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A study on the development of long-acting injectable formulation of rotigotine

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Rotigotine 의 장기 지속형 주사제 개발에 대한 연구

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로티고틴은 파킨슨병과 하지불안증후군에 사용되는 선택적 도파민 작용제이다. 현재 경피 투여 제제인 Neupro® 만이 시판되고 있다. Neupro® 는 많은 장점을 가지고 있지만 종종 홍반, 가려움증, 및 도포 부위의 피부염 등을 야기하고, 부착 부위에 따라 흡수 정도가 달라지는 문제점이 있다. 이러한 문제를 해결하고 환자의 편의성을 향상시키기 위해 장기간 주입 할 수 있는 fluidal lipid matrix(FLM)을 설계했다. FLM 은 castor oil, cottonseed oil, span 80 및 ethanol 로 구성되어있다. 제형 설계 시 최적의 조성비를 기존의 경험적 방법이 아닌 보다 과학적이고 통계적으로 결정하기 위하여 실험 설계법 (Design of Experiment)을 활용하였다. 본 연구에서는 혼합물 설계법 (Mixture designs) 중에서 구성 성분이 정해진 상한치, 하한치를 지니거나 제약 조건이 존재할 때 사용하기 적합한 꼭지점 설계법 (Extreme vertices design)을 바탕으로 실험을 구성하였다. 제제의 조성을 최적화하기 위해 실험 설계법을 기반으로 11 가지 제형을 제조하고, 그들의 점도 및 120 시간 동안의 누적 방출 백분율을 평가하였다. 120 시간 동안의 방출 프로파일 및 주사 가능한 점도를 고려하여 제형 조성 최적화를 진행하였다. 최적화된 제형에 대하여 광학 현미경 관찰, 점도 변화 측정 및 형태 변화 관찰 등의 특성 평가를 진행하였으며 제시된 결과들은 본 제형이 rotigotine 을 장기간 방출하기에

적합한 주사제제로서의 가능성이 있음을 시사하였다. 시간적 제약으로 인해 미쳐 진행하지 못한 실험들에 대해 정리하였으며 추후 이 실험들을 수행해서 본 연구를 완성하기로 계획하였다.

2. Introduction

Parkinson's disease (PD) is a slowly progressive, neurodegenerative disease characterized by muscular rigidity and bradykinesia[1]. The death of dopaminergic neurons is the major cause of PD but the exact cause, whether genetic or environmental remains unknown. Because of the longer life expectancy, the incidence of PD is expected to increase[2]**.** In the United States, an estimated 630,000 to 1 million people were diagnosed with PD in 2010. With approximately 50,000 new cases diagnosed each year, recent estimates project that the number of people diagnosed with PD in the United States will double by the year 2040 compared to 2010[4]. The prevalence of PD is higher in males compared with females with a ratio of 1.6: 1[1]. At present, after about 30 years of efforts in drug development, levodopa is regarded as a standard therapy for the treatment of PD[3]. However, patients were gradually tolerated to the therapeutic benefits of levodopa, and its causes some harmful side effects, including response fluctuations (wearing off) and levodopa-induced dyskinesia (LID). Therefore, new strategies that can release drug continuously are required to decrease the occurrence of LID[2].

Rotigotine was developed for the once daily treatment of idiopathic Parkinson's disease[5]. Rotigotine is an agonist of dopamine D3/D2/D1 receptors for the therapy of PD which has similar structure to dopamine. It is a lipid-soluble agonist with a molecular weight of 315. Due to an extensive first-pass effect, rotigotine shows a very low bioavailability through oral administration[6]. Neupro® was developed by Schwarz Pharma (Brussels, Belgium) for the once daily treatment of Parkinson's disease using a transdermal delivery system. Neupro® releases rotigotine steadily over 24 h, reducing side effects, such as dyskinesia and resting tremor, resulting from fluctuating concentration-versus-time profiles[5]. The rotigotine transdermal system achieved sustained administration of the drug to overcome some of the limits of short-acting dopaminergic PD treatments by providing stable 24 h steady-state plasma concentrations of rotigotine[4]. The most common adverse events observed during rotigotine clinical studies were application site reactions. Approximately half of rotigotine patients had application site reactions, including dermatitis, pruritus, and erythema, compared with

about 15% of patients receiving placebo[5]. There is also a fatal disadvantage that the rate of absorption of the drug varies depending on the patch attachment site[6]. Given the problems associated with transdermal routes of administration, it is crucial to develop a more effective routes of administration which can be an alternative transdermal delivery route[7].

Implant drug delivery systems have provided meaningful results in the delivery of various drugs in vivo[6]. For example, leuprolide is one of the most successful drugs in SR depot injection formulation of PLGA microsphere[8-12]. Though PLGA microspheres are useful for clinical application, they are difficult and expensive to prepare and need some exclusive tools[13-15].

In order to defeat these problems and enhance patient's convenience, we designed fluidal lipid matrix (FLM) for long-acting injection that can maintain the long efficacy of rotigotine, minimize initial burst and release rotigotine at a constant rate for a long period. We adopted organogel as FLM. Utilizing Design of Experiments(DoE), which can give a combination of variables that result in near-maximum output, are more efficient in multi-factor designs compared with one-factor-at-a-time experimentation. We utilized DoE to construct experiments and conducted experiments to find the optimum composition ratio based on this. FLM generally comprise appropriate proportions of an oil phase, surfactant and organic solvent. FLM has specific physicochemical property. When FLM is administered into the body, the organic solvent is released to surrounding tissues and the viscosity is increased to form a matrix.

