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Original Article

Quality by Design Optimization of Alendronate Sodium Proniosomes Gel for Enhanced Transdermal Drug Delivery

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ABSTRACT

Introduction: Alendronate Sodium has low bioavailability when taken orally and may lead to side effects. Gastrointestinal irritation, such as esophagitis and ulceration. The research aims to investigate the potential of a Proniosomes-based vesicular carrier gel for improving transdermal delivery. Permeation of ALS, controlled drug release and drug bioavailability while minimising gastrointestinal adverse effects. **Materials and Methods:** Alendronate Sodium-loaded Proniosomes gel was formulated using the phase-separation technique, known as the coacervation phase-separation technique. A Box-Behnken experimental design was employed to systematically assess the influence of critical formulation variables, including cholesterol, span 40, and Span 60 concentrations, on key response parameters such as vesicle size and entrapment efficiency. **Results:** The developed novel formulation exhibited a particle size of 19.55±0.20 nm and net entrapped drug content of 82.81±0.20% efficiency. Physical characterisation of the gel was performed, and the surface characteristics of the optimised formulation were examined using Scanning Electron Microscopy (SEM). The drug release and diffusion studies confirmed the effectiveness of the novel drug gel in delivering ALS. Furthermore, stability studies demonstrated that the formulation remained stable under different temperature conditions for up to three months. **Discussion:** The optimised formulation demonstrated improved drug release and diffusion compared with the plain drug gel formulation. The drug release kinetics were evaluated using the Higuchi model, which indicated sustained therapeutic activity. The Proniosomes gel formulation: the drug release remained constant over 12 h, indicating prolonged drug release. **Conclusion:** The formulated gel containing ALS Proniosomes is found to have improved vesicular permeation. The formulation developed using nanotechnology has proven to have the following properties: promise for the age-related issue of osteoporosis, better treatment and patient care compliance.

Keywords: Alendronate Sodium, Box-Behnken design, Gel, Osteoporosis, Proniosomes, Quality by Design (QbD), Transdermal drug delivery.

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Bisphosphonates are a major category of drugs that are commonly used for the treatment of osteoporosis. Alendronate Sodium (ALS), an amino bisphosphonate, is a synthetic analogue of pyrophosphate.[1] that has proven antiresorptive activity.[2] It has a high affinity for hydroxyapatite crystals of bone, and tends to localise in areas of bone with active bone resorption, specifically those that are osteoclastic.[3] The drug safely prevents bone loss by inhibiting the action of osteoclasts, and also helps, to some degree, with the growth of new bone.[4] Alendronate Sodium is moderately lipophilic and causes gastrointestinal irritation (esophagitis and ulceration). Its molecular structure does not give optimal balance of lipophilicity/hydrophilicity needed for gastrointestinal absorption. In other countries, these restrictions can be overcome in other ways. Transdermal Drug Delivery Systems (TDDS) provide a means of delivering drugs at a controlled rate, maintaining stable plasma concentrations, reducing dosing frequency, improving patient adherence, and avoiding gastrointestinal complications.[5,6] TDDS have come to the fore as a useful alternative for drug administration because they permit self-administration and allow therapy to be discontinued whenever necessary.[7,8] Vesicular drug delivery systems reduce toxicity and increase solubility, permeability and bioavailability and can also be used for controlled release of the drug; thus, they can be used for BCS class III drugs.[9,10] Of these, the vesicular carriers like Proniosomes have shown great promise in transdermal applications because they penetrate the stratum corneum, thereby enhancing the permeation of drugs and modulating their release.[11] The formulation and development of the system followed a Quality by Design (QbD) approach in alignment with ICH Q8 guidelines. Following

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this approach, a systematic framework emerges that is based on predefined objectives, comprehensive product and process understanding, scientifically based process control, and quality risk management.[12–14] The final product was developed as a Proniosomes gel for transdermal administration, with the aim of enhancing and regulating alendronate sodium permeation across the skin. Proniosomal systems have been reported to enhance transdermal flux and enable controlled novel drug release relative to conventional formulations. Furthermore, such vesicular systems exhibit adequate physicochemical stability under specified storage conditions, maintaining vesicle integrity and drug content over time.[15]

Although proniosomal formulations have shown promise for transdermal drug delivery, limited studies have explored the Quality by Design (QbD)-based development of alendronate sodium-loaded proniosomal gel for improving transdermal permeation. Therefore, the present study aimed to find feasibility to develop and optimise an alendronate sodium-loaded proniosomal gel using a QbD approach and evaluate its physicochemical characteristics and transdermal performance.

