FORMULATION AND EVALUATION OF TOPICAL SLN BASED GEL OF CROMOLYN SODIUM FOR ANTI- INFLAMMATORY ACTIVITY

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Abstract: The main objective of present work was to prepare and evaluate SLN based topical gel of Cromolyn sodium for treatment of inflammation. SLN were formulated by hot homogenization technique, by use of design expert software. The influence of independent variables lipid and surfactant on properties of SLN such as particle size and drug release, was studied using central composite design. The in-vitro drug release rate of gel was evaluated using franz diffusion cell containing cellophane membrane with phosphate buffer pH 6.8 as a medium. From all formulations and the results batch 7 was selected as an optimized batch that showed 95.04% drug release after 8 hr.
The different parameters like pH, viscosity, spreadability are also evaluated. It is concluded that Cromolyn sodium loaded SLN based gel containing cocoa butter as a lipid and tween 80 as a surfactant was suitable for topical application and shows effective anti-inflammatory activity.

Index terms: SLN, optimization, topical gel.

INTRODUCTION

One of the pathophysiological conditions of living tissue is an inflammation. It leads to the accumulation of plasmatic fluid during an infection or injury and upholds tissue homeostasis in injurious situations. Inflammation is the major response of the body in conflict of an infection on the tissue damage. Mediators are any messenger that acts on blood vessels, inflammatory cells or other cells to contribute to an inflammatory response. Allergic or chemical irritation, injury/damage or infection may activate the mediator [1].
Severity of the injury is depending upon the extent of the injury. These are pro-inflammatory fundamental factors. They bind to specific target receptors present on the cells and increases the vascular permeability, support neutrophil chemotaxis, excite smooth muscle contraction, expand direct enzymatic activity, and induce pain. Chemical mediators include prostaglandins, nitric oxide, cytokines such as interleukin-3 (IL-3), IL-4, IL-5, IL-6, IL-10, and IL-13, histamine, leukotrienes, and serotonin [1,2].
There are many problems in the formulation of drugs such as low bioavailability, low solubility, and some stability issues. To conquer these problems, drug should be formulated as a novel drug delivery system. One of the novel approaches is nanotechnology [3]. Solid lipid nanoparticles (SLN), which were invented in 1991. It is one of the conventional approaches of nanotechnology. It is used for developing new therapeutics with various uses [4,5]. They are included in controlled and targeted drug delivery systems. Combination of drugs into nanocarriers sets a unique example in drug delivery system which could be used for drug targeting. Hence, solid lipid nanoparticles hold great potential for site-specific drug delivery systems and hence attract researchers [6-9].

MATERIALS AND METHODS

Materials

SLN were prepared using are Cocoa butter was obtained from (New Neeta Chemicals) as solid lipid, Tween 80 was obtained from (Loba Chemie Pvt., Ltd.) as surfactants, and Cromolyn sodium was obtained from (Micro Labs) as an active ingredient. For formulating SLN into gel Carbopol 945 (Loba Chemie Pvt., Ltd.) as a gel base and triethanolamine (Loba Chemie Pvt., Ltd.) as a viscosity modifier, propylene glycol, and glycerol (Loba Chemie Pvt., Ltd.) as a stabilizer.

Methods

Preparation of SLN

Hot homogenization technique was used for the preparation of Cromolyn sodium loaded SLNs. Optimization studies were performed by using Design Expert 11 software. Central composite factorial design was selected for the optimization studies. Consist of 20 runs and 2 levels and 3 factors. Optimization was performed to find out the level of independent variables. And that will yield a smallest value of Particle size (Response 1), and Drug release (Response 2).

Initially, solid lipid (cocoa butter) was melted. And then for the formation of lipid phase Cromolyn sodium was added. The surfactant was added to hot distilled water to form an aqueous phase. Both these phases were mixed together. And they were subjected to high shear homogenizer. Temperature was maintained for three hours. After three hours they were allowed to cool down. [9,10]

Independent variables and their corresponding levels of Cromolyn sodium loaded SLN preparation for Central composite factorial design are stated in Table 1 and the formula table of preparing Cromolyn sodium based SLNs is stated in Table 2 formulation of SLN based gel.

