https://doi.org/10.31925/farmacia.2022.2.4 **ORIGINAL ARTICLE**

DEVELOPMENT AND CHARACTERIZATION OF LORATADINE LIPOSOMAL GEL USING QbD APPROACH

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Abstract

This study aimed to formulate, prepare and characterize a loratadine liposomal gel (LOR-L-G) using the Quality by Design (QbD) approach. The formulation variables and process parameters that influence the Critical Quality Attributes (CQAs) were identified using risk assessment. Five dependent variables were considered relevant for this study: particle size before and after extrusion, polydispersity index (PDI) before extrusion, loratadine (LOR) concentration and encapsulation efficiency (EE). A two-level full factorial design was performed in order to develop loratadine-loaded liposomes (LOR-L) with the desired Quality Target Product Profile (QTPP). Based on the results, the design space was generated and the optimum formulation was prepared. The *in vitro* drug release study aimed at measuring drug availability and revealed a prolonged release profile of LOR with a maximum concentration at 48 hours. LOR-L were incorporated into a Carbopol 940 gel base in order to obtain the final pharmaceutical product. The liposomal gel was characterized by viscosity, texture, spreadability and it was compared with a commercial product. The results indicated the applicability of the QbD approach in the development of LOR-L, and the obtained gel characteristics were suitable for a product useful in skin allergies.

Rezumat

Obiectivele studiului au fost formularea, prepararea și caracterizarea unui gel lipozomal cu loratadină (G-L-LOR) utilizând conceptul de calitate prin design (QbD). S-au identificat variabilele de formulare și parametrii de proces responsabili de afectarea atributelor critice de calitate ale lipozomilor cu loratadină (L-LOR) prin efectuarea analizei de risc. Au fost luate în considerare 5 variabile dependente, anume mărimea particulelor înainte și după extrudare, indicele de polidispersie (PDI) înainte de extrudare, concentrația de loratadină (LOR) și eficiența încorporării (EE). S-a folosit un plan experimental cu două niveluri pentru dezvoltarea L-LOR care să dețină caracteristicile de calitate dorite. Pe baza rezultatelor obținute s-a determinat domeniul optim și a fost preparată formularea optimă. S-a efectuat studiul de cedare *in vitro*, care a demonstrat o cedare prelungită a LOR din formulările lipozomale, cu o concentrație maximă la 48 ore. În vederea obținerii produsului farmaceutic final, lipozomii au fost încorporați într-o bază de gel de Carbopol 940. Gelul lipozomal a fost caracterizat din punct de vedere al vâscozității, texturii, capacității de întindere și a fost comparat cu un gel existent pe piață. Rezultatele au demonstrat aplicabilitatea conceptului de calitate prin design în dezvoltarea L-LOR, iar caracteristicile gelului obținut au fost potrivite pentru aplicarea acestuia în alergiile cutanate.

Keywords: liposomes, quality by design, ethanol injection method, loratadine

Introduction

The topical application of active pharmaceutical ingredients (API) in skin diseases may overcome the systemic side effects compared to other routes of administration due to the reduction of the required doses. Moreover, drugs with a short half-life can be topically applied to avoid frequent dosing [1]. Generally, the optimal molecular weight of the API for a topical agent should be less than 500 Da for an efficient penetration into the skin [2].

Antihistamines are therapeutic agents, which improve itching symptoms caused by the release of histamine from mastocytes and basophils. Second-generation antihistamines represent the first-line treatment for skin allergies [2, 3]. Also, for treating chronic urticaria, second-generation antihistamines are recommended as first-line therapy because of their efficacy and safety [3, 4]. Loratadine (LOR) is a lipophilic drug, a second-generation H_1-antihistamine, which belongs to the Biopharmaceutics Classification System class II [2, 5]. Oral administration is the most common route of administration for LOR, but it is associated with side effects and poor bioavailability. Thus, the topical application of LOR could be beneficial in the treatment of cutaneous conditions defined by a localized allergic reaction. LOR can be used topically for disorders characterized by hypersensitivity, itching, redness and insect bites [2]. Despite its therapeutic potential, LOR has limited topical application due

to poor skin penetration and low water solubility, but incorporating it into different carriers could overcome these limitations [5].

Liposomes are lipid vesicles composed of one or more phospholipid bilayers enclosing an inner aqueous core [6, 7]. Due to the similarity of the liposome composition to that of the epidermal membranes, this type of nanosized vesicles can penetrate the epidermal barrier to a higher extent compared with other topical formulations, and may result in prolonged or sustained drug release of the payload [8]. Furthermore, liposomes demonstrated the potential to increase the permeation of drugs through the skin and also to provide a localized depot effect or targeted delivery through skin annexes [9, 10]. Liposome particle size represents an important feature for penetration into the skin layers [7]. Studies showed that vesicles with sizes less than 300 nm are able to penetrate further into skin layers [11]. Liposomes applied topically present increased penetration of drugs, high permeability through skin layers and reduced side effects due to the lower doses of API [12].

