JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Design of a buccal mucoadhesive, nanoparticles based delivery system of fluoxetine

Abhay S. Sapre [1*] and Rajesh K.Parikh [1]

Department of pharmaceutics and pharmaceutical technology, L.M.College of pharmacy, Ahmedabad, India, 380 015

ABSTRACT:

The study was attempted to develop an alternative oral mucosal delivery of nanoparticles based system for antidepressant drug. The aim was to formulate a novel, transmucosal (buccal), polymeric nanoparticles based mucoadhesive system (diskettes) that could deliver fluoxetine hydrochloride with bypass first pass effect, with relative rapid onset, higher absorption and sustain release effect to increase bioavailability compared to oral absorption. In this study, the drug was encapsulated into poly (methyl vinyl ether / maleic anhydride) (Gantrez MS-955) mucoadhesive polymer nanoparticles. The drug bearing nanoparticles were prepared by emulsion solvent evaporation method. The effect of critical formulation variables like, polymer concentration, emulsifier concentration and process variable like rate of homogenization were studied for the particle size distribution; drug entrapment efficiency and mucoadhesion. The dependent variables of the formulations were optimized using 3^2 full factorial designs and defined in mathematical equations. The desired values of response variables were found by contour plots generated using the design-expert® 8 version 8.0.6.1 software. The drug encapsulated polymeric nanoparticles were gently compacted along ethyl cellulose layer into small round shaped diskettes for facilitating buccal application in such a way that polymer layer adhere with buccal mucosa. The gently compacted diskettes gave dose precision through uniform drug distribution, high surface areas for better drug releases with sustain effect and without disrupter of nanoparticles. The in vitro studies of the diskettes included mucoadhesion and drug release profile were characterized. The in vivo studies were performed on rats. A significant improvement in the pharmacokinetic parameters of bioavailability like C_{max}, T_{max} and AUC was observed when compared with oral solution. The stability study was conducted on the optimized formulation at accelerated conditions.

Key words: nanoparticles, mucoadhesive diskettes, fluoxetine hydrochloride, factorial designs and Gantrez MS-955.

Article history:
Received 16 May , 2012
Accepted 01 June 2012
Available online 13 Jun 2012

For Correspondence:

Abhay S. Sapre,

Department of Pharmaceutics and Pharmaceutical Technology,

L.M.College of Pharmacy, Ahmedabad, India, 380 015

E-mail: abhaysapre@rediffmail.com

(www.jpsbr.org)

INTRODUCTION:

Among the various routes of drug delivery, oral route is perhaps the most preferred to the patients and the clinician alike. However, peroral administration of drugs has primary disadvantages such as (a) hepatic first pass metabolism and (b) enzymatic degradation within the gastro intestinal tract (GIT), that prohibit oral administration of certain classes of drugs (c) ph depended solubility in the GIT (d) drug degradation in acidic environment (e) presence of food etc. Affects rate and extent of drug absorption in the GIT. Consequently, other absorptive mucosa is considered as potential sites for drug administration.[1] transmucosal routes of drug delivery (i.e. The mucosal linings of the nasal, buccal/sublingual (oral cavity), rectal, vaginal, and ocular) offer distinct advantages over peroral administration of systemic drug delivery. These advantages include possible (a) bypass of first pass effect, (b) avoidance of presystemic elimination within the GIT and (c) depending on the particular drug, a better enzymatic flora for drug absorption (d) rapid onset for drug absorption (e) convenient route (f) modified rate for systemic absorption.[2-4] the oral mucosal route besides being convenient, accessible and robust is is highly vascularized and hence permits rapid absorption. In recent years mucoadhesive polymer / copolymer have attracted much attention in the design of delivery systems, oral mucosal routes in particular.[5-7]

An oral mucoadhesive delivery system is known to enhance the drug bioavailability by prolonging residence time at the specified regions and an optimal contact with the adsorbing biological membrane. Thus, oral mucosal mucoadhesive systems additionally permit an opportunity for modulated drug release as long as the system remains adherent to the mucosal membrane. These facilitate high drug concentration in the local area based on drug solubility in the saliva at modified rate and may even increase the total permeability of many drugs due to thin and rich blood absorption site.[8-10] Furthermore, oral transmucosal drug delivery bypasses first pass effect and avoids presystemic elimination in the GIT. These factors make the oral mucosal cavity a very attractive and feasible site for systemic drug delivery. Within the oral mucosal cavity, delivery of drugs can be through (a) sublingual delivery, which is systemic delivery of drug through the mucosal membranes lining the floor of the mouth and (b) buccal delivery, which is drug administration through the mucosal membranes lining the cheeks (buccal mucosa).[11-13]

The present work is an effort to develop oral mucoadhesive delivery system for anti depressant drugs like fluoxetine hydrochloride. In this study, the drug was encapsulated into poly (methyl vinyl ether/maleic anhydride) (Gantrez MS-955) nanoparticles. Poly (methyl vinyl ether/maleic anhydride) is reported to have excellent mucoadhesive characteristics and used in oral mucosal (buccal), ophthalmic, intestinal, nasal, vaginal and rectal applications. Hence it was selected on account of its well-established mucoadhesive, biocompatible and stable nature. [14]

MATERIALS AND METHODS:

Materials:

Fluoxetine hydrochloride (BP, USP, Ph.Eur.) Was received as gift samples from Dr. Reddy's laboratory, India. Poly (methyl vinyl ether/maleic anhydride) (Gantrez MS-955) was purchased from international specialty products (ISP). Light mineral oil (IP, NF, Drakeol 7 LT) was purchased from calumet Penreco, USA. Ethylcellulose (BP, USP, Ph.Eur., Aqualon, Ethocel std. 4 premium N7) was purchased from Dow chemicals, USA. Diethyl ether and Methanol (BP, USP, Ph.Eur.) Were purchased from Merck, Germany and Sorbitan mono oleate (USP, span 80 hp-lq-mh) from Croda chemicals, USA. All other analytical grade chemicals were obtained from commercial sources.

Methods:

Preparation of the nanoparticles and diskettes:

The emulsion solvent evaporation technique[15] was selected for preparation of nanoparticles. Drug and polymer were dissolved in methanol (polar internal phase) and sorbitan mono oleate (span 80) (nonionic surface active agent, HLB-4.3, stabilizer) was dissolved in light mineral oil (non polar external phase) under constant stirring to get clear solution. The polar phase was slowly emulsified with non polar phase using sonication. The w/o emulsion so formed,

was homogenized (Ultra Turrex, IKA) continuously at room temperature till complete evaporation of the internal phase (methanol). The polymer precipitated around the nanodroplets of solubilised drug, formed a drug encapsulated nanoparticles based stabilized matrix system in colloidal range and uniformly suspended in non polar oil phase under controlled homogenization. The nanoparticles are fairly stable and uniform in size identified by microscopy. [4,16] The suspended nanoparticles were filtered through vacuum filtration process using 1000nm millipore filters (inert with stable porosity) to filter off the aggregated particles (if any). The oil filtrate contained suspended colloidal particles below 1000nm, which was again filtered through vacuum filtration process using 200nm millipore filters (inert with stable porosity) to filter off the nanoparticles above 200nm in size. The oil filtrate was discarded and the nanoparticles retained over 200nm filter were washed with diethyl ether to remove traces of light mineral oil adsorbed on the nanoparticles surface. Now, the washed, uniform sized drug encapsulated nanoparticles in the range of 200nm to 1000nm were collected and dried at 40±5˚c for about 2 hrs. The dried particles were gentle sieved using 635 mesh ASTM sieve to break soft aggregates (if any) and 'drug encapsulated nanoparticles' were collected and stored in glass bottles for

Table 1: Composition with different polymer and drug concentration

Batch no.	Polymer concentration (%w/v)	Polymer qty. (mg)	Drug base qty. (mg)	Drug salt qty. (mg)	Methanol (ml)	Light mineral oil (ml)	Span 80 concentration (%w/v)	Span 80 qty. (g)	Homogenization speed (rpm)
FG1	5	750	100	111.8	15	150	2.5	3.750	20000
FG2	5	750	300	335.4	15	150	2.5	3.750	20000
FG3	5	750	500	558.9	15	150	2.5	3.750	20000
FG4	3	450	100	111.8	15	150	2.5	3.750	20000
FG5	3	450	300	335.4	15	150	2.5	3.750	20000
FG6	3	450	500	558.9	15	150	2.5	3.750	20000
FG7	1	150	100	111.8	15	150	2.5	3.750	20000
FG8	1	150	300	335.4	15	150	2.5	3.750	20000
FG9	1	150	500	558.9	15	150	2.5	3.750	20000

