33	7.29	24.3
3.	45	0.43	2.15	6.46	8.16	27.2
4.	60	0.46	2.33	7	9.15	30.5
5.	120	0.75	3.78	11.35	13.68	45.6
6.	180	0.78	3.92	11.76	15.54	51.8
7.	240	0.94	4.73	14.2	18.21	60.7
8.	300	1.06	5.32	15.97	20.7	69.0
9.	360	1.15	5.76	17.29	22.59	75.3
10.	420	1.2	6	18	23.76	79.2
11.	480	1.27	6.4	18.9	24.2	80.5

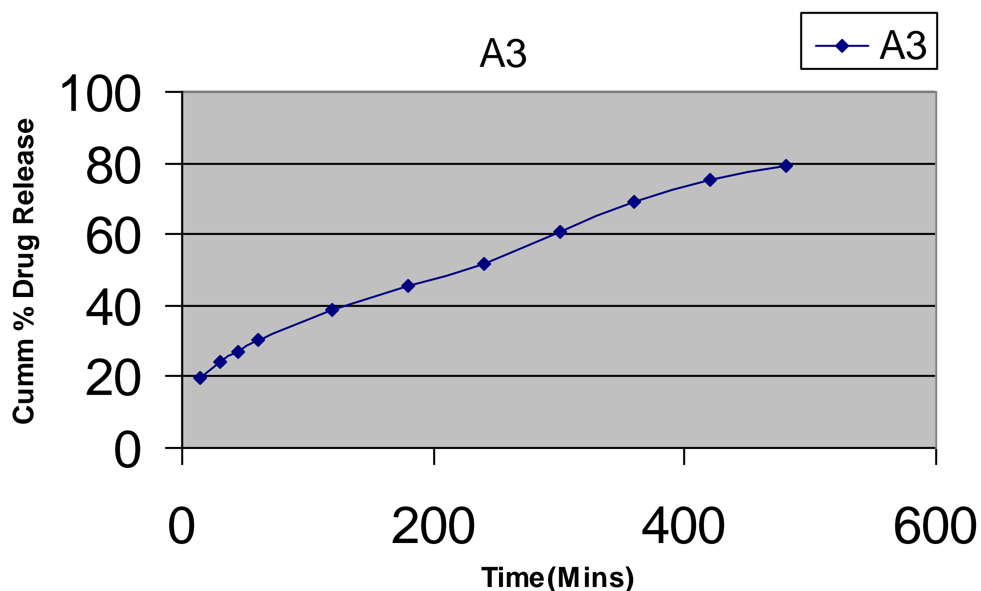

Figure. 2.9 In vitro release profile of batch A3

TABLE 3.10 In vitro release profile of batch A4

Sl. no	Time (mins)	Conc. (mg/ml)	Conc. (In 5ml)	Conc. (In 15ml)	Cumulative Amount	Cumulative %Released
1.	15	0.32	1.64	4.92	4.92	16.4
2.	30	0.26	1.34	4.03	5.67	18.9
3.	45	0.37	1.89	5.68	7.02	23.4
4.	60	0.42	2.1`4	6.44	8.34	27.8
5.	120	0.45	2.29	6.89	9.09	30.3
6.	180	0.67	3.35	10.07	12.27	40.9
7.	240	0.76	3.81	11.43	14.73	49.1
8.	300	0.78	3.78	11.23	15.15	50.5
9.	360	0.94	4.72	14.16	17.94	59.89
10.	420	1.05	5.27	15.82	20.52	68.4
11.	480	1.12	5.61	16.85	22.05	73.5

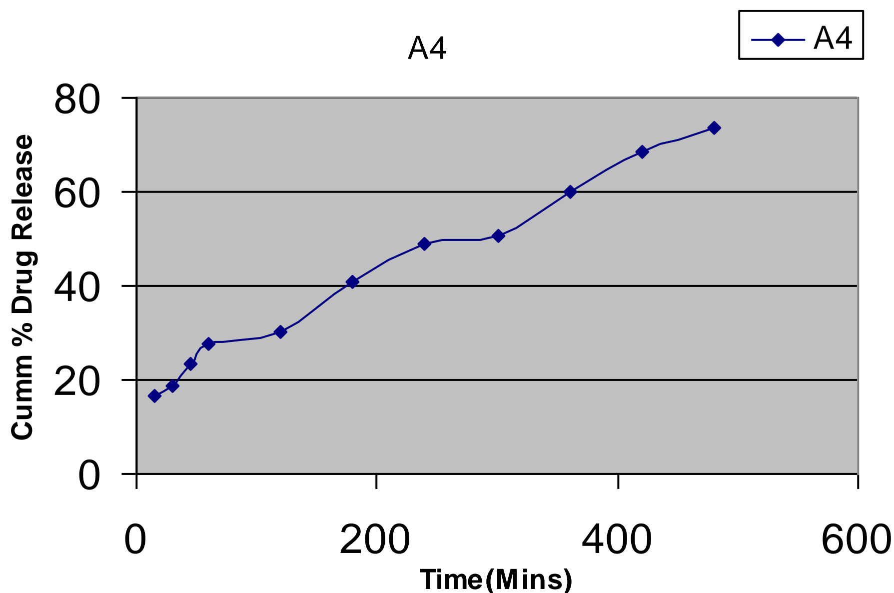

Figure. 2.10 In vitro release profile of batch A4

TABLE 3.11 In vitro release profile of batch P1

Sl. no	Time (mins)	Conc. (mg/ml)	Conc. (In 5ml)	Conc. (In 15ml)	Cumulative Amount	Cumulative %Released
1.	15	0.47	2.35	7.05	7.05	23.5
2.	30	0.40	2.04	6.14	8.49	28.3
3.	45	0.52	2.62	7.86	9.9	33
4.	60	0.54	2.74	8.24	10.86	36.2
5.	120	0.79	3.8	11.4	14.1	47
6.	180	0.85	4.25	12.76	16.56	55.2
7.	240	1.07	5.39	16.18	20.43	61.5
8.	300	1.12	5.6	16.9	22.2	68.1
9.	360	1.18	5.9	17.8	23.4	74
10.	420	1.27	6.36	19.09	24.9	75
11.	480	1.34	7.2	20.1	25.3	76.5