Table 1 levels of Cromolyn sodium loaded SLN preparation for central composite factorial design

VARIABLES	LEVELS	
	-1	+1
Concentration of lipid (mg)	300	600
Concentration of Surfactant (%)	2	4
Stirring speed (rpm)	1200	1600

Table 2 Formulation table of SLN

Batch no.	Factor 1 (Lipid Concentration/ X_1) mg	Factor 2 (Surfactant Concentration/ X_2) %	Factor 3 (Stirring Speed/ X_3) rpm
1	1	-1	1
2	-1	-1	-1
3	+1	1	1
4	1	1	1
5	-1	+1	-1
6	-1	1	1
7	1	1	1
8	1	1	1
9	-1	+1	+1
10	1	1	1
11	1	1	1
12	+1	+1	-1
13	1	+1	1
14	1	1	+1
15	+1	-1	+1
16	+1	+1	+1
17	-1	-1	+1
18	+1	-1	-1
19	1	1	1
20	1	1	-1

1-2% Cromolyn sodium loaded SLN was prepared. 0.3% of Carbopol was dispersed in water for 24 hrs to avoid the formation of lumps. After 24 hrs add 1-2% SLN dispersion and was stirred it using mechanical stirrer. Sufficient quantity of propylene glycol and glycerol was added to it. 2–3 drops of triethanolamine was added. As soon as, the triethanolamine was added the solution turned viscous and gel was formed. [11]

Characterization of SLN

Particle size

Digital microscope (Labomed Lx 300 Binocular) was used for the determination particle size analysis. The software used was PixelPro. It was cleaned properly. Stage micrometer was used for the calibration of microscope. A drop of SLN dispersion was placed on a clean glass slide. This slide was placed on the stage. The microscope was adjusted. The image of visual SLN was captured. PixelPro software was used for determination of size of nanoparticles. The particle size was checked in a triplicate manner. [12,13]

In vitro drug release studies

Drug release studies were done using the artificial cellophane membrane of molecular weight 10,000 - 12,000. Apparatus used was vertical Franz diffusion cell. Phosphate buffer of pH 6.8 was prepared. The prepared buffer was filled into the diffusion cell up to the mark. This compartment is called a receptor compartment. The cellophane membrane was dipped in hot water and then was placed between two halves of the cell. The upper half part of the cell is called donor compartment. The temperature of the receptor compartment was maintained at 37±5°C and was stirred continuously using magnetic stirrer. SLN dispersion equivalent to 5-10 mg was placed on the cellophane membrane facing donor compartment. 1 ml of the sample from the receptor compartment was withdrawn and was diluted up to 10 ml with distilled water. UV analysis was done to calculate concentration. Similarly, after every 1 h, the sample was withdrawn and was subjected to UV analysis to calculate concentration. The results of Spreadability and drug release are stated. The UV analysis was done in triplicate manner. The study was carried out for 8hrs [7,9,13, 14,15].

pH measurement

pH measurement was done using a pH meter. Dissolve SLN equivalent to 1 g in 100 ml of distilled water. Keep aside for 2 h. Using standard buffers, check out the pH by dipping the rods into the buffers. After this, dip the rod in solution of SLNs. It was done in triplicate manner. [16, 17]

Stability study

For the stability study, the optimized formulation is stored at 5°C and 45°C for 3 months. After 3 months, the formulations were tested for particle size, entrapment efficiency, and drug release. [18]

Characterization of gel formulated from an optimized batch of Cromolyn sodium loaded SLN based gel [19,20]

The physical evaluation of gel was tested for homogeneity, color, and appearance.

pH

This was checked using pH meter. One gram of the gel was dissolved in 100 ml of distilled water. It was kept aside for 2 h. The rod was dipped in solution of gel.

Viscosity

This was checked using Brookfield's viscometer. The gel was tested for its viscosities at ambient temperature. They were tested by considering revolutions per minute (r.p.m). They were tested at 5, 10, 20, 50, and 100 rpm.

Stability study

For the stability study, the gel formulation is stored at 5°C and 45°C for 3 months. After 3 months, the formulations were tested for physical parameters, viscosity, and spreadability.

Spreadability

Clean glass slides (Chromatography plates) were taken. One gram of the gel was weighed and was placed on the glass slide. The other glass slides were placed over it. Place 100 g of weight over it. The diameter of the spread gel was calculated. It was done in a triplicate manner.