Table 2: Composition with different emulsifier concentration and homogenization speed

Batch no.	Span 80 concentration (%w/v)	Span 80 qty. (g)	Homogenization speed (rpm)	Polymer concentration (%w/v)	Polymer qty. (mg)	Drug base qty. (mg)	Drug salt qty. (mg)	Methanol (ml)	Light mineral oil (ml)
FG10	1	1.5	5000	4	600	400	447.1	15	150
FG11	2	3.0	5000	4	600	400	447.1	15	150
FG12	3	4.5	5000	4	600	400	447.1	15	150
FG13	1	1.5	15000	4	600	400	447.1	15	150
FG14	2	3.0	15000	4	600	400	447.1	15	150
FG15	3	4.5	15000	4	600	400	447.1	15	150
FG16	1	1.5	25000	4	600	400	447.1	15	150
FG17	2	3.0	25000	4	600	400	447.1	15	150
FG18	3	4.5	25000	4	600	400	447.1	15	150

further characterization and analysis. The polymeric nanoparticles were prepared at different polymer concentration (1 to 5% w/v) and drug concentration (100 to 500mg) (considered 100mg fluoxetine base = 111.8mg fluoxetine hydrochloride). The other critical formulation/process variables were kept constant in the composition given in Table 1.

Similarly, the polymeric nanoparticles were also prepared at different emulsifier concentration (Span 80) (1 to 3% w/v) and homogenization speed (5000 to 25000 rpm). The other critical formulation/process variables were kept constant with optimized polymer and drug concentration given in following composition Table 2.

Diskettes were prepared, comprised of ethylcellulose layer and drug encapsulated nanoparticles layer. These layers were gently compacted using KBR press (used in infrared spectroscopy instrument). About 20mg of ethylcellulose and added into 5mm die fitted in KBR press. The ethylcellulose was gently compacted at force 40 ±5 kg/cm^2 to make uniform layer of ethylcellulose. About 50mg of the drug encapsulated nanoparticles (equivalent to 20mg drug base) from optimized formulations batches and added into 5mm die, over ethylcellulose layer. The nanoparticles were gently compacted at force 40 ±5 kg/cm^2 to make an uniform layer of nanoparticles (layer 2) which was adhered to ethylcellulose layer to form uniform, distinct bilayer diskettes. The compaction force was optimized in such a way that no breakage of nanoparticles observed microscopically and gives uniform diskettes. Thus the final small, round, uniform discs of 5 mm diameter mucoadhesive diskette (finished product) for oral mucosal (buccal) application were formulated and the diskettes were stored in glass bottles.

Determination of particle size and shape:

The particle size of drug encapsulated nanoparticles was measured by zetasizer (Malvern- nano ZS). This instrument is based on non-invasive back scatter (NIBS) technology which takes particle sizing to levels of sensitivity in the 0.6 nm to 8.9 micron range. It gives accurate, reliable and repeatable size analysis of particles. The shape and surface characteristics were examined by scanning electron microscopy (SEM, Philips Holland).

Determination of drug content:

The drug content (assay) determination was carried out using stability indicating HPLC method. The buffer solution was prepared by dissolving 3.0 g of ammonium acetate and 0.7 g of 1-octane sulfonic acid sodium salt in 630 ml of water. The mobile phase was prepared by dissolving a mixture of buffer solution, acetonitrile, tetrahydrofuran (stabilized) and triethylamine in the ratio of (630: 320: 50: 2). Adjusted the pH to 7.0 ± 0.1 with glacial acetic acid. Filtered the solution through 0.2 μm filter and degas. The HPLC details are column (Zorbax sb c8, (7.5 cm x 4.6 mm), 3.5 μm; wavelength (227 nm); flow rate (1.8 ml/minute); injection volume (20 μl) and column temperature (40°C). Chromatograph the standard preparation and recorded the peak responses. Injected the mobile phase, standard preparation and sample preparation in sequences into the chromatograph, recorded the chromatograms and measured the responses for the analyte peaks. Calculated the fluoxetine per average mass sample preparation based on area obtained compared to standard area.

Mucoadhesion method:

A simple quantitative and realistic in situ method[17] to test mucoadhesive potential of polymer was used. The buccal mucosa (cheek area) of rat was cut and cleaned. The buccal mucosal was placed on polyethylene support at inclined position with the help of pins. The weighed amount of mucoadhesive polymer based drug encapsulated nanoparticles(approx. 50mg) was spread uniformly on the buccal mucosa. It was placed in a desiccators maintained at > 80% relative humidity at room temperature for 1 hr to allow the nanoparticles to hydrate. After 1hr, the buccal mucosa was gentle washed with purified water and washing was collected into a pre weighed petri dish. The petri dish was dried at 100˚C ± 2˚C, cooled it at room temperature (25˚C ± 2˚C) and weighed. The percent mucoadhesion was determined and recorded. The same method was used to test diskette sample in such a way that nanoparticles based layer 2 should in contact with it for 8hrs. The ethylcellulose layer 1 was kept on outer side.

In vitro drug release method:

The in vitro drug release from diskettes was performed in USP

5, paddle over disc apparatus in 1000 ml dissolution media-simulated saliva solution[4] (potassium chloride -0.4g; sodium chloride -0.4g; sodium sulphide -0.02g; magnesium pyrophosphate--0.02g; calcium chloride-0.6g; Di sodium hydrogen phosphate-0.6g; mucin-2g; purified water q.s. 1000 ml) at agitation speed 100 rpm and samples were withdrawn at 30,60,120,180,240,300 min and analysed using HPLC method. To the test sample, apply sufficient amount of hydrophobic silicon adhesive to the surface of a diskette containing ethylcellulose layer and affix it on a 50 mesh round sieve disk towards center. Placed the sieve gently into the dissolution vessel in such a way that "drug bearing nanoparticle layer" should be faced towards up side. Repeat it for six different vessels and operate the instrument exactly for the specified time. At the end of specified time withdrew about 10 ml of the solution from middle zone and replaced with 10ml with fresh dissolution media. Filtered the sample solution through 0.2 μm millipore HVLP filter and use it for analysis on HPLC column (zorbax cn- (15 cm x 4.6 mm), 5 μm; wavelength (227 nm); flow rate (1.0 ml/minute); injection volume (50 μl) and column temperature (30°C). Chromatograph the standard solution preparation and recorded the peak responses. Inject mobile phase, dissolution medium, standard preparation and sample preparation in sequence into the chromatograph and measure the responses for the analyte peak. Calculated the fluoxetine per average mass sample preparation based on area obtained compared to standard area.

In vivo study method:

The in vivo study was performed on mucoadhesive diskettes of optimized formulation using available animal model (wistar rats) (250-350g) under fasting conditions. The animals were divided into two groups of six animals in each group. The rats were fasted overnight before initiating experiments and had access to water. To the first group, fluoxetine solution (batch no.: FS) (1 ml) (22.4mg fluoxetine hydrochloride equivalent to 20mg fluoxetine base dissolved in simulated saliva solution, pH 6.5) was administered orally. The rats of second group were anesthetized by administration of urethane (0.5 mg/ kg body weight) till study period of 8 hrs prior to the mucoadhesion of diskettes (batch no.: FG18) into the buccal cavity (cheek) of rats. Carefully placed one diskette (drug base 20 mg) into the buccal cavity in each rat and ensured that the diskettes were adhered to the buccal cavity during study period of 8 hrs. After 8hrs, the mucoadhered diskettes were removed carefully. All rats were visually monitored for 24 hrs and their blood samples were collected at 0 (before drug administration), 1,2,3,4,5,6,7,8,10,12 and 24hrs. The blood samples (0.3 ml) were centrifuged at 3000 rpm for 15 mins to separate plasma and samples were stored at -20˚C before analysis. The drug concentration on separate plasma was determined by HPLC for fluoxetine.[18] the measured drug concentration was expressed as ng / ml and C_{max}, T_{max}, $AUC_{t=24}$ were calculated.