FLM has significant advantages, such as simple preparation, controlling drug delivery rate, and improved bioavailability of lipophilic drugs like rotigotine via injectable routes. FLM will be a promising method for long-injectable drug delivery, and a mechanism has been identified to explain the advantages of FLM for injectable drug delivery. The concept of the target formulation using FLM is shown in Fig.1.

In this study, FLM were developed after selection of oil, surfactant, and organic solvent. Using DoE, the composition ratios of the optimized FLM was determined after a more scientific and effective process. The properties of the optimized formulation were identified by characterization and the suitability as a long-term injectable formulation platform was determined.

3. Materials and Methods

3.1. Materials

Rotigotine was purchased from Sigma-Aldrich (Oakville, Canada). Span 80 and tween 80 were purchased from Daejung (Gyeonggi-do, South Korea). Dipotassium hydrogen orthophosphate was purchased from Junsei chemical (Tokyo, Japan) Orthophosphoric acid was purchased from Dongyang chemical (Jeollanam-do, South Korea). Castor oil, cottonseed oil, safflower oil, sesame oil, soybean oil, triethylamine, methanol were purchased from Sigma-Aldrich (Oakville, Canada) Molecularporous membrane tubing was purchased from Spectrum Laboratories (Rancho Dominguez, USA). Male Sprague–Dawley (SD) rats (4 weeks old, about 150 g body weight) were obtained from Orient Bio (Seongnam, South Korea).

3.2. Preparation of rotigotine-loaded FLM

Castor oil, cottonseed oil, span 80 and ethanol were mixed over-night in an appropriate ratio to prepare FLM using hot plate with magnetic stirring(37℃, 200 rpm). After preparation of FLM, rotigotine was dissolved in FLM at a concentration of 9 mg / ml, and after 30 min in water bath sonication, the mixture was stirred for 2 h (25℃, 200 rpm).

3.3. Design of experiment(DoE)

Minitab® 16 package software was used for mixture design. In order to create a mixture design, number of components, degree of design and type of design had to be set into Minitab® 16 package software. The type of design was an extreme vertices design which is one of three design types the software can create for mixtures. The Extreme vertices design is suitable for use when components have upper and lower limits. By selecting a design degree, augmenting the center and axial points, and setting the upper and lower limits of each component, Minitab® 16 package software could present the points needed for the experiment. Also, in order to find an estimate of the experimental error in the process and get estimates of the components effect more

accurately, the center point was set to 3. Independent variables were castor oil (x_1), cottonseed oil (x_2) and span 80 (x_3). The established dependent variables were cumulative release of rotigotine for 120 h (y_1) and viscosity (y_2). The software suggested 11 points for experimentation. Judging from the experimental results, the 11 experimental test points provided adequate data for fitting and validating a full quartic regression model.

3.4. Measurement of solubility

20 mg of rotigotine was added to 2 g of each oil (castor oil, cottonseed oil, safflower oil, sesame oil, soybean oil) and stirred for 12 h (25℃, 200rpm). One gram of the stirred oil was centrifuged at 12,000 rpm for 30 min. 100 uL of the upper layer and 900uL of methanol are mixed, sonicated for 20 min, and vortexed for 30 sec. The mixture was then centrifuged again at 12000 rpm for 30 min, and 100 uL of the supernatant and 900 uL of methanol are mixed. The solubility of rotigotine in each oil was measured by HPLC system (Azura, Germany).

3.5. Optical microscopy

Optical microscopy (DS-12, Nikon, Japan) was used to investigate the structure of the FLM. Two types of pictures were taken that were in contact with or not in contact with PBS. First, 0.5 mL of FLM is dropped onto 10 ml of PBS. After 10 min, 20 μL of FLM in the upper layer of PBS was dropped and spread on the glass slide. And, the cover glass was placed on the glass slide to prevent air bubbles forming. Additionally photographs without exposure to PBS were taken. 20 μL of FLM was directly dropped and thinly spread on the glass slide. The cover glass was also placed on the glass slide.

3.6. Viscosity

The viscosity of the prepared formulations was determined using a viscometer (DV-I, Brookfield, USA) on 10 ml of the sample. The viscosity of the samples was measured at 25℃, 10 rpm (Cone Spindle CPA-42Z, Brookfield, USA). Samples were recorded after 10 min to stabilize temperature and rotating state of the samples in the chamber.

3.7. *In vitro* release study

The release medium was prepared by adding 0.02% (w/v) sodium azide and 2% (w/v) tween 80 to PBS. The rotigotine-loaded FLM of 0.5 mL and the release medium of 2 mL were transferred into dialysis tubes (Standard RC Tubing, MWCO: 6-8 Kd, Spectrum Labs, USA). The bag, sealed with clamps to prevent leakage, was then immersed into 200 mL of the release medium in a dissolution tester (DST-600A, Fine scientific instruments, South Korea). The entire system was kept at 37℃ with continuous stirring at 100 rpm. Two milliliter of the solutions was taken at determined time 0.5 h, 1 h, 2 h, 3 h, 6 h, 12 h 24 h, 48 h and 120 h to measure the drug concentrations in the dialysate with HPLC. Then, 2 mL fresh solution was added after sampling to keep the constant volume of the solution as 200 mL. The cumulative release was calculated as a percentage of the ratio of the amount of drug injected into the membrane to the amount of drug released outside the membrane.

