Diskettes of Mucoadhesive Polymeric Nanoparticles for Oral (Buccal) Transmucosal Delivery of Fluoxetine Hydrochloride: Formulation And Characterization.

A.Sapre, R.Parikh and M.Gohel
Department of Pharmaceutics and Pharmaceutical Technology, L.M.College of Pharmacy, Ahmedabad 380 015, India, abhaysapre@rediffmail.com

ABSTRACT

The study was attempted to develop an alternative oral mucosal delivery of nanoparticles based system for Fluoxetine hydrochloride (antidepressant). The drug bearing nanoparticles were prepared by emulsion solvent (internal phase) evaporation method. The critical variables like, polymer concentration, emulsifier concentration and rate of homogenization were characterized for the particle size distribution; drug entrapment efficiency and mucoadhesion. The formulations were optimized using 3^2 full factorial design and contour plots were drawn. The nanoparticles were compacted into small diskettes for facilitating oral mucosal application. The in vitro studies of the diskettes included mucoadhesion and drug release profile were performed. The in vivo studies were performed on white male albino rats. The stability study was conducted on the optimized formulation at accelerated conditions. A significant improvement in the pharmacokinetic parameters like C_{max}, T_{max} and AUC was observed.

Keywords: fluoxetine hydrochloride, gantrez MS-955, diskettes, buccal, nanoparticles.

1 INTRODUCTION

Among the various routes of drug delivery, oral route is perhaps the most preferred route. However, peroral administration of drugs has primary disadvantages such as: first pass, gastro intestinal tract degradation, pH dependent solubility and presence of food on rate and extent of drug absorption etc. Consequently, other absorptive mucosa is considered as potential sites for drug administration [1]. Transmucosal routes (i.e. the mucosal linings of the nasal, rectal, vaginal, ocular, and oral cavity) offer distinct advantages over peroral administration of systemic drug delivery. These advantages include possible by pass of first pass effect, rapid onset for drug absorption, avoidance of presystemic elimination, convenient route [2]. The oral mucosal route permits rapid absorption [3]. An oral mucoadhesive delivery system is known to enhance the drug bioavailability by prolonging residence time at the specified region and an optimal contact with the adsorbing biological membrane [4-6]. The present work is an effort to develop oral mucoadhesive delivery system for anti depressant drug like fluoxetine hydrochloride. In this study, the drug was encapsulated into polymer (Gantrez MS-955) nanoparticles. Gantrez is well-established mucoadhesive, biodegradable, biocompatible and stable [7-8].

2 EXPERIMENTAL

2.1 Preparation of the nanoparticles and diskettes

Gantrez MS-955 and drug were dissolved in methanol (15 ml). This phase was emulsified by sonication with light liquid paraffin (150 ml) containing Span 80 (emulsifier) (2.5% v/v) for 15min. The emulsion was homogenized continuously (Ulta Turrex, IKA) at room temperature for complete evaporation of the internal phase. The colloidal stabilized drug loaded polymer particles were stirred with 100 ml of ether. The colloidal dispersion was passed through a Millipore filter (1 micron) to eliminate particles above 1 micron in size. The filtrate was again filtered through a Millipore filter (0.20 micron). The nanoparticles retained over the filter were washed with sufficient quantity of ether to remove traces of oil and dried. A series of nanoparticles were prepared by varying the polymer concentration; emulsifier concentration and homogenization rate. The nanoparticles were gently compacted into diskettes using IR disc forming press. The final mucoadhesive diskette for oral mucosal application was a small, round, disc of 5 mm diameter of approx 50 mg weight.

2.2 Characterization

2.2.1 Size and shape

The dried nanoparticles were analyzed by laser diffractrometry (Malvern 2600 D laser sizer). The shape and surface characteristics were examined by scanning electron microscopy (SEM 515 Philips Holland) (Figure 1).

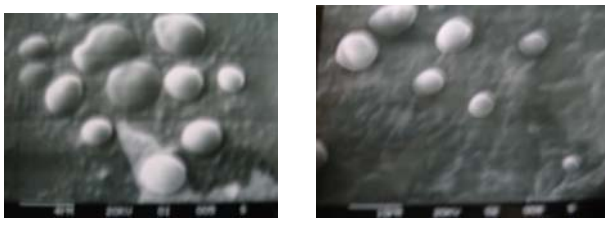

Figure 1: SEM images of nanoparticles (300 to 600 nm)

2.2.2 Drug content determination

The drug content (assay) determination was carried out using HPLC method. Ten diskettes were dispersed in methylene chloride (40 ml). The dispersion was sequentially

sonicated with 0.1 N HCl (10 ml) and methanol (150 ml). The drug solution was filtered and analyzed by HPLC.