In vitro drug release

Phosphate buffer of pH 6.8 was prepared. The prepared buffer was filled into the diffusion cell up to the mark. This compartment is called a receptor compartment. The cellophane membrane was dipped in hot water and then was placed between two halves of the cell. The upper half part of the cell is called donor compartment. The temperature of the receptor compartment was maintained at 37±5°C and was stirred continuously using magnetic stirrer. One-gram LEO loaded SLN based gel was placed on cellophane membrane facing donor compartment. 1 ml of the sample from the receptor compartment was withdrawn and was diluted up to 10 ml with ethanol. UV analysis was done to calculate concentration. Similarly, after every 1 h, the sample was withdrawn and was subjected to UV analysis to calculate concentration. The UV analysis was done in triplicate manner. The study was carried out for 8 h.

RESULTS AND DISCUSSION

Particle size and drug release of Cromolyn sodium loaded SLNs.

Response 1: Particle size

Final equation in terms of coded factors

Particle size = 35.08-0.0143*A+1.26*B+0.0956*C+0.1437*AB-0.9513*AC-0.3013*BC

The particle size parameter showed that as the concentration of surfactant increases, the particle size increased. The particle size of all the batches ranges from 30.91±0.30 to 38.36±0.13 nm. Batch 7 was found to have the lowest particle size.

Effect of concentration of lipid and surfactant on particle size

When Increased concentration of cocoa butter (lipid) after an increase in particle size. The fact that the size of lipid nanoparticles is highly reliant on lipid concentration can be described in terms of the propensity of lipid to coalesce or unite at high lipid concentration. According to Stake's law, this kind of behavior can be described by the difference in density between internal and external phases. Increase in particle size of SLNs because of a reduction in the diffusion rate of the solute molecules in the outer phase as a result of viscosity increases in the lipid-solvent phase. Moreover, the increase in particle size might be due to increased quantity of lipid which provides additional space for drug molecules to get entrapped. [21]

And increasing the concentration of surfactant, the particle size was found to get decreased. This might be due to the surfactant-induced decrease in surface tension between the aqueous phase and organic phase. Besides, the surfactant helps to stabilize the fresh generated surfaces and avoids particle aggregation. Higher concentrations of surfactants permit improved stabilization of the smaller droplets of lipids and thus prevent them from aggregating into larger droplets [22] (Figs. 1 and 2).