3.8. HPLC

For HPLC (Azura, Germany) analysis, a reversed-phase Phenomenex, Luna C-18 column (250 x 4.6mm, Merck, India) was used. The mobile phase was consisting of phosphate buffer: MeOH (30:70, v/v%). One liter of phosphate buffer was composed of 5.22g disodium hydrogen phosphate, 0.726 g trimethylamine and adjusted to pH 5.5 with phosphoric acid. The injection volume was 20 µL. The flow rate was adjusted to 1.0 ml/min (Azura P 6.1L HPLC pump) and the wavelength was set to 225 nm (Azura DAD 2.1L UV/VIS detector). Samples were injected using an autosampler (Azura AS 6.1L sampler) after suitable dilution with the methanol, and the chromatograms were analyzed using ClarityChrom® software (Version 6.1.0.130, Knauer, Germany) provided with the system.

3.9. Animal study

Rats were acclimated for approximately 7 days before dosing. They had free access to food and water. All rats were taken care of by following the OECD guidelines for testing chemicals.

3.10. Morphology study in animal model

The morphology of the optimized FLM was evaluated using rat. First, 10 mg of coumarin was dissolved in 20 mL of LM-001, stirred at 37℃ for 30 min, and sonicated for 10 min. It was then cooled at room temperature for 1 h. One milliliter of coumarin-dissolved FLM was injected into the back of the rat with 23 gauge injection, and the morphological changes after 6 h and 7 days were observed.

4. Results and Discussion

4.1. Preliminary study for experimental design

The targeted formulation should be capable of slowly releasing the drug over a long period of time and at the same time being able to pass through a 23 gauge syringe and be naturally lost in the body over time. Five oil candidates were selected that could satisfy these conditions and the solubility of rotigotine in oils was analyzed (Fig. 2). Solubility of rotigotine in five oils was similar. Castor oil, which has the highest viscosity and low cost, span 80 to be used as a gelator, and ethanol to improve the ease of injection were combined in various ratios. Experiments were conducted to determine the viscosity of this pre-formulation and the possibility of passing through a 23 gauge syringe In this preliminary experiment, it was found that the viscosity should be 100-120 cP or less in order to pass through the 23 gauge syringe, and castor oil-only formulation could not pass a 23 gauge syringe. It was decided to make a formulation by adding cottonseed oil, which is relatively low viscosity oil. However, as the viscosity was greatly reduced due to the addition of cottonseed oil, ethanol was fixed at 8%, which is lower than the maximum value used in the preliminary experiment, and only the remaining castor oil, cottonseed oil and span 80 were selected as independent variables for DoE.

4.2. Experiment of extreme vertices design

This experiment is based on DoE using Minitab® 16 package software. We set the minimum and maximum values of each oil to be 20% and 70%, respectively, so that each oil can play a meaningful role based on the preliminary experiment results. Also, the span 80 used as gelator was set at the upper limit value of 18% and the minimum value was set at 2% based on the references. Based on the upper and lower limits of the set independent variable (Table.2), the extreme vertices design was constructed to have three center points. By setting the center point to 3, errors that occur experimentally can be evaluated. For each formulation FLM-001 to FLM-011 corresponding to these composition ratios, the cumulative release for five days and viscosity were set as dependent variables y_1 and y_2 to measure the significance of the formulation. The

cumulative release rate is a value associated with the goal of this experiment, and the current goal is to release over 90% over 30 days. We planned to optimize through the release profile for a shorter period. Also, in order to achieve the goal of passing 23 gauge, it was confirmed through a preliminary experiment that the viscosity should be less than 100-120 cP. Viscosity is also an important factor for achieving the goal. For each of FLM-001 to FLM-011, cumulative release rates for 5 days and (Fig.3) and viscosity (Fig.4) were measured, respectively. The results of the extreme vertices design based on the measured values are shown in Table.4. In order to assume a statistically significant model based on the results, the analyze process sets the terms as full quartic for each of y_1 and y_2, where x_1, x_2, x_3, x_2x_3, $x_1x_2(x_1-x_2)$, $x_1x_3(x_1-x_3)$, $x_2x_3(x_2-x_3)$ and $x_1x_1x_2x_3$ were selected in y_1, and x_1, x_2, x_3 and x_1x_2 were selected in y_2. The statistical analysis results obtained based on these selected terms are shown in Table 5 and Table 6 for y_1 and y_2, respectively. R^2 is the change degree in observed response value as described in the model. Assuming a model in the analysis of the experiment, if some of the interactions are omitted, it becomes a reduced model. If the p-value of the lack of fit in the reduced model is less than 0.05, then there is a problem with the assumed model. If the p-value is greater than 0.05, it is assumed that the hypothetical model is correct. For each of y_1 and y_2, the p value of all terms does not exceed 0.05, R^2 approximates to 1, and rack of fit exceeds 0.05, all of which suggest that it is a statistically significant model. The polynomials based on this results are as follows.