MATERIAL AND METHODS

Material

Alendronate Sodium (ALS) was received as a benevolent gift sample from Ambrosia Remedies Pvt. Ltd., Vadodara, India. Span 60 and Span 40 were purchased from Vishal Chemicals, Mumbai. Lecithin, cholesterol, and Carbopol 940 were procured from Dolphin Pharmacy Pvt. Ltd., Mumbai. The remaining ingredients were of analytical grade.

Methods

Formulation development of ALS Proniosomes was through the QbD approach. The Quality Target Product Profile (QTPP) was established to define the desired characteristics of the ALS-loaded Proniosomes gel formulation. The predefined objectives and critical quality attributes are presented in Table 1.

Formulation development and optimisation

Proniosomes were prepared by a modified coacervation-phase separation method following previously reported procedures.[16–18] Precisely weighed amounts of the non-ionic surfactants (Span 40 and Span 60), cholesterol, and soya lecithin were transferred to a clean, dry beaker, and a small volume of ethanol was added to obtain the organic phase. In parallel, alendronate sodium was dissolved in phosphate-buffered saline (PBS, pH 7.4) to prepare the aqueous phase. The organic phase was heated in a water bath at 60-70°C for approximately 5 min to ensure complete dissolution and uniform mixing of the components. Subsequently, the aqueous drug solution was added slowly in a dropwise manner with continuous stirring under mild heating. After the addition was completed, the preparation was cooled to room temperature, resulting in formation of the Proniosomes suspension. The final Proniosomes gel was obtained by incorporating the suspension into 1% Carbopol 940 gel in a 1:1 ratio.[19,20]

Table 1: Quality Target Product Profile (QTPP) and Critical Quality Attributes (CQAs) of ALS-Loaded Proniosomes Gel.

A. Quality Target Product Profile (QTPP).		
Predefined Objectives (QTPP elements)	Target	Reason
Dosage form	Proniosomes gel	Enhances drug permeation through the skin, bypasses hepatic first-pass metabolism, and improves bioavailability.
Route of administration	Transdermal	Provides sustained drug delivery, improves patient compliance, and reduces systemic side effects.
Stability	3 months	Ensures short-term shelf life and initial evaluation of formulation stability.
B. Critical Quality Attributes (CQAs).		
CQA	Target Range	Reason
Vesicle Size	<100 nm	Smaller size enhances dermal penetration and bioavailability
Zeta Potential	> ±30 mV	Indicates good physical stability of vesicles.
Drug Content	85-95%	Maintains dosage accuracy for therapeutic effectiveness.

Design of experiment for the optimisation of formulations

Design-Expert software 13.0 was employed to optimise the selected factors according to the Box-Behnken design for ALS-loaded Proniosomes.[21] The influence of these factors on the response variables, namely mean particle size (Y1) and percentage drug entrapment efficiency (Y2), was evaluated. Table 2 describes the experimental design together with the factors and responses coded for 2^3 Design.

Optimisation of Proniosomes suspension formulation using Design-Expert (DOE):

Goal of Optimization: To optimise the formulation for proniosomal suspension by balancing:

(Y1) Mean vesicle size (nm): Minimise.

