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Lamotrigine Ultrafine Particles Preparation by Antisolvent Precipitation Technique with Salting Out Effect

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Abstract
Minimizing a drug's size is an effective means to increase its dissolution and hence the bioavailability, which can be achieved by specialized dispersion techniques. This strategy results in increased surface area, the potential to increase saturation solubility, and decreased diffusional distance, all of which lead to an increase in the extent and the rate of dissolution. The purpose of this research was to develop antisolvent precipitation system for the preparation of stable aqueous suspension from ultrafine particles of lamotrigine as a poorly water soluble drug. Use of high stream velocities enhances mixing of lamotrigine acetic acid solution with water, that water was containing of polymer or surfactant inhibitor, was prepared a solution with lamotrigine particles with size <250 nm. Several experimental parameters, such as the type of stabilizer, the concentration of stabilizer, the concentration of salt (NaCl) and the concentration of drug that affected on size of the particles were optimized by undertaking Taguchi experimental design methodology. Using different analytical tools, such as X-ray diffraction (XRD), dynamic light scattering (DLS) and differential scanning calorimetry (DSC), the effect of different parameters on the size of the produced particles was investigated. The results showed that the best stabilizer is PEG (poly ethylene glocal) 4 mg ml^{-1}, concentration of lamotrigine 10 mg ml^{-1} and NaCl concentration: 2 mol L^{-1}, that the produced submicrometer suspension had a mean particle size of 248.5 nm and size distribution 243.9 – 252.2 nm.

Keywords
Lamotrigine, Submicrometer Suspension, Antisolvent, Precipitation, Poorly Water Soluble Drug

1.Introduction

Many existing and new drugs fail to be fully utilized because of their limited bioavailability due to poor solubility in aqueous media. With the increasing number of new drug candidates, the number of those that have poor aqueous solubility is also on the rise. Poorly water soluble drugs, are preferably designed as oral dosage forms if the dissolution limit can be broken through. Micronization of drugs plays an important role in improving the drug dosage and therapeutic efficacy. The bioavailability of pharmaceuticals presented in a solid formulation strongly depends on the size, particle size distribution (PSD) and morphology of the particles. The particle size reduction is one of the methods which can achieve desired bioavailability of poorly soluble drugs, as the dissolution rate can be enhanced by reducing the particle size [1]. Reducing the size of drug particles as a result of increasing the surface area, leads to an increase in the dissolution rate of these drugs in aqueous environments such as body fluids. [2]. Lamotrigine [lamictal, 3, 5- diamino- 6- (2, 3- dichlorophenyl)- 1, 2, 4- triazine] is a poorly soluble drug in water used increasingly as an adjunctive and monotherapy in the management of a wide range of epilepsy syndromes. It has also been assessed for the treatment of various unrelated neurological conditions [3]. Precipitation of a solid solute is achieved in the LAS (liquid antisolvent) process through an increase in the molar volume of solution and, hence, a decrease in the solvent power for the solute by addition of a nonsolvent (antisolvent). Nucleation due to supersaturation attained by mixing of solute solution and antisolvent and simultaneous growth of nuclei by coagulation and condensation. Higher nucleation rates result in low or negligible growth and, hence, can potentially produce submicrometer particles. The stability of these particles in colloidal solution further depends on agglomeration or flocculation, driven by hydrophobic effects, electrostatic interaction and weak van der waals attractive forces. To control the particle size and PSD and to improve the

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stability, it is necessary to increase the nucleation rate, inhibit the particle growth, and control the agglomeration of particles by steric or electrostatic stabilization [4-6]. The use of a polymeric or oligomeric stabilization agent could protect the produced Lamotrigine particles from agglomeration [7- 9]. Among commonly used stabilization agent is the water soluble polymer. In this work the Lamotrigine in acetic acid solution may be mixed with an aqueous antisolvent solution in the presence of stabilizing agents to form homogeneous solution, but in presence of NaCl such as reagent with salting out effect, antisolvent precipitation system occurred to form submicrometer particles of lamotrigine that covered with stabilizer agent. Several experimental parameters, such as the type and concentration of stabilizer, the concentration of salt and the concentration of Lamotrigine were investigated with Taguchi experimental design. The obtained suspension was characterized by dynamic light scattering (DLS). The obtained suspension was then dried and the samples were taken for FT-IR, TG and XRD analysis. The results were compared with the unprocessed drug.

