

FORMULATION AND *IN VIVO* EVALUATION OF ONCE A DAY PUSH-PULL OSMOTIC TABLET**RAJESHRI WAKODE¹ AND AMRITA BAJAJ *²**¹KLE Academy of Higher Education and Research Belgaum, College of Pharmacy Bangalore, 560 010, India.²C.U.Shah College of Pharmacy, SNDT, Women's University, Juhu Tara Road, Santacruz, Mumbai 400049, India.* *Corresponding author* bajajamrita@rediffmail.com**ABSTRACT**

Once a day push pull osmotic tablets containing pramipexole as an antiparkinson's agent were developed and evaluated for *in vivo* efficacy. Push pull osmotic tablets are bilayered tablets consisting of pull layer (drug layer) and push layer (polymer layer) coated with semipermeable cellulose acetate membrane containing water leaching pore forming agents. To determine the *in vivo* efficacy of formulation, symptoms of Parkinson's disease were induced in male Wistar rats using reserpine 5mg/kg, (i.p) and induction of disease was confirmed with the help of *in vivo* tests. Striatal dopamine content was decreased from 12 ng/gm of tissue in normal control to 0.6 ng/gm in disease induced rats. Dopamine content was found to increase to 6ng/gm after intragastric administration of push pull osmotic tablet and even the motility in rats was improved. Various pharmacokinetic parameters were estimated and the studies revealed that the developed formulation maintained plasma levels of pramipexole for a period of 24 hrs.

KEYWORDS

Parkinson's disease, Push pull osmotic tablets, reserpine, dopamine, pramipexole

INTRODUCTION

It is widely accepted that the primary cause of Parkinson's disease is a progressive degeneration of nigral dopamine neurons^{1,2,3,4}. Which leads to a substantial decrease in the dopamine levels in the caudate nucleus and putamen^{5,6,7}. A deficit of this neurotransmitter is directly linked to the appearance of numerous symptoms of this disease, such as akinesia, muscle rigidity and tremors^{8,9,10}. Substitutive administration of levodopa (L-DOPA) is the most effective and commonly used treatment in Parkinson's disease. However, this therapeutic strategy is often complicated by severe side-effects such as

psychoses, dyskinesia and on-off phenomena^{11,12}. Continuous treatment with levodopa causes reduced activity after 5 to 7 years of treatment. This disadvantage of L-DOPA therapy imposes major limitations on long-term, effective application of this drug. Dopamine receptor agonists comprise a class of drugs which are efficacious in the treatment of both early and advanced stages of Parkinson's disease¹³. Pramipexole (2-amino-4,5,6,7-tetrahydro-6-propyl-amino-benzthiazole-dihydrochloride) is a novel, highly active, full dopamine receptor agonist which acts on the D2 receptor family with a preferential affinity for the D3 type^{14,15}. Pramipexole antagonizes the reserpine-

induced akinesia and neuroleptic-induced catalepsy^{16,17,18}. The above data show that due to stimulation of dopamine D2 postsynaptic receptors, pramipexole appears to have a high antiparkinsonian potential^{19,20,21}.

It has been investigated as a monotherapy in the treatment of PD. In advanced PD the usual dose of Pramipexole is as high as 1.5 mg three to four times a day^{22,23}. Pramipexole has been associated with episodes of somnolence during the daytime (referred to as sleep attacks) and other adverse effects such as abnormal behaviour, drowsiness, dizziness, fainting, hallucinations and many more. The side effects are highly dose dependent^{24,25,26}. Currently there are no controlled release formulations available for pramipexole. So the major aim of the research work was to develop once a day controlled release formulations that can deliver pramipexole for period of 24hrs and to investigate its efficacy in animal model. This reduction in dose frequency is expected to improve patient compliance and maintain the therapeutic level of pramipexole over a prolonged period of time. This may result in reduced severity of motor fluctuations and other side effects caused by pramipexole.

Osmotic drug delivery systems have many advantages over other controlled release formulations like drug release from this system is independent of external pH and agitation. The drug is released in solution form ready for absorption. Hence an attempt was made to incorporate pramipexole in osmotic delivery systems.