$$y_1=-(487x_1)+561x_2+7075x_3-21227x_2x_3+4071(x_1-x_2)x_1x_2-16933(x_1-x_3)x_1x_3+21636(x_2-x_3)x_2x_3+16984x_1x_1x_2x_3 \quad (1)$$

$$y_2=186x_1+76.1x_2-2.3x_3-121.5x_1x_2 \quad (2)$$

x_1, x_2, x_3 : independent variables
y_1, y_2 : dependent variables

The targeted y_1 value is 15% and the y_2 value is 90 cP. Assuming that the release consists of a time-proportional primary function, it is a value that can release 90% per

month, and y_2 is set to a relatively high viscosity value that allows the 23 gauge to pass smoothly. Based on this completed model, the range of y_1 and y_2 is set to 15±4% and 90±20 cP, and the area that satisfies the range is shown in the mixture contour plot in Fig.5. A common range satisfying all of the areas set in y_1 and y_2 is shown in the overlaid contour plot in Fig 6. Any composition within the white area shown here can suggest y_1, y_2 within the set area range.

4.3. Validation of the DoE model

We set the upper and lower bounds of y_1 and y_2 for extreme vertices design in Table 2, respectively, and obtained a contour plot for each of y_1 and y_2 (Fig.5). The white area of the overlaid contour plot suggests an area that commonly meets both upper and lower boundaries of these two dependent variables (Fig.6). Within this range, we obtained a formulation ratio that satisfies our target of y_1 as 15%, y_2 as 90 cP. The optimized formulation ratio of castor oil, cottonseed oil, span 80 and ethanol were 33, 46, 13 and 8 (%) respectively. The cumulative release for 120 h and viscosity of KJ-001 were 13.7% and 91 cP respectively, which was similar to the predicted value. Accuracies are 91.3% and 101.1%, respectively. In addition, an experiment was conducted in which one arbitrary composition for further verification. The arbitrary formulation ratio of castor oil, cottonseed oil, span 80, ethanol were 33, 46, 13 and 8% respectively. The predicted y_1 of arbitrary composition was 19% and the predicted y_2 of arbitrary composition was 83 cP. As a result of the actual experiment, y_1 of this composition was 19.5% and y_2 was 85 cP. Accuracy was 102.6 and 102.4% respectively. KJ-001 and arbitrary composition were all within 100±10% of the accuracy test, so that the set DoE model was validated well.

4.4. Optical microscopy

Optical microscopy photographs of the optimized formulation, KJ-001, were taken before and 1 h after application into PBS. (a) of Fig.7 was a photograph of only the formulation. It could be seen that the components were well mixed. (b) was a photograph of KJ-001 taken 1 h after application to PBS. Spherical shape of 10 μm in

diameter was observed. Each of them had multi-layers, and the inside and outside of the layer had the same color. It had been shown that vesicles can bind to each other to produce larger aggregates, suggesting that gel formation was composed of interconnections between small branches. As the ethanol spread into the PBS, the viscosity of the formulation increased as well (Fig.8). This showed that the formulation was in a form that allowed the ethanol to escape into the surrounding tissue after it had been injected into muscle. Fig.9 showed that KJ-001 was agglomerated like a gel 5 min after it was added to PBS. It could be expected that FLM at the in vivo injection site would also become harder.

4.5. Morphology study in animal model

The appearance of the KJ-001 formed in the subcutaneous region was observed after an autopsy. On the first day of injection, the formulation immediately flowed out after the incision and showed a less hardened state. However, at one week after the injection, the formulation hardened at the injection site and the viscosity increased to some extent. (Fig.10). This was probably due to the fact that ethanol had spread to the surrounding tissue and increased viscosity. In fact, the viscosity of the formulation after application of the formulation to PBS was about 1.4 times higher than that of the formulation alone (Fig.8). At 7 days, special indications at injection site and surrounding tissues were not observed with the naked eye, and no pathological changes or inflammation were observed.

5. Summary

We developed long acting injectable system for rotigotine. Rotigotine- loaded KJ-001 can release rotigotine for over 28 days without initial burst theoretically, indicating a good potential treatment of Parkinson's disease. The developed FLM has several advantages for long acting injectable system, such as simple preparation process, high encapsulation concentration of non-soluble drug, controlled drug release rate without initial burst. The FLM is a promising platform technology for not only rotigotine but also other drugs that need sustained release over a long period.

`6. Future study

So far, we have been able to confirm the possibilities and prospects of the FLM. However, in order for the FLM to appreciate its value, some additional experiments have to be performed. First, *in vitro* release test should be performed to confirm that rotigotine-loaded KJ-001 was actually releasing more than 90% of the rotigotine for one month. Secondly, stability test should be performed to confirm that there was no change in properties or drug solubility during storage of rotigoitne-loaded KJ-001 at various temperature conditions. Third, the morphology test using rat should be carried out for one month. Finally, by administrating rotigotine-loaded KJ-001 to rats, pharmacokinetic study should be performed to measure blood levels of rotigoitne. We are planning to complete this study by performing the above four additional experiments.

