Original Article

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In situ thermosensitive gel of levodopa: Potential formulation for nose to brain delivery in Parkinson disease

Shohreh Alipour[1], Hamideh Azari[1], Fatemeh Ahmadi[1,*]

[1]Department of Pharmaceutics, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.

Abstract

Parkinson's disease is a disabling neurodegenerative disease which limits many functional activities of the patients. Treatment of the disease by oral formulations is not successful and even not applicable in some patients because of difficulty in swallowing. Levodopa is one of the commonly used drugs for Parkinson's disease which bioavailability of its oral formulations is variable between patients. The aim of the present study was to develop an in-situ gel forming formulation for nasal delivery of levodopa. Poloxamer 407, chitosan and alginate were used as thermosensitive and mucoadhesive polymers for formulating nasal gel. Different concentrations of polymers and drug were tested to optimize the gelation temperature. Based on the gelation temperature, two formulations (PL7 and PAL10) which respectively contained poloxamer 20% and levodopa 0.3% w/v and poloxamer 18%, Alginate 0.4% and levodopa 0.3% w/v presented desirable properties for a nasal gel. The gels showed suitable pH, clarity and strength as well as sustained release profile which is necessary for a formulation designed for retention in the nasal cavity. It seems that thermosensitive nasal gel of levodopa with poloxamer could be a potentially successful formulation for delivery of levodopa to the brain.

Keywords: In-situ gels, Levodopa, Nasal delivery, Parkinson's disease, Poloxamer 407

1. Introduction

Parkinson's disease (PD) is a progressive neurological disorder results from reduced dopamine level in central nervous system (CNS) (1). Levodopa (LVD) is a dopamine precursor which is widely used in PD treatment because LVD despite dopamine can cross blood brain barrier (2). LVD circulation metabolism results in low bioavailability and hence low brain uptake (3). Considering LVD conversion to dopamine in extra-cerebral tissues specially in gastrointestinal tract following oral administration, small doses of LVD may reach to the CNS (4). Oropharyngeal dysphagia is another problem of patients with PD which reduces patient compliance for oral LVD dosage forms (5). Although, ease of administration makes oral rout the most desirable and preferred route for drug delivery, but fluctuation of LVD plasma concentration and different rate and extent of absorption happens due to variation in gastric factors (6).

Intranasal drug delivery is one of the attractive alternatives for oral delivering of therapeutic agents preferentially to the brain. There is a unique anatomical connection between nasal cavity and CNS through olfactory bulb which makes it a great opportunity for rapid drug access to the brain following intranasal administration (7). Nose to brain drug delivery through olfactory and trigeminal nerves pathway benefits are numerous including bypassing hepatic first pass metabolism, rapid onset, similar plasma profile to intravenous administration and less systemic side effects (8). However, intranasal delivery drawbacks for ex-

Corresponding Author: Fatemeh Ahmadi, Department of Pharmaceutics, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran.
Email: ahmadi_f@sums.ac.ir

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ample rapid mucocilliary clearance of common liquid dosage forms could not be ignored. Therefore, some strategies have been introduced to improve the efficacy of intranasal drug delivery. One of these strategies is formulating in situ gels. The special advantage of in situ thermosensitive gelling systems is their ease of administration, since they are liquid at room temperature while can form semisolid gel at nasal cavity temperature. These thermosensitive gels provide longer residence time with possibility of sustained drug release (8-10). Poloxamer (PLX) 407 is a commercial polymer with low toxicity and thermoreversible gel forming ability. Considering insufficient mucoadhesive properties of PLX for nasal delivery, it is usually mixed with other mucoadhesive polymers to provide more desirable characteristics (9). Alginate (ALG) and chitosan (CTS) are two most common natural polymers with no toxicity and remarkable mucoadhesive properties (11). ALG and CTS have been tested in many mucoadhesive formulations especially those for nasal delivery (12-15).

Many studies focused on developing new formulations for LVD to improve PD patient compliance and treatment outcomes. The aim of the present study was to design and evaluate an in situ gel composed of PLX and two mucoadhesive polymers. The pharmaceutical desirability of this gel for sustained delivery of LVD to the nasal cavity was also studied.

2. Materials and methods

2.1. Materials

Levodopa was purchased from Alborz Daru Co. (Iran). Poloxamer 407 (average molecular weight about 12,600) and Chitosan (low molecular weight) were purchased from Sigma-Aldrich. Sodium alginate was purchased from Samchun (Korea). Potassium dihydrogen phosphate and di-potassium hydrogen phosphate were obtained from Merck. All other chemicals and solvents were purchased from domestic suppliers.