2.Experimental

2.1.*Materials*

Acetic acid with purity of 99.8% was prepared from Merck (Darmstat, Germany) as Lamotrigine solvent. Lamotrigine was a gift received from Shafa (Tehran, Iran) pharmaceutical company at a purity of 97% and was used without further purification. PVP (poly n-vinyl -2- pyrolidone) from lobachem (India), PEG (poly ethylene glocal) and SDS (sodium dodecyl sulfate) from Merck with purities better than 99.5 % were prepared as stabilizer agent. Dieonized Millipore water was used as an antisolvent. NaCl with purity of 99.9 % was purchased from Merck Company.

2.2.Production of submicrometer suspension

Antisolvent precipitation technique by using of agent with salting out effect was used to produce the lamotrigine submicrometer particles. The theoretical basis of this method is that a solution of Lamotrigine in acetic acid with certain concentration is rapidly dispersed through a needle of syringe with pressure of plunger in an aqueous saline antisolvent solution in the presence of stabilizing agents to form a suspention solution of submicrometer particles of lamotrigine in water.

2.3.Analysis

Dynamic light scattering (DLS, Brookhaven model 90 plus, USA) was used to measure the mean particle hydrodynamic diameter and PSD of suspension. Measurements were made using 666 nm laser light with a 90 ˚ scattering angle at room temperature. Before measurement, the suspension solution was dispersed by ultrasonic waves with power 100 W for 1 min. analytic software was used automatically used to assay the mean particle size and PSD. At least the scattering intensity of 10000 counts/s is the mean of three measurements of 120 s each. The polydispersity index that is a measure of dispersion homogeneity and ranges from 0 to 1 was recorded, the values close to 0 indicate a homogeneous dispersion while those greater than 0.3 indicate high heterogeneity [10].

The XRD pattern of the unprocessed drug and the processed substances were evaluated using an XRD (STOEMP, Germany). The sample was irradiated using a Cu target tube, and exposed to all lines. A monochromator was used to select the $k\alpha_1$ line (λ=1.54 Å). The scanning angle ranged from 0 to 35 ˚ of the diffraction angle (2θ), and the counting time used was 0.6 s/step in steps of 2θ=0.05˚. The scanning rate used was 5˚/min. the excitation current used was 30 mA and the excitation voltage used was 40 kV.

The IR spectra (400-4000 cm^{-1}) in KBr were recorded with the aid of a Vertex 70 spectrometer. Thermogravimetric analysis was carried out using the Rheometric scientific simultaneous thermal analyzer.

2.4.Optimization of procedure

Taguchi method has been known as a powerful tool for DOE in the various fields of sciences and engineering. It is used to investigate the effects of various factors on process when the factors are engaged in that process. Therefore, with a limited number of tests, the sensitivity of the process to each parameter is determined. In addition, it becomes possible to identify synergistic effects between parameters. In Taguchi method, all factors affecting the process quality can be divided into two types: control factors and noise factors. Control factors are those that are adjustable while noise factors, such as the ambient humidity and aging of parts, are those undesired variables that are difficult, impossible or expensive to control. Taguchi method uses signal to noise (S/N) ratio for robust quality maintenance from the influence of noise factors. The S/N ratio means the ratio of the power of signal input to the power of noise output. If a factor corresponding to a large value of S/N ratio of each control factor is selected, it will be robust when subjected to noise. The definition of S/N ratio is different according to on objective function that is a characteristic value. There are three kinds of characteristic values nominal is best (NB), lower is better (LB) and higher is better (HB). Since the small particle size of lamotrigine is the aim of DOE, here in LB must be chosen. The S/N ratio of LB is calculated by:

$$\frac{S}{N} = -10 \times Log(\frac{1}{n}\sum_{i=1}^{n} Y_i^2) \qquad (1)$$

Where n is the sampling size and Yi is the response at each sampling point. In this work, L9 orthogonal array with four factors at three levels was chosen as follows: stabilizer type and concentration of stabilizer, concentration of drug and concentration of salt (Table 1).
It consisted of nine experiments corresponding to the nine rows and four columns. Each experiment was repeated twice under the same conditions to determine the effects of noise sources on the process (Table 2).

Analysis of variance (ANOVA) is one of the important statistical tools, which used to uncover the main and interaction effects of variables. It is also used to identify the process for parameters that are statistically significant. In ANOVA, F ratio is employed to recognize these significant parameters from other ones. It is the ratio of the variance estimate for the treatment effect to the variance estimate of the error. The large value of F ratio means that the selected parameter has a significant effect on the quality characteristics compared with the error variation.

Table 1. The four factors involved in the antisolvent process and three levels used in the design of experiments

Factor	Variable name	levels		
		1	2	3
A	Stabilizer type	PEG	SDS	PVP
B	Concentration of stabilizer (mg ml^{-1})	0	2	4
C	Concentration of lamotrigine (mg ml^{-1})	5	10	20
D	Concentration of salt (mol L^{-1})	2	4	6

Table 2. Arrangement of the nine experiments according to the L_9 orthogonal array design

Run	Factors				Series	Average size as response for DOE(nm)	S/N ratio	Particle size distribution (nm)	Poly dispersity
	A	B	C	D					
1	1	1	1	1	Series 1	351.2	-51.01	345.9-357.4	0.060
					Series 2	359.1		340.7-374.1	0.081
					Mean Y	355.15			
2	1	2	2	2	Series 1	327.7	-50.36	226.4-435	0.435
					Series 2	331.5		216.5-440	0.352
					Mean Y	329.6			
3	1	3	3	3	Series 1	414.4	-52.46	330.4-594.8	0.468
					Series 2	425.3		335-600.1	0.521
					Mean Y	419.85			
4	2	1	2	3	Series 1	488.3	-53.81	391.0-589.1	0.108
					Series 2	492.1		375.2-620.2	0.204
					Mean Y	490.2			
5	2	2	3	1	Series 1	604.2	-55.52	465.8-736.9	0.362
					Series 2	589.1		473.7-750.7	0.409
					Mean Y	596.65			
6	2	3	1	2	Series 1	487.1	-53.69	420.8-1360.5	0.367
					Series 2	479.9		404.1-1420.1	0.427
					Mean Y	483.5			
7	3	1	3	2	Series 1	720.4	-57.35	506.7-1420.6	0.405
					Series 2	753.1		550.8-1505.7	0.478
					Mean Y	536.75			
8	3	2	1	3	Series 1	502.1	-54.10	494.7-511	0.0931
					Series 2	512.3		487-535.1	0.164
					Mean Y	507.2			
9	3	3	2	1	Series 1	248.5	-47.94	243.9-252.2	0.093
					Series 2	250.7		223.1-272.7	0.085
					Mean Y	249.6			

3.RESULT AND DISCUSSION

Figure 1 shows the particle size distribution and cumulative distribution obtained by DLS in test conditions 9 and 5, which are reported in Table 2 in the first series of runs. The results of average size, particle size distribution (PSD) and

polydispersity was taken from DLS experiments, with duplicate measurement, were given in Table 2. The average size of particles was selected as response.