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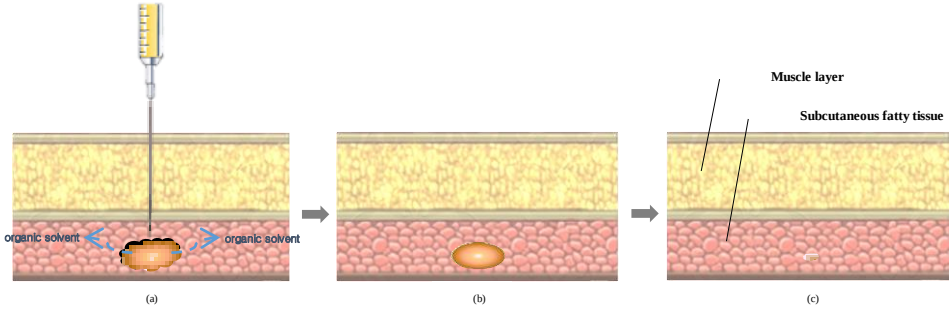


Fig. 1. Schematic illustration of the FLM. (a) Pre-solidification before diffusion of the solvent; (b) formation of FLM after diffusion of the solvent around tissues; (c) degradation of FLM for treatment period.

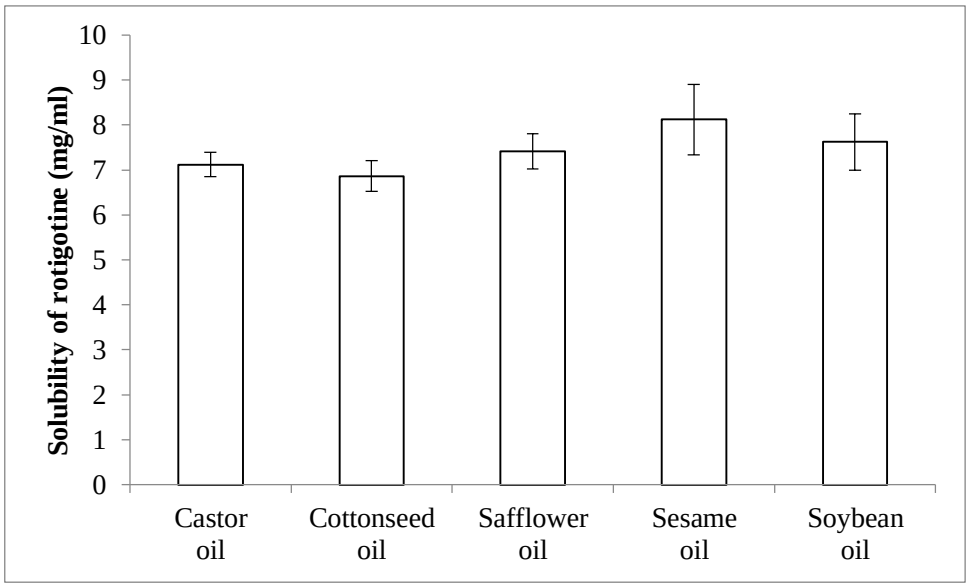


Fig. 2. Solubility of rotigotine in various oil.

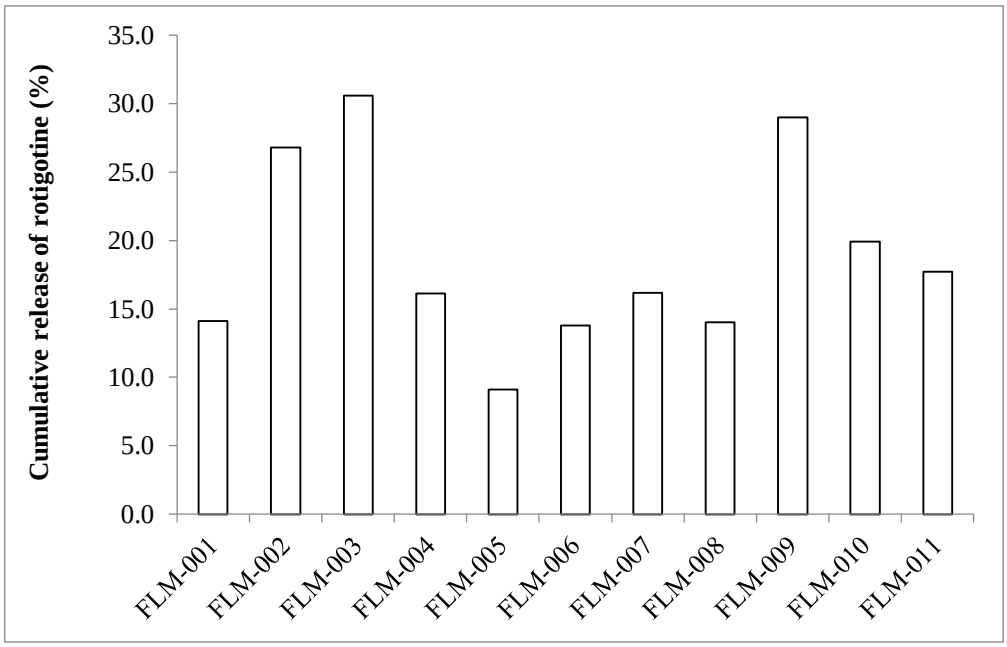


Fig. 3. Cumulative release of rotigotine from rotigotine-loaded FLM formulations for 120 h.