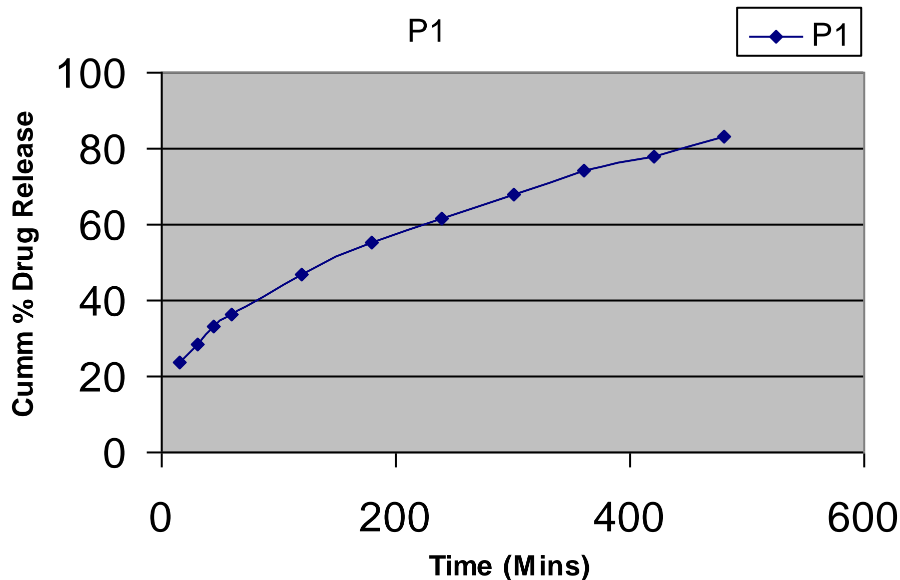

FIGURE 2.11: In vitro release profile of batch P1

TABLE 3.12 In vitro release profile of batch P2

Sl. no	Time (mins)	Conc. (mg/ml)	Conc. (In 5ml)	Conc. (In 15ml)	Cumulative Amount	Cumulative %Released
1.	15	0.39	1.96	5.88	5.88	19.6
2.	30	0.30	1.50	4.52	6.42	21.4
3.	45	0.40	2.03	6.09	7.59	25.3
4.	60	0.42	2.13	6.4	8.43	28.1
5.	120	0.61	3.06	9.18	11.31	37.7
6.	180	0.75	3.79	11.37	14.43	48.1
7.	240	0.89	4.46	13.4	17.19	57.3
8.	300	1.12	5.62	16.8	21.33	66.4
9.	360	1.15	5.79	17.3	23.01	71.1
10.	420	1.19	6.22	18.66	24.45	76.7
11.	480	2.1	6.84	19.1	25	80.0

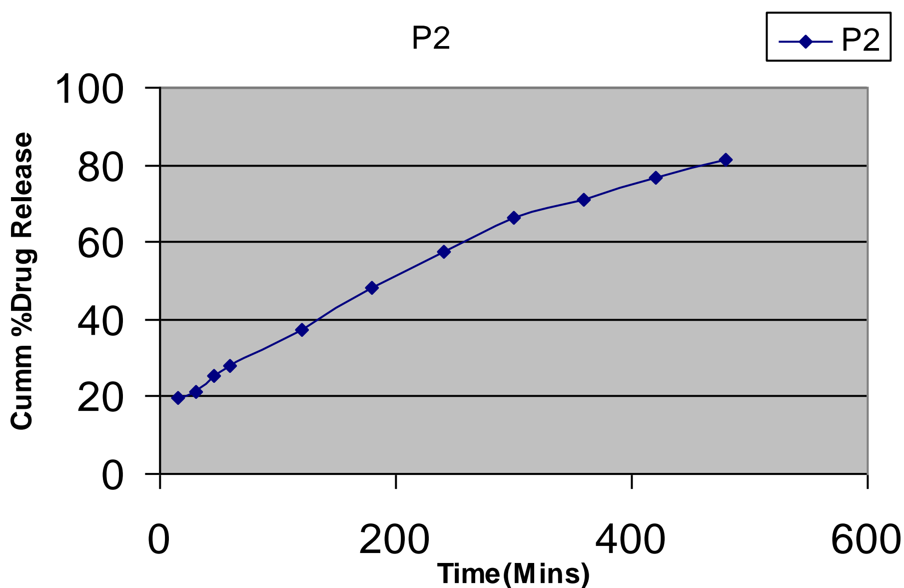

FIGURE 2.12: In vitro release profile of batch P2

TABLE 3.13 In vitro release profile of batch P3

Sl. no	Time (mins)	Conc. (mg/ml)	Conc. (In 5ml)	Conc. (In 15ml)	Cumulative Amount	Cumulative %Released
1.	15	0.35	1.79	5.37	5.37	17.9
2.	30	0.36	1.79	5.38	7.17	23.9
3.	45	0.43	2.19	6.58	8.37	27.9
4.	60	0.48	2.4	7.2	9.39	31.3
5.	120	0.84	4.21	12.63	15.03	41.9
6.	180	0.85	4.29	12.87	17.07	50.1
7.	240	0.85	4.29	12.87	17.07	56.9
8.	300	0.97	4.85	14.55	18.84	62.8
9.	360	1.04	5.22	15.67	20.52	68.4
10.	420	1.12	5.61	16.83	22.05	73.5
11.	480	1.23	5.82	17.1	23.1	78.1