MATERIAL AND METHODS

Pramipexole dihydrochloride monohydrate was obtained as a gift sample from Sekhsaria Chemicals Ltd. (Mumbai, India). Sodium bicarbonate, sodium chloride, mannitol and magnesium stearate were used as osmogens and were obtained from S.D. Fine-Chem Ltd. (Mumbai, India). Lactose and microcrystalline

cellulose were a gift from Signet Chemicals (Mumbai, India). Hydroxypropyl methyl cellulose of various grades were obtained as gift samples from Colorcon Asia Pvt. Ltd. (Mumbai, India) Cellulose Acetate with 39.8 % acetyl content and reserpine were purchased from Sigma Chemicals (Bangalore, India). Polyethylene glycol 400 (PEG 400), dibutylphthalate (obtained from supplier S.D. Fine-Chem Ltd), were employed as pore formers. Titanium dioxide and talc were gifts of Sekhsaria Chemicals Ltd. High-performance liquid chromatography (HPLC) grade acetonitrile (Ranbaxy), potassium dihydrogen orthophosphate and sodium hydroxide were procured from S.D.Fine-Chem.Ltd. All the chemicals used were of analytical grade.

1. Formulation development

(i) Preparation of core tablets: Microporous membrane-coated bilayered osmotic tablets of pramipexole dihydrochloride monohydrate were prepared using conventional wet granulation technology. Drug and excipients were blended together to produce 500 tablets. The alcoholic solution of PVP K 30 M was added to produce a damp mass, which was passed through a # 16 sieve and dried in a hot air oven at 60°C for 2 h. The dried granules were then passed through a # 22 sieve and mixed with lubricants. Granules for the push compartment were prepared in a similar fashion and for identification a coloring agent was added to the push layer. The tablets were compressed at an average weight of 250 mg. The weight of the push compartment was adjusted to 110 mg and the pull compartment weight was adjusted to 140 mg. Prepared granules were compressed as bilayer tablets using 8 mm deep concave punches on a single-punch tablet machine. The different formulations tried are given in table 1.

Table 1
Various core formulations of push pull osmotic tablets containing pramipexole.

Ingredients (mgs)	Core (mg/tablet)				
	I	II	III	IV	V
Pull compartment					
Pramipexole	4.5	4.5	4.5	4.5	4.5
Microcrystalline cellulose	30	30	25	30	30
Lactose	45	90	90	90	90
Sodium chloride	-	5	5	-	-
Sodium bicarbonate	5	-	-	-	-
Mannitol	45	-	5	-	-
Potassium carbonate	-	-	-	5	-
PVP K30 M	10	10	10	10	10
Magnesium stearate	0.5	0.5	0.5	0.5	0.5
Push compartment					
Microcrystalline cellulose	25	25	25	25	25
HPMC K4M	45	45	45	45	45
Sodium bicarbonate	29	29	29	29	29
PVP K30 M	10	10	10	10	10
Magnesium stearate	1	1	1	1	1

(ii) Coating of bilayer tablets

Tablets were coated with cellulose acetate (2% w/v in acetone) along with a suitable pore former such as PEG 400 (20% w/w of total polymer weight). Talc and titanium dioxide in concentration of 1% were used as antiadherent and opacifier respectively. The coating process parameters were optimized as follows: - pan diameter, 9 inch; spray gun (pilot scale); baffles, 4; speed of pan, 25 rpm; nozzle diameter, 1mm; spray pressure, 40-50 lb/sq.in; drying temperature, 55°C. After being coated, the bilayer tablets were dried overnight at 60°C to remove any residual solvent. The residual solvent content was determined by headspace gas chromatography and was found to be within limits (2000 ppm for acetone). No change in membrane characteristics was observed after drying. The coated tablets had smooth, uniform surfaces without any defects.

(iii) Drug Content

For determining the drug content, 20 tablets were crushed and powdered in a mortar. The powder equivalent to 4.5 mg of the drug was accurately weighed and transferred to a 50 mL volumetric flask. The drug was extracted into diluent (phosphate buffer pH 7.4: acetonitrile in a ratio of 40:60) by sonication for 30 min. The solution was filtered through a 0.45µm nylon filter after making up the volume. One milliliter of this solution was diluted to 10 mL with diluent and analyzed using HPLC system [Tosho Co, Tokyo, Japan].

(iv) In Vitro Release

In vitro drug release studies were carried out using the USP type II dissolution test apparatus. Operating conditions were maintained at 37± 0.5°C, paddle speed was 100 rpm, and the dissolution medium was phosphate buffer at a pH of 7.4 with a volume of 900 mL. Since the solubility of the drug is not pH-sensitive, the pH change method was not used. Samples of 5 mL were withdrawn

once every hour, and the same amount of dissolution medium was replenished. Samples were filtered and analyzed using developed, validated HPLC method. The experiments were performed in triplicate.