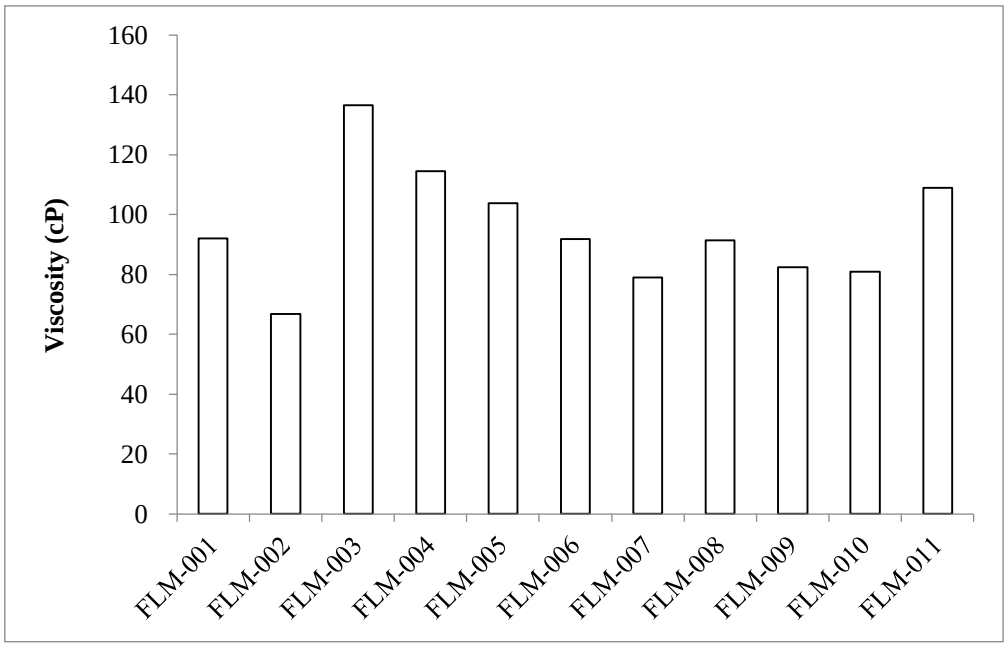
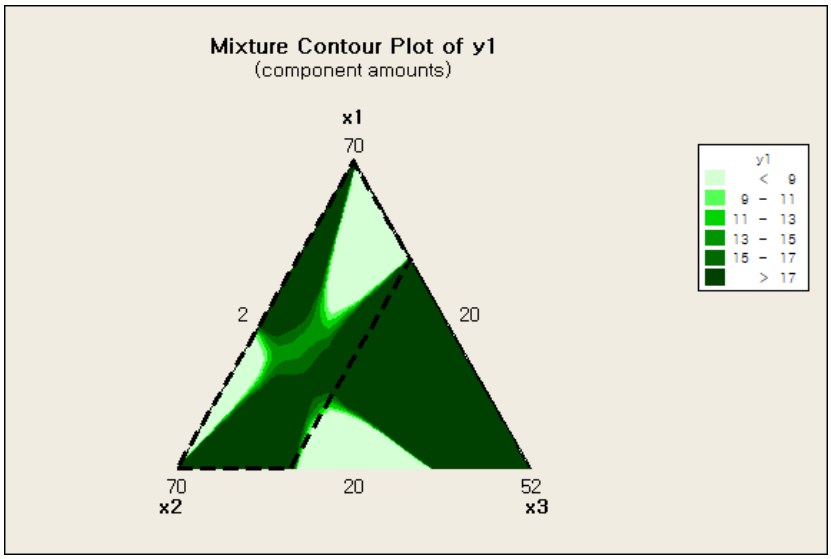
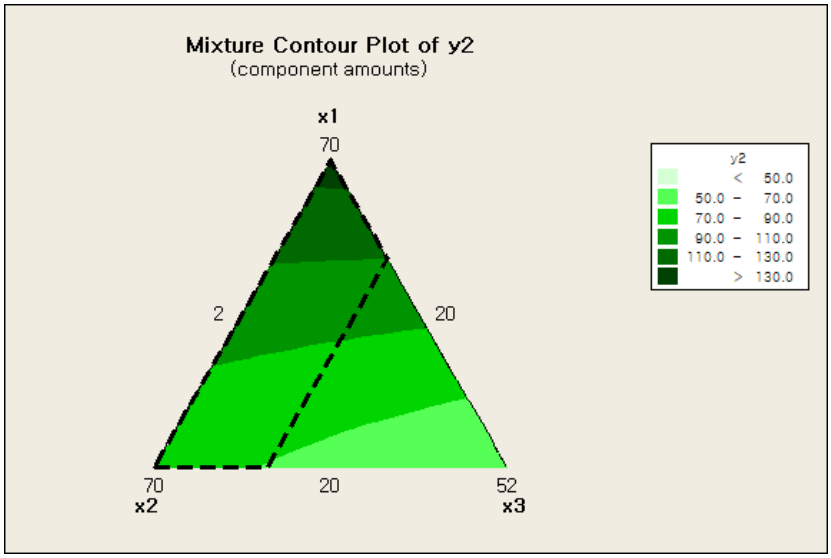


Fig. 4. Viscosity of FLM formulations.



(a)



(b)

Fig. 5. Mixture contour plots of cumulative release of rotigotine for 120 h and viscosity from DoE. (a) y_1 is cumulative release of rotigotine for 120 h;(b) y_2 is viscosity.