The Quality by Design (QbD) approach includes all the elements of pharmaceutical development that will lead to a quality product and its production process to constantly provide the aimed efficacy and safety. The main goal of the QbD concept is to achieve robustness, cost-effective analytical methods and to reach regulatory flexibility [13]. Defining the Quality Target Product Profile (QTPP) refers to establishing all the quality characteristics that a product must achieve to ensure safety and efficacy [13, 14]. Critical Quality Attributes (CQAs) are all the characteristics that a final product should own and are associated with the API, excipients, intermediates and the final product [14]. The CQAs need to be identified in the QTPP and have to be controlled within a certain limit to ensure the quality and performance of the final product [13, 15, 16].

The risk assessment represents a systematic organization and measurement of the impact that each variable or quality attribute of raw materials has on the final product [17, 18]. The Ishikawa diagram is a qualitative risk assessment tool that illustrates the cause-effect relationship between all potential critical materials attributes (CMAs) and critical process parameters (CPPs) that may influence the CQAs of the final product [13, 16, 17, 19]. The failure mode effects analysis (FMEA) prioritizes the CPPs by calculating the Risk Priority Number (RPN) [18].

The traditional development of pharmaceutical products implies changing one factor at a time. To overcome the limitations of this method, the Design of Experiments (DoE) approach for determining the relationship between the input factors and output responses has been developed [13].

This study aimed to develop loratadine liposomal gel (LOR-L-G) using the QbD approach by identifying all the factors that influence the final product characteristics. As an element of novelty, the study performed the preparation of loratadine-loaded liposomes which were afterward incorporated in a Carbopol gel. Liposomes were selected as nanocarriers due to their biodegradability, biocompatibility, lack of toxicity and immunogenicity. Moreover, the natural phospholipids used in the formulation of liposomes are derived from renewable sources and are available on a larger scale at relatively low costs compared to synthetic phospholipids and are also described in pharmacopoeias and relevant regulatory guidance documentation of the Food and Drug Administration (FDA) and European Medicines Agency (EMA). Also, the study followed the *in vitro* drug release from the loratadine-loaded liposomes' (LOR-L) optimal formulation and the characterization of the LOR-L-G. Furthermore, the formulation of the LOR-L-G represents an innovative approach with multiple advantages over conventional topical formulations: the entrapment of LOR into nanocarriers may enhance the solubility of the lipophilic compound and protect the entrapped drug while the inclusion in a gel provides prolonged retention of the drug on the skin surface. To the best of the authors' knowledge, no other research papers reported the preparation of liposomal loratadine gel formulation.

Materials and Methods

Materials

LOR was purchased from Quimica Sintetica (Alcalá de Henares, Spain). The phospholipid (PL) used was Lipoid E80® from Lipoid GmbH (Ludwigshafen, Germany). Cholesterol (CHOL) was purchased from Sigma-Aldrich (St. Louis, USA), Carbopol 940 from Lubrizol (Wickliffe, USA) and acetonitrile from Merck (Darmstadt, Germany). Ethanol, o-phosphoric acid 85% and propylene glycol were obtained from Chemical Company SA (Iași, Romania). Sodium hydroxide was purchased from Chimreactiv SRL (Bucharest, Romania). Methyl p-hydroxybenzoate and propyl p-hydroxybenzoate were purchased from Fluka Chemie GmbH (Buchs, Switzerland).

Methods

Defining the Quality Target Product Profile (QTPP) and Critical Quality Attributes (CQAs)

In conformity with the regulatory agencies' recommendations, the QTPP should identify the objectives of the product and should also explain how these objectives can be achieved [20]. The QTPP and the CQAs of the LOR-L-G were identified based on the literature research on topical semisolid nano-formulations. The QTPP for nanosized topical formulations mainly includes dosage form, route of administration, physical form, drug release, therapeutic indication, site of activity and biological effects [17, 21-23]. Table I contains the QTPP related to LOR-L-G.

Table I

Quality Target Product Profile of LOR-L-G

Element	Target	Justification
Dosage form	Liposomal gel	To improve the efficacy and bioavailability of loratadine
Route of administration	Topical	Localized delivery, non-invasive, avoidance of systemic side effects
Drug release	Prolonged release	-
Therapeutic indication	Skin allergies	Useful in irritation, itching, allergies, eczemas
Site of activity	Topical	Topical formulations are designed to allow the dermal penetration of the drugs into the deeper layers of the skin such as the viable epidermis and dermis where mast cells are located

According to several types of research, particle size, polydispersity index (PDI), entrapment efficiency (EE), drug concentration and zeta potential are the most important features of topical nano-formulations [4, 15, 17, 19, 24]. Therefore, some of these CQAs were considered relevant for this study (Table II).