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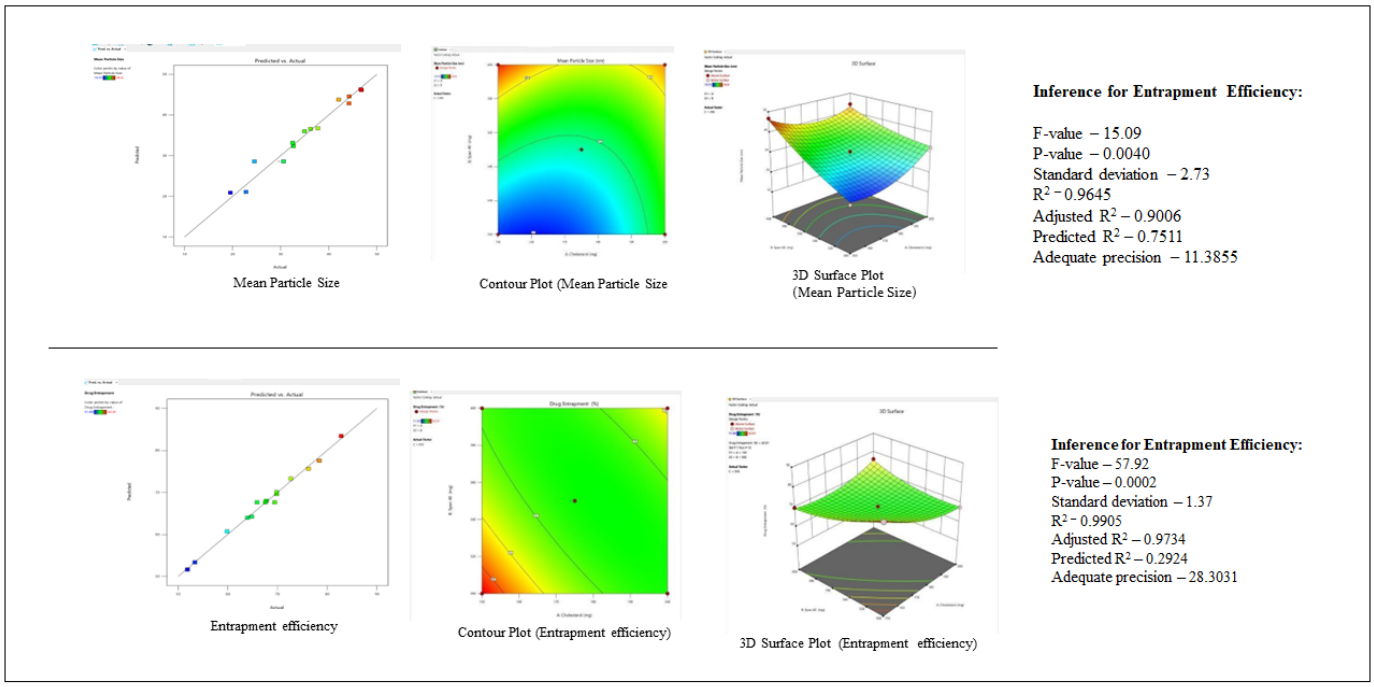


Figure 1: Effect of surfactant and cholesterol on mean particle size (Y1) and entrapment efficiency (Y2), illustrated through predicted versus actual plots, contour plots, and 3D response surface plots generated using the Box-Behnken design.

Table 2: Factors and Levels in Design-Expert.

Factors	Levels		
Independent variables (factors)	-1.0	0.0	+1.0
X1: Amount of cholesterol (mg)	150	175	200
X2: Amount of Span 40 (mg)	300	350	400
X3: Amount of Span 60 (mg)	300	350	400

(Y2) Drug entrapment efficiency (%): Maximise.

Selection of Independent Variables

Based on preliminary studies, the amounts of cholesterol, span 40, and span 60 were selected as independent variables for optimisation. The factors and their levels used in the Box-Behnken design are presented in Table 2.

Fixed Components: ALS (API): 100 mg, Soya Lecithin: 100 mg.

Optimisation and statistical analysis of experimental results using software developed by design experts

The Box-Behnken design was executed for 15 runs. The achieved responses for each run are listed in Table 3. The mean vesicle size for all runs was found to be in the range of 19.55-46.8 nm and the drug entrapment was found to be in the range of 51.89-82.81%.

Effect of surfactant, cholesterol on Entrapment Efficiency% and vesicle size.