Stability study:

The diskettes of optimized formulation (batch no.: FG18) were packed in glass bottles and placed for 6 months at 40± 2˚C and 75 % ± 5 % RH along with plain API packed in glass bottles as control samples. The samples were analysed for stability indicating tests like physical description and drug assay along with controls at initial, 1,3 and 6 months interval using HPLC method. [18]

RESULTS AND DISCUSSION:

The effect of formulation/process variables with different compositions were characterized and evaluated. The effect of critical independable formulation variables like polymer concentration, drug concentration, emulsifier concentration and process variable like homogenization rate during nanoparticle preparation were indentified and studied based on design of experiments (DOE). These variations were measured for drug entrapment efficiency, particle size distribution and mucoadhesion and analysed with using 3^2 full factorial designs of design-expert 8 software as given in Table 3 and 10. The responses were optimized using statistical methods of simple linear regression analysis as given in Table 4 to 19. The results plotted through contour graph shown in Figure 1 to 7 and SEM photographs shown in Figure 8.

Effect of drug and polymer concentration study:

Table 3: 3^2 full factorial design: effect of polymer and drug concentration on response variables for nanoparticles

Run	Batch no.	Factors/ independent variables		Response / dependent variables	
		Polymer concentration (%w/v) (X_1)	Drug concentration (mg) (X_2)	Drug entrapment efficiency (%w/w) (Y_1)	Mean particle Size ± SD (nm) (Y_2)
1	FG1	5	100	97.2	825 ± 14.1
2	FG2	5	300	97.5	856 ± 16.5
3	FG3	5	500	99.0	871 ± 18.6
4	FG4	3	100	92.5	655 ± 11.5
5	FG5	3	300	95.5	675 ± 12.0
6	FG6	3	500	96.3	693 ± 13.7
7	FG7	1	100	90.0	352 ± 7.9
8	FG8	1	300	52.4	341 ± 8.2
9	FG9	1	500	45.5	378 ± 9.4

Response -1: Percent drug entrapment efficiency (Y_1)

Analysis of variance (ANOVA):

Table 4: Anova for response surface 2FI model for % drug entrapment efficiency in nanoparticles

Source	Sum of square	Df	Mean square	F value	P-value Prob>F	
Model	2653.73	3	884.58	5.60	0.0469	Significant
Polymer concentration X_1	1865.61	1	1865.61	11.82	0.0185	
Drug concentration X_2	252.20	1	252.20	1.60	0.2620	
$X_1 * X_2$	535.92	1	535.92	3.39	0.1248	

Regression analysis:

Table 5: Regression analysis with regular model for % drug entrapment efficiency in nanoparticles

Details	Coefficients	Std. Error	P –value	Lower 95% CI	Upper 95% CI
Intercept	91.47	7.70	0.0013	66.97	115.96
Polymer concentration (X1)	17.63	4.22	0.02	4.22	31.05
Drug concentration (X2)	-6.48	4.22	0.22	-19.90	6.93
X1*X1	-14.50	7.30	0.14	-37.74	8.74
X2*X2	4.95	7.30	0.55	-18.29	28.19
X1*X2	11.58	5.16	0.11	-4.86	28.01

Table 6: Regression analysis with reduced model for % drug entrapment efficiency in nanoparticles

Details	Coefficients	Std. Error	P –value	Lower 95% CI	Upper 95% CI
Intercept	85.10	4.95	0.000002	72.98	97.22
Polymer concentration (X_1)	17.63	6.07	0.03	2.79	32.48
Drug concentration (X_2)	-6.48	6.07	0.33	-21.33	8.36

The three levels (low, medium and high) values of two critical independable variables (polymer-X_1:1,3,5%w/v and drug concentration – X_2:100,300,500 mg) were evaluated to measure two independable variables (percent drug entrapment efficiency-Y_1 and mean particle size nm/std.dev -Y_2)(response surface values) using appropriate model of ANOVA (analysis of variance) for significant value. The nine runs provided by software were performed as per composition given in Table 3 and compiled the measured response surface values Y_1 and Y_2. Percent drug entrapment efficiency was calculated as percentage of total drug content in nanoparticles to the initial drug quantity taken. The statistical analyses for different formulations are discussed below:

Response -1: Percent drug entrapment efficiency (Y_1): It was observed that as polymer concentration (X_1) increased from 1% to 5% w/v, the drug entrapment efficiency was increased up to 97.2 -99.0 % for different drug concentration (X_2) levels 100-500mg. It was also observed that as drug concentration (X_2) increased from 100mg to 500mg, the drug entrapment efficiency was varies in the range 45.5 -99.0% at different polymer concentration. It can be explained as when polymer quantity was similar or higher to the drug quantity dissolved in the methanol (internal phase), the drug entrapment efficiency was higher than 90%. When polymer quantity was less than drug quantity dissolved in the methanol (internal phase), the drug entrapment efficiency was in the range approx 45.5 – 52.4 % as indicated in Table 3. Hence for better drug entrapment

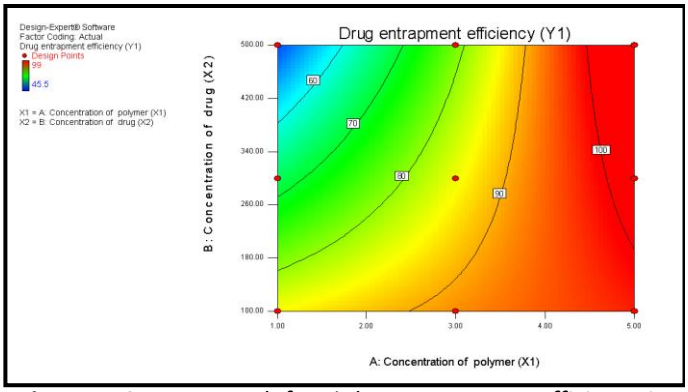


Figure 1: Contour graph for % drug entrapment efficiency in nanoparticlesusing different concentration of drug and polymer

within polymer matrix, drug quantity should be similar or less than polymer quantity dissolved in internal phase. The software analysed the ANOVA for response surface with 2FI model. The Model F-value of 5.60 implies the model was significant. There was only a 4.69 percent chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms were significant. In this case, Polymer concentration X1 was significant model terms.**Regression analysis:** The effect of independable variables was evaluated using simple linear regression analysis given in Table 5. The process data was evaluated at 95% confidence interval with regular model showed that for polymer concentration, the p-value (0.02)

Response -2: Mean particle size (nm) (Y_2)

- **Analysis of variance (anova):**

Table 7: Anova for response surface linear model for mean particle size (nm) of nanoparticles

Source	Sum of square	Df	Mean square	F value	P-value Prob>F	
Model	377700	5	75533.47	598.42	0.0001	Significant
Polymer concentration X_1	365600	1	365600	2896.16	<0.0001	
Drug concentration X_2	2016.67	1	2016.67	15.98	0.0281	
X_1 * X_2	100.00	1	100.00	0.79	0.4390	
X_1 * X_1	9940.50	1	9940.50	78.75	0.0030	
X_2 * X_2	50.00	1	50.00	0.40	0.5738	

- **Regression analysis:**

Table 8: Regression analysis with regular model for mean particle size (nm) of nanoparticles

Details	Coefficients	Std. Error	P-value	Lower 95% CI	Upper 95% CI
Intercept	671.00	8.37	0.0000043	644.35	697.65
Polymer concentration (X_1)	246.83	4.59	0.00	232.24	261.43
Drug concentration (X_2)	18.33	4.59	0.03	3.74	32.93
X_1*X_1	-70.50	7.94	0.00	-95.78	-45.22
X_2*X_2	5.00	7.94	0.57	-20.28	30.28
X_1*X_2	5.00	5.62	0.44	-12.88	22.88

Table 9: Regression analysis with reduced model for mean particle size (nm) of nanoparticles

Details	Coefficients	Std. Error	P-value	Lower 95% CI	Upper 95% CI
Intercept	674.33	5.94	0.0000	659.07	689.59
Polymer concentration (X_1)	246.83	4.20	0.00	236.04	257.62
Drug concentration (X_2)	18.33	4.20	0.01	7.54	29.12

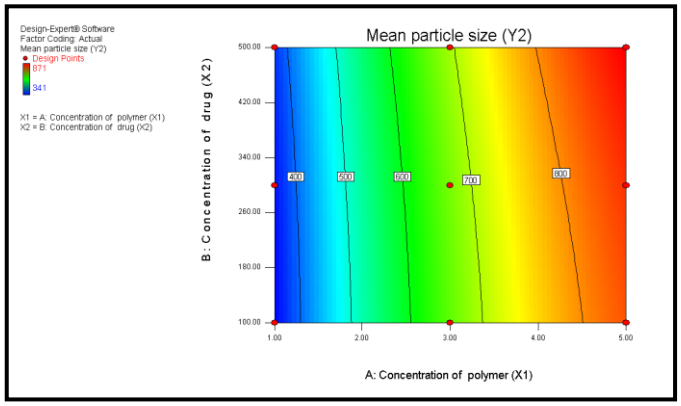


Figure 2: Contour graph for mean particle size (nm) of nanoparticles using different concentration of drug and polymer