Table II

Critical quality attributes for LOR-L and LOR-L-G

CQA	Target	Is it critical?	Justification
	LOR-L		
Particle size	Less than 300 nm	Yes	Improved penetration
PDI	Less than 0.2	Yes	Homogeneity of liposomal dispersion
EE	More than 80%	Yes	To ensure sufficient nano-system drug loading
	LOR-L-G		
Aspect	White or off-white gel	No	Adequate aspect
Homogeneity	Homogenous gel	Yes	To ensure uniformity
Physical properties	Gel with desired rheological properties	Yes	To improve patient compliance
pH	4.5-6	Yes	Compatible with skin pH

CQA - Critical Quality Attribute; LOR-L - loratadine liposomes; LOR-L-G - loratadine liposomal gel; PDI - polydispersity index; EE - entrapment efficiency

Risk assessment

A risk analysis was performed by using the Ishikawa diagram and the FMEA which allowed to identify and analyse risk factors. For both the LOR-L and the LOR-L-G an Ishikawa diagram was constructed and contained the formulation factors and process parameters that can influence the quality of these products. The CPPs taken into consideration have been divided into two categories: formulation factors and process parameters, respectively. For LOR-L, the concentration of PL, CHO and LOR were considered as critical formulation factors, while the injection time and temperature, the stirring speed, the extrusion membrane porosity, the evaporation temperature, pressure and time were the essential process parameters. For LOR-L-G, the chosen CPPs were the stirring speed, and the homogenization time and temperature. FMEA evaluation followed three criteria: frequency of occurrence (O), the severity of consequences (S) and difficulty of detection (D), allowing the identification and prioritization of the failure modes that can cause product failure. Each of these criteria was attributed to each CPP and was evaluated on a scale from 1 to 5 as follows: the occurrence was ranked as 5 for frequent, 4 for probable, 3 for occasional, 2 for remote, and 1 for improbable; the second attribute, the severity, was ranked as 5 for catastrophic, 4 for critical, 3 for serious, 2 for minor, and 1 for negligible; the detectability was ranked as 5 for hard to detect, 4 for low chance to be detected, 3 for moderately detectable, 2 for highly detectable and 1 for easily detectable. The failure risk was calculated as RPN by multiplying the three attributes (O × S × D).

Experimental design

A two-level full factorial design matrix was developed by the Modde 12.1 software (Sartorius Stedim Biotech, Göttingen, Germany). Based on the risk analysis three independent variables were identified: PL concentration (X_1, %), CHOL concentration (X_2, %) and LOR concentration (X_3, %), respectively. The responses (dependent variables) investigated were the size before extrusion (Y_1, nm), PDI before extrusion (Y_2), size after extrusion (Y_3, nm), LOR concentration (Y_4, µg/mL) and EE (Y_5, %). Table III summarises the experimental quantitative formulation factors.

Table III

Quantitative formulation factors of LOR-L

Exp. name	Run order	X_1	X_2	X_3
N1	1	10	0.25	0.5
N2	2	10	0.25	1
N3	5	2	0.25	1
N4	4	2	0.25	0.1
N5	3	10	0.5	0.5
N6	8	10	0.25	0.5
N7	7	10	0.25	0.5
N8	6	10	1	0.5
N9	9	10	0.5	1
N10	10	2	0.5	1

Exp. – Experiment; X_1 – PL concentration (%); X_2 – CHOL concentration (%); X_3 – LOR concentration (%)

Liposomes preparation

The liposomes were prepared using the ethanol injection method. The required amounts of LOR, PL and CHOL were dissolved in ethanol. The aqueous phase was preheated at 55°C. The organic phase was injected into the aqueous phase using a syringe with a 23G needle under magnetic stirring on a MultiStirrer 6 from VELP Scientifica (Usmate Velate, Italy) at 400 rpm for 30 minutes at 55°C. After the spontaneous formation of the liposomes, the ethanol was removed by rotary evaporation with a HEI-VAP Advantage equipment from Heidolph (Schwabach, Germany) under reduced pressure at 80 rpm for 3 minutes at 55°C. Finally, the dispersion was adjusted to 10 g with distilled water and homogenized.

The size of liposomes was reduced by extrusion using a LiposoFast LF-50 extruder from AVESTIN Europe GmbH (Mannheim, Germany) connected to a water bath (Julabo GmbH, Seelbach, Germany). The liposomes were sequentially passed through three different polycarbonate membranes Whatman International Ltd (Maidstone, UK) with pore diameters of 0.6, 0.4 and 0.2 µm. For each formulation, this procedure was repeated three times at 55°C.

Liposomes characterization

Particle size and size distribution

The mean vesicle size and PDI of LOR-L were determined by Dynamic Light Scattering (DLS) using a Zetasizer Nano ZS90 (Malvern Instruments Ltd, Malvern, UK) before and after extrusion. Each sample was diluted with distilled water in a 1:100 ratio.

Purification of liposomes

The separation of unloaded LOR from the liposomes was performed using a SIGMA 3-30KS Centrifuge (Osterode am Harz, Germany) for 15 minutes at 2000 rpm. After centrifugation, the supernatant was separated from the sediment.