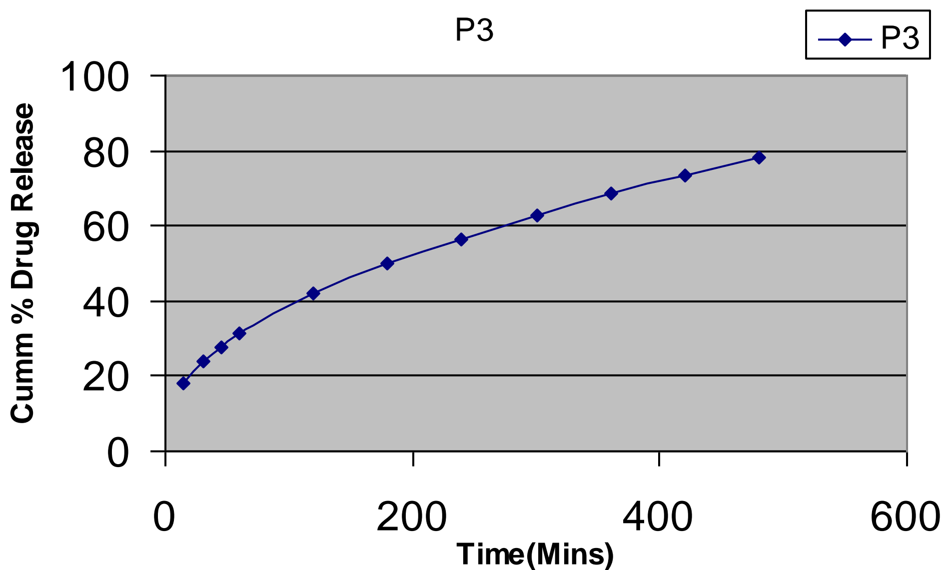

FIGURE 2.13: In vitro release profile of batch P3

TABLE 4.1 In vitro release profile of batch A3R1

Sl. no	Time (mins)	Conc. (mg/mll)	Conc. (In 5ml)	Conc. (In 15ml)	Cumulative Amount	Cumulative %Released
1.	15	0.37	1.85	5.55	5.55	18.5
2.	30	0.26	1.32	3.97	5.82	19.4
3.	45	0.37	1.88	5.64	6.96	23.2
4.	60	0.38	1.93	5.8	7.68	25.6
5.	120	0.51	2.57	7.73	9.63	32.1
6.	180	0.63	3.19	9.5	12.15	40.5
7.	240	0.96	4.8	14.4	17.62	40.9
8.	300	1.05	5.25	15.75	20.55	58.75
9.	360	1.09	5.49	16.47	21.72	68.5
10.	420	1.20	6.01	18.05	23.55	72.4
11.	480	1.34	6.2	18.7	24.0	78.5

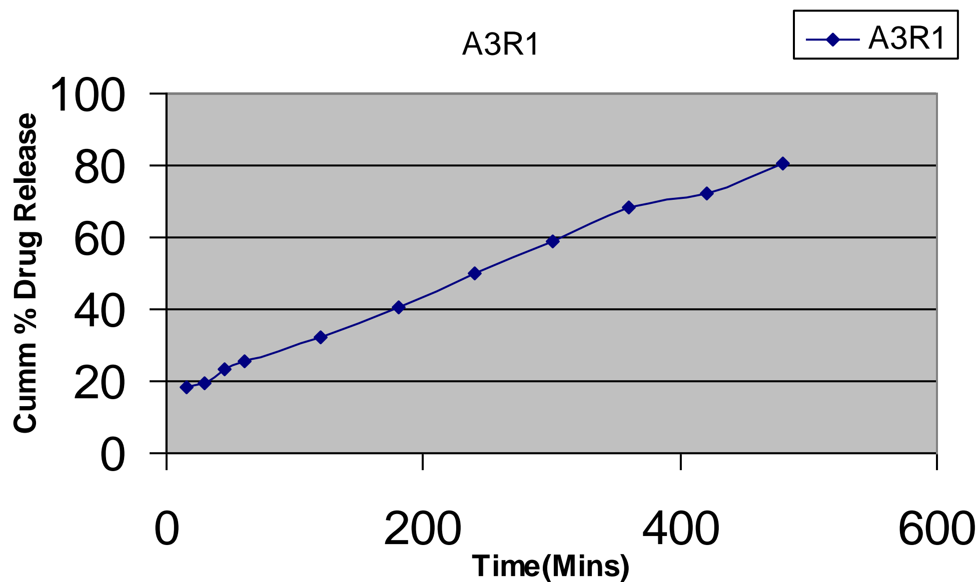

FIGURE 2.14: In vitro release profile of batch A3R1

TABLE 4.2 In vitro release profile of batch A3R2

Sl. no	Time (mins)	Conc. (mg/ml)	Conc. (In 5ml)	Conc. (In 15ml)	Cumulative Amount	Cumulative %Released
1.	15	0.32	1.64	4.92	4.92	16.4
2.	30	0.34	1.20	3.61	5.25	17.5
3.	45	0.32	1.63	4.89	6.09	20.3
4.	60	0.39	1.95	5.85	7.48	24.96
5.	120	0.45	2.29	6.87	8.82	39.5
6.	180	0.63	3.18	9.55	11.85	44.5
7.	240	0.67	3.38	10.15	13.35	55.5
8.	300	0.88	4.42	13.27	16.65	67
9.	360	1.04	5.22	15.68	20.1	70.5
10.	420	1.06	5.31	15.96	21.15	72.9
11.	480	1.21	6.07	18.23	23.53	78.48