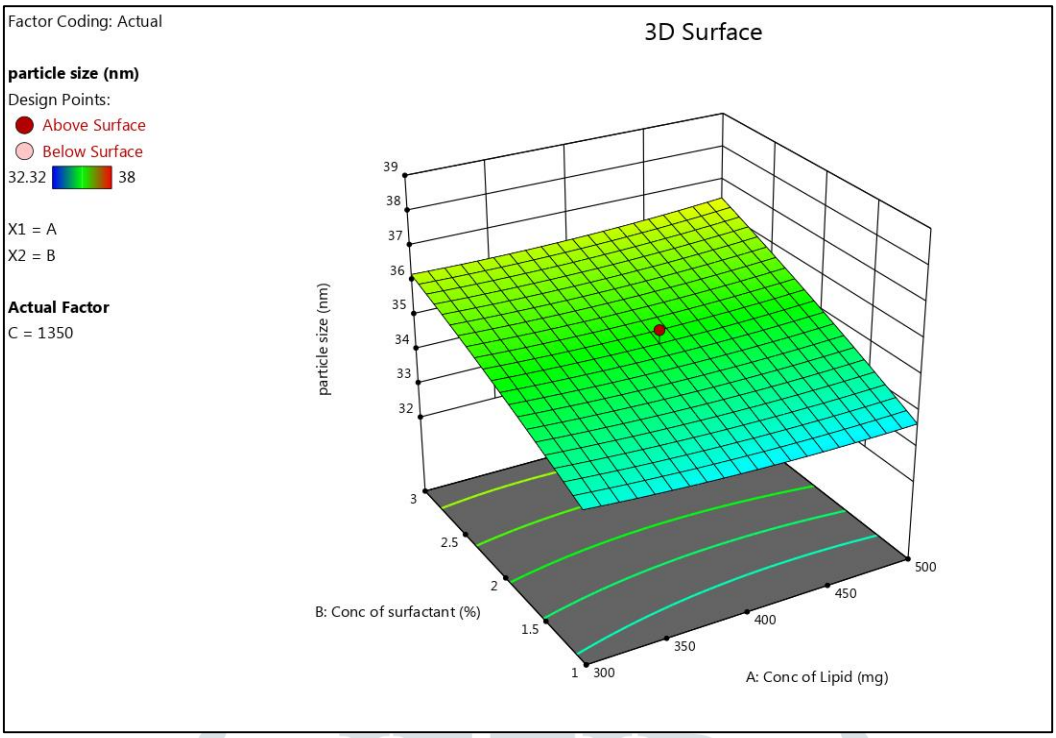

Figure no.1 Three-dimensional plot for effect of concentration of lipids and concentration of surfactant on particle size

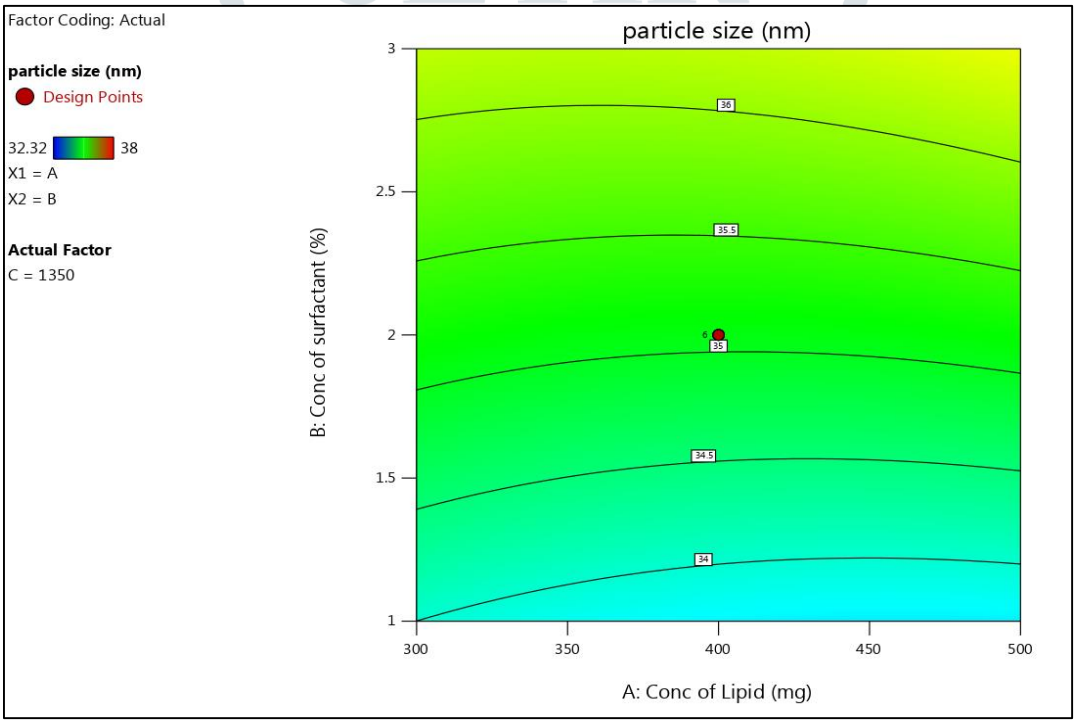

Figure no. 2 Contour plot for effect of concentration of lipids and concentration of surfactant on particle size

Response 2: In vitro drug release studies

Final equation in terms of coded factors

Drug release = 93.74-0.0176*A+0.1628*B-0.6854*C-0.1275*AB-0.6825*BC

Drug release parameter shows that drug release of all the eight formulation ranges from 80.48±0.69 to 90.41±0.55. Batch 7 was found to have the highest drug release.

Effect of concentration of lipid and surfactant on drug release

The RPM decreases the drug release rate increases. And also, the lipid concentration increases, the drug release rate decreases; this might be due to the higher concentration of drug present in the low rmp speed. Drug release might also get decreased due to differences in the partition coefficient of the active material and lipophilicity of lipids. This lipophilicity decreased the diffusion of the drug from the cocoa butter to the aqueous medium of dissolution. As the surfactant concentration increases, the drug release rate increases due

to the increased solubility of drugs in the external phase. This might be due to the high affinity of surfactants toward aqueous environments. [22,23,24] (Fig. 3 and 4).