(v) HPLC Analysis

The drug content was determined using an HPLC system. The HPLC system used was the same as that employed in the above experiments and equipped with a UV-visible detector and a data management system. The column used was C18 hypersil gold (250cm x 4.6 mm, 5 μ). The mobile phase consisted of a phosphate buffer at a pH of 7.4: acetonitrile (40:60 v/v) at a flow rate 1 mL/min and an injection volume of 100 μ L. Analysis was performed at a wavelength of 262 nm and the peaks obtained were integrated using EZChrom chromatography data system (Scientific Software Inc.San Ramon, CA.)

(vi) Determination of osmotic pressure

Drug release from osmotic systems depends upon osmotic pressure generated in the tablet. The effect of osmotic pressure on drug release was assessed by performing *in-vitro* release studies. Osmotic pressure generated within the tablet was determined using 3D3 Freezing point osmometer (Model 3D3 Advanced Instruments Osmometer). The readings obtained were in mosmol/kg²⁷. Before measurement osmometer was calibrated using standards of 100 mosm/kg and 1500 mosm/kg.

(vii) Scanning Electron microscopy:

To evaluate the surface morphology of the coating membrane, surfaces of the coated tablets were examined using Scanning Electron microscopy both before and after dissolution. Samples of the coating membrane were cut from the exhausted shells (after 24hr dissolution studies) dried at 50°C for 12-16 hrs and mounted on the stub with double sided adhesive tape. Whereas the coated tablet (before dissolution) was placed as such on specimen stub and examined under SEM Model FEI Quanta 200F.

(viii) In vivo studies

Male Wistar rats weighing 200-250 gm were used. Animals were housed in polypropylene cages and had free access to food and water. Reserpine was dissolved in 50 μ l of acetic acid and distilled water (vehicle used as control solution). Reserpine (5 mg/kg) and vehicle were administered intraperitoneally in constant volume of 10 ml/kg to induce symptoms of Parkinson's disease. The dose of pramipexole was calculated as per the body weight of animals and push pull osmotic tablets were formulated considering the calculated dose. The animal protocol was approved by the Animals Ethical Committee of Bombay Veterinary College, (Resolution 2107 dated 25/05/07), Mumbai, India.

2. Experimental Procedures

(i) Sucrose preference test

Anhedonia is a depressive state in which there is diminished responsiveness to rewards. It can be measured by intracranial self stimulation, by a place preference test, and by a palatable (e.g., sucrose solution) preference test². Sucrose preference test is the most used hedonic measurement because it is easier to perform, more practical and rapid.

The animals were first habituated to consume sucrose solution (2%) by being exposed for 48 h to two bottles, one containing potable water and the other containing the sucrose solution. After an initial sucrose preference test the animals were divided into three matched groups (n = 12) according to preference for sucrose solution. Immediately before beginning of the second sucrose preference test, two groups were first treated with reserpine (5 mg/kg) and second with vehicle. Reserpine was dissolved in 50 μ l acetic acid to get clear solution. The animals were then tested for spontaneous locomotion. The third reserpine treated group was administered with developed push-pull osmotic tablet to observe any changes in total fluid consumption. The sucrose preference test was carried out by measuring the consumption of the contents of the two bottles (potable water and sucrose solution) placed at the same time in the home

cage over a period of 24 h. Consumption of solution by animals was measured by weighing the bottle before and after the test. Percentage of preference for sucrose was calculated according to the formula:

Percent sucrose preference = $\frac{\text{Sucrose solution intake} \times 100}{\text{Total intake}}$

Where, Total intake = Sucrose solution intake + Water intake.