As results of Table 2 indicates polydispersity in four batches 1, 4, 8 and 9 are in the acceptable range <0.3. In other batches a high polydispersity index is seen that indicate high heterogeneity in these batches [10]. As the Table 2 shows, the mean particle size of Lamotrigine varies from one run to another. For instance, the maximum and minimum values for mean particle size were 736.75 and 249.6 (nm) respectively (experiment number 7 and 9). It suggests that one or some of the factors can be markedly significant in the process.

From Table 2, it is clear that S/N ratio possess the optimal condition for experiment number 9. As illustrated in Fig. 2, for factor A and D the lower levels and for factor B, the higher level and for factor C the middle level are most desirable for reducing the particle size of lamotrigine because of their large S/N ratio values. The concentration of lamotrigine (C factor) is the most effective factor in minimizing the particle size of lamotrigine.

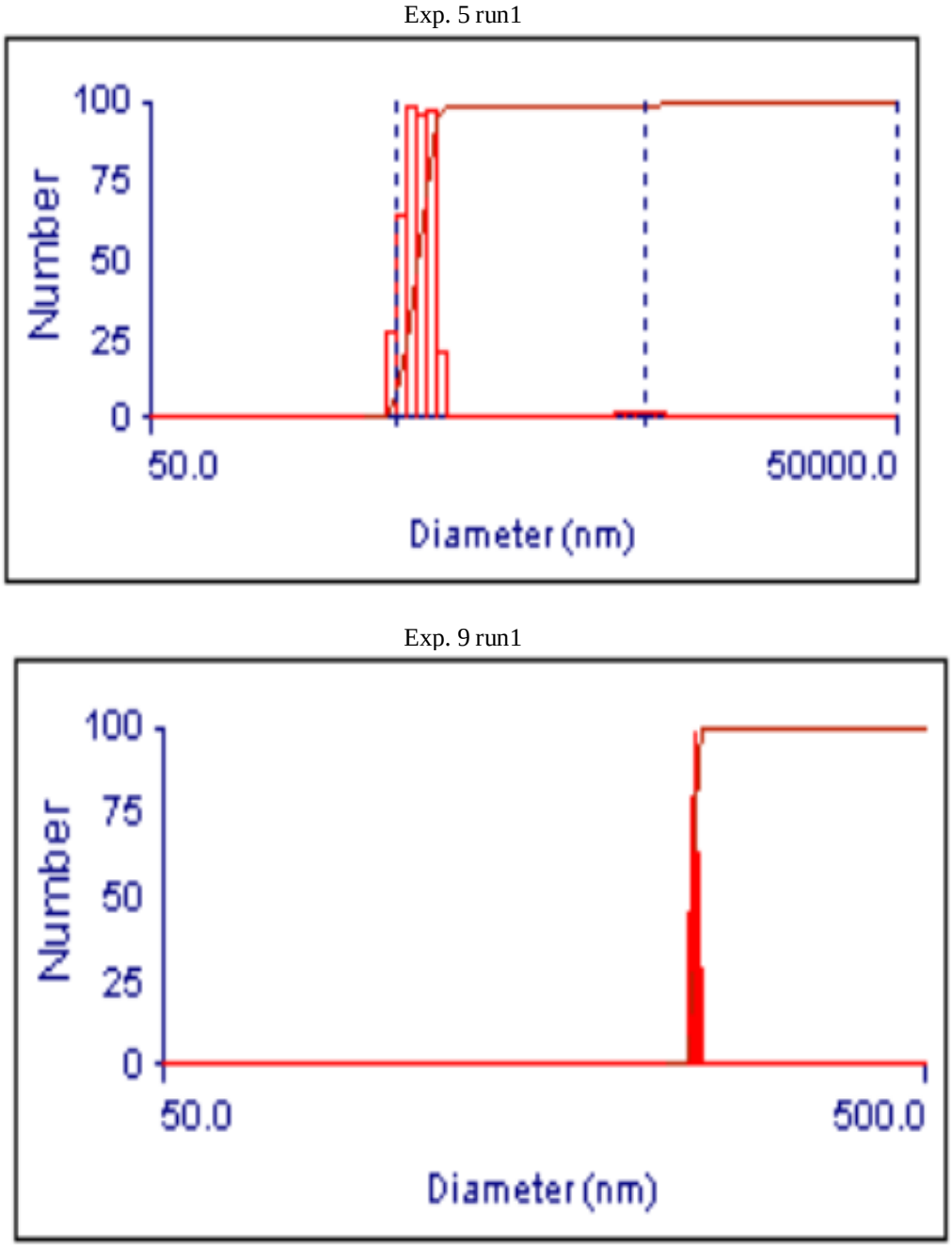

Fig. 1. Dynamic light scattering analysis (gussion and cumulative distribution) of lamotrigine particles suspended in water

The second important factor is stabilizer type (A factor). The third factor is the concentration of stabilizer (B factor) and the followed by the concentration of the salt (D factor).

According to Taguchi, the S/N ratio is employed to find the optimal condition for a process or a product. Therefore, as is shown by Table 3, smallest the absolute values of the S/N ratios for each factor are devoted to the optimal condition of that factor among three levels. As a result, the optimal condition of levels for each factor is to be A1, B3, C2 and D1.

The results of complementary experiment show that the practical optimal condition is in good agreement with predicted values of S/N ratios which obtained by the software. Results in Table 2 shows that the smallest particles obtained from the test number 9. The condition of levels for each factor in this run is to be A3, B3, C2 and D1 that are very similar to the optimal conditions. Table 4 is analysis of variance for standard results as the average value. The results of Table 4 have shown that the all of the factors have significant effects in the process with regards to larger values of F ratios. In typical FT-IR spectra of the pure unprocessed lamotrigine and prepared from experiment number 9, the intensity of the spectra of the submicronized lamotrigine was weakly compared with the unprocessed lamotrigine but was similar. In Fig. 3 thermogram of the pure unprocessed lamotrigine (a) and submicrometer particles of lamotrigine prepared from experiment number 9 (b) and experiment number 8 (c) from first series are reported. As a result from Fig. 3-a, melting point for lamotrigine is 220 ˚C, in Fig 3-b melting point increases toward 300 ˚C. Masking of lamotrigine with PVP is reason for this observation. In Fig. 3-c compared to 3-b, due to the increase in the size of the particles prepared in experiment number 8 compared to experiment number 9, the melting point increases by more than 300 °C.