Effect of surfactant

Span 60 (Sorbitan monostearate) and Span 40 (Sorbitan monopalmitate) are non-ionic surfactants widely used in Proniosomes formulations.[22] They influence the formation, size, stability, and Entrapment Efficiency (EE %) of vesicles due to their Hydrophilic-Lipophilic Balance (HLB) and alkyl chain length. A higher proportion of Span 60 significantly enhances entrapment efficiency due to better vesicle formation and membrane rigidity. An optimal ratio of Span 60 to Span 40 helps achieve smaller, more stable vesicles with higher EE%. Span 40 is more appropriate for hydrophobic drugs such as ALS, and the excess Span 40 can be used to do this. Lower EE% because of less rigid formation of the membrane.

Effect of cholesterol

Cholesterol plays an important role in regulating the structure of bilayer systems. Its interaction with lipid molecules promotes tighter molecular packing. This results in a larger bilayer thickness

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Table 3: BBD matrix with obtained results for the responses.

Run	Formulation Code	Span-60 (mg)	Span-40 (mg)	Cholesterol (mg)	Drug (mg)	Drug Entrapment Efficiency (%)	Mean Particle Size (nm)
1	F1	350	350	175	100	65.91± 0.33	30.6± 0.14
2	F2	300	350	200	100	51.89± 0.21	34.97± 0.23
3	F3	400	350	200	100	72.68± 0.15	46.65± 0.26
4	F4	300	350	150	100	64.85± 0.20	36.22± 0.17
5	F5	300	400	175	100	59.87± 0.25	44.24± 0.20
6	F6	400	400	175	100	63.97± 0.21	42.07± 0.25
7	F7	350	400	150	100	69.85± 0.18	46.8± 0.11
8	F8	400	350	150	100	69.84± 0.26	37.75± 0.26
9	F9	350	350	175	100	67.57± 0.16	24.55± 0.18
10	F10	350	400	200	100	76.25± 0.22	44.2± 0.22
11	F11	350	350	175	100	69.45± 0.25	30.6± 0.16
12	F12	300	300	175	100	53.38± 0.16	22.8± 0.10
13	F13	350	300	150	100	82.81± 0.20	19.55± 0.20
14	F14	350	300	200	100	67.78± 0.22	32.5± 0.15
15	F15	400	300	175	100	78.41± 0.25	32.655± 0.25

Each result is the mean±S.D (n=3).

and smaller molecular mobility. The effect of cholesterol is also seen on membrane phase behaviour and organisation. It is unable to self-assemble bilayers; however, it does function in association with lipids or surfactants.[23] With the ALS Proniosomes, 150 mg of cholesterol seems to be the best number. Excess cholesterol (above 175-200 mg) may reduce EE% by competing with the drug for space within the bilayer or by increasing membrane rigidity, which can hinder vesicle formation. Balance is A key factor to maximise entrapment while maintaining vesicle structure.

Experimental responses were analysed with a Quadratic model and model adequacy was determined by mathematical parameters, such as R^2, adjusted R^2, and predicted R^2 values (Table 4). The concentrations of cholesterol (A), Span 40 (B), and the equality of the two (C) were the independent variables chosen. (B), and Span 60 (C). The significance of the formulation variables on mean particle size and drug entrapment efficiency was assessed using Analysis of Variance (ANOVA) (Table 4), with $p<0.05$ considered statistically significant. Span 40 (B) and Span 60 (C) showed statistically significant effects on the mean particle size of the proniosomal formulations. In addition, cholesterol (A), Span 40 (B), and Span 60 (C) significantly influenced drug entrapment efficiency. The "3D" plots of the response surfaces using (Figure 1), demonstrate the interactions of these variables on the two primary responses. Furthermore, the actual vs. predicted plots (Figure 1) shows an excellent agreement between the actual and the predicted plots. Experimental data were compared with the predicted values from the model, and the model was found to be accurate in predicting the experimental values. The accuracy and dependability of the applied quadratic model.