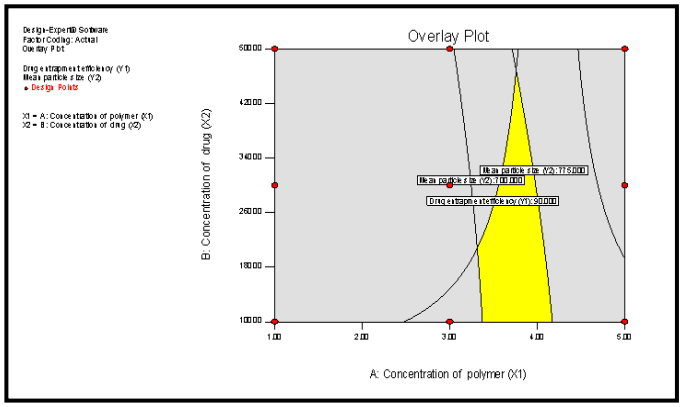


Figure 3: Contour overlay graph for optimum desired response variables for different concentration of drug and polymer.

was less than 0.05 and for drug concentration, the p-value (0.22) was higher than 0.05. Therefore it again interpreted with reduced model to ensure all p-value < 0.050. The process data of reduced model given in Table 6 indicated that percent drug entrapment efficiency (Y_1) linearly depends upon variation in polymer concentration (X_1) and it is defined by following equation:

Percent drug entrapment efficiency (Y1) = intercept + coefficient *X_1 –coefficient*X_2

= 85.10 + 17.63 * X_1 – 6.48 * X_2

The regression statistics provided Multiple R 0.9524 and 0.7843; R Square 0.9071 and 0.6151 for regular and reduced model respectively. The **contour graph** between polymer and drug concentration indicated higher drug entrapment efficiency (90-100%) can be obtained with higher polymer concentration (approx 3.5 to 4.5% w/v) at different 100-500 mg drug concentration range as indicated in Figure 1.

Response -2: Mean particle size (Y_2): It was observed that as polymer concentration (X_1) decreased from 5% to 1% w/v, the mean particle size was also decreased from ~825-871 nm to ~341-378 nm range at different drug concentration (X_2) levels

100-500mg. It was also observed that as drug concentration (X_2) decreased from 500mg to 100mg, the mean particle size also decreased with decrease in standard deviation as indicated in trend, but not significantly changed at different polymer concentration. It can be well explained when polymer and drug concentration decreased, the polymer and drug quantity in methanol (internal phase) also decreased to give Relative thin or less viscous solution. During nano-emulsion stabilization, the sub-division of internal phase droplets (polymer and drug solution in methanol) could be possible to stabilize into further smaller droplets relatively easy leading to stabilization of drug encapsulated particle(s) into smaller size with narrow standard deviation on evaporation of methanol. Therefore, the result indicated that there is directly proportion relationship between polymer and drug concentration to the mean particle size and size distribution of nanoparticles given in Table 3. The software analysed the ANOVA for response surface with Quadratic model. The Model F-value of 598.42 implies the model was significant. There was only a 0.01 percent chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms were significant. In this case X_1, X_2, X_1^2 were significant model terms given in Table 7.

Regression analysis: The effect of independable variables was evaluated using simple linear regression analysis given in Table 8. The process data was evaluated at 95% confidence interval with regular model showed that for polymer concentration, the p-value (0.00) was very less than 0.05 and for drug concentration; the p-value (0.003) was also very less than 0.05. The process data of reduced model given in Table 9 indicated that mean particle size (Y_2) linearly depends upon variation in polymer concentration (X_1) and drug concentration (X_2) and it is defined by following equation:

Mean particle size (nm) (Y_2) = intercept + coefficient * X_1 + coefficient * X_2

$$= 674.33 + 246.83 * X_1 + 18.33 * X_2$$

The regression statistics provided Multiple R 0.9995 and 0.9993; R Square 0.9990 and 0.9986 for regular and reduced model respectively. The **contour graph** between polymer and drug concentration indicated higher particle size (700-800 nm) can be obtained with polymer concentration (approx 3.5 to 4.5 % w/v) at different 100-500 mg drug concentration range to get higher drug entrapment efficiency (90-100%) as shown in Figure 2.

Optimization graph: The contour overlay graph (Figure 3) for optimum desired value (yellow shaded region) gave variety of combinations to get better drug entrapment efficiency and narrow particle size range. Based on peak value, approx 4% w/v polymer and 400 mg drug concentration were considered an optimum concentration to give percent drug entrapment efficiency ~(90-100%) and mean particle size ~(700-775 nm) range. Further, emulsifier concentration and homogenization speed were optimized to get desired mean particle size and its distribution; percent drug entrapment efficiency and percent mucoadhesion.

Effect of emulsifier concentration and rate of homogenizer study:

Table 10: 3^2 full factorial design: effect of emulsifier concentration and homogenization speed on response variables for nanoparticles

Run	Batch no.	Factors/ independent variables		Response / dependent variables		
		Emulsifier concentration (%w/v) (X_1)	Homogenization speed (rpm) (X_2)	Drug entrapment efficiency (%w/w) (Y_1)	Mean particle Size ± SD (nm) (Y_2)	Mucoadhesion (%w/w) (Y_3)
1	FG10	1	5000	82.5	970 ±20.1	82
2	FG11	2	5000	84.1	954 ±19.3	84
3	FG12	3	5000	86.7	920 ±17.0	87
4	FG13	1	15000	95.1	885 ±15.6	92
5	FG14	2	15000	96.4	870 ±15.0	93
6	FG15	3	15000	96.9	835 ±14.2	95
7	FG16	1	25000	98.1	600 ±11.7	96
8	FG17	2	25000	98.5	575 ±10.8	98
9	FG18	3	25000	98.8	530 ±6.9	99

Response -1: Percent drug entrapment efficiency (Y_1):

- **Analysis of variance (ANOVA):**

Source	Sum of square	Df	Mean square	F value	P-value Prob>f	
Model	349.81	5	69.96	505.42	0.0001	Significant
Emulsifier concentration (X_1)	7.48	1	7.48	54.05	0.0052	
Homogenization speed (X_2)	295.40	1	295.40	2134.01	<0.0001	
$X_1 * X_2$	3.06	1	3.06	22.12	0.0182	
$X_1 * X_1$	55560	1	55560	4013	0.9535	
$X_2 * X_2$	43.87	1	43.87	316.90	0.0004	

- **Regression analysis:**

Table 12: Regression analysis with regular model for % drug entrapment efficiency in nanoparticles

Details	Coefficients	Std. Error	P-value	Lower 95% CI	Upper 95% CI
Intercept	96.12	0.28	0.00000005	95.24	97.00
Emulsifier concentration (X_1)	1.12	0.15	0.0052	0.63	1.60
Homogenization speed (X_2)	7.02	0.15	0.000022	6.53	7.50
X_1*X_1	0.02	0.26	0.95347167	-0.82	0.85
X_2*X_2	-4.68	0.26	0.00038	-5.52	-3.85
X_1*X_2	-0.87	0.19	0.01818	-1.47	-0.28

Table 13: Regression analysis with reduced model for % drug entrapment efficiency in nanoparticles

Details	Coefficients	Std. Error	P-value	Lower 95% CI	Upper 95% CI
Intercept	96.13	0.19	0.00000000	95.62	96.65
Emulsifier concentration (X_1)	1.12	0.13	0.00105847	0.75	1.48
Homogenization speed (X_2)	7.02	0.13	0.00000074	6.65	7.38
X2*X2	-4.68	0.23	0.00003317	-5.32	-4.05
X1*X2	-0.87	0.16	0.00558873	-1.32	-0.43

Response -3: percent mucoadhesion (Y_3)

- **Analysis of variance (ANOVA):**

Table 17: Anova for response surface linear model for % mucoadhesion of nanoparticles