2.2.3 Mucoadhesion determination

The polymeric nanoparticles were evaluated for their mucoadhesion by the in situ novel method. About 50 mg of nanoparticles were spread uniformly on the freshly cut piece of albino rat buccal mucosa, previously cleaned using simulated saliva solution [3]. The piece of rat buccal mucosa was placed in a dessicator maintained at > 80% relative humidity at room temperature (28ºC ± 2ºC) for 30 minutes to allow for the hydration of polymeric nanoparticles. Then the mucosal piece was washed carefully with 50 ml simulated saliva at the rate 10ml per min. The washing was collected in the glass petri dish, dried at 105ºC and estimated for residue by weight method to compute percent mucoadhesion.

2.2.4 In vitro dissolution study

The apparatus 2 of Ph. Eur. was used to determine drug release profile from the diskettes of optimized formulation with some modifications. The diskette was fixed on to a glass plate with the aid of a silicone adhesive. This assembly was placed at the bottom of the vessel prior to the rotation of the paddle to keep the diskette in contact with simulated saliva solution (900 ml) [2]. The apparatus was operated immediately at 50 rpm and maintained it at a temperature of 37ºC ± 0.5ºC. At periodic intervals, samples were withdrawn with the help of auto sampler and analyzed.

2.2.5 In vivo absorption study

In vivo studies were performed on diskettes of optimized formulation and evaluated on white albino rats (250–350g) under fasting condition. The animals were divided into three groups with six animals in each group. The rats were fasted overnight before experiments and had access to water. One group was kept as control. To the second group, plain drug aqueous solution (5ml) of (20 mg/ 250 g body weight) Fluoxetine hydrochloride was administered orally with the help of canula. Prior to the administration of diskettes into the oral mucosal (buccal) cavity, rats from the third group were anesthetized by administration of pentobarbitone (30 mg/ kg body weight) and then the optimized formulation was administered in the buccal cavity. Blood sample were collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14 and 24hr. The blood samples were centrifuged at 3000 rpm for 3 mins to separate serum and drug concentration was measured. The measured drug concentration was expressed as ng / ml and C_{max}, T_{max}, AUC_t and AUC_∞ were calculated.

2.2.6 Stability study

The diskettes of nanoparticulate system from optimized formulation were packed in glass bottles and placed for 6 months at 40± 2ºC and 75 %± 5 % RH along with plain drug packed in glass bottles as controls. At the end of six months, the diskettes were analyzed for physical description and drug content along with controls.

3 RESULTS AND DISCUSSION

The present study was an attempt to develop alternative oral mucosal delivery nanoparticles based system for anti depressant drug fluoxetine hydrochloride. The formulation parameters were optimized using 3^2 full factorial designs and contour plots were drawn.

3.1 Effect of polymer concentration

It was observed that with an increase in polymer concentration (PC) (Gantrez MS-955), there was a significant increase in the mean particle size (MPS) of nanoparticles. The increase in MPS with constant polymer / drug ratio (3:2) can be attributed to an increase viscosity of the internal phase (methanol). The formulations were optimized using 3^2 factorial designs to evaluate the effects of polymer and drug concentration (DC) on the % drug entrapment efficiency (EE) and mean particle size of nanoparticles. The optimum combination of the two independent variables (X_1 and X_2) were studied to obtain desired values of response variables (Y_1 and Y_2) in contour plots generated using the Sigma plot® software. The polymer concentration and drug concentration was optimized by overlapping of contour plots (Figure 2). It was found that the optimized polymer concentration range (3.5% to 4.0%) consistently produced nanoparticles in the mean particle size range (700 nm to 800 nm) with always above 90% drug entrapment efficiency at different drug concentration range (100 mg to 500 mg) (Table 1).

X_1	X_2	Y_1	Y_2
PC (%w/v)	DC (mg)	EE (%)	MPS (nm)
1	100	93.0	352
1	300	52.4	341
1	500	45.5	378
3	100	93.5	655
3	300	95.5	675
3	500	98.3	693
5	100	91.2	825
5	300	92.5	856
5	500	95.0	871

Table 1: 3^2 full factorial design: Effect of independent variables on response variables

3.2 Effect of emulsifier concentration

As emulsifier (span 80) concentration (EC) increased, a slight decrease in mean particle size (MPS) was observed, but insignificant (Table 2). The increment in the amount of emulsifier led to a decrease in the interfacial tension, thereby assisting small droplet formation. It also prevented droplet aggregation / coalescence. Thus the nanoparticles

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were stabilized at narrow particle size range. The entrapment efficiency (EE) of drug was improved.