Entrapment efficiency (EE)

The quantitative measurement of the loaded LOR in the vesicles was performed by using a high-performance liquid chromatography (HPLC) system Agilent 1100 Series (Santa Clara, USA) equipped with an ultra-violet-visible (UV-VIS) detector. 100 µL from the supernatant obtained from the centrifugation was diluted with 900 µL ethanol. 500 µL from the mixture were diluted with 500 µL mobile phase. The chromatographic column used was LUNA C18(2) (5 µm, 100 Å, 150 x 4.6 mm) from Phenomenex (Torrance, USA). The mobile phase consisted of a mixture of acetonitrile and 0.1% phosphoric acid (40:60; v/v) at a flow rate of 1.5 mL/min. 50 µL of the sample were injected in the chromatographic system at 30˚C and the wave-length detection was set at 242 nm. The drug EE was expressed as the percentage of the encapsulated amount of drug from the total amount of drug, by using the following formula:

$$\text{EE (\%)} = \frac{\text{amount of LOR in the liposomes}}{\text{total amount of LOR}} \text{ x } 100$$

In vitro drug release study

The *in vitro* release study of LOR from the robust setpoint formulation was performed in triplicate using the dialysis method according to an adapted method from the literature [25]. The LOR-L were added into the dialysis cassette (Slyde-A-Lyzer™ Dialysis Cassettes, 10k MWCO, Thermo-Fisher Scientific, Waltham, MA, United States) which was inserted into a dissolution medium of 100 mL of phosphate buffer pH 5.5 and ethanol mixed in a 1:1 ratio (v/v), maintained at the temperature of 32 ± 0.5°C and stirred at 80 rpm. Samples of 1 mL were withdrawn at predetermined time intervals for 72 h and were replaced with an equal volume of fresh dissolution medium. The amount of drug released was determined by the HPLC method previously described.

Preparation of LOR-L-G

The gel base was prepared using appropriate quantities of propylene glycol, Carbopol 940, methyl para-hydroxybenzoate, propyl parahydroxybenzoate which were dispersed in distilled water, under homogenization using an IKA RW11 "Lab egg" (IKA-Werke GmbH & Co. KG, Staufen, Germany), at 50°C. 30% sodium hydroxide was used as a neutralizing agent for carbomer up to pH 6, using a pH meter Hanna HI-2002 Edge (Woonsocket, USA). The liposomal gel was prepared by mixing an adequate amount of the robust setpoint liposomal formulation with the gel base and the obtained LOR-L gel had a final concentration of 1 mg/g LOR.

Characterization of the LOR-L-G

The evaluation of the texture profile of the gel was performed compared to a commercially available product containing carbomer and propylene glycol, as well. CT3 Texture Analyzer (Brookfield, Middleborough, USA) equipped with a TA-BT-KIT fixture base table was used for the determination of the following texture parameters: firmness and consistency using TA-DEC accessory and spreadability, adhesiveness and stringiness using TA-SF accessory. The obtained results were registered by Texture Pro CT Software v 1.9 Brookfield Engineering (Middleborough, USA). The viscosity of the gels was determined with DV-III Ultra Rheometer (Brookfield, Middleborough, USA) equipped with LV-4 spindle at 0.2 rpm speed and a temperature of 21 ± 1°C. All the parameters were measured in triplicate.

Results and Discussion

Defining the Quality Target Product Profile (QTPP) and Critical Quality Attributes (CQAs)

Based on previous studies and literature research, the QTPP elements were identified (Table I). The CQAs for LOR-L and for LOR-L-G, derived from the QTPP,

with impact on the final product quality were framed in Table II and some of these CQAs were chosen as dependent variables for the DoE.

Risk analysis

In order to establish the factors that influence the variability of the LOR-L (Figure 1) and LOR-L-G (Figure 2), two Ishikawa diagrams were generated.

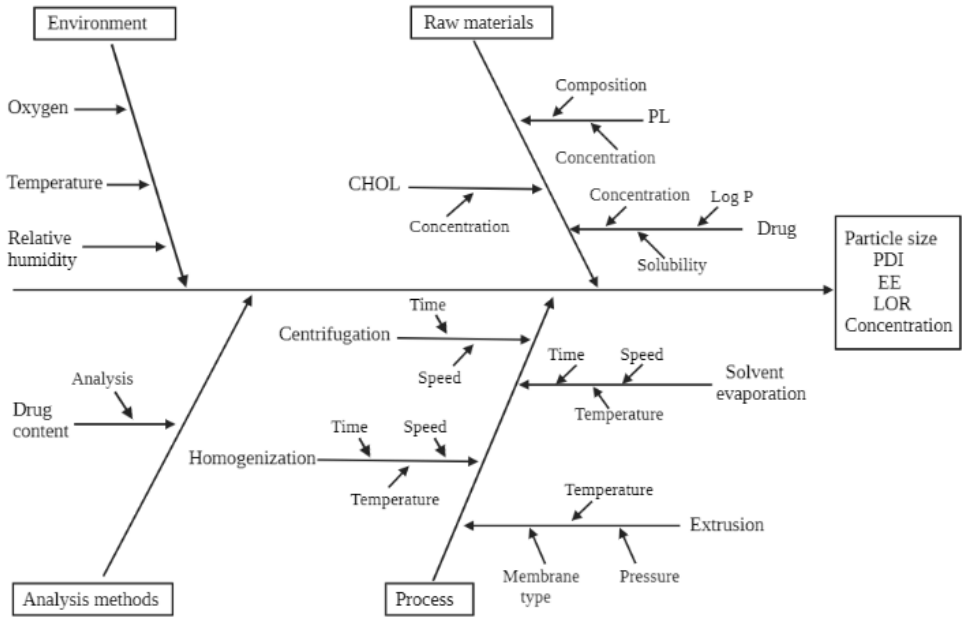

Figure 1.
Ishikawa diagram of LOR-L
PL – phospholipid; CHOL – cholesterol; PDI –polydispersity index; EE – entrapment efficiency; LOR – loratadine