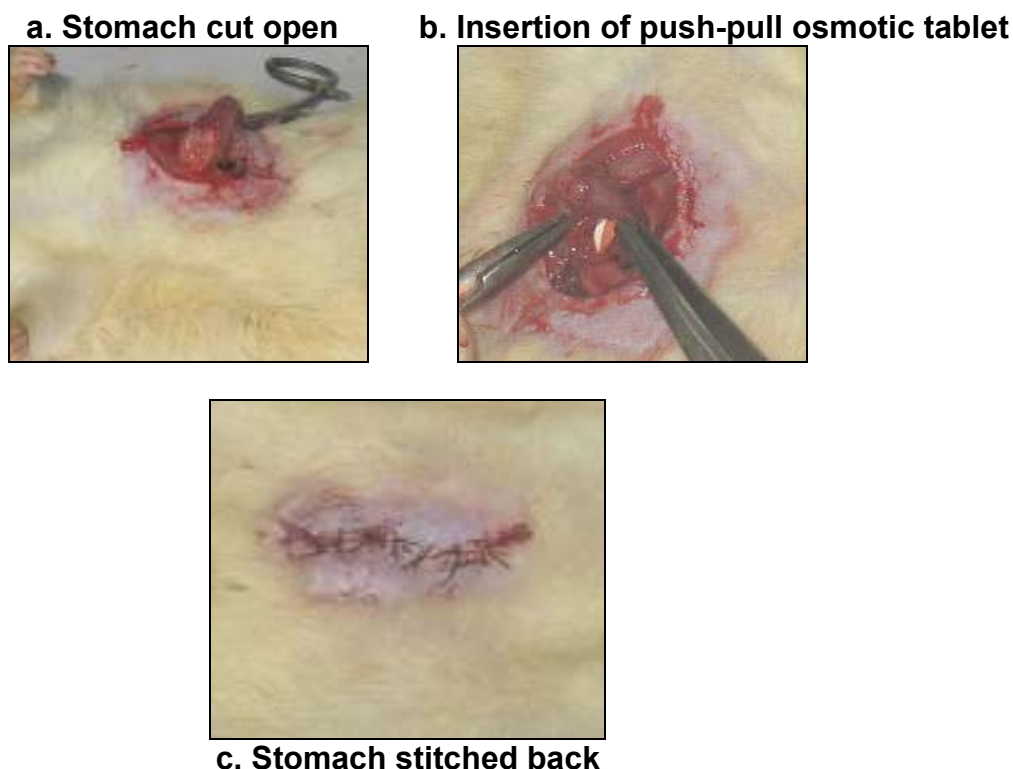
(ii) **Spontaneous locomotor activity**

Photoactometer was used to assess the effect of reserpine on movements. The photocell was equipped 40 x 25 x 20-cm activity chambers, with a grid floor made of stainless-steel bars (spaced 1.0 cm) and provided with three photocells (spaced 10 cm apart and at a height of 1.5 cm). The luminosity of the chamber during the experiments was 0.57lx. Numbers of beam interruptions were cumulatively recorded over a period of 15 mins².

(iii) **Administration of tablet**

The optimized push-pull osmotic tablets were administered intragastrically. Animals were anesthetized with xylazine and ketamine. The stomach was cut open by giving a small incision. The osmotic tablet was inserted into the stomach and was sutured back using 0-6 absorbable catgut as shown in figures 4 a, b and c. After 24 hrs again the animals were tested for sucrose preference and locomotor activity. Animals were then euthanized, brains were removed and striatal tissues were separated and the tissue extract were assessed for dopamine content by HPLC. The test was carried out in both normal control and Parkinson's disease induced rats. Placebo tablets (without drug) were administered to control group.

Figure 4 (a,b,c)
Intragastric administration of Push pull osmotic tablet



(iv) **Striatal dopamine content**

Dopamine is necessary for normal muscle movements; depletion of this causes rigidity,

tremors and bradykinesia. To investigate the effect of reserpine on dopamine depletion, striatal dopamine content was estimated.

Animals were divided into three groups, 6 animals per group. The two groups were treated with reserpine, the third was control. The reserpine treated second group was then administered with developed push-pull osmotic tablet intragastrically. All the animals were then sacrificed to determine the striatal dopamine content in brain.

The rats were euthanized by cervical dislocation, brains were rapidly removed and stored at -75°C until analysed. The rat brains were homogenized in 0.1 M, 500 μl of perchloric acid and sonicated for 20 mins. The resulting homogenate was centrifuged at 1500 rpm for 20 min. The supernatant solution was filtered through 0.2 μ filter and analysed by HPLC.

HPLC method was developed using Inertsil C18 column (250 x 4.6 mm, particle size 5 micron. The mobile phase consisted of 1.72 gm of dipotassium hydrogen orthophosphate, 84 μM n-octylsodium sulfate, 10-15 mg/l, disodium EDTA, 5 mg SLS in 400 ml filtered distilled water (pH 4.1, adjusted with orthophosphoric acid) and 100ml acetonitrile. The mobile phase was delivered at a flow rate of 0.8 ml/min. This method was found to be sensitive in the concentration range of 2-10 μg .

(v) **Pharmacokinetic studies**

To investigate the peak plasma concentration pharmacokinetics studies were carried out. The push pull osmotic tablets were administered to rat intragastrically. The dose was adjusted as per the body weight of rats. At different time intervals the blood was collected and concentration of drug in blood was estimated by HPLC. Various pharmacokinetic parameters like C_{max} , T_{max} , AUC, bioavailability and $t_{1/2}$ were determined. From the plots of plasma drug concentration vs time the $t_{1/2}$

of the developed formulations was found to be 10 hrs.

STATISTICAL ANALYSIS

The data obtained was statistically analysed by one way ANOVA using Sigma statistical software. Multiple comparisons versus control group (Holm-Sidak method) and Kruskal-Wallis one way analysis of variance on ranks were performed when appropriate, to assess the significance of spontaneous locomotor activity and dopamine content with significant level set at 0.05. All values reported were mean \pm S.E.M, the data is shown in Tables 6 and 7 respectively.