2.2. LVD Analysis

For LVD quantification, UV-Vis spectrophotometry method was used at maximum absorbance wavelength of the drug. To determine the maximum absorbance wavelength, a working standard solution with a known concentration was prepared in phosphate buffer solution (PBS) 200 mM, pH 7.4. Then, the solution was scanned in the UV-Vis spectrophotometer (UV- 1650, Shimadzu, Japan) in the range 200-400 nm and the UV spectrum was recorded.

2.2.1. LVD analysis method validation

LVD calibration curve was constructed by analyzing 5 different LVD concentrations (6.25, 12.5, 25, 50 and 100 µg/mL) which were prepared in phosphate buffer at pH 7.4 using serial dilution technique. Calibration curve concentrations were prepared in three different days. Each concentration was tested in triplicate. Calibration curve was validated for linearity, intraday and inter-day precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ).

2.3. LVD-PLX Gel Preparation

Blank PLX gels (17-22 % w/v) without LVD (P1-P5) and with different LVD concentrations (0.03, 0.06 and 0.3 % w/v) were prepared in phosphate buffer pH 6 (PL1-PL11). Blank PLX gels (17-22 % w/v) in presence of CTS (0.1 % w/v) without LVD (PC1-PC5) and with different LVD concentrations (0.03, 0.06 and 0.3 % w/v) were prepared in phosphate buffer pH 6 (PLC1-PLC7). Blank PLX gels (17-22 % w/v) in presence of different ALG concentrations (0.15 and 0.4%) without LVD (PA1-PA5) and with different LVD concentrations (0.03, 0.06 and 0.3% w/v) were also prepared in phosphate buffer pH 6 (PAL1-PAL10). Samples were stirred for 1 h and kept at 4 ºC for 24 h.

2.4. Pharmaceutical evaluation

2.4.1. Gelation Temperature

Gelation temperature is a specific temperature in which a solution is converted into the gel. Gelation temperature was determined by test tube inversion method. It is the temperature at which upon inverting the test tube, formulation would not move. The tubes containing the formulations were heated in a water bath with a rate of 1°C/min from 25 to 40 °C and each time the gel formation was examined by inverting the tube (16). Gelation temperature was the initial parameter for nasal gel

optimization in the present study.

2.4.2. Appearance of nasal gel

Visual observation under black and white background was performed to determine clarity of nasal gels (10). Appearance was graded as follows: turbid: +, clear ++, very clear (glassy): +++.

2.4.3. pH of nasal gel

pH of nasal gel was determined at room temperature using pH meter (Metrohm, Switzerland) to investigate the biocompatibility of formulations. Standard solutions of pH 4.5 and pH 7.0 were used to calibrate pH meter.

2.4.4. Nasal gel strength

Gel strength was presented as the time it takes for a special weight to penetrate 5 cm deep into a gel. Nasal gel was put in a 100 ml graduated cylinder and converted to gel in water bath at 35 °C. A weight of 35 g was placed onto the gel. The gel strength was determined by the time in seconds that the weight penetrated into the gel (17).

2.4.5. LVD Content

LVD content was defined by diluting 1ml of in-situ gel formulations 1:100 using PBS pH 6. The LVD amount was determined using the validated analysis method.

2.5. Rheology Evaluation

Selected gel formulations were evaluated for viscosity and rheological parameters. Cone and plate viscometer (R/S Plus, Brookfield, USA) was used with shear rate between 0 to 100 rpm. Rheological parameters were evaluated at 32, 33, 34, and 35 ºC.

2.6. In vitro Release Study

Dialysis bag (cutoff: 1200 Da) on a diffusion cell was used to determine LVD *in vitro* release from gel formulations. Dissolution medium was phosphate buffer pH 6 and gel formulation was applied on the donor part. The whole system was maintained at 34±1 ºC and samples were withdrawn at 5, 10 15, 30, and 60 min form the receptor compartment.

2.7. Statistical analysis

All experiments were performed in triplicate and mean ± SD were reported for statistical analysis. All statistical tests of the present study were performed using SPSS 16 software and Microsoft Excel 2016. The one-way ANOVA test was used to compare results. p-value<0.05 was considered as the significant difference.

3. Results and Discussion

3.1. LVD analysis

Based on the UV spectroscopy results, maximum wavelength of LVD was 280 nm. Further analytical tests on the samples were performed in this wavelength. UV spectrum recorded for blank gel showed no interfering peak in the range of LVD maximum wavelength.