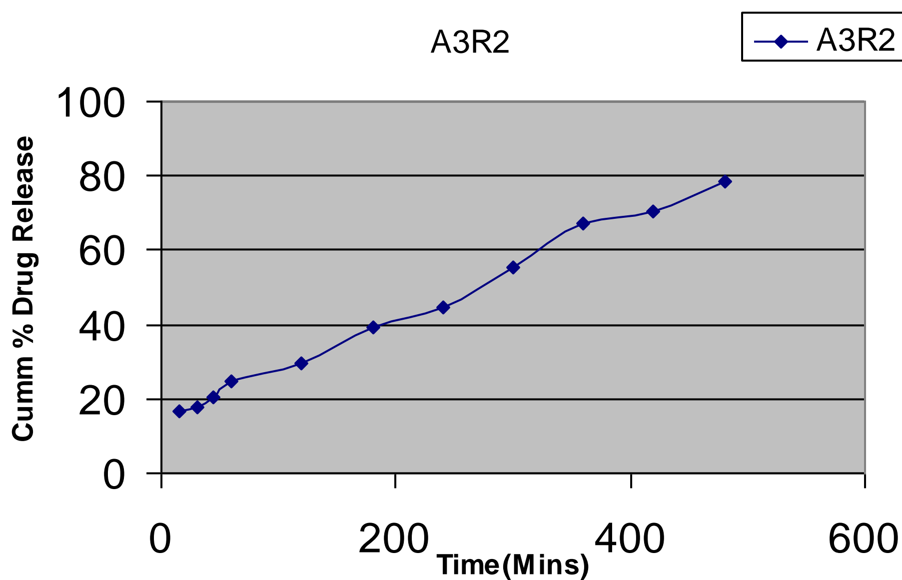

FIGURE 2.15: In vitro release profile of batch A3R2

TABLE 4.3 In vitro release profile of batch A3G1

Sl. no	Time (mins)	Conc. (mg/mll)	Conc. (In 5ml)	Conc. (In 15ml)	Cumulative Amount	Cumulative %Released
1.	15	0.33	1.69	5.07	5.07	16.9
2.	30	0.28	1.44	4.34	6.03	20.1
3.	45	0.35	1.79	5.37	6.81	22.7
4.	60	0.42	2.06	6.19	7.98	26.6
5.	120	0.59	2.97	8.92	10.92	36.4
6.	180	0.71	3.5	10.5	13.5	45
7.	240	0.79	3.99	11.98	15.48	51.6
8.	300	0.89	4.48	13.46	17.46	58.2
9.	360	0.97	4.87	14.61	19.11	63.7
10.	420	1.04	5.23	15.69	20.49	68.3
11.	480	1.08	5.42	15.9	21.1	73.1

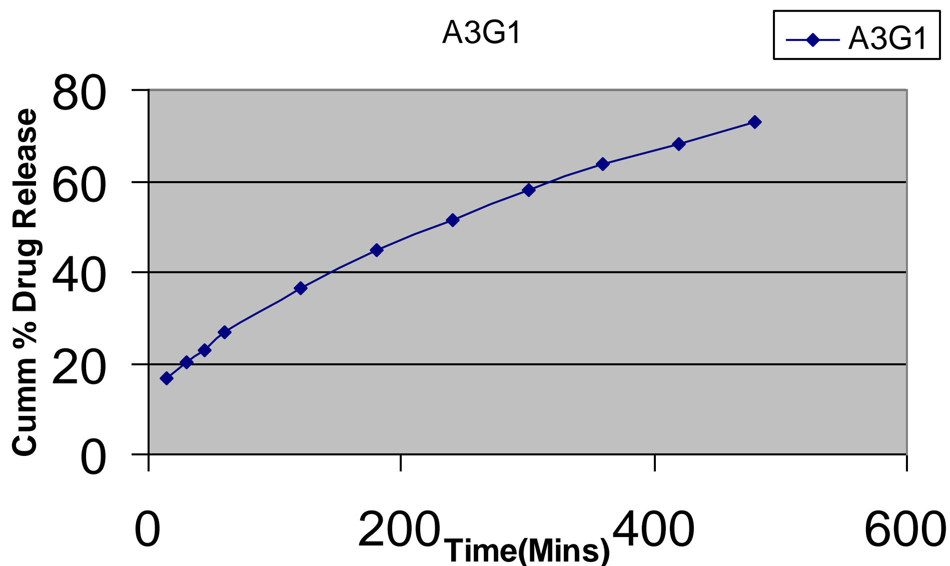

FIGURE 2.16: In vitro release profile of batch A3G1

TABLE 4.4 In vitro release profile of batch A3G2

Sl. no	Time (mins)	Conc. (mg/ml)	Conc. (In 5ml)	Conc. (In 15ml)	Cumulative Amount	Cumulative %Released
1.	15	0.31	1.55	4.65	4.65	15.5
2.	30	0.31	1.57	4.72	6.27	20.9
3.	45	0.32	1.62	4.88	6.45	21.5
4.	60	0.38	1.92	5.76	7.38	24.6
5.	120	0.54	2.71	8.15	10.05	33.5
6.	180	0.61	3.05	9.15	11.85	39.5
7.	240	0.79	3.95	11.85	14.85	49.5
8.	300	0.85	4.26	12.78	16.68	55.6
9.	360	0.93	4.65	13.95	18.15	60.5
10.	420	1.00	5.01	15.05	19.7	65.67
11.	480	1.03	5.18	15.54	20.55	68.5