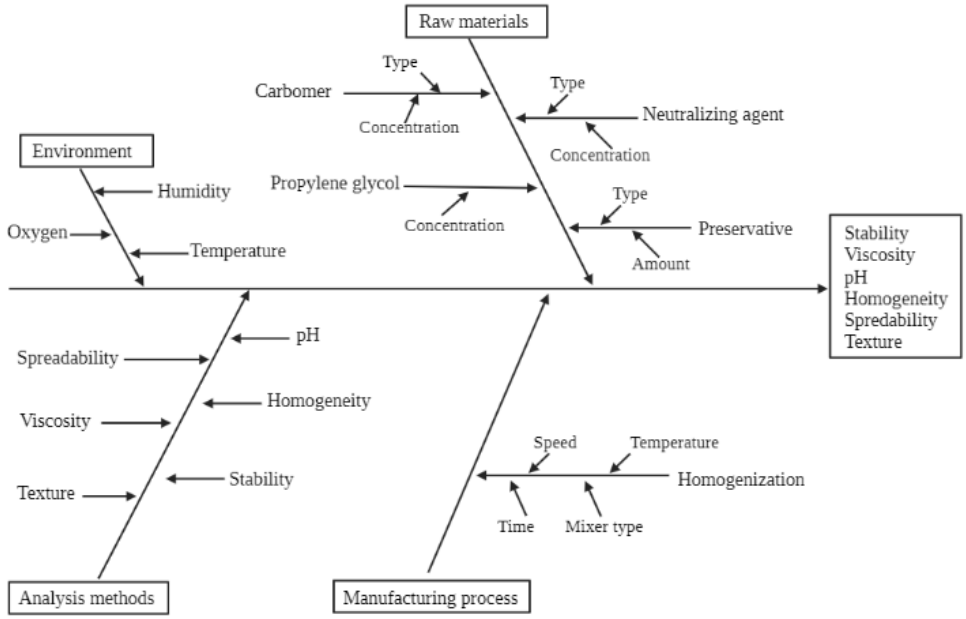

Figure 2.
Ishikawa diagram of LOR-L-G
LOR-L-G – loratadine liposomal gel

The FMEA approach is widely used for risk assessment in nanoformulation development. Therefore, Table IV shows the FMEA for the LOR-L and LOR-L-G, respectively. The formulation factors chosen for the experimental design were PL concentration, CHOL concentration and LOR concentration, respectively. The selection was made based on the RPN, meaning that the factors with the highest RPN were considered relevant for the study.

Table IV

Failure mode effects analysis of LOR-L and LOR-L-G

CPPs	Failure Mode	Failure effects	O	S	D	RPN
Critical formulation factor		**LOR-L**				
PL concentration	Improper PL concentration	Particle size, PDI	5	4	4	80
CHOL concentration	Improper CHOL concentration	Particle size, PDI, rigidity, stability	5	4	4	80
LOR concentration	Improper LOR concentration	Efficiency	5	4	3	60
Critical process parameter		**LOR-L**				
Injection time	Improper time	Particle size, PDI, stability	4	5	2	40
Injection temperature	Unsuitable temperature	Particle size, PDI, stability	5	3	3	45
Stirring speed	Improper speed	Particle size, PDI, aggregation	4	4	3	48
Evaporation temperature	High temperature	EE, stability	4	3	2	24
Evaporation pressure	High pressure	EE, stability	3	5	3	45
Evaporation time	Short time	EE, stability	3	4	3	36
Extrusion membrane pore size	Unsuitable size	Particle size, PDI	3	3	4	36
Critical process parameter		**LOR-L-G**				
Homogenization time	Short time	Consistency, viscosity	4	4	3	48
Homogenization temperature	Low temperature	Homogeneity	3	2	2	12
Stirring speed	Improper speed	Homogeneity	3	4	3	36

CPPs – Critical Process Parameters; O – occurrence; S – severity; D – detectability; RPN – risk priority number; PDI – polydispersity index; EE – entrapment efficiency; LOR – loratadine; CHOL – cholesterol; PL – phospholipids; LOR-L – loratadine liposomes; LOR-L-G – loratadine liposomal gel

Experimental data fitting

Figure 3 shows the model summary of fit, which revealed a good fit of the experimental data. The multiple linear regression fitting evaluated the determination coefficient (R^2), the prediction coefficient (Q^2), the model validity and the reproducibility. R^2 and Q^2 had a score greater than 0.5 for all the responses which indicated a good fit and a proper predictive power of the model. Also, the other statistical parameters had values high enough for the chosen model to be considered appropriate.