3.1.1. LVD Calibration curve validation

Calibration curve construction was in the range of the expected concentrations of 6.25 to 100µg/mL in PBS pH 6. Validation parameters including linearity, inter-day and intraday precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ) were calculated and are reported in Table 1. The correlation coefficient (r^2) of the standard curve (0.9997) indicated linear relationship at selected range of LVD concentrations. Precision, accuracy, LOD and LOQ are in the acceptable range for this analysis (18).

3.2. LVD-PLX Gel Preparation

Blank PLX and LVD loaded PLX (PL) nasal gel formulations were prepared and optimized on the basis of their gelation temperature and results are presented in Table 2.

For optimizing the formulation of nasal

Table 1. Calibration curve validation parameters (n=3).

Precision (%) (Inter-day)	Precision (%) (Intra-day)	Accuracy (%)	LOD (µg/ml)	LOQ (µg/ml)	Regression coefficient	Regression Equation
98.5±1.1	99.3±0.6	97±3.3	1.44	4.84	0.9997	Y=0.0156x-0.0194

Table 2. PLX and PLX-LVD nasal gel formulations gelation temperature (n=3).

	PLX% (w/v)	LVD % (w/v)	Gelation Temperature ºC
P1	22	-	27.2±0.4
P2	20	-	28.6±0.5
P3	19	-	31.1±0.9
P4	18	-	31.8±0.5
PL1	20	0.03	28.3±0.3
PL2	19	0.03	30.8±0.1
PL3	18	0.03	33.5±0.1
PL4	20	0.06	30.7±0.6
PL5	19	0.06	32.1±0.2
PL6	18	0.06	37.3±0.3
PL7	20	0.3	33.8±0.1
PL8	19	0.3	39.2±0.2
PL9	18	0.3	40.1±0.1

PLX: Poloxamer, LVD: Levodopa

in situ forming gel, gelation temperature of PLX was the most important parameter. Considering previous reports acceptable gelation temperature was in the range of 30-35 ºC (16). As it is seen in Table 2, gelation temperature of PLX in concentration of 18 and 19% w/v were in the acceptable range while addition of LVD in nasal gel gradually increased gelation temperature of PLX. PL7 formulation, which contained 0.3%w/v LVD in nasal gel was selected as the most optimum gel with the most desired gelation temperature according to the nasal cavity temperature. Previous studies confirmed that presence of soluble drugs in PLX gel structure increases its gelation temperature since it can be solubilized and acts as a salt in PLX gel network (19, 20).

The major problem of oral administration of levodopa is that the stability in blood and level of drug in brain is very low. Therefore, different routes have been suggested for administration of drug. Nose to brain delivery could be a useful alternative, since the drug is uptaken by the brain directly and passage from the blood brain barrier is not a challenge. Therefore, it seems this low dose of drug in the gel would still present satisfying drug concentration in the blood and brain (6).

Blank PLX and LVD loaded PLX nasal gel formulations in presence of CTS (0.1%w/v) were prepared and optimized on the basis of their gelation temperature and results are reported in Table 3. Table 3 results indicate that presence of chitosan in PLX gel resulted in higher gelation temperature

Table 3. PLX-CTS and PLX-LVD-CTS nasal gel formulations gelation temperature (n=3)

	PLX% (w/v)	CTS % (w/v)	LVD%(W/V)	Gelation Temperature ºC
PC1	20	0.1	-	28.03±0.1
PC2	19	0.1	-	37.1±0.2
PC3	18	0.1	-	39.1±0.1
PC4	17	0.1	-	41.2±0.2
PCL1	20	0.1	0.03	26.9±0.1
PCL2	19	0.1	0.03	31.2±0.1
PCL3	18	0.1	0.03	33.1±0.1
PCL4	17	0.1	0.03	40.2±0.2
PCL5	19	0.1	0.3	28.2±0.1
PCL6	18	0.1	0.3	29.3±0.2

PLX: Poloxamer, CTS: Chitosan, LVD: Levodopa

Table 4. PLX-ALG and PLX- LVD-ALG nasal gel formulations gelation temperature (n=3).