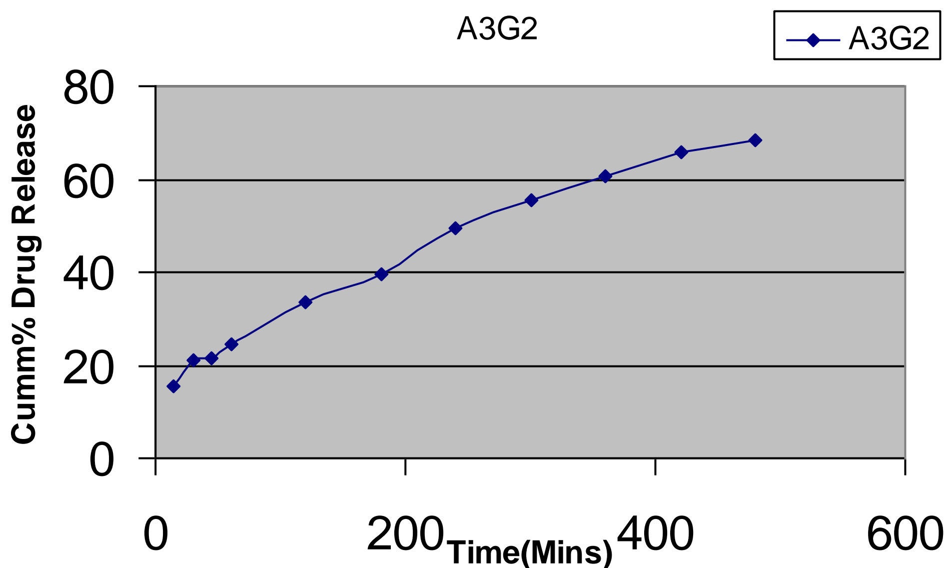

FIGURE 2.17: In vitro release profile of batch A3G2

TABLE 4.5 In vitro release profile of batch A3EP1

Sl. no	Time (mins)	Conc. (mg/ml)	Conc. (In 5ml)	Conc. (In 15ml)	Cumulative Amount	Cumulative %Released
1.	15	0.30	1.96	5.8	5.8	19.6
2.	30	0..35	1.79	5.39	7.35	24.5
3.	45	0.41	2.07	6.22	8.01	26.7
4.	60	0.47	2.36	7.08	9.15	30.5
5.	120	0.59	2.96	8.89	11.25	37.5
6.	180	0.69	3.49	10.48	13.38	44.67
7.	240	0.78	3.91	11.75	15.15	50.5
8.	300	0.91	4.55	13.65	17.55	58.5
9.	360	0.91	4.56	13.68	18.18	60.67
10.	420	1.1	5.56	16.65	21.15	64.5
11.	480	1.64	6.1	16.91	21.32	70.5

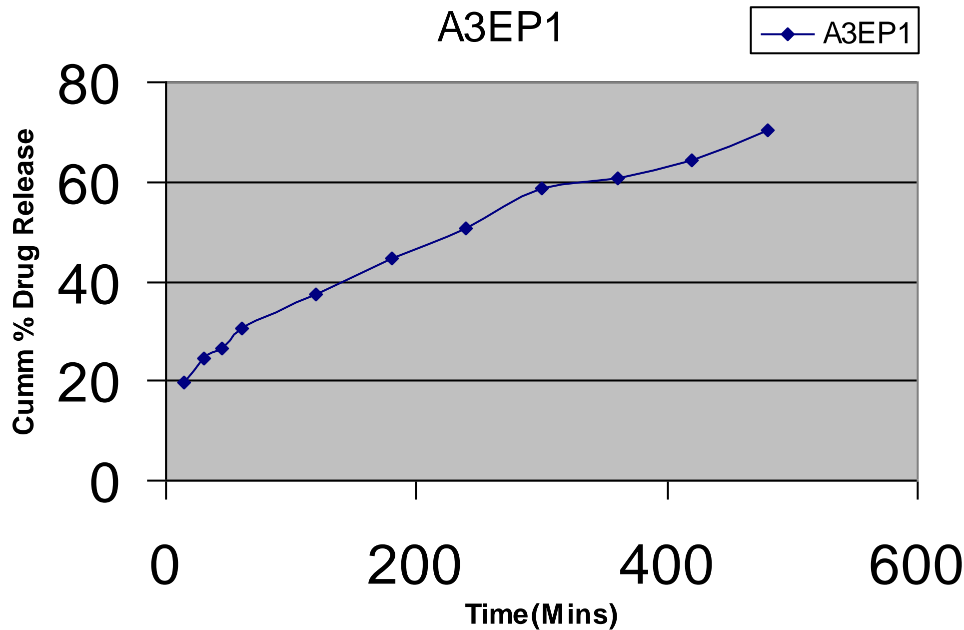

FIGURE 2.18: In vitro release profile of batch A3EP1

TABLE 4.6 In vitro release profile of batch A3EP2

Sl. no	Time (mins)	Conc. (mg/ml)	Conc. (In 5ml)	Conc. (In 15ml)	Cumulative Amount	Cumulative %Released
1.	15	0.30	1.96	5.8	5.8	19.6
2.	30	0.35	1.79	5.39	7.35	24.3
3.	45	0.42	2.12	6.37	8.16	27.2
4.	60	0.47	2.35	7.05	9.15	30.5
5.	120	0.62	3.13	9.4	11.7	39
6.	180	0.70	3.52	10.58	13.68	45.6
7.	240	0.80	4.01	12.04	15.54	51.8
8.	300	0.94	4.73	14.21	18.21	60.7
9.	360	1.06	5.3	16	20.7	69
10.	420	1.15	5.76	17.29	22.59	75.3
11.	480	1.20	6.02	18.06	23.76	72.9