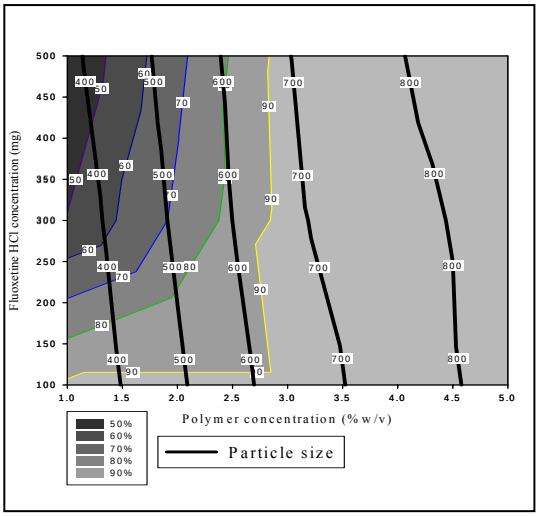

Figure 2: Contour plot for optimization of polymer concentration

3.3 Effect of rate of homogenizer

At low speed (5000 rpm) of homogenization, the mean particle size (MPS) was found to be higher (900 nm to 1000 nm). As the homogenization speed (HS) was increased, a significant decrease in mean particle size was evident (Table 2). The increase in the homogenization speed decreases droplet size of an emulsion that assisted in particle stabilization at lower and uniform size range. Thus the nanoparticles were stabilized at narrow particle size range. The entrapment efficiency (EE) was also improved.

3.4 In situ mucoadhesion study:

It was observed that as mean particle size of nanoparticles decreased, the % mucoadhesion increased (Table 2). The significant mucoadhesion was attained due to hydrated, viscous anionic nature of mucin in buccal mucosa and cationic nature of polymer-based nanoparticles, which gave stable mucoadhension due to opposite charge.

The formulations were optimized using 3^2 factorial design for studying the effects of emulsifier concentration (EC)(X1) and homogenizer speed (HS)(X2) on the % drug entrapment efficiency (EE) (Y1), mean particle size (MPS) (Y2) and % mucoadhesion (Y3). The combinations of the two independent variables (X1 and X2) were studied to obtain desired values of response variables (Y1, Y2 and Y3) by contour plots. It was observed that as increase in emulsifier concentration (X1) and homogenizer speed (X_2), the entrapment efficiency (Y_1) of drug slightly increased, the mean particle size (Y_2) of nanoparticles decreased significantly and % mucoadhesion (Y_3) of nanoparticles improved (Table 2). The emulsifier concentration and homogenizer speed were optimized by overlapping of contour plots (Figure 3). It was found that the optimized emulsifier concentration range (2.5% to 3.0%) and homogenizer speed (25000 rpm) consistently produced nanoparticles in the mean particle size range (500 nm to 600 nm) with above 98% drug entrapment efficiency and 98% mucoadhesion. Thus the optimized formulation was selected for further in vitro and in vivo studies.

X_1	X_2	Y_1	Y_2	Y_3
EC (%w/v)	HS (rpm)	EE (%)	MPS (nm)	Mucoadhesion (%)
1	5000	82.5	970	82
2	5000	84.1	954	84
3	5000	86.7	920	87
1	15000	95.1	885	92
2	15000	96.4	860	93
3	15000	96.9	815	95
1	25000	98.1	600	96
2	25000	98.5	575	98
3	25000	98.8	530	99

Table 2: 3^2 full factorial design: Effect of independent variables on response variables

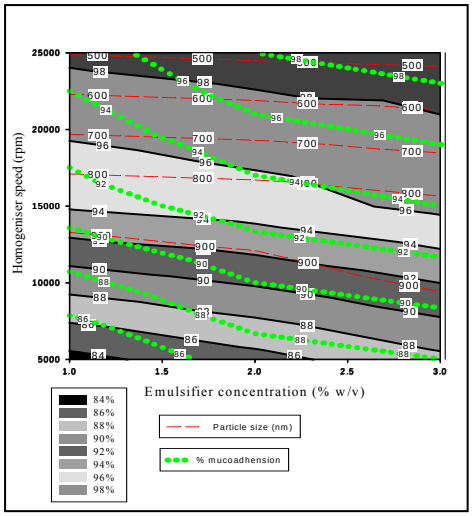

Figure 3: Contour plot for optimization of emulsifier concentration and homogenization speed

3.5 In vitro dissolution study

In vitro release studies were performed on diskettes of optimized formulation to record the % cumulative amount of drug dissolved in simulated saliva solution. It is apparent from the plot that a sustained drug release profile was achieved (Table 3 and Figure 4).

Time in hr	% Cumulative drug dissolved & RSD
0.5	44.3 ± 9.9
1	57.9 ± 7.6
2	67.5 ± 7.7
3	82.0 ± 7.6
4	92.4 ± 2.1
5	99.9 ± 0.8

Table 3: In vitro profile of optimized formulation in simulated saliva solution.