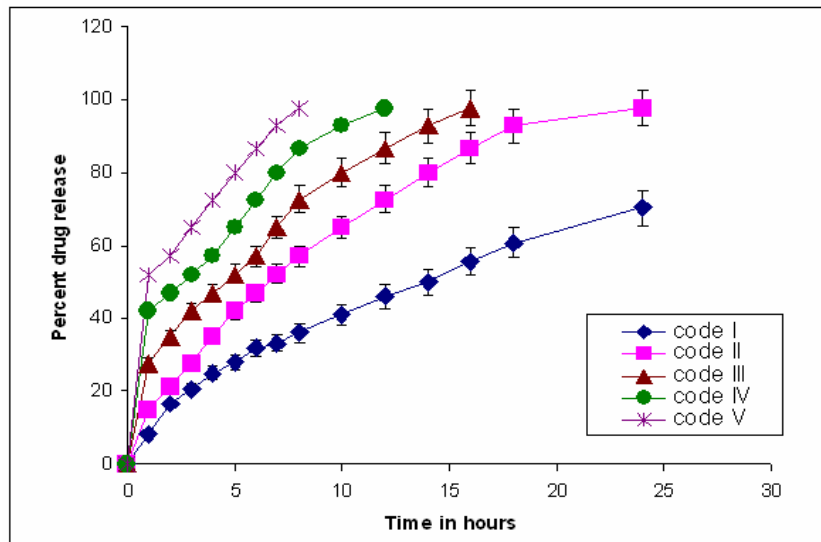
RESULTS

1. ***In vitro* drug release studies**

In vitro release data showed that formulation code II containing sodium chloride as osmotic agent in pull layer and sodium bicarbonate as osmogen in push layer gave the desired once a day release of pramipexole over a period of 24 hrs. Almost 95 % of drug was released in 18 hrs (Figure 1). Formulation code I released 80% of pramipexole in 16 hrs. Formulations code III, IV and V released drug at much faster rate, about 90 % of pramipexole was released in 10-12 hrs. From the *in vitro* release data formulation code II was selected as optimized formulation for further *in vivo* studies.

The drug content of the tablets was found to be 99.99 %.

Figure I
In vitro release profiles of different formulations

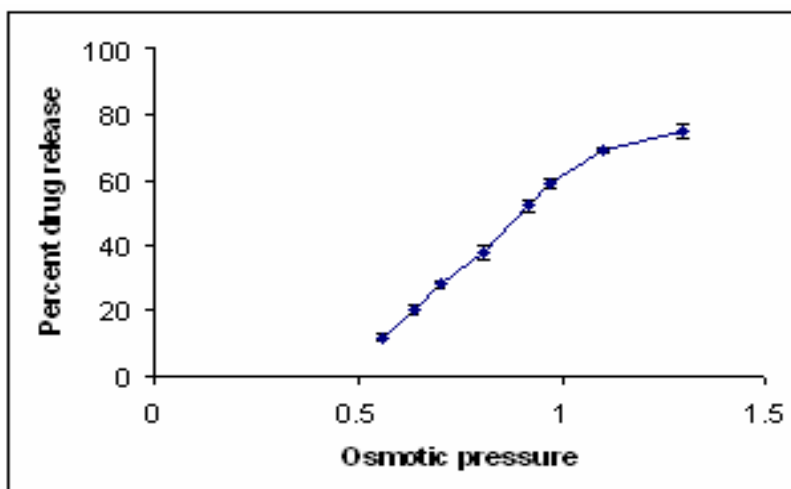


2 Effect of Osmotic Pressure

It was observed from the Figure 2 that the drug release from the push pull osmotic tablet was

directly proportional to the osmotic pressure generated in the tablets. The drug release was increased with the osmotic pressure.

Figure II
Effect of Osmotic pressure on in-vitro drug release studies performed on formulation (code II)



3 Surface morphology

To investigate the change in the membrane structure, surface of coated tablets (both before and after dissolution studies) was studied using Scanning Electron Microscopy Photomicrophotographs are showed in (Figures 3a-b). Figure 3a shows membrane structure before dissolution, initially the surface of coated

tablets was smooth, before coming into contact with aqueous environment the coats appeared to be free of point defects. A microporous structure of the membrane after dissolution was observed in (Figure 3b), which shows SEM of membrane after dissolution. This significant porosity has resulted due to leaching of water soluble additive i.e PEG 400

during dissolution through which drug release takes place.