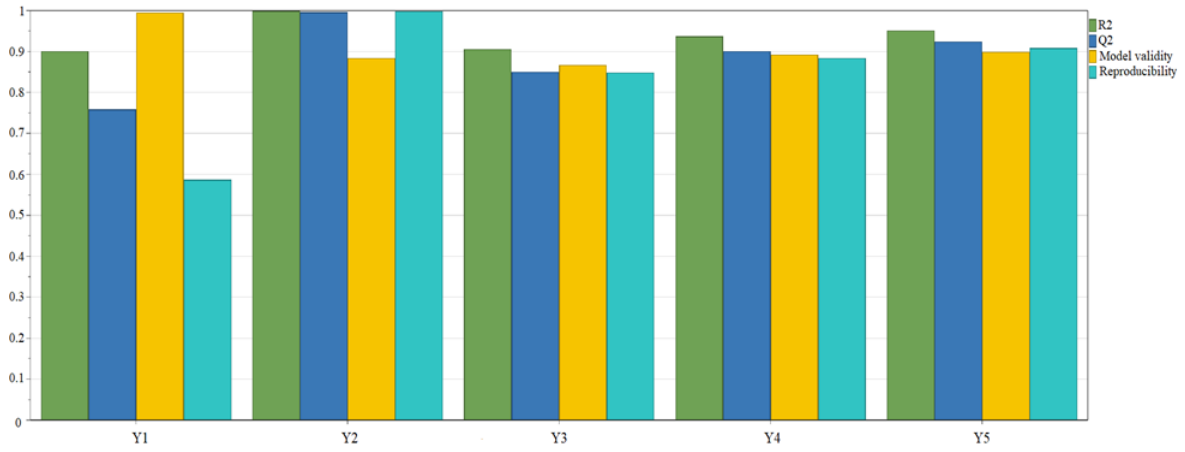

Figure 3.

Y_1 – size before extrusion (nm); Y_2 – PDI before extrusion; Y_3 – size after extrusion (nm); Y_4 – LOR concentration (µg/mL); Y_5 – EE (%)

Characterization of LOR-L

Table V reveals all the responses (dependent variables) for LOR liposomal formulations. The results were reported as mean ± SD. The particle size before extrusion (Y_1) and polydispersity indices (Y_2) of the liposome formulations ranged from 210.5 to 986.2 nm and from 0.216 to 0.716, respectively. All the LOR-L presented particle sizes under 200 nm after extrusion (Y_3). Goindi *et al*. developed topical levocetirizine-loaded liposomes and reported monodisperse vesicles with a mean size of 129.1 nm and the PDI of 0.180, similar to our results [26]. With respect to LOR concentration (Y_4), it can be observed that for the formulations with 2% PL (N3, N4, N10), the LOR concentration had lower values. The EE (Y_5) varied widely from a minimum of 3.408% (for N10 with 2% PL) to a maximum of 96.156% (for N7 with 10 times higher amount of PL). Also, it was observed that all the formulations with EE higher than 60% (N1, N4, N5, N6, N7 and N8) had the PL:CHOL ratio of 20:1. Qiang *et al*. reported a particle size of 358 ± 3.5 nm and EE of 65.8 ± 0.9% for the uncoated fexofenadine-

loaded liposomes formulated with DPPC (1,2-ditetradecanoyl-sn-glycero-3-phosphocholine) and DPPG (1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol sodium salt) as phospholipids, and cholesterol [27]. In another study, Shrestha *et al*. prepared fexofenadine-loaded liposomes using the ethanol injection method, and the particle size reported ranged between 73 and 1573 nm, and the EE was 65% [28]. The differences in results are probably caused by the presence of Tween 80 in the liposomal formulation mentioned by Shrestha *et al*., the different type of phospholipids used by Qiang *et al*., or the different final pharmaceutical products. Elzainy *et al*. described the preparation of another second-generation antihistamine drug, cetirizine, which was also incorporated in liposomal nanocarriers and the reported EE was 92.8 ± 0.3% [29].

Table V

Results for LOR liposomal formulations' characterization

Exp. Name	Y_1	Y_2	Y_3	Y_4	Y_5
N1	458.2 ± 12.52	0.216 ± 0.062	182.0 ± 2.12	4670.60	81.292 ± 8.10
N2	706.1 ± 23.94	0.4160.012	177.5 ± 2.00	2638.38	22.748 ± 0.58
N3	344.5 ± 83.29	0.606 ± 0.082	160.0 ± 2.00	522.975	4.553 ± 0.05
N4	210.5 ± 2.40	0.275 ± 0.013	153.1 ± 1.34	988.069	84.915 ± 2.82
N5	447.3 ± 3.13	0.237 ± 0.023	180.7 ± 2.76	3809.22	66.286 ± 0.28
N6	703.6 ± 17.95	0.231 ± 0.079	184.7 ± 0.64	4269.43	74.116 ± 3.71
N7	421.6 ± 19.38	0.219 ± 0.034	192.7 ± 1.41	5571.14	96.156 ± 5.43
N8	428.3 ± 3.46	0.248 ± 0.011	195.3 ± 0.40	5360.11	91.370 ± 0.53
N9	986.2 ± 19.99	0.354 ± 0.048	179.5 ± 0.35	2637.96	22.888 ± 0.50
N10	443.6 ± 190.50	0.716 ± 0.187	159.9 ± 1.00	393.348	3.408 ± 0.37

Exp. – Experiment; Y_1 – size before extrusion (nm); Y_2 – PDI before extrusion; Y_3 – size after extrusion (nm); Y_4 – LOR concentration (µg/mL); Y_5 – EE (%)