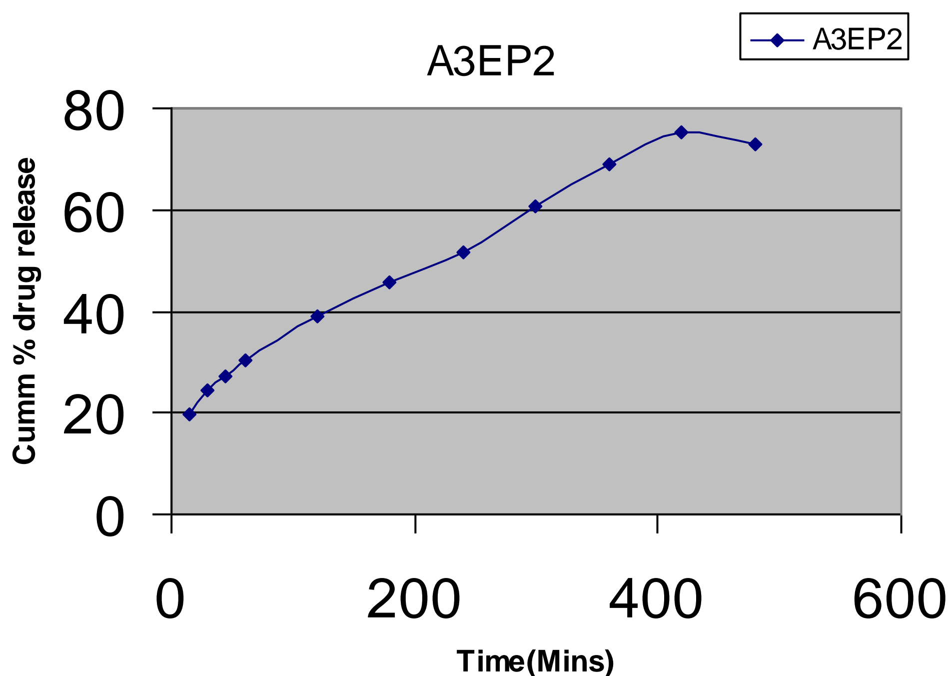

FIGURE 2.19: In vitro release profile of batch A3EP2

TABLE 4.7 In vitro release profile of batch A3EP3

Sl. no	Time (mins)	Conc. (mg/ml)	Conc. (In 5ml)	Conc. (In 15ml)	Cumulative Amount	Cumulative %Released
1.	15	0.28	1.44	4.32	4.32	14.4
2.	30	0.25	1.28	3.85	5.25	17.5
3.	45	0.32	1.64	4.92	6.12	20.4
4.	60	0.52	2.59	7.78	9.42	31.4
5.	120	0.60	3.0	9.05	11.55	38.5
6.	180	0.65	3.25	9.75	12.75	42.5
7.	240	0.77	3.89	11.64	14.94	49.8
8.	300	0.85	4.28	12.8	16.6	55.5
9.	360	0.95	4.75	14.25	18.45	61.5
10.	420	0.99	4.95	14.87	19.62	65.4
11.	480	1.00	5.04	15.14	20.04	66.8

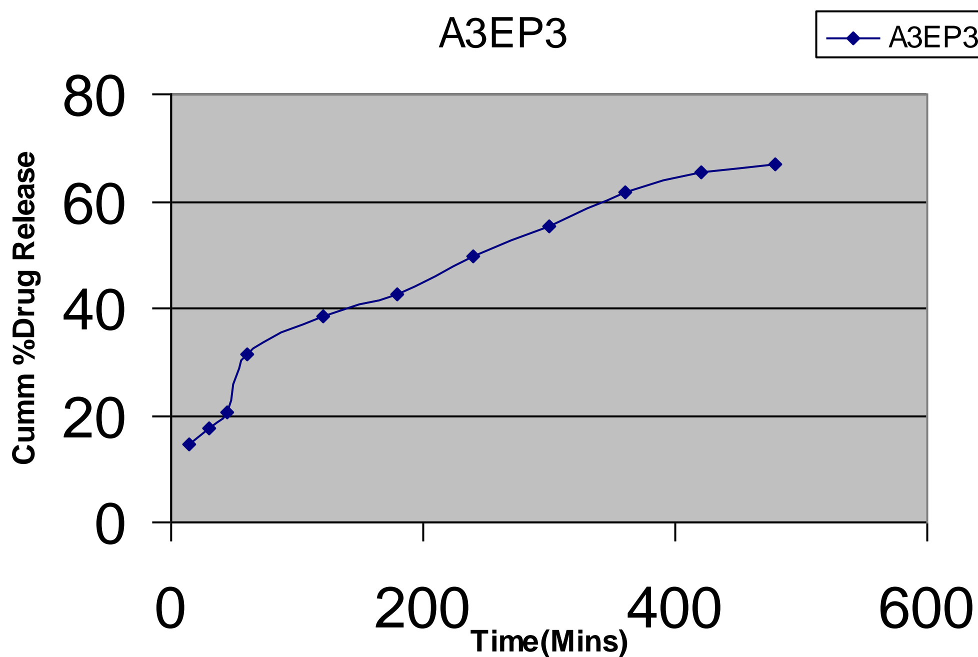

FIGURE 2.20: In vitro release profile of batch A3EP3

TABLE 4.8 In vitro release profile of batch A3DOSS1

Sl. no	Time (mins)	Conc. (mg/ml)	Conc. (In 5ml)	Conc. (In 15ml)	Cumulative Amount	Cumulative %Released
1.	15	0.37	1.85	5.5	5.5	18.5
2.	30	0.26	1.32	3.97	5.82	19.4
3.	45	0.376	1.88	5.64	6.96	23.2
4.	60	0.39	1.96	5.88	7.68	25.6
5.	120	0.51	2.56	7.7	9.6	32.1
6.	180	0.63	3.19	9.59	12.15	40.5
7.	240	0.79	3.95	11.87	14.97	49.9
8.	300	0.91	4.58	13.74	17.64	58.8
9.	360	1.07	5.35	16.05	20.5	68.5
10.	420	1.09	5.45	16.37	21.72	72.4
11.	480	1.25	6.25	18.75	24.15	80.00