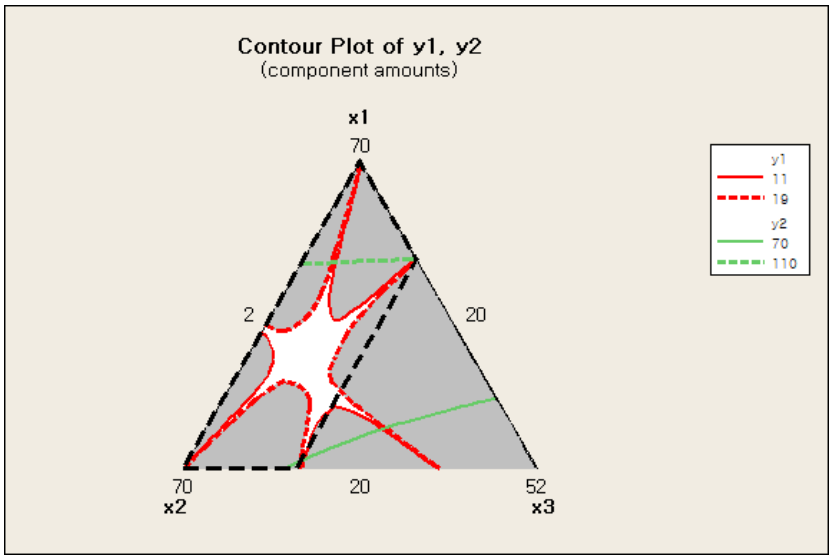
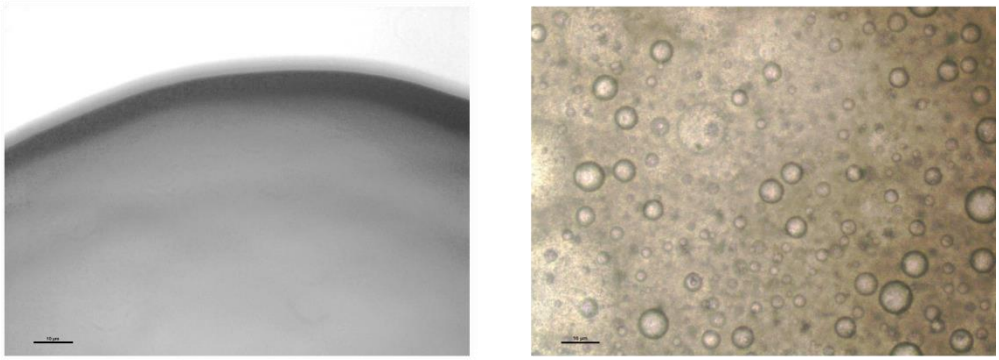


Fig. 6. Overlaid contour plots of cumulative release of rotigotine for 120 h and viscosity from DoE.



(a) (b)

Fig. 7. Optical microscope images of KJ-001. All images were taken at 400x magnification and the scale bar is 10 μm. (a) Photograph taken with only KJ-001; (b) photograph of KJ-001 which was in PBS for 1 h.

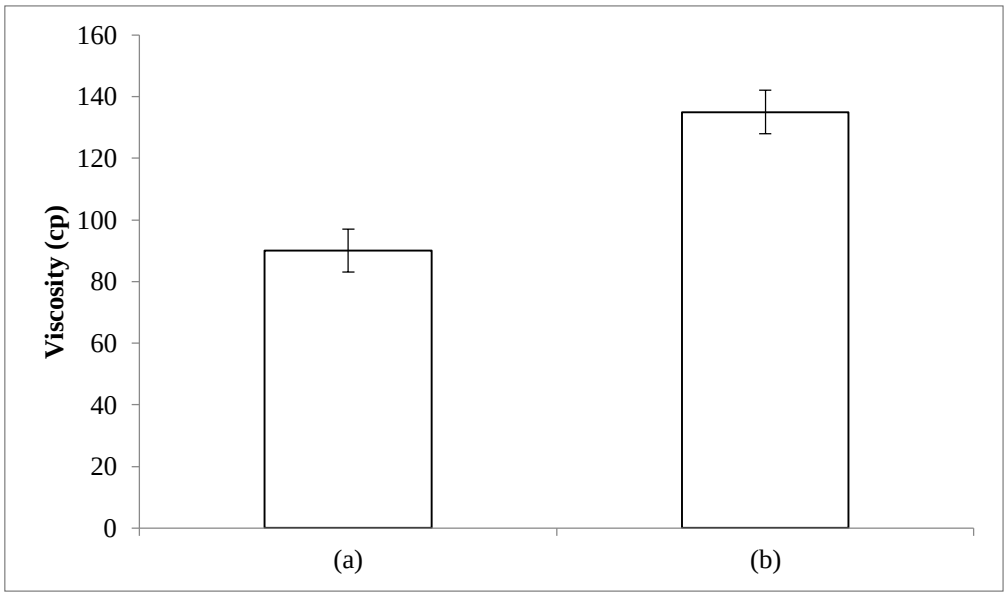


Fig. 8. Viscosity change of KJ-001. (a) The initial viscosity of KJ-001; (b) The viscosity of KJ-001 after 2 h in PBS. The viscosity of KJ-001 was increased after the organic solvent had spread into PBS.

Fig. 9. Image of KJ-001 applied into PBS. Five min after the addition of KJ-001 into PBS, KJ-001 clumped into one place and showed a gel shape.

(a) (b)

Fig. 10. Morphologies of KJ-001 and tissue around the injection site. (a) 6 h after the injection; (b) 7 days after the injection.