Effects of independent variables on the responses

Model regression coefficient plots are presented in Figure 4, which show the effect of the formulation variables on the responses. The liposome particle size (Y_1, Y_3) was positively influenced by the PL concentration (X_1) and LOR concentration (X_3). As reported by other authors, increasing the amount of CHOL or PL led to an increase in the liposome particle size [30, 31]. The PL concentration was the only significant variable that influenced the size of liposomes after extrusion in a positive manner, as discussed above. The PDI (Y_2) was influenced by the CHOL concentration (X_2) and the LOR concentration (X_3), noting a positive effect on the homogeneity of the liposomal dispersion, while the PL concentration (X_1) had an opposite effect. Concerning the LOR concentration (Y_4), the main variables which influenced this response were the PL concentration (X_1) with a positive influence and the LOR concentration (X_3) with a negative influence. The PL concentration (X_1) had a positive influence on the EE (Y_5), whereas the LOR concentration (X_3) had a superior influence but in a negative manner. It is noteworthy that the ratio between PL and LOR had a significant impact on the EE. As the PL:LOR ratio increased, the EE also increased at a constant 0.25% CHOL concentration.

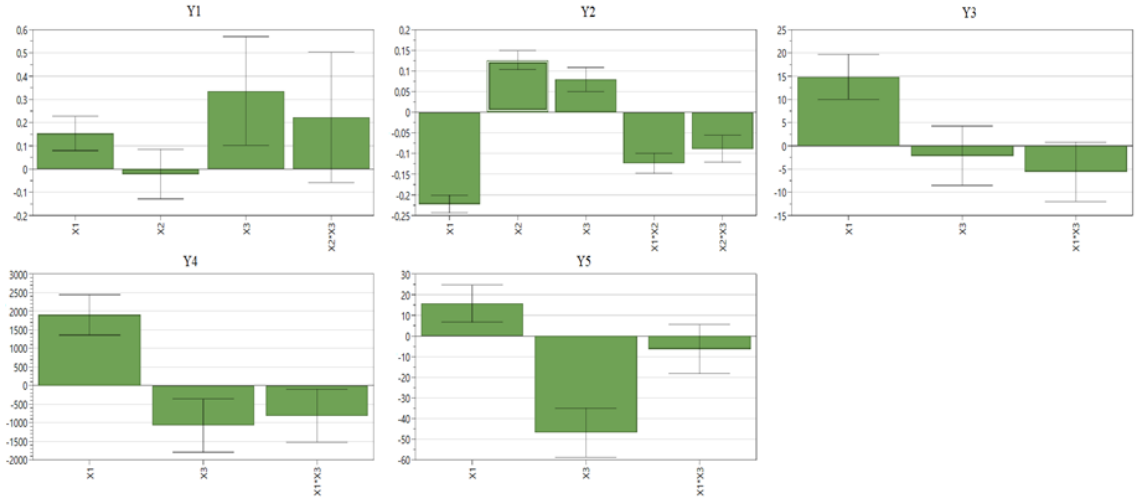

Figure 4.

Regression coefficients plots

X_1 – PL concentration (%); X_2 – CHOL concentration (%); X_3 – LOR concentration (%); Y_1 – size before extrusion (nm); Y_2 – PDI before extrusion; Y_3 – size after extrusion (nm); Y_4 – LOR concentration (µg/mL); Y_5 – EE (%)

Design Space and optimization

The design space was identified (Figure 5), where all the conditions were provided to obtain liposomal formulations with required CQAs. The Modde software generated the optimal formulation (the robust setpoint) with the desired properties, and this was set at the intersection of the two axes, according to Figure 5. For validation, the robust setpoint formulation was prepared in triplicate (R_1, R_2 and R_3), and the results are expressed as mean ± SD (Table VI). The objective was to minimize the PDI and the particle size before and after extrusion and to maximize LOR concentration and EE, respectively. The mean particle size and the PDI before extrusion of the optimal formulation were 490.4 ± 8.44 nm and 0.237 ± 0.011, respectively, indicating the homogeneity of the LOR-L formulation. The mean EE of 80.508 ± 2.98% and the mean LOR concentration of 4341.42 µg/mL demonstrated a satisfying entrapment of the API. To confirm the validity of the design space, a formulation outside the optimal experimental domain was prepared (O), and the results are indicated in Table VI.

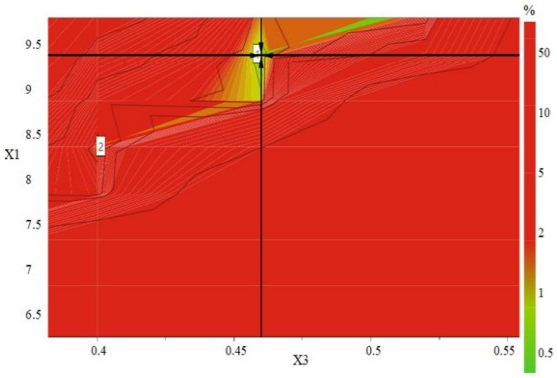

Figure 5.
The obtained design space of LOR-L by plotting the PL concentration (X_1) against the LOR concentration (X_3), at a constant CHOL level of 0.25%

Table VI

Results for LOR-L optimal formulation and LOR-L formulation outside the optimal domain