Source	Sum of square	Df	Mean square	F value	P-value Prob>f	
Model	298.78	5	59.76	230.49	0.0004	Significant
Emulsifier concentration (X_1)	20.17	1	20.17	77.79	0.0031	
Homogenization speed (X_2)	266.67	1	266.67	1028.57	<0.0001	
X1 * X2	1.00	1	1.00	3.86	0.1443	
X1 * X1	0.056	1	0.056	0.21	0.6749	
X2 * X2	10.89	1	10.89	42.00	0.0075	

- **Regression analysis:**

Table 18: Regression analysis with regular model for % mucoadhesion of nanoparticles

Details	Coefficients	Std. Error	P-value	Lower 95% CI	Upper 95% CI
Intercept	93.22	0.38	0.0000001	92.01	94.43
Emulsifier concentration (X_1)	1.83	0.21	0.0030717	1.17	2.49
Homogenization speed (X_2)	6.67	0.21	0.0000666	6.01	7.33
X_1*X_1	0.17	0.36	0.6749412	-0.98	1.31
X_2*X_2	-2.33	0.36	0.0074571	-3.48	-1.19
X_1*X_2	-0.50	0.25	0.1442936	-1.31	0.31

Table 19: Regression analysis with reduced model for % mucoadhesion of nanoparticles

Details	Coefficients	Std. Error	P-value	Lower 95% CI	Upper 95% CI
Intercept	93.33	0.35	0.0000000	92.43	94.23
Emulsifier concentration (X_1)	1.83	0.25	0.0007020	1.20	2.47
Homogenization speed (X_2)	6.67	0.25	0.0000013	6.03	7.30
X2*X2	-2.33	0.43	0.0028272	-3.43	-1.23

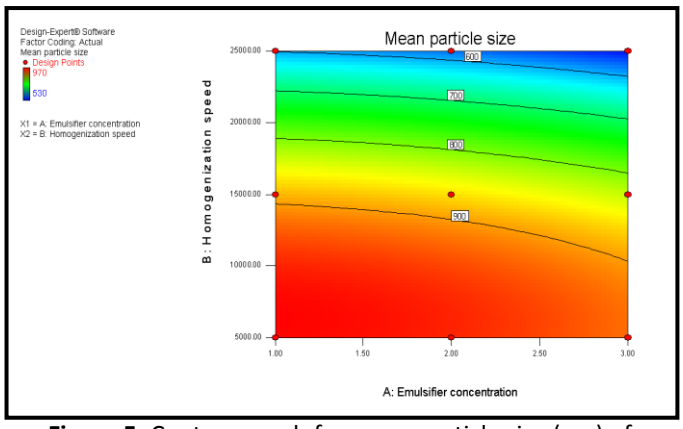


Figure 5: Contour graph for mean particle size (nm) of nanoparticles using different emulsifier concentration and homogenization speed

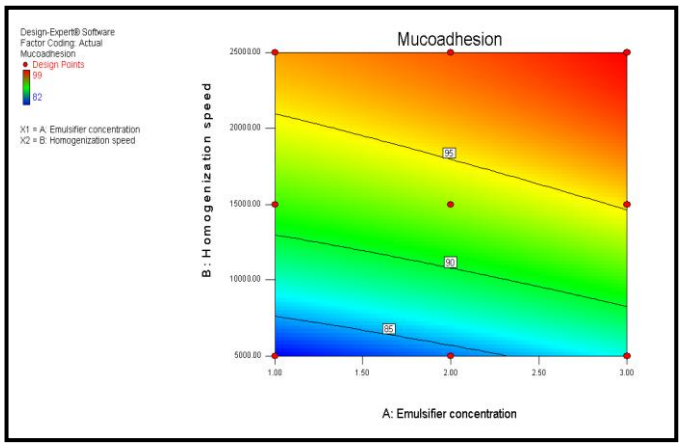


Figure 6: Contour graph for % mucoadhesion of nanoparticles using different emulsifier concentration and homogenization speed

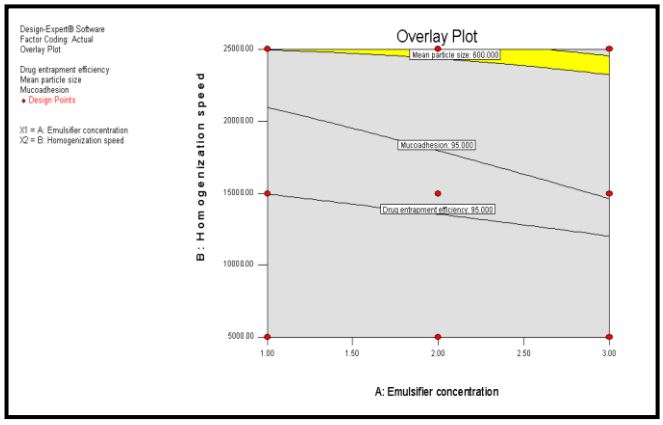


Figure 7: Contour overlay graph for optimum desired response variables using different emulsifier concentration and homogenization speed

The effect of critical formulation variables like emulsifier concentration and process variable like homogenization speed on drug entrapment efficiency; particle size distribution and mucoadhesion were studied. 3 level full factorial design (3^2) using design-expert® 8 software, was adopted to find out the optimum combination of two independent variables (emulsifier concentration-% w/v and homogenization speed-rpm) to obtain desired (response) values of three dependable variables like percent drug entrapment efficiency; mean particle size and size distribution in nm and percent mucoadhesion of nanoparticles. The three levels (low, medium and high) values of two independable variables (emusifier-X_1: 1,2,3% w/v and homogenization speed –X_2: 5000,15000,25000 rpm) were evaluated to measure three independable variables (percent drug entrapment efficiency-Y_1 ; mean particle size nm/std.dev -Y_2 and percent mucoadhesion-Y_3 of nanoparticles)(response surface values) using appropriate model of ANOVA (analysis of variance) for significant value. The nine runs provided by software were performed as per composition (keeping constant polymer concentration at 4%w/v and drug concentration 400mg) and compiled the measured response surface values Y_1; Y_2 and Y_3 in table 10. Percent drug entrapment efficiency was calculated as percentage of total drug content encapsulated in nanoparticles to the initial drug quantity taken. Percent mucoadhesion was determined and the statistical analyses for different formulation are discussed below.

Response -1: Percent drug entrapment efficiency (Y_1): It was observed that as emulsifier concentration (X_1) increased from 1% to 3%w/v, the percent drug entrapment was also increased not significantly, but in trend from (~82.5% to 86.7%); (~95.1% to 96.9%) and (~98.1% to 98.8%) at different homogenization speed (X_2) of 5000 rpm, 15000 rpm and 25000 rpm respectively. It was also observed that as homogenization speed (X_2) increased from 5000 rpm to 25000 rpm, the percent drug entrapment increased significantly up to ~98.1-98.8% with decrease in standard deviation (from 17-20.1 to 11.7-6.9) as indicated in trend, at different emulsifier concentration (1-3%w/v). It can be explained as emulsifier quantity and homogenization speed increased, droplets size of the methanol (internal phase) in w/o emulsion reduced due to reduction of interfacial surface tension at interface of methanol-light mineral oil, hence total surface area of internal phase increased. The small sized droplets of the methanol contained drug and polymer which was well stabilized through nano sized o/w emulsion, hence the encapsulation of drug within polymer increased significantly. Therefore it was evident that percent drug entrapment increased from 82.5% to 98.8% as emulsifier quantity and homogenization speed increased. Hence, the result indicated that there is directly proportion relationship between emulsifier concentration and homogenization speed to the percent drug entrapment efficiency of nanoparticles given in Table10.

The software analysed the ANOVA for response surface with Quadratic model. The Model F-value of 505.42 implies the model was significant. There is only a 0.01 percent chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms were significant. In this case, X_1, X_2, X_1*X_2 and X_2*X_2 are significant model terms. Values greater than 0.1 indicate the model terms were not significant given in Table 11.