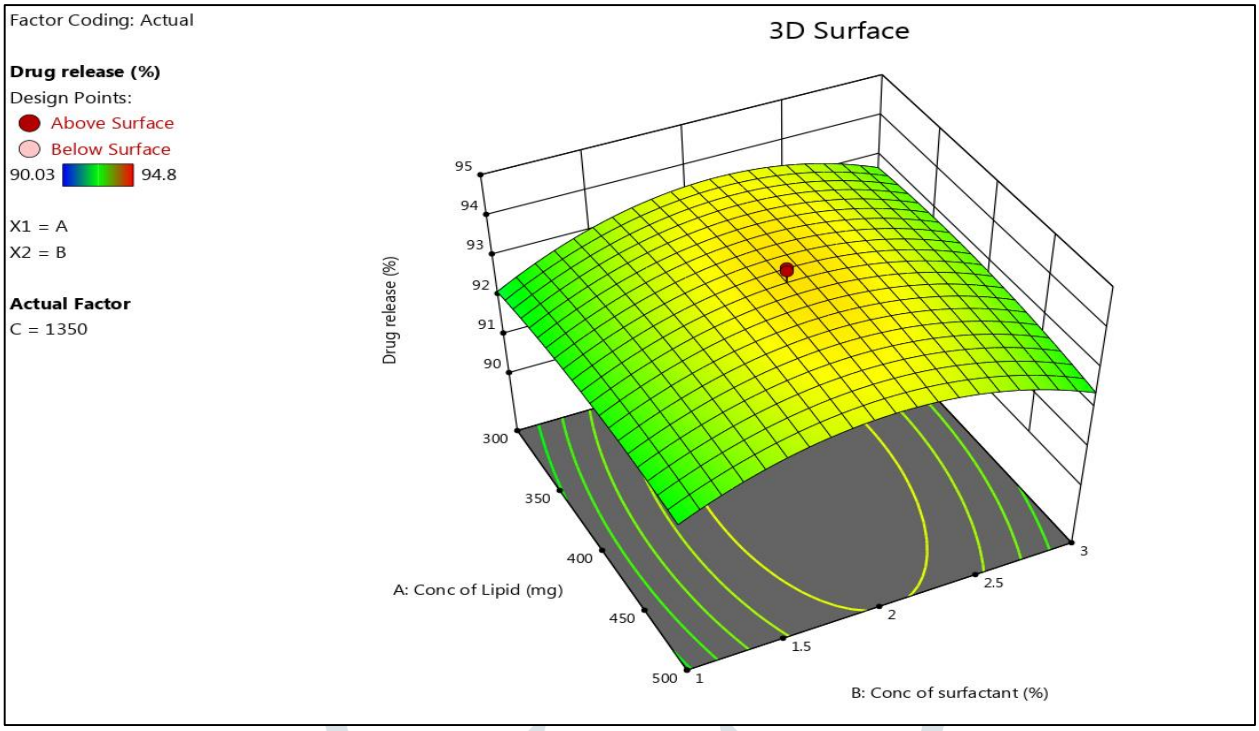

Figure no. 3 Three-dimensional plot for effect of concentration of lipids and concentration of surfactant on drug release