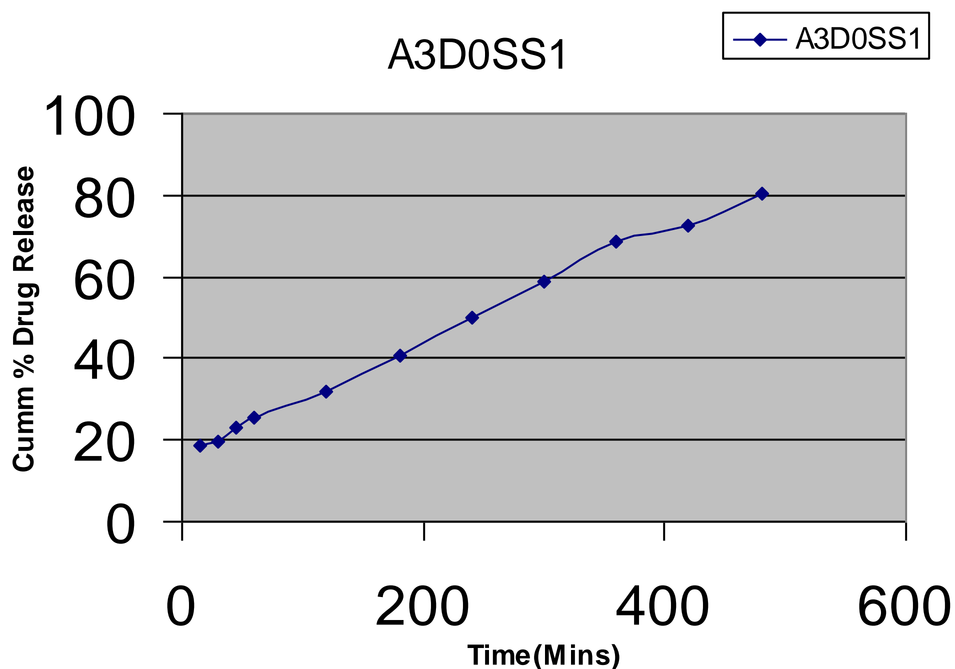

FIGURE 2.21:In vitro release profile of batch A3DOSS1

TABLE 4.9 In vitro release profile of batch A3DOSS2

Sl. no	Time (mins)	Conc. (mg/ml)	Conc. (In 5ml)	Conc. (In 15ml)	Cumulative Amount	Cumulative %Released
1.	15	0.28	1.44	4.32	4.32	14.4
2.	30	0.25	1.28	3.85	5.25	17.5
3.	45	0.32	1.64	4.92	6.12	20.4
4.	60	0.52	2.59	7.78	9.42	31.4
5.	120	0.60	3.0	9.05	11.55	38.5
6.	180	0.65	3.25	9.75	12.75	42.5
7.	240	0.77	3.89	11.64	14.94	49.8
8.	300	0.85	4.28	12.8	16.6	55.5
9.	360	0.95	4.75	14.25	18.45	61.5
10.	420	0.99	4.95	14.87	19.62	65.4
11.	480	1.00	5.04	15.14	20.04	66.8

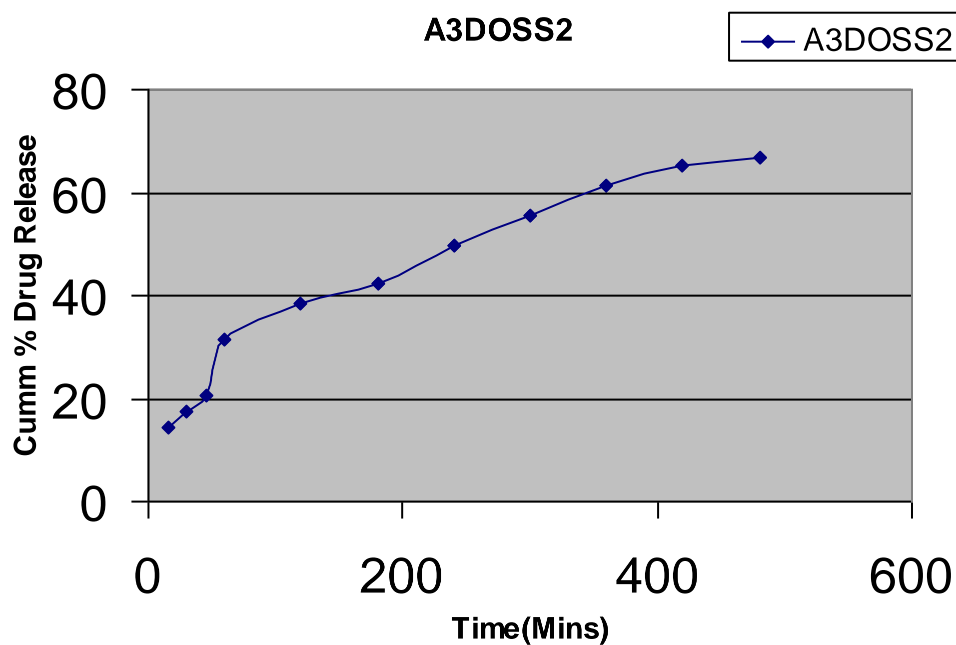

FIGURE 2.22:In vitro release profile of batch A3DOSS2

3.10 ACCELERATED STABILITY STUDIES:

- **Accelerated stability studies as per ICH guidelines:**

The optimized formulation was wrapped in aluminum foils and kept in petri dish at 40ºC ±2ºC/75% RH ±5%in humidity chamber. The stability studies were conducted after 30 and 60 days.

Following parameters were evaluated:

- **Drug content:**

UV-Visible spectroscopy is used for carrying
out stability indicating assay. The optimized formulation A3was kept at 40ºC ±2ºC/75% RH ±5% and the content of Tizanidine HCL in it was determined using Shimadzu UV-Visible spectrophotometer (UV-1700) after 10 days interval for 60 days.

TABLE 4.10: Degradation of Tizanidine HCl at 40ºC ±2ºC/75% RH ±5%

Time (days)	Abs	Conc.(µg/ml)	% drug remaining	% drug remaining
0	0.349	9.98	99.88	1.999
30	0.347	9.93	99.31	1.997
60	0.346	9.90	99.02	1.996
90	0.345	9.87	98.74	1.994

The log of drug remaining was plotted against time (in days). Slope of line was obtained and degradation rate constant was calculated by the formula

Slope = -K/2.303

Where K is the degradation rate constant.

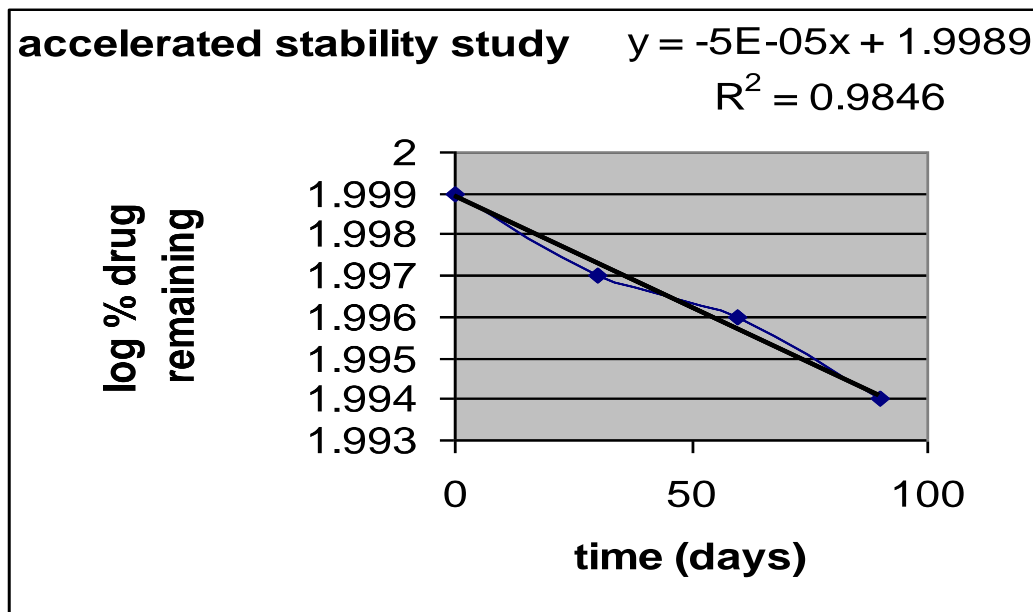

Figure 2.23: log % Drug remaining Vs Time (Days)

log percent of drug remaining was plotted against time. 1.26 % drug was degraded in 2 months.