Surface Morphology Studies coating membranes of push pull osmotic tablets

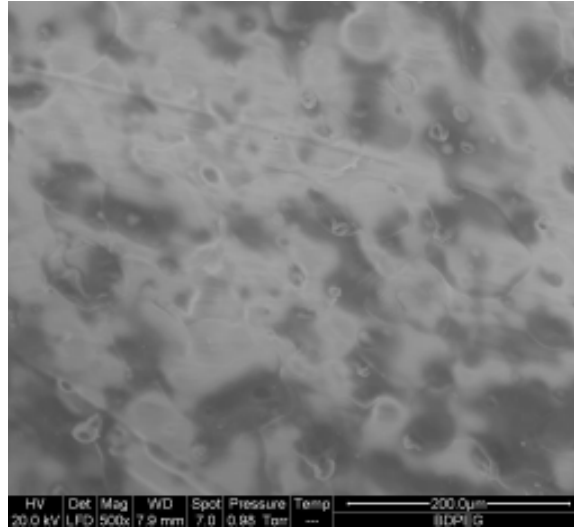


Figure 3 a

Scanning Electron Microscopy before dissolution (No pore formation)

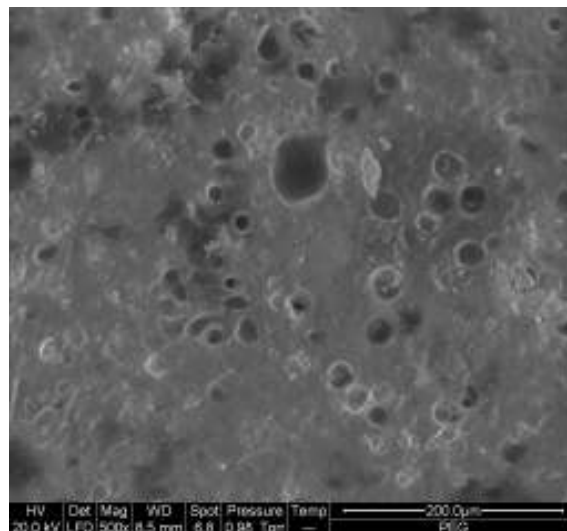


Figure 3b

Scanning Electron Microscopy after dissolution (pore formation)

4 Sucrose preference test

Parkinson disease induced animals showed less preference for sucrose and the total fluid consumption was also reduced to 90 % as

seen in table 2. After intragastric administration of push pull osmotic tablet there was significant increase in sucrose consumption which was increased to 60%.

Table 2

Sucrose Preference Test of rats treated with push pull osmotic tablets

Test	Sucrose consumed	Potable water	Total fluid consumption
Control group	480 ± 50 ml	80 ± 40 ml	560 ± 50 ml
After reserpine administration	<10 ml	50 ± 10 ml	60 ± 10 ml
Treatment with push-pull osmotic tablet	250 ± 50 ml	50 ± 10 ml	250 ± 20 ml

5 Spontaneous locomotor activity

Locomotor activity was found to decrease in animals treated with reserpine. The motility was completely lost due to muscle rigidity.

Normal control group was highly active as seen from (Tab 3). After intragastric administration of push pull osmotic tablets the activity was increased when observed after 24 hrs of treatment.

Table 3

Spontaneous Locomotor activity in rats treated with push pull osmotic tablets

Test	Number of beams interruption observed per 15 mins
Control group	50 ± 4
After reserpine administration	6 ± 3
Treatment with push-pull osmotic tablet	35 ± 2

6 Striatal dopamine content

Striatal dopamine content was decreased to 0.6 ng/gm of tissue after reserpine administration. Since depletion of dopamine causes abnormality in movements and depression, the rats were found to be less active. When the Parkinson's disease induced

animals were treated with developed formulation the dopamine content was found to increase and the animals seemed to be more active. Almost 50% increase in dopamine content was seen after 24 hrs of treatment, the data is given in (Tab 4).

Table 4

Striatal Dopamine Content in rats treated with push pull osmotic tablets

Test	Dopamine content in brain (ng/gm of tissue)
Control group	12 ± 0.9
After reserpine administration	0.6 ± 1.5
Treatment with push-pull osmotic tablet	6 ± 2

7. Pharmacokinetic studies

The pharmacokinetic parameters obtained are given in (Tab 5). The developed push pull osmotic tablets maintained concentration of

pramipexole in plasma over a period of 24 hrs. The relative bioavailability was found to be 85 %.