	PLX% (w/v)	ALG % (w/v)	LVD%(W/V)	Gelation Temperature ºC
PA1	24	0.15	-	23.1±0.2
PA2	20	0.15	-	27.7±0.2
PA3	19	0.15	-	31.4±0.2
PA4	18	0.15	-	33.7±0.1
PA5	17	0.15	-	35.2±0.2
PAL1	24	0.15	0.03	24.1±0.2
PAL2	20	0.15	0.03	26.1±0.2
PAL3	19	0.15	0.03	31.2±0.1
PAL4	18	0.15	0.03	33.1±0.1
PAL5	17	0.15	0.03	40.2±0.2
PAL6	20	0.15	0.3	31.1±0.2
PAL7	19	0.15	0.3	33.1±0.2
PAL8	20	0.4	0.3	30.4±0.2
PAL9	19	0.4	0.3	31.7±0.1
PAL10	18	0.4	0.3	34.3±0.2

PLX: Poloxamer, ALG: Alginate, LVD: Levodopa

which is out of acceptable range for nasal formulation. In comparison, addition of LVD decreased gelation temperature, so gelation temperature of PLC3 and PLC4 were in suitable range. Increasing LVD concentration to 0.3% w/v decreased gelation temperature which was not acceptable, therefore PLX/CTS gel evaluation was not continued.

Blank PLX and LVD loaded PLX nasal gel formulations in presence of ALG (0.15 and 0.4%w/v) were prepared and optimized on the basis of their gelation temperature and results are reported in Table 4. Gelation temperature of PLX in the presence of ALG gel (0.15%) decreased while presence of LVD (0.03%) increased it again. Increasing ALG concentration up to 0.4% did not present any further effect on gelation temperature. Therefore, PAL8, PAL9 and PAL10 were selected as the most desirable formulations. Considering the lowest concentration of PLX in PAL10, it was selected for further experiments.

3.3. Pharmaceutical evaluation

3.3.1. Gelation temperature

Gelation temperature was the initial parameter for nasal gel optimization. Since the physiological temperature of the nasal cavity is about 32-34 ℃, the desirable gelation temperature for nasal gels is between 30-36 ℃ (21, 22). All formulations showed gelling temperature in the range 23-41 ℃.

Adding LVD increased the gelation temperature which is related to the water soluble nature of the drug which changes micellar association (22). Presence of mucoadhesive polymers within PLX network decreased the gelation temperature. This observation was predictable, because of the increasing viscosity and higher entanglement between polymeric chains which facilitates micelle formation (22, 23). Based on the gelation temperature, PL7 and PAL10 were selected as optimum formulations for further experiments.

3.3.2. Clarity of nasal gel

Clarity of nasal gel is a pharmaceutical property which affects patient attraction to the dosage form. It was graded as follows: turbid: +, clear ++, very clear (glassy): +++. Clarity of optimized formulations are presented in Table 5. As it is seen, presence of ALG deceased PLX gel clarity, which is again related to the higher entanglement of the polymer chains and dehydration of the polymers. Both formulations were clear enough to be acceptable.

3.3.3. pH of nasal gel

pH of both optimized formulations was

Table 5. Pharmaceutical evaluation of optimized nasal gels (n=3).

	Clarity	pH	Gel strength (sec)	LVD content%
PL9	+++	6.4±0.5	43±3	95.8±3.1
PAL10	++	5.8±0.3	32±2	98.2±2.5

within nasal physiological pH range 5.5-6 (17). Results are reported in Table 5.

3.3.4. Nasal gel strength

Gel strength of optimized nasal gels was determined using the method mentioned in section 2.4.4. All measurements were done in triplicate and results are shown in Table 5.

3.3.5. LVD gel Content

LVD content of the optimized nasal gel formulations was also determined. As reported in Table 5, drug content is in the acceptable range.

3.4. Rheological evaluation

Rheological parameters of optimized nasal gels PL7 and PAL10 were evaluated at 32, 33, 34, and 35 ºC. Shear stress and viscosity versus shear rate profiles were obtained at mentioned temperatures. Results are presented in Figure 1. Considering temperature dependent PLX gelation process, significant difference was observed in viscosity of PL7 at mentioned temperatures. As it was predictable, the higher viscosity would be obtained at higher temperatures (34 and 35ºC) that reveals gel formation temperature. However, for PAL10, ALG presence eliminated the differences between lower temperatures. For the gelling temperature point at 34 ºC the difference reveals gel formation temperature.

As it is seen in Figure 1, rheograms based on the shear stress show similar trends. Rheology profiles show that the formulations are liquid at room temperature and can form gels at nasal physiological temperature (17).