- **Conclusion:** The drug content in the formulation A3 is degraded by 1.26 %

SURFACE MORPHOLOGY AND SHAPE

These microspheres were spherical with smooth surface, glossy in nature and were not aggregated.

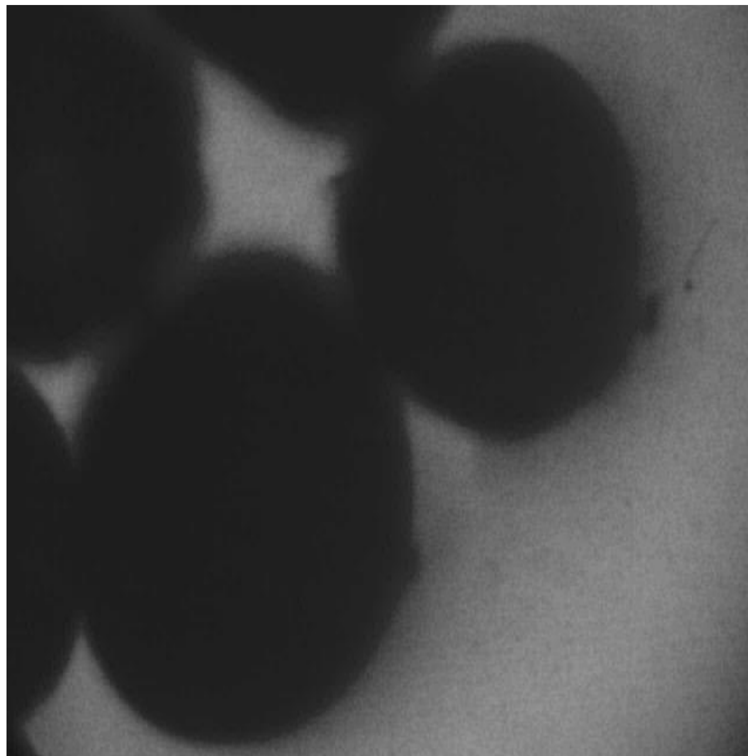

Figure. 2.24: Optical Micrograph of microspheres after stability study

SIZE AND SIZE DISTRIBUTION

The mean particle diameter of the prepared microspheres batch A3 was found to be 65.57 µm.

SWELLING ABILITY

The swelling ability of microspheres in phosphate buffer pH6.6 was found to be 1.1.

IN VITRO BIOADHESION TEST

TABLE 4.11 In vitro bioadhesion test of batch A3

Batch No. Time (h)	% Mucoadhesion				
	1	2	4	6	8
A3	68	60	50	42	38

- **MICROMETRICS PROPERTIES OF CHITOSAN MICROSPHERES**

TABLE 4.12 Micrometrics properties of batch A3

PROPERTIES	A3
ANGLE OF REPOSE	30.5
CARR'S INDEX	20.3
HAUSNER RATIO	1.24

- ## IN VITRO NASAL PERMEATION STUDIES:

TABLE 4.13 In vitro permeation study of batch A3

Sl. no	Time (mins)	Conc. (mg/ml)	Conc. (In 5ml)	Conc. (In 15ml)	Cumulative Amount	Cumulative %Released
1.	15	0.396	1.98	5.94	5.94	19.8
2.	30	0.286	1.43	4.29	6.27	20.9
3.	45	0.402	2.01	6.04	7.47	24.9
4.	60	0.401	2.00	6.02	8.03	26.79
5.	120	0.604	3.02	9.07	11.07	36.9
6.	180	0.772	3.86	11.59	14.61	48.7
7.	240	0.844	4.22	12.67	16.53	55.1
8.	300	1.00	5.04	15.13	19.35	64.5
9.	360	1.02	5.11	15.33	20.37	67.9
10.	420	1.10	5.53	16.61	21.72	72.4
11.	480	1.11	5.58	16.76	22.29	74.3

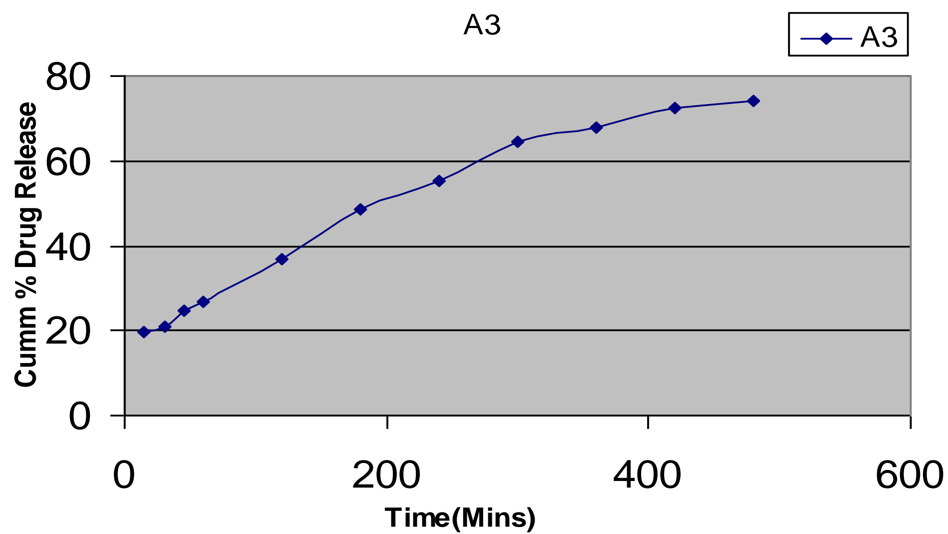

Figure 2.26: In vitro release profile of batch A3