Table 5

Pharmacokinetics data of developed Push pull osmotic tablets administered to rats

Observations	Results
C _{max}	0.34 ng/ml
T _{max}	6.5 hrs
T _{1/2}	10 hrs
AUC ₍₀₋₂₄₎	0.40 ng.hr/ml
V _d	500 ml

8 Statistical analysis

The differences in the median values among the treatment groups for both locomotor activity and striatal dopamine content were found

greater than would be expected. Hence it was concluded that there is a statistically significant difference in control and treated groups.

Table 6

Multiple Comparisons versus Control Group (Holm-Sidak method) for spontaneous locomotor activity

Comparison	Difference of Means	t	Unadjusted P	Critical level	Significant
Control Vs Reserpine Treated groups	15	9.231	0.000000142	0.025	Yes
Control vs Pramipexole Treated groups	5.833	3.590	0.00268	0.050	Yes

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001).

Power of performed test with alpha = 0.050: 1.000

Data represents mean ± S.E.M (n = 10/group)

Table 7

Kruskal-Wallis One Way Analysis of Variance on Ranks (Striatal dopamine content in brain)

Group, N = 6	Medians	25 %	75%
Control	16	14	18
Reserpine Treated	0.520	0.410	0.6
Pramipexole Treated	1.915	1.720	2.12

H = 15.158 with 2 degrees of freedom. (P = <0.001). The differences in the median values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference (P = <0.001)

DISCUSSION

The results obtained from *in vitro* release data showed that formulation code II containing sodium chloride and sodium bicarbonate as osmotic agents could generate optimum osmotic pressure within the bilayered tablet, which resulted in desired once a day release of pramipexole over a period of 24 hrs (Fig 1). The osmotic pressure developed by these osmotic agents was found to be linear with the drug release.

Surface morphology studies revealed that significant porosity was resulted due to leaching of water soluble additive i.e PEG 400 during dissolution through which drug release takes place as seen in (Fig 3a and b). It is evident from the sucrose preference test that there was a significant increase in sucrose consumption after treatment with push pull osmotic tablet (table 2). This was due to anhedonic status (a diminished responsiveness to rewards) due to the action of reserpine. Anhedonia is probably related to a postsynaptic decrease of dopamine type 2 receptors in the nucleus accumbens. There was almost 5 fold increase in spontaneous locomotor activity after intragastric administration of pull osmotic tablet that can be seen in (tab 3). As observed from the data given in (Tab 4) the striatal dopamine content was significantly increased from 0.6 ng/gm of tissue (in reserpine treated rat) to 6 ng/gm of tissue in rats treated with developed push pull

osmotic tablet administered intragastrically. The developed osmotic tablet maintains the drug concentration in plasma for 24 hrs as revealed by pharmacokinetic data given in (Tab 5). The statistical data obtained from the *in vivo* studies confirms that there was a significant increase in locomotor activity as well as increase in dopamine content in rat brain after intragastric administration of push pull osmotic tablet.

CONCLUSION

Once a day push pull osmotic drug delivery system based on controlled porosity membrane for pramipexole was successfully developed and evaluated. Symptoms of Parkinson's disease could be successfully induced in rats using reserpine, making the studies more reliable which could be performed in laboratory. Results of pharmacokinetic studies indicated that the developed push pull osmotic tablet maintained drug concentration in plasma for 24 hrs as compared to conventional tablets. The push pull osmotic system was developed without using laser drilling technology which makes it more cost effective and simple and could be applied on large scale.

ACKNOWLEDGEMENT

Authors are thankful to Sekhsaria Chemicals Ltd. India, for the support.