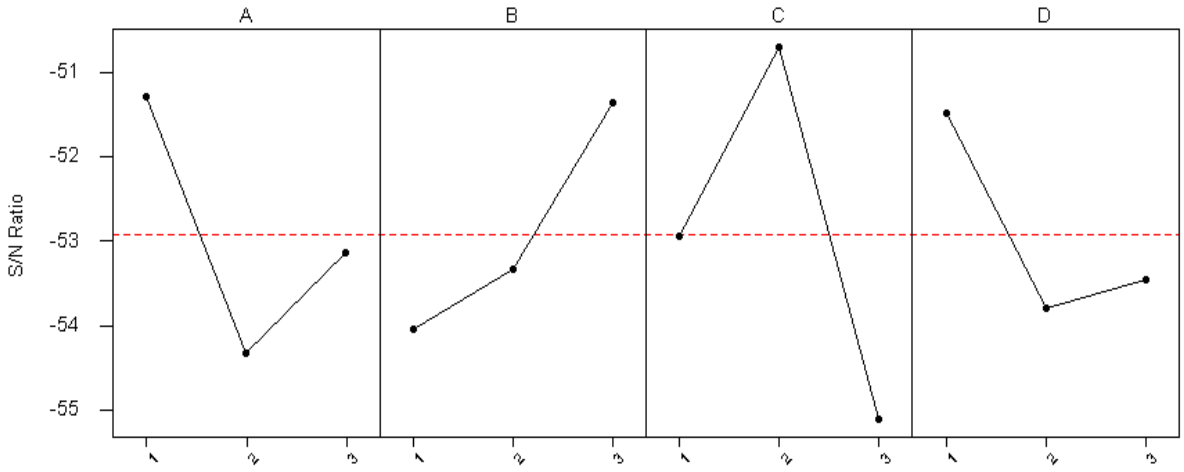

Fig 2. Main effects of factors for mean particle size

Table 3. The S/N ratio values for each of the factors with related levels

Level	A	B	C	D
1	-51.28	-54.05	-52.93	-51.49
2	-54.34	-53.33	-50.70	-53.80
3	-53.13	-51.37	-55.11	-53.46
Rank	2	3	1	4

Table 4. Analysis of variance for standard results as the average value

Factors	DOF	Sum Of Squares	Variance	F- Ratio	Pure Sum	Percent
A	2	83,133.819	41,566.909	448.516	82,948.466	23.951
B	2	63,321.464	31,660.732	341.626	63,136.111	18.230
C	2	157,789.252	78,894.626	851.290	157,603.899	45.508
D	2	41,242.261	20,621.130	222.506	41,056.908	11.855
Other/Error	9	834.088	92.676			0.456
Total	17	346,320.887				100.000

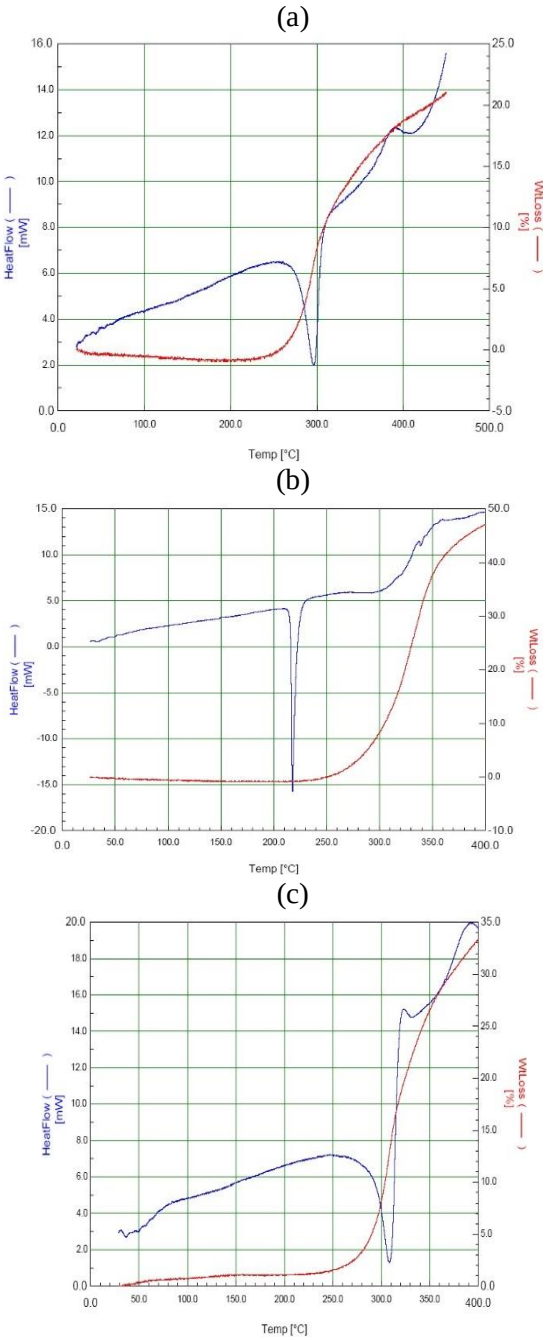

Fig. 3. Thermogram of (a) unprocessed Lamotrigine (b) prepared submicrometer particles of Lomotrigine from experiment number 9 first series (c) prepared submicrometer particles of Lomotrigine from experiment number 8 first series