Regression analysis: The effect of independable variables was evaluated using simple linear regression analysis given in Table 12. The process data was evaluated at 95% confidence

confidence interval with regular model showed that for emulsifier concentration, the p-value (0.00024) was less than 0.05 and for homogenization speed; the p-value (0.00000083) was also less than 0.05. The process data of reduced model given in Table 16 indicated that mean particle size (Y_2) linearly depends upon variation in emulsifier concentration (X_1) ; homogenization speed (X_2) and square of homogenization speed $(X_2)^2$, it is defined by following equation:

Mean particle size (nm) (Y_2) = intercept + coefficient * X_1 + coefficient * X_2 + coefficient * X_2^2

$$= 869.78 - 28.33 * X_1 - 189.83 * X_2 - 105.17 * X_2^2$$

The regression statistics provided Multiple R 0.9999 and 0.9997; R Square 0.9999 and 0.9995 for regular and reduced model. The **contour graph** between emulsifier and homogenization speed indicated lower mean particle size in nm obtained with increase in homogenization speed at different 1-3% w/v emulsifier concentration range. Hence effect of homogenization speed on particle size distribution was more significant than emulsifier concentration given in Figure 5.

Response -3: Percent Mucoadhesion (Y_3): It was observed that as emulsifier concentration (X_1) increased from 1% to 3%w/v, the percent mucoadhesion was increased not significantly, but in trend from (82% to 87%); (92% to 95%) and (96% to 99%) at different homogenization speed (X_2) of 5000 rpm, 15000rpm and 25000 rpm respectively. It was also observed that as homogenization speed (X_2) increased from 5000 rpm to 25000 rpm, the percent mucoadhesion increased significantly from ~(82% to 87%) to (96% to 99%) as indicated in trend, at different emulsifier concentration (1-3%w/v). It can be well explained when emulsifier concentration and homogenization speed increased, the mean particle size and size distribution decreased significantly. Mucoadhesion is surface adsorption property; hence small particles will give higher surface adsorption with mucus membrane due to higher surface area. Hence, the results also indicated higher percent mucoadhesion with increase of emulsifier concentration and homogenization speed. Therefore, the result indicated that there is directly proportion relationship between emulsifier concentration and homogenization speed to percent mucoadhesion given in Table 10.

The software analysed the ANOVA for response surface with Quadratic model. The Model F-value of 230.49 implies the model was **significant.** There is only a 0.01 percent chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms were significant. In this case X_1, X_2 and X_2^2 were significant model terms given in Table 17.

Regression analysis: The effect of independable variables was evaluated using simple linear regression analysis given in Table 18. The process data was evaluated at 95% confidence interval with regular model showed that for emulsifier concentration, the p-value (0.0030717) was less than 0.05 and

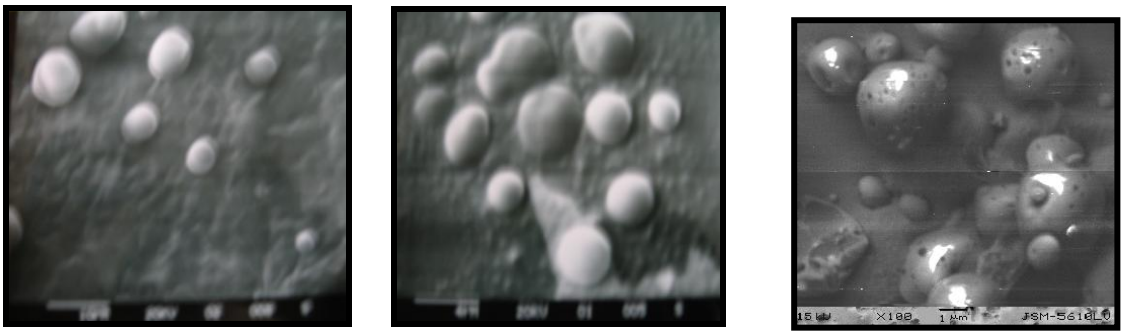
Table 20: Estimated quantity of 'fluoxetine encapsulated Gantrez MS-955 nanoparticles' required for diskette based on drug assay values.

Batch no.	Drug entrapment efficiency (%w/w)	Mean particle Size ± SD (nm)	Fluoxetine base per 50mg nanoparticles (label claim)# (mg)	Assay of fluoxetine base per 50mg nanoparticles (mg)	Estimated quantity## of nanoparticles per 20mg of fluoxetine base (mg)
FG1	97.2	825 ± 14.1	5.80	5.64	177.3
FG2	97.5	856 ± 16.5	13.82	13.47	74.2
FG3	99.0	871 ± 18.6	19.10	18.91	52.9
FG4	92.5	655 ± 11.5	8.90	8.23	121.5
FG5	95.5	675 ± 12.0	19.10	18.24	54.8
FG6	96.3	693 ± 13.7	24.78	23.86	41.9
FG7	90.0	352 ± 7.9	19.10	17.19	58.2
FG8	52.4	341 ± 8.2	30.90	16.19	61.8
FG9	45.5	378 ± 9.4	35.26	16.05	62.3
FG10	82.5	970 ±20.1	19.10	15.76	63.5
FG11	84.1	954 ±19.3	19.10	16.06	62.3
FG12	86.7	920 ±17.0	19.10	16.56	60.4
FG13	95.1	885 ±15.6	19.10	18.16	55.1
FG14	96.4	870 ±15.0	19.10	18.41	54.3
FG15	96.9	835 ±14.2	19.10	18.51	54.0
FG16	98.1	600 ±11.7	19.10	18.74	53.4
FG17	98.5	575 ±10.8	19.10	18.81	53.2
FG18	98.8	530 ±6.9	19.10	18.87	53.0

Note: example—calculation done for B.No. FG1

[(100mg (drug-base) / 861.8mg (polymer-750mg+111.8drug salt)]*50 (for 50mg diskette) = 5.8mg drug base

[20 * 50 / actual assay] = 20*50 / 5.64 = 177.3mg (nanoparticles)



(FG8, mean particle size341nm)(FG18, mean particle size530nm)(FG10, mean particle size970nm)

Figure 8: SEM photographs: Fluoxetine hydrochloride encapsulated Gantrez MS-955 nanoparticles at magnification: 15000x

Table 21: Composition of diskettes and optimized characteristics of 'drug encapsulated nanoparticles'

Batch no.	Composition			Optimized characterized		
	Quantity of nanoparticles in layer 2 (mg)	Drug-base and estimated quantity (mg)	Quantity of ethyl cellulose in layer 1 (mg)	Mean particleSize of nanoparticles ± SD (nm)	Drug entrapment efficiency (%w/w)	% mucoadhesion of nanoparticles
FG3	52.9	Fluoxetine-20mg	20	871 ± 18.6	99.0	95
FG18	53.0	Fluoxetine-20mg	20	530 ±6.9	98.8	99

for homogenization speed; the p-value (0.0000666) was also less than 0.05. The process data of reduced model given in Table 19 indicated that percent mucoadhesion (Y_3) linearly depends upon variation in emulsifier concentration (X_1) , homogenization speed (X_2) and squares of homogenization speed (X_2) of it is defined by following equation:

Percent mucoadhesion (Y_3) = intercept + coefficient * X_1 + coefficient * X_2 + coefficient * X_2^2

$$= 93.33 + 1.83 * X_1 + 6.67 * X_2 - 2.33 * X_2^2$$

The regression statistics provided Multiple R 0.9987 and 0.9969; R Square 0.9974 and 0.9902 for regular and reduced model respectively. The **contour graph** between emulsifier and homogenization speed indicated higher percent mucoadhesion (95-100%) obtained at higher homogenization speed (20000 to 25000 rpm) and higher emulsifier concentration (1.5-3% w/v) range given in Figure 6.

Optimization graph: The contour overlay graph for optimum desired value (yellow shaded region) gave variety of combinations to get higher percent drug entrapment efficiency, optimized particle size range and higher percent mucoadhesion. Based on optimized area through graph (Figure 7), following formulation and process parameters were established i.e. emulsifier concentration range (2-3% w/v) ; homogenization speed range (23000 to 25000 rpm); polymer concentration (4%w/v) and drug concentration (400mg) to get optimum percent dug entrapment efficiency (95-100%), optimum mean particle size range in nm (550-600nm) and optimum percent mucoadhesion (95-100%).

The two optimum formulations (FG3 and FG18) were selected for further studies based on maximum actual assay value and two different sizes of nanoparticles based diskettes. The composition of diskettes and optimized characteristics of 'drug encapsulated nanoparticles' are summarized in Table 21.