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Table 1. Composition ratio of the pre-formulations of FLM

Castor oil (%)	Span 80 (%)	Ethanol (%)	Viscosity (cP)	23gage syringability
85	5	10	246.1	X
80	10	10	235.3	X
70	20	10	210.6	X
65	25	10	191.1	X
60	30	10	196.2	X
55	35	10	194.4	X
40	50	10	161.4	X
30	60	10	153	X
20	70	10	171.6	X
10	80	10	151.1	X
60	25	15	126.6	O
55	30	15	142.8	O
50	35	15	145.8	O
45	40	15	129.6	O

Table 2. Range of independent and dependent variables

Independent variable	**Symbol**	**Minimum**	**Medium**	**Maximum**
Castor oil	x_1	20	45	70
Cottonseed oil	x_2	20	45	70
Span 80	x_3	2	10	18
Dependent variable	**Symbol**	**Minimum**	**Medium**	**Maximum**
Cumulative release	y_1	11	15	19
Viscosity	y_2	70	90	110

Table 3. Composition ratio of FLM formulations

Formulation	Castor oil (%)	Cottonseed oil (%)	Span 80 (%)	Ethanol (%)
FLM-001	20	70	2	8
FLM-002	20	54	18	8
FLM-003	70	20	2	8
FLM-004	54	20	18	8
FLM-005	41	41	10	8
FLM-006	31	56	6	8
FLM-007	31	48	14	8
FLM-008	56	31	6	8
FLM-009	48	31	14	8
FLM-010	41	41	10	8
FLM-011	41	41	10	8

Table 4. Design of FLM formulations in extreme vertices design

StdOrder	RunOrder	PtType	Blocks	Castor oil (%)	Cottonseed oil (%)	Span 80 (%)	Cumulative release of rotigotine for 120 h (%)	Viscosity (cP)
5	1	0	1	41	41	10	15.1	93.1
2	2	1	1	20	54	18	26.8	66.9
3	3	1	1	70	20	2	30.6	136.5
8	4	-1	1	56	31	6	16.1	114.6
9	5	-1	1	48	31	14	9.1	103.8
11	6	0	1	41	41	10	13.8	91.8
6	7	-1	1	31	56	6	16.2	78.9
10	8	0	1	41	41	10	13	90.5
7	9	-1	1	31	48	14	21.2	82.5
1	10	1	1	20	70	2	19.9	81.0
4	11	1	1	54	20	18	17.7	108.9

Table 5. Statistical analysis results of the optimal models in cumulative release of rotigotine for 120 h (y_1)

Term	Cumulative release of rogitoine for 120 h (y_1)	
	coefficient	p
x_1	-487	*
x_2	561	*
x_3	7075	*
x_2*x_3	-21227	0.017
$x_1*x_2*(-)^1$	4071	0.019
$x_1*x_3*(-)^2$	-16933	0.016
$x_2*x_3*(-)^3$	21636	0.018
$x_1*x_1*x_2*x_3$	16984	0.025
R^2 (%)	99.36	
Lack of fit	0.707	

[1] (x_1-x_2)

[2] (x_1-x_3)

[3] (x_2-x_3)

Table 6. Statistical analysis results of the optimal models in viscosity (y_2)

Term	Viscosity (y_2)	
	coefficient	**p**
x_1	186.0	*
x_2	76.1	*
x_3	-2.3	*
x_1*x_2	-121.5	0.019
R^2 (%)	98.39	
Lack of fit	0.132	

Table 7. Evaluation of DoE

Term	Cumulative release of rotigotine for 120 h (%)	Viscosity (cP)	Accuracy of cumulative release (%)	Accuracy of viscosity (%)
Formula 1(measured)	13.7	91	91.3	101.1
Formula 1(predicted)	15.0	90		
Formula 2(measured)	19.5	85	102.6	102.4
Formula 2(predicted)	19.0	83		

ABSTRACT

A study on the development of long-acting injectable formulation for rotigoitne

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Rotigotine is a selective dopamine agonist that is used in Parkinson's disease and restless leg syndrome. Currently, only transdermal dosage form – Neupro® is available. Although Neupro® has many advantages, it frequently causes side effects such as erythema, pruritus, and dermatitis on the application site, and there is a problem that the absorption difference varies depending on the patch attachment sites. In order to defeat problems and enhance patient's convenience, we designed the fluidal lipid matrix (FLM) for long-acting injection that can maintain the long efficacy of rotigotine that focused on minimizing initial burst and releasing rotigotine at constant rate for long period. The fluidal lipid matrix consists of castor oil, cottonseed oil, span 80 and ethanol. In order to find out the optimal composition for designing the formulations using these excipients, Design of Experiment (DoE) was conducted. In this study, we used Mixture designs among the various methods of experimental design method, and the optimization process is done through the Extreme vertices design which is suitable for use when the constituents have upper and lower limits. To optimize the compositon of the formulation, 11 formulations were prepared and their viscosity and *in vitro* release pattern for 120 h were evaluated. We evaluated the characteristics of optimized FLM and the results suggest that the developed formulation is potential as long-acting injection for rotigotine. Experiments that have not progressed due to time constraints have been summarized and planned to be completed later by carrying out these experiments.

Keywords: Rotigotine, Parkinson’s disease, Fluidal lipid matrix, Long-acting injection, Design of experiments (DoE)