Exp. Name		Y1	Y2	Y3	Y4	Y5
Target		235	0.199	150	4950	90
Predicted values (R)		482.2	0.222	185.638	4628.75	84.298
Experimental values	R_1	509 ± 12.15	0.259 ± 0.007	163.9 ± 1.28	4292.42	75.726 ± 6.08
	R_2	540.2 ± 7.72	0.193 ± 0.018	179.5 ± 1.15	4355.62	82.377 ± 0.42
	R_3	422 ± 5.45	0.259 ± 0.010	174.2 ± 1.94	4376.24	83.420 ± 2.44
Predicted values (O)		-	-	-	6856	-
O		650.8 ± 32.85	0.540 ± 0.017	164.6 ± 2.35	4764.90	61.406 ± 3.08

Exp. – Experiment; Y_1 – size before extrusion (nm); Y_2 – PDI before extrusion; Y_3 – size after extrusion (nm); Y_4 – LOR concentration (µg/mL); Y_5 – EE (%)

In vitro drug release study

The *in vitro* release study gives information about the drug release behaviour from the liposomal formulation and is illustrated in Figure 6. The LOR-L showed a fast release of LOR in the initial stage, followed by a sustained release phase that lasted up to 72 hours. A cumulative 96.83 ± 2.87% of LOR was released from the liposomes within 48 hours, and the concentration remained constant at 72 hours (96.46 ± 2.95%). The increased residence time of LOR entrapped inside the liposomes and slow diffusion is attributed to the structure of the liposomes, allowing the slow release of the drug.

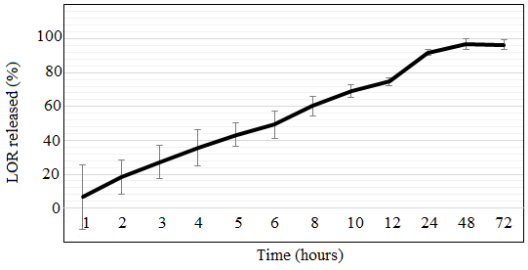

Figure 6.
In vitro drug release rate of LOR from the optimal liposomal formulation

LOR-L-G characterization

Currently, texture analysis is a broadly used method to collect data about semisolid pharmaceutical products [32]. The product's acceptability and efficacy depend on several factors such as its mechanical properties, spreadability, adhesiveness and rheological behaviour [33]. Thus, an optimal topical formulation should have an acceptable consistency that allows the easy pick-up of the product from the container, satisfactory spreading capacity and adequate adhesiveness [32]. Table VII displays the mean values determined during the texture profile analysis and viscosity measurements of LOR-L-G. The hardness of the samples was determined as the necessary force to attain a 25 mm deformation. More precisely, the firmness and consistency of the gels were determined through the back extrusion test, the forward extrusion indicating the necessary force to squeeze the gel from the tube packaging.

The spreadability, adhesiveness and stringiness of the samples were determined during the spreadability test by using the TA-SF probe. The spreadability of the gels was measured as the firmness of the sample at the specified depth, a higher value indicating a less spreadable product. Generally, the adhesiveness values give information about the work necessary to overcome

the attractive force between the surface of the gel and the surface of the skin. As shown in Table VII, the LOR-L gel displayed higher values for both firmness and consistency parameters, but also for the adhesive properties which presumably favour the increased remanence on the skin surface. Consistent with the texture parameters, the results showed a higher viscosity of the LOR-L gel than the commercial product.

Table VII

Comparative physical properties of the gel formulations

Parameter	LOR-L-G	Conventional gel
Texture TA-DEC Accessory		
Firmness - Hardness (g)	1440.00 ± 87.90	1096.00 ± 19.70
Consistency - Hardness work (mJ)	296.50 ± 11.89	223.40 ± 1.37
Texture TA-SF Accessory		
Spreadability - Hardness (g)	486.80 ± 25.50	310.80 ± 25.30
Adhesive Force (g)	302.00 ± 24.30	198.80 ± 18.80
Adhesiveness (mJ)	13.27 ± 0.77	8.37 ± 0.70
Stringiness length (mm)	0.67 ± 0.06	0.62 ± 0.01
Viscosity (cP)	$2.13 \cdot 10^6 \pm 2.08 \cdot 10^4$	$8.20 \cdot 10^5 \pm 1.05 \cdot 10^4$

LOR-L-G – loratadine liposomal gel

Conclusions

In this study, based on the QbD approach, various formulations of LOR-L were prepared and characterized. The optimal formulation presented the ideal attributes in terms of particle size, PDI, LOR concentration, and EE. The design space was constructed by taking into account the key variables that have been demonstrated to affect product quality the most, aiming to obtain a high LOR concentration and EE, and reduced size and PDI of the liposomes. One of the most important product quality parameters for all dosage forms is the *in vitro* release behaviour that revealed a high drug cumulative concentration after 72 hours, and a sustained release of LOR from the liposomes. The LOR-L gel showed higher values for both texture and viscosity parameters, describing a firm gel with high consistency, appropriate for topical application. Overall, our research suggests that the LOR-L-G could be a feasible candidate for the topical treatment of allergic skin conditions, and it is the first research paper reporting the preparation and characterization of a loratadine liposomal gel.

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Conflict of interest

The authors declare no conflict of interest.

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