Diskettes were selected as a novel terminology to prepare small, round, flat, thin disc like dosage form to facilitate drug delivery with the advantages of its simplicity in preparation, stability, accuracy of dosage, compactness, portability, conformity and ease in buccal route of administration. It comprised of 'ethylcellulose layer-1' which has no drug and 'drug encapsulated polymeric nanoparticles layer-2'. Both layers were adhered properly. The hydrophobic ethylcellulose layer 1 could be barrier to moisture and provide unidirectional drug release during *in vitro* drug release and *in vivo* studies. The drug encapsulated layer-2 made up of mucoadhesive polymer based nanoparticles, which could facilitate mucoadhesion with mucus layer to increase residence time for better and consistent drug delivery in unidirectional way through buccal route. The compaction force (40 ±5 kg/cm^2) was optimized to ensure no breakage of nanoparticles took place during diskette preparation and proper binding of two layers without any physical visual defects. The rational was (a) to prepare diskettes with estimated quantity of nanoparticles per 20mg drug-base (label claim) per diskette so that average weight per diskette should near to theoretical weight (i.e. 50mg) which could be based on optimum percent drug entrapment efficiency (DEE) in nanoparticles and (b) diskettes consists of two different mean nanoparticle size (MPS) range i.e. 400-600 nm and 800-1000 nm to check the effect particle size on drug release. The drug content was determined and based on results given in Table 20 and 21, two optimum formulations were selected. (a) FG3 (DEE-99%; weight of diskette-52.9mg and MPS-871nm) and (b) FG18 (DEE-98.8%; weight of diskette-53mg and MPS-530nm).

Mucoadhesion study:

The mucoadhesion was carried out as per the established procedure[7] to test mucoadhesive potential of polymer, polymeric nanoparticles and its diskettes. By weighed method, quantitative results of mucoadhesion were find out for nanoparticles and its diskettes and recorded in Table 21 and Table 22 respectively.

Table 22: Percent mucoadhesion of diskettes

Batch no. Of diskettes	Weight of diskettes (mg)			% mucoadhesion
	Initial (a)	Washed (b)	Residual on buccal mucosa (a-b)	(a-b) / a *100
FG3	72.9	0.3	72.6	99.6
FG18	73.2	0.4	72.8	99.5

The optimized formulation diskettes were tested for mucoadhesion on buccal mucosa of rat as per established procedure. The adherence of diskettes to the buccal mucosa at > 80% relative humidity at room temperature 25˚C ± 2˚C for 8 hr was studies and observed adhesion scores at 2hrs interval which were recorded in Table 23.

Table 23: Adhesion scores v/s time of diskettes

Batch no. Of diskettes	Observations with adhesion score* v/s time			
	2hrs	4hrs	6hrs	8hrs
FG3	0	0	0	0
FG18	0	0	0	0

*Scores: 0: complete visually adhered to the buccal mucosal surface.

The results of mucoadhesion study of nanoparticles given in Table 10 and 21 indicated that as mean particle size of nanoparticles decreased, percent mucoadhesion increased. This was due to higher surface area of nanoparticles and its polymeric surface forms an adhesion bonds (physical) with mucus membranes to increase residence time and resist moving during gentle washing. The optimized formulation diskettes were tested for mucoadhesion on buccal mucosa of rat. The results of Table 22 indicated that all diskettes were strongly mucoadhered to buccal mucosa after 1hr of hydrated condition at high humidity condition. The percent mucoadhesion was recorded >99% w/w for both the formulations. The same condition was continued till 8hrs (based on application time during *in vivo* study), and observed the adhesion scores based on scale rating. The results of Table 23 indicated that all diskettes were mucoadhered strongly with zero (0) score, mean no lift of diskettes observed visually.

In vitro drug release study:

The *in vitro* drug release on optimized diskette formulation (FG3 and FG18) was determined using USP-apparatus 5 as per defined method for fluoxetine. The results are given in Table 24 and graph shown in Figure 9. It is apparent from the plot that a sustained drug release profile was achieved.

Table 24: Percent average cumulative drug release from diskettes In simulated saliva v/s time

Time hrs.	% average cumulative drug release (% of label claim) ±SD (n=6)	
	B.No. FG3	B.No. FG18
0	0	0
0.5	27±14.5	35±11.5
1	48±8.3	54±10.2
2	65±6.8	70±7.2
3	80±5.7	84±6.2
4	94±2.5	95±2.2
5	102±1.1	101±1.2

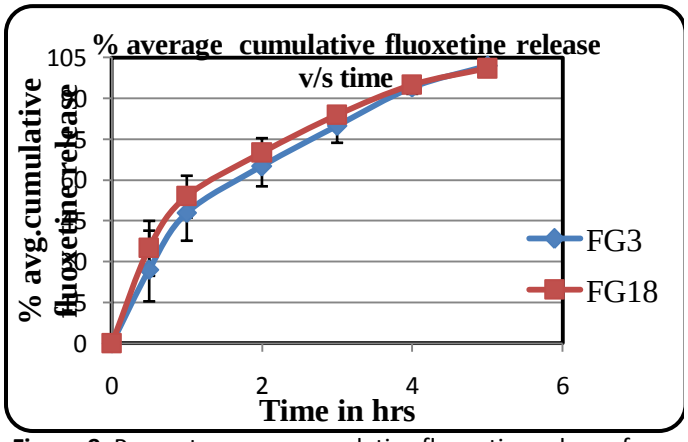


Figure 9: Percent average cumulative fluoxetine release from Gantrez MS-955 nanoparticle diskettes in simulated saliva v/s time

The drug release was measured between the formulations with different nanoparticle size in diskettes. The results given in Table 24 and Figure 9 indicated that formulations made from lower mean particle size (mps) nanoparticle (FG18) (MPS 530 nm ± SD 6.9) by released higher drug by approx (5-10% of label claim). At 4 and 5 hrs, the drug release was found similar with similar standard deviation. It was observed that more than >85% drug released at 4hrs and complete drug release was observed at 5hrs. The discrimination in the drug release pattern was observed up to 3hrs, hence it could happen due to slow and constant drug released from formulation with maintained sink condition in simulated saliva solution. Based on better drug release characteristics with extended release pattern, formulations batch no. FG18 were selected for further *in vivo* studies and stability studies.

***In vivo* studies:**

The *in vivo* study was performed on mucoadhesive diskettes of optimized formulation (batch no.:FG18) using available animal model under fasting conditions. The plasma drug concentration (fluoxetine)–time profile is given in **Table 25 and Figure 9.**

Table 25: Invivo plasma drug concentration – time profile and data analysis

Time hrs.	Drug solution (FS)	Drug polymer nanoparticles diskettes (FG18)
0	0	0
1	37.1±15.4	30.5±25.4
2	56.0±13.5	90.5±18.5
3	75.4±11.6	155.3±14.6
4	99.9±10.5	175.3±11.5
5	112.6±9.4	200.1±4.4
6	163.6±7.5	187.2±3.6
7	174.2±6.8	169.8±4.6
8	90.5±8.4	136.7±2.4
10	45.3±10.5	144.3±8.5
12	49.2±11.0	74.3±6.6
24	12.4±12.5	34.3±8.5
No. of subject	6	6
Mean plasma conc.(ng/ml)	90.4	136.4
Mean Tmax(hrs)	7.0	5.0
Mean C_{max}(ng/ml)	174.2	200.1
Mean AUC $_{last(t=24)}$ (hr*ng/ml)	124.01	202.59

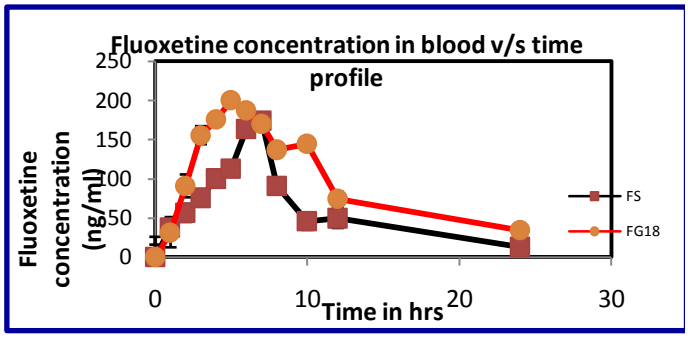


Figure 10: In vivo plasma fluoxetine concentration – time profile

The results of plasma drug concentration – time profile indicated that drug the rate and extent of drug absorption from test formulation diskettes from buccal route is significantly higher than reference drug solution from oral GIT route.