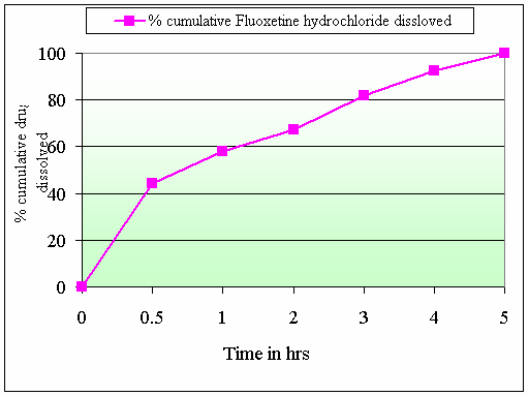

Figure 4: In vitro dissolution profile of optimized formulation

3.6 In vivo study

In vivo (fasting condition) data were evaluated for C_{max}, T_{max}, AUC_t and AUC_{inf} (Table 4). It is evident that drug encapsulated in nanoparticles and intentionally absorbed through buccal route has shown better bioavailability than conventional route. The C_{max}, $AUC_{t=24}$, AUC_{inf} with nanoparticle based mucoadhesive diskette system from buccal route was found to be significant higher than drug solution absorbed from conventional oral route with comparative lower T_{max} (Figure 5). It was observed that the drug was available for the systemic circulation at a modulated rate, without any dose dumping. The probable reason for improved bioavailibity could be avoidance of first pass effect and quicker absorption through oral mucosa.

Pharmacokinetic parameters	Fluoxetine HCl solution	Fluoxetine HCl in diskette
C_{max} in ng/ml	174	200
T_{max} in hr	7	5
$AUC_{t=24}$(hr*ng/ml)	1232	1559
AUC_{inf} (hr*ng/ml)	1241	1582

Table 4: Observed pharmacokinetic parameters

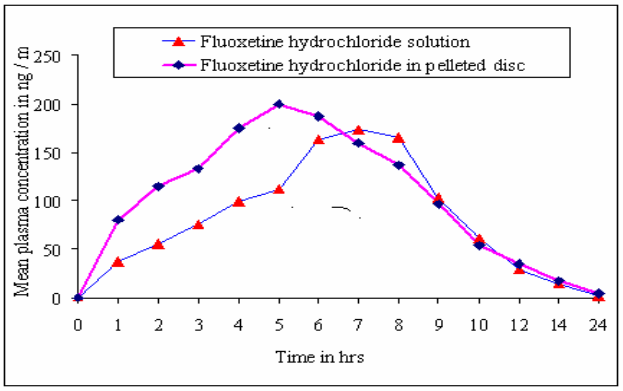

Figure 5: In vivo drug absorption profile of optimized formulation

3.7 Stability studies

The stability data (6M) at accelerated conditions showed insignificant changes in the physical appearance and assay (Table 5) when compared to API as control.

Tests	Fluoxetine HCl (API as control)		Diskettes of Fluoxetine HCl	
	Initial	6 M	Initial	6 M
% Assay	99.9	99.2	99.5	98.7

Table 5: Accelerated Stability studies data

4 CONCLUSION

It is possible to develop a novel mucoadhesive system for buccal route that could be effectively maintain the drug release at modulated rate compared to oral dosage forms for better antidepressant response with rapid onset and avoid first pass effect to the patients. Such novel formulated system could have significant advantage in terms of bioavailability.

REFERENCES

[1] A. Shojael, "Buccal mucosa as a route for systemic drug delivery: A review", J Pharm Pharmaceut Sci, 1, 15-30, 1998.

[2] P. Venugopalan, A. Sapre, N. Venkatesan and S. Vyas, "Pelleted bioadhesive polymeric nanoparticles for buccal delivery of insulin: preparation and characterization", Pharmazie, 56 (3), 217-219, 2001.

[3] N. Peppas and P. Buri, "Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues", J Control Release, 2, 257-262, 1985.

[4] M. Rathbone and J. Hadgraft, "Absorption of drugs from the human oral cavity", Int J Pharm, 74, 9-24, 1991.

[5] R. Birudaraj, R. Mahalingam, X. Li and B. Jasti, "Advances in buccal drug delivery", Crit Rev Ther Carrier Syst, 22 (3), 295-330, 2005.

[6] C. Squier, "The permeability of oral mucosa", Crit Rev Oral Biol Med, 2, 13-32, 1991.

[7] T. Owens, R. Dansereau and A. Sakr, "Development and evaluation of extended release bioadhesive sodium fluoride tablets", Int J Pharm, 06288 (1), 109-122, 2005.

[8] S. Kockisch, D. Gareth, J.Tsibouklis and J. Smart, "Mucoadhesive, triclosan-loaded polymer microspheres for application to the oral cavity: preparation and controlled release characteristics Eur. J. Pharm. Biopharma, 59(1), 207-216, 2005.