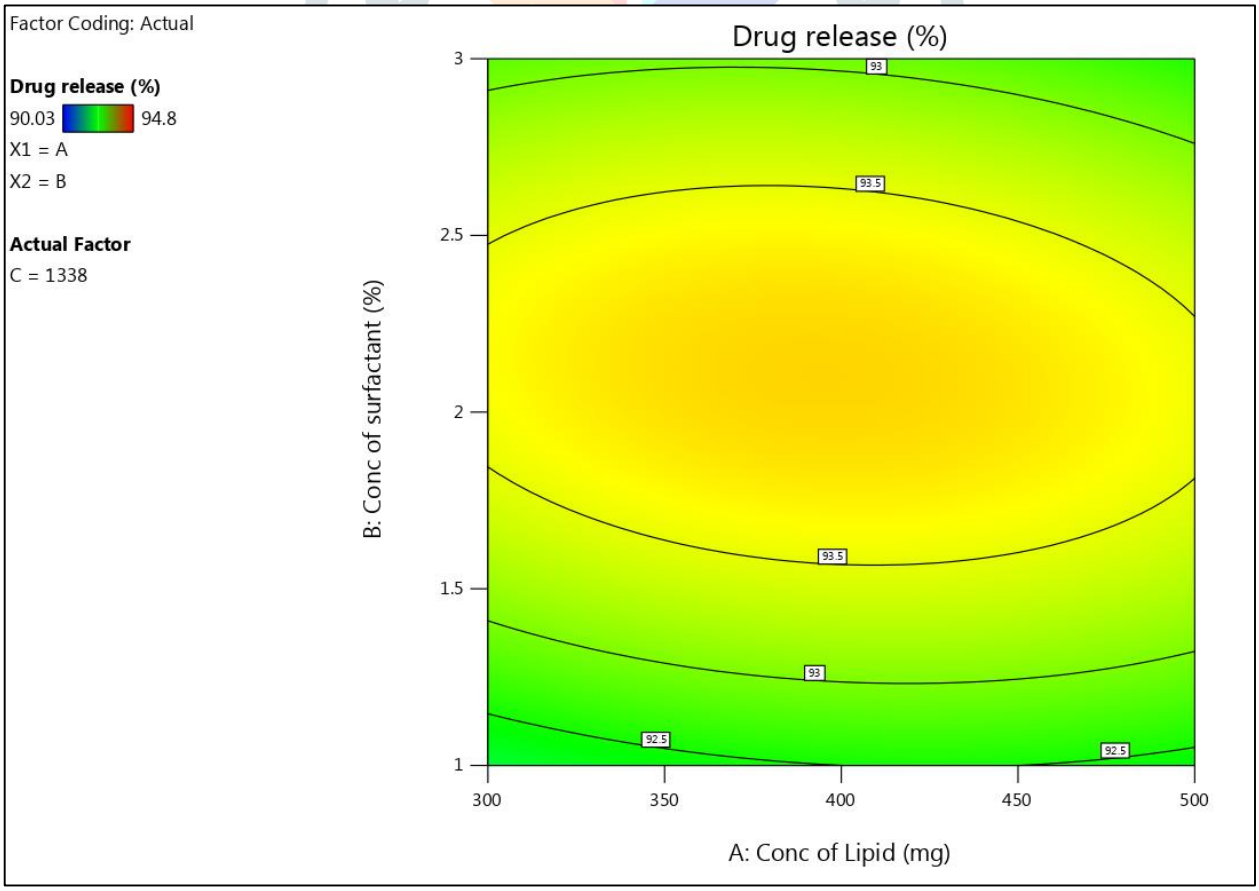

Figure no. 4 Contour plot for effect of concentration of lipids and concentration of surfactant on drug releases

From the above observation, batch 7 was found to be optimized. Hence, it was further evaluated for zeta potential and was then formulated into the gel. Image of SLNs of batch 7 is shown in Fig. 7 which was observed in digital microscope.

pH

The pH of the optimized formulation batch 7 was found to be 6.1. The skin pH is found to be in the range of 4–6.8. Hence, the formulation prepared was compatible with skin pH.

SLNs covered by a non-ionic surfactant like Tween 80 tend to remain stable regardless of having a lower zeta potential. Greater steric stabilization and less electrostatic stabilization are responsible for this kind of behavior. Surface encapsulation of the SLNs lessens the electrophoretic mobility of the particles and thus lowers the zeta potential. Hence, zeta potential measurement was not taken into consideration as a primary parameter in the selection of the optimal formulation [25].

Stability study

The stability studies were studied over different storage conditions of 5°C and 25°C as per the International Conference on Harmonization (ICH) guidelines. Both physical and chemical changes were studied after 3 months. Physical stability was checked in terms of appearance and particle size, whereas chemical studies were checked in terms of entrapment efficiency and drug release profile. The results show that there was no significant change in particle size, entrapment efficiency and drug release of the SLN formulation stored at 5°C and 25°C after 3 months (Table 3).

Table no. 3 stability study of optimized batch

Evaluation parameter	Initial	After 3 months (°C)	
		5±3	25/60% RH
Particle size (nm)	35.01	34.87	33.23
% Drug release	93.48	93.83	92.25

Characterization of gels

Physical evaluation

The SLN based gel of LEO was found to be homogenous, smooth, and consistent.

pH measurement

The pH of the formulation was found to be 6.8. It was found to be compatible with the skin pH. [26]

Viscosity study

The viscosity of the gel at different rpm is stated in Table 5. The viscosity was found to decrease with the increase in the rpm, i.e., rate of shear, showed with non-Newtonian flow. This behavior might be due to its low flow resistance when applied at the high shear condition. The results show that as the rpm increases, there was decrease in viscosity of the gel (Table 4).