REFERENCES

1. Lorenc-Koci E, Wolforth S, Efficacy of pramipexole a new dopamine receptor agonist, to relieve the parkinsonian-like muscle rigidity in rats, *European Journal of Pharmacol*, 385, 39-46, (1999).
2. Skalisz LL, Benjamin V, Joca LS ,Vital MA ,Cunha CD, Andreatini R, Evaluation of the face validity of reserpine administration as an animal model of depression- Parkinson's disease association, *Progress in Neuro-PsychoPharmacology and Biological Psychiatry*, 26, 878-883, (2002).
3. Bernheimer, H, Birkmayer, W, Hornykiewicz, O, Jellinger, Seitelberger F, Brain dopamine and the syndromes of Parkinson and Huntington, *Journal of the Neurological Sci*, 20, 415-455, (1973).
4. Forno LS, Pathology of Parkinson's disease. In: Marsden, Fahn, S. Ed, *Movement Disorders*. Butterworth, London, 25-40, (1982).
5. Hornykiewicz O, Brain neurotransmitter changes in Parkinson's disease. In: Marsden, D., Fahn, S. Ed., *Movement Disorders*. Butterworth, London, 41-58, (1982).
6. Hornykiewicz O, Parkinson's disease and the adaptive capacity of the nigrostriatal dopamine system: possible neurochemical mechanism. *Advances in Neurology*, 60, 140-147, (1993).
7. Hornykiewicz O, Kish SJ, Biochemical pathophysiology of Parkinson's disease. *Advances in Neurology*, 45, 19-34, (1986).
8. Hornykiewicz O, Ageing and neurotoxins as causative factors in idiopathic Parkinson's disease — a critical analysis of the neurochemical evidence. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 13, 319- 328, (1989).
9. Hornykiewicz O, Biochemical aspects of Parkinson's disease, *Neurology*, 51 (suppl 2), S2- S9, (1998).
10. McGeer PL, Itagaki S, Akijama H, McGeer EG, Comparison of neuronal loss in Parkinson's disease and aging. In: Calne,
11. D.B.(Ed.), *Parkinsonism and Aging*, Raven Press, New York, 25-34, 1989.
12. Fahn S, Adverse effects of levodopa. In: Olanow, C.W., Lieberman, A.N. Eds., *The Scientific Basis for the Treatment of Parkinson's Disease*, Parthenon, 89-112, (1992).
13. Hagan JJ, Middlemiss DN, Sharpe PC, Poste GH, Parkinson's disease: prospects for improved drug therapy. *TIPS* 18, 156-163, (1997).
14. Lieberman A, Goldstein M, Gopinathan G, Neophytides A, D-1 and D-2 agonists in Parkinson's disease. *Canadian Journal of Neurological Science*, 14, 466-473, (1987).
15. Mierau, J, Pramipexole: a dopamine-receptor agonist for treatment of Parkinson's disease. *Clinical Neuropharmacology*, 18, 195-206, (1995).
16. Carter A.J., Muller RE, Pramipexole, a dopamine D2 autoreceptor agonist, decreases the extracellular concentration of dopamine in vivo, *European Journal of Pharmacology*, 200, 65-72, (1991).
17. Mierau J, Schingnitz G, Biochemical and pharmacological studies on pramipexole, a potent and selective dopamine D2 receptor agonist. *European Journal of Pharmacology*, 215, 161-170, (1992).
18. Mierau J, Schneider FJ, Ensinger HA, Chio CL, Lajiness, ME ,Huff RM, Pramipexole binding and activation of cloned and expressed dopamine D2, D3 and D4 receptors. *European Journal of Pharmacology*, 290, 29-36, (1995).
19. Ellenbroek B., Schwarz M, Sontag K, Jaspers R, Cools A, Muscular rigidity and delineation of dopamine specific neostriatal subregion: tonic EMG activity in rats, *Brain Research*, 345, 132-140, (1985).
20. Elliott PJ, Close S.P., Walsh DM, Hayes AG, Marriott, AS, Neuroleptic-induced catalepsy as a model of Parkinson's disease: I.Effect of dopaminergic agents. *Journal of Neural Transmission*, 2, 91-100, (1990).

21. Elverfors A, Nissbrandt H, Reserpine — insensitive dopamine release in the substantia nigra, *Brain Research*, 557, 5-12, (1991).
22. LaHoste GJ, Marshall JF, Rapid development D1 and D2 dopamine receptor supersensitivity as indicated by striatal and pallidal Fos expression. *Neuroscience Letter*, 179, 153-156, (1994).
23. Lieberman A, Ranhosky A, Korts D, Clinical evaluation of pramipexole in advanced Parkinson's disease: results of a doubleblind, placebo-controlled, parallel-group study. *Neurology*, 49, 162-168, (1997).
24. Maj J, Rogo Z, Skuza G, Kolodziejczyk K, The behavioural effects of pramipexole, a novel dopamine receptor agonist. *European Journal of Pharmacology*, 324, 31-37, (1997).
25. Maj J, Rogo Z, Skuza G, Kolodziejczyk K, The behavioural effects of pramipexole, a novel dopamine receptor agonist. *European Journal of Pharmacology*, Volume 324, 1997, 31–37.
26. Lorenc-Koci, E, Ossowska, K, Wardas, J, Wolfarth, S, Does reserpine induce parkinsonian rigidity? *Journal of Neural Transmission*, 9, 211-223, (1995).
27. Lorenc-Koci, E, Wolfarth, S, Ossowska, K, Haloperidol-increased muscle tone in rats as a model of parkinsonian rigidity. *Experimental Brain Research*, 109, 268-276, (1996).
28. Wakode RR, Bajaj AN, Development and evaluation of Push-Pull based osmotic delivery system for Pramipexole. *PDA Journal of Pharmaceutical Science and Technology*, 62, 22-31, (2008).