The obtained aqueous suspension from experiment number 9 first series after evaporation and pulverizing and the obtained aqueous suspension from experiment number 8 first series after centrifuging, drying and pulverizing were analyzed by XRD and compared with the unprocessed lamotrigine and PVP. The intensity of the XRD- peaks of the processed lamotrigine are lower and broader to compare with the unprocessed lamotrigine. This result indicates a reduction in the degree of crystallinity of lamotrigine after processing. This decrease in crystallinity can be helpful for the better dissolution of lamotrigine particles.

Also, reduce particle size increase the surface area of particles significantly may be another reason to explain the decreasing intensity and broadening in peak in comparison between XRD patterns obtained from powder of experiment number 9 relation to experiment number 8.

4.CONCLUSIONS

Antisolvent precipitation technique with salting out effect was successfully used to produce the lamotrigine particles in the submicrometer size particles (<250 nm). The influences of the factors in this process on the size and size distribution were optimized by Taguchi experimental design methodology. The results show that the concentration of the lamotrigine is most effective operation parameter and PEG is best stabilizer.

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تهیه ذرات فوق ریز لاموتروجین با تکنیک رسوب‌دهی به روش ضد حلال با اثر نمک

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چکیده

به حداقل رساندن اندازه ذرات دارو یک وسیله موثر برای افزایش انحلال آن و در نتیجه افزایش دسترسی در محیط بیولوژیک می‌باشد که می‌تواند با تکنیک‌های پراکندگی تخصصی بدست آید. این روش منجر به افزایش سطح تماس، پتانسیل افزایش حلالیت اشباع و کاهش فاصله انتشار می‌شود که همه این موارد موجب افزایش مقدار و سرعت انحلال می‌گردد. هدف از این کار تحقیقاتی، توسعه سیستم رسوب‌دهی به روش ضدحلال برای تهیه سوسپانسیون آبی پایدار از ذرات بسیار ریز لاموتروجین به عنوان یک داروی کم محلول در آب می‌باشد. استفاده از سرعت جریان بالا، باعث افزایش سرعت اختلاط محلول اسید استیکی حاوی لاموتروجین با آب می‌گردد. آب حاوی پلیمر یا سورفکتانت بازدارنده بود، در نتیجه محلول با ذرات لاموتروجین با اندازه کمتر از ۲۵۰ نانومتر تهیه شد. چندین پارامتر تجربی که بر اندازه ذرات تاثیر می‌گذارد مانند نوع تثبیت‌کننده، غلظت تثبیت‌کننده، غلظت نمک (NaCl)، و غلظت دارو به روش طراحی آزمایش تاگوچی بهینه شدند. با استفاده از تجهیزات تجزیه‌ای مختلف مانند پراش پرتو ایکس (XRD)، پراکندگی نور دینامیکی (DLS) و کالریمتری اسکن تفاضلی (DSC)، تاثیر پارامترهای مختلف بر اندازه ذرات تولید شده بررسی گردید.

نتایج نشان داد که بهترین تثبیت‌کننده PEG (پلی اتیلن گلیکول) با غلظت ۴ میلی‌گرم در میلی‌لیتر، بهترین غلظت لاموتروجین ۱۰ میلی‌گرم در میلی‌لیتر و غلظت NaCl ۲ مول در لیتر می‌باشد، که سوسپانسیون با اندازه کمتر از میکرومتر تولید شد. میانگین اندازه ذرات ۲۴۸/۵ نانومتر و توزیع اندازه ذرات ۲۵۲/۵– ۲۴۳/۹ نانومتر است.

واژه‌های کلیدی

لاموتروجین؛ اندازه ذرات کمتر از میکرومتر؛ ضد حلال ؛ رسوب دهی؛ داروی کم محلول در آب.