The **mean plasma concentration** of fluoxetine from fluoxetine solution (FS)(reference) on 6 rat subjects was found to be 90.4ng/ml. The mean plasma concentration of fluoxetine from mucoadhesive diskette FG18 (test diskettes) was found to be 136.4ng/ml which was significantly higher compared to reference FS by approx 50.9%. These data indicated that higher drug was absorbed and bioavailable in plasma at various time points. This could be explain by (a) higher drug solubility within nano sized matrix, (b) higher permeability through prolonged residence time due to mucoadhesion of diskettes, (c) rich vascular bed, but highly keratinized buccal route in rat which bypassed the first pass effect and (d) slow, unidirectional and extended drug release from the nanoparticulate based matrix for better bioavailability, hence increased drug concentration in plasma over period of 24hrs reported compared to drug solution through GIT route. The **mean Tmax** (time required to get peak plasma fluoxetine concentration-Cmax) from fluoxetine solution (FS) (reference) on 6 rat subjects was found to be 7 hrs. The mean Tmax for fluoxetine from mucoadhesive diskette FG18 (test diskettes) was found to be 5 hrs which was also significantly lower compared to reference FS by 2hrs. The **mean Cmax** (peak plasma fluoxetine concentration) from fluoxetine solution (FS)(reference) on 6 rat subjects was found to be 174.2ng/ml. The mean Cmax for fluoxetine from mucoadhesive diskette FG18 (test diskettes) was found to be 200.1ng/ml which was also significantly higher compared to reference FS by approx 14.9%. These data indicated that the rate of drug absorption was significantly higher from formulation FG18 through buccal route compared to drug solution through oral GIT route. The higher Cmax with lower Tmax indicated higher rate of drug delivery from novel nanoparticulate system. The Figure 10 indicated that early drug released from both formulations and absorbed efficiently through buccal route compared slow drug absorption from solution in GIT. The rate of drug release was faster (onset) from formulation FG18. This could be due to polymeric drug dissolution within matrix and release property. The **mean AUC $_{last\ (t=24)}$** (area under the curve of drug concentration-time 24hrs profile) from fluoxetine solution (FS) (reference) on 6 rat subjects was found to be 124.01 hr*ng/ml. The mean AUC $_{last\ (t=24)}$ for fluoxetine from mucoadhesive diskette FG18 (test diskettes) was found to be 202.59 hr*ng/ml which was also significantly higher compared to reference FS by approx 63.4%. The AUC data indicated bioavailability of drug in plasma at different time points. The data indicated that higher drug bioavailable from formulation FG18 through buccal route compared to drug solution through oral GIT route. This could be due to higher drug absorption from buccal route and bypassing first pass effect metabolism.

The novel diskette formulations were adhere to mucus membrane of buccal tissues and released the dissolved drug at slow and extended rate form dosage form to make it absorbed through high vascular rich buccal bed. Hence it can be concluded that with novel formulation diskettes, fluoxetine was absorbed with higher rate and extent from buccal route compared to solubilize drug from GIT route. This was made possible due to increase in residence time during mucoadhesion of diskettes up to 8 hrs and slow, but continues unidirectional drug release from nano-sized matrix bed to increase drug absorption from oral mucosal buccal route to increase bioavailability of fluoxetine.

Stability study:

Accelerated stability studies were carried out on the diskettes of optimized formulation as per ICH conditions. The results are given in **Table 26.**

The six months stability results given in Table 26 showed no significant changes occurred in the physical appearance and drug content at accelerated conditions 40 ± 2˚C and 75 % ± 5 %

Table 26: Stability study data at accelerated conditions

Time points	Stability testing at accelerated conditions (40± 2˚C and 75 % ± 5 % RH)			
	Fluoxetine hydrochloride (drug) (as control)		Test formulations (FG18)	
Tests	Description	Assay (%)	Description	Assay (%)
Specifications	*	98 to 102% of LC	**	95 to 105% of LC
Initial	*	99.9	**	99.5
1 month	No change	99.6	No change	99.1
3 months	No change	99.3	No change	98.9
6 months	No change	99.2	No change	98.7

LC = label claim

** White to off-white crystalline powder*

*** Off white to light tan colour (ethylcellulose layer 1) and white to off white colour (nanoparticle layer 2)*

RH when compared to plain drugs as control. As per the ICH guidelines, maximum 24 months shelf life can be given to the formulations in the glass bottle pack.

CONCLSION:

Finally, it is concluded that anti depressant drug like Fluoxetine hydrochloride which show first pass effect when orally administered through GIT, can be successfully administered through oral mucosal (buccal) route with increased drug efficacy to the patients. It was successfully designed an acceptable, stable, alternative novel mucoadhesive system for oral mucosal (buccal) route that could be effectively maintain the drug release at extended release rate compared to oral dosage forms for better antidepressant response with rapid onset, avoid first pass effect, with increase rate and extent of drug absorption for better drug efficacy to the patients. This was encouraging results for further reduction of doses to get desired pharmacological effect which definitely reduces the side effects of psychotropic drugs.

The nanoparticle technology consist of drug carriers in which the active ingredient is dissolved, dispersed, entrapped, encapsulated, adsorbed or chemically attached to have an innovative drug delivery system. It could be a good pharmaceutical nanotechnology based model, with wide application to many drugs to improve their efficacy by increasing their potential to reach the target site of therapeutic actions. Such novel formulated system could have significant advantage in terms of bioavailability and reduction in dose regime. Such effective effective and safe alternative product could significantly improve the treatment of depressant disorders.

REFERENCES:

1. Harris D and Robinson JR. Drug delivery via the mucous membranes of the oral cavity, J Pharm sic, 1992; 81:1-10.
2. Shojael AH. Buccal mucosa as a route for systemic drug delivery: a review, J Pharm pharmaceut sci, 1998; 1:15-30.
3. Mashru RC, Sutariya VB, Sankalia MG and Sankalia JM. Effect of pH on in vitro permeation of ondensatron hydrochloride across porcine buccal mucosa, Pharm dev technol, 2005; 10: 241-47.
4. Venugopalan P, Sapre A, Venkatesan N and Vyas SP. Pelleted bioadhesive polymeric nanoparticles for buccal delivery of insulin: preparation and characterization. Pharmazie, 2001; 56: 217-19.
5. Peppas NA and Buri PA. Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues, J control release, 1985; 2: 257-62.
6. Aungst BJ and Rogers NJ. Site dependence of absorption promoting actions of laureth-9, Na salicylate, Na_2EDTA, and aprotinin on rectal, nasal, and buccal insulin delivery. Pharm res, 1988; 5: 305-08.
7. Yoncheva K, Gomez S, Campanero MA, Gomazo C and Irache JM. Bioadhesive properties of pegylated nanoparticles, Expert opin drug deliv, 2005; 2: 205-18.
8. Rathbone MJ and Hadgraft J. Absorption of drugs from the human oral cavity. Int J Pharm, 1991; 74: 9-24.
9. Arbos P, Wirth M, Arangoa MA, Gabor F and Irache JM. Gantez AN as a new polymer for the preparation of ligand-nanoparticle conjugates, J Control Release, 2002; 83:321-30.
10. Birudaraj R, Mahalingam R and Li X and Jasti BR. Advances in buccal drug delivery, Crit Rev Ther Drug Carrier Syst, 2005; 22: 295-30.
11. Bodde HE, De Vries ME and Junginger HE. Mucoadhesive polymers for the buccal delivery of peptides-structure-adhesiveness relationships, J Control Release, 1990; 13: 225-31.
12. De Vries ME, Bodde HE, Verhoef JC and Junginger HE. Developments in buccal drug delivery, Crit Rev Ther Drug Carrier Syst, 1991; 8: 271-03.
13. Squier CA. The permeability of oral mucosa, Crit Rev oral biol med, 1991; 2:13-32.
14. Ei-samaligy MS, Yahia SA and Basalious EB. Formulation and evaluation of diclofenic sodium buccoadhesive discs, Int. J Pharm, 2004; 286: 27-39.
15. Vyas SP and khar RK. Targeted and controlled drug delivery, CBS publisher and distributer, New Delhi, First Edition (2002): 331-386.
16. Julienne MC, Alonso MJ, Gomez JL and Benoit JP. Preparation of poly(dl-lactide/glycolide) nanoparticles of controlled particle size distribution: application of experimental designs. Drug Dev. Ind. Pharm, 1992; 18: 1063-77.
17. Peppas NA and Buri PA. Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues, j. Control. Rel., 1985, (2), 257-275.
18. Ibrahim AD. Development and validation of spectrophotometric methods for determination of fluoxetine, sertraline, and fluoxetine in pharmaceutical dosage forms, Journal of AOAC international, 2005; 88: 38-45.