Table no. 4 viscosity study of Cromolyn sodium SLN loaded

S. No.	R.P.M	Viscosity at 0.2% concentration (cps)
1.	5	2559
2.	10	2487
3.	20	2412
4.	50	2301
5.	100	2311

Spreadability

Spreadability plays an important role in patient compliance and helps in the uniform application of the gel to the skin. A good gel spreads easily and takes less time to spread on the skin. The Spreadability of the gel was found to be 6.48±0.4 cm.

In vitro drug release of optimized SLN based gel

The drug release of the gel after 8 h was found to be 95.04% (Fig. 5).

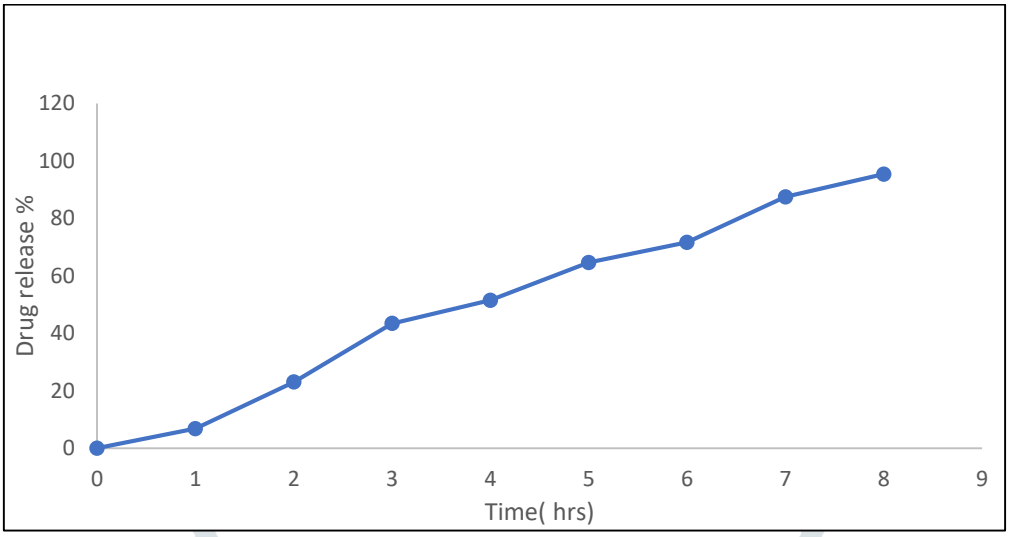

Figure no. 5 In vitro drug release of optimized based gel

Stability studies

The stability studies were studied over different storage conditions of 5°C and 25°C as the per the ICH guidelines. Physical and chemical changes were studied after 3 months. Physical studies were checked in terms of visual examination, whereas the chemical changes were checked in terms of pH, particle size, and drug release and viscosity. The results show that there was no significant change found in stability stored at 5°C and 25°C after 3 months. The results are stated in Table 6.

Table no. 5 Stability study of optimized Cromolyn sodium loaded SLN based gel

S. No.	R.P.M	Viscosity (cps) (Before 3 months)	Viscosity (cps) (After 3 months)
1.	5	2559	2609
2.	10	2487	277
3.	20	2412	2603
4.	50	2301	2561
5.	100	2311	2453

Conclusion

In the present work, Cromolyn sodium loaded SLNs were successfully prepared by the hot homogenization technique. The various physicochemical properties, particle size, and drug release were affected and can be controlled using the optimization technique. Alteration in the concentration of lipid, concentration of surfactant and stirring time can overcome the problems associated. Central composite factorial design was used for the experiment and 20 formulations were developed. Batch 7 formulation was found to be optimized. Its particle size was smaller as desired and drug release were found to be maximum. SLN based gel was also developed successfully. Stability studies were conducted after 3 months, which did not show any remarkable change. Hence, the desired aim and objective could be achieved properly by lavender oil loaded SLN based gel.

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