3.5. In vitro drug release

The *in vitro* drug release profiles of the optimized nasal gel formulations in comparison with free LVD are presented in Figure 2. Significant slower LVD release ($p<0.05$) from PL7 and PAL10 compared with free LVD confirmed that LVD is maintained in nasal gels matrix network which can prevent rapid release of LVD. Adding

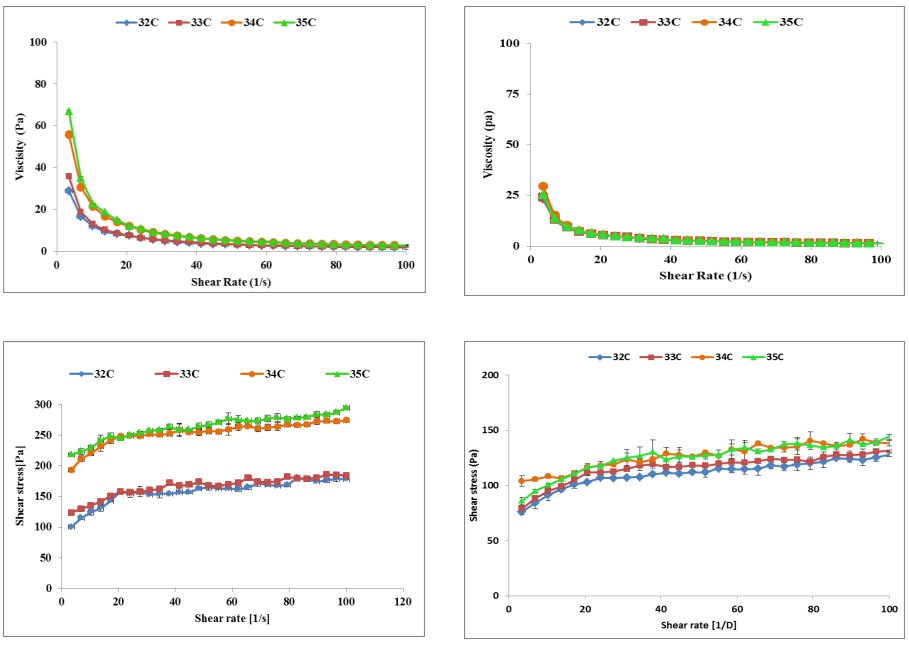

Figure 1. Rheograms of optimized PL7 (Left) and PAL10 (right), Mean±SD (n=3).

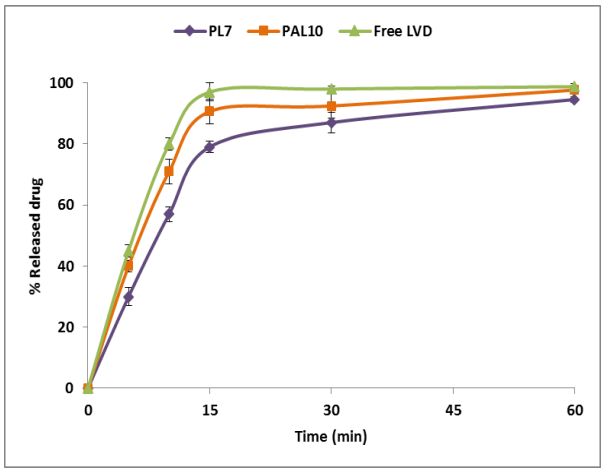

Figure 2. Release profiles of the optimized formulations PL7 and PAL10 in PBS pH 6 at 34±1 ºC (n=3).

ALG to PLX increased the release rate which may be related to the soluble nature of ALG that facilitates dissolving and release of drug (24). The drug release profile is sustained in PL7 formulation that is acceptable for a nasal gel designed for delivery from nose to brain. Meanwhile, it should be mentioned that the ultimate goal of nose to brain delivery is to increase the chance of brain uptake and bypassing blood brain barrier. Therefore, sustained release property is not of much value in these cases and the brain concentration should be monitored.

4. Conclusion

In this study, a series of in-situ thermosensitive gel forming polymer combinations of poloxamer, chitosan and alginate were developed for delivery of levodopa from nose to brain. Polymer combinations were tested to study the effect of mixing mucoadhesive polymers with thermosensitive ones on retention time in the nasal cavity. Formulation PL7 which contained poloxamer 20% and levodopa 0.3% w/v and PAL10 which contained poloxamer 18%, Alginate 0.4% and levodopa 0.3% w/v presented desirable properties for a nasal gel. These properties include suitable gelation temperature (34 ºC), sufficient gel strength and clarity as well as sustained release of drug compared with the drug solution. The results showed that in situ gel forming formulation could be of value for longer retaining of the drug in nasal cavity and enhancing the chance for delivery to the brain.

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Conflict of Interest

None declared.

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