Optimisation of the model

Optimisation was conducted to achieve a formulation which showed the desired CQAs. The primary objective was to achieve a vesicle size of less than 100 nm and to achieve as high a drug entrapment as possible. efficiency. The formulation was optimised with three independent variables: the amount of cholesterol (X1) at 151.541 mg, span 40 (X2) at 364.295 mg, and Span 60 (X3) at 303.313 mg. The predicted mean particle size (Mpz) for this combination was 46.29 nm, and the observed value was the size during the confirmation run was 46.65 nm. In the same manner, the drug entrapment efficiency was also predicted as 83.4288%, and the observed value was 82.81%. The close agreement between the predicted and observed values confirms that the optimisation model is reliable and accurate.

CHARACTERISATION OF THE ALS-LOADED PRONIOSOMES

Drug excipient compatibility study

Preformulation compatibility of Alendronate Sodium (ALS) with excipients (1:1 ratio) was assessed by storing samples at 25°C/60% RH and 30°C /75% RH for 30 days. Evaluation was carried out through visual inspection and Fourier Transform Infrared (FTIR) analysis.

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Table 4: BBD matrix results of different models for regression analysis of dependent variables (responses) and ANOVA table for the mean particle size and drug entrapment efficiency.

BBD matrix results of different models for regression analysis of dependent variables (responses).					
Responses	R^2	Adjusted R^2	Predicted R^2	Standard deviation	Coefficient of variation (%)
Mean Vesicle Size	0.9645	0.9006	0.7511	2.73	7.79
Drug Entrapment	0.9905	0.9734	0.9347	1.37	2.03
Mean particle size					
Source	Sum of Squares	d_f	Mean Square	F-value	*p*-value
Model	1013.82	9	112.65	15.09	0.0040(Significant)
Residual	37.32	5	7.46		
Lack of Fit	12.92	3	4.31	0.3529	0.7963(not significant)
Pure Error	24.40	2	12.20		
Cor Total	1051.14	14			
Drug entrapment efficiency					
Source	Sum of Squares	d_f	Mean Square	F-value	*p*-value
Model	985.26	9	109.47	57.92	0.0002(significant)
Residual	9.45	5	1.89		
Lack of Fit	3.18	3	1.06	0.3376	0.8051(Not significant)
Pure Error	6.27	2	3.14		
Total	994.71	14			

Vesicle Size and Morphology

0.1 g sample of the proniosomal suspension was diluted with 10 mL of Phosphate Buffer (pH 6.8) and subjected to sonication for 2 min. A drop of the diluted suspension was placed on a glass slide and examined under an optical microscope at 10× and 45× magnifications, with the corresponding images captured.[24] The mean vesicle size was measured using a digital optical microscope (Nilpa LCD S150 HDMI). The surface morphology of the optimised formulation (F13) was further characterised by Scanning Electron Microscopy (SEM). The zeta potential of the optimised formulation (F13) was determined at 25°C using a Malvern Zetasizer to assess its surface charge and stability.

Entrapment Efficiency (%EE)

Proniosomes suspension (0.1 g) was reconstituted with 10 mL of isotonic phosphate buffer (pH 6.8) and sonicated. The dispersion was centrifuged at 15,000 rpm for 30 min. The supernatant was collected to determine the amount of unentrapped drug using a UV-visible spectrophotometer at 240 nm after complex formation with copper (II) reagent. The encapsulated drug was calculated accordingly.

$$EE\ (\%) = \frac{(C_t - C_r) * 100}{C_t}$$

Where C_t is the total drug concentration and C_r is the concentration of unentrapped drug.

Physical appearance and homogeneity

The prepared gels were visually inspected for color, clarity, and homogeneity, along with the presence of any aggregates after being allowed to set in the containers.[25]

Viscosity

The viscosity of the prepared gel was determined using a Brookfield Viscometer (DV-E). Approximately 10 g of the gel sample was subjected to analysis with spindle 64 at a rotational speed of 12 rpm. All measurements were carried out in triplicate.

pH Determination

The apparent pH of the formulation was measured in triplicate using a pH meter at 25±1°C using a calibrated digital pH meter.

Spreadability

The spreadability was assessed by placing 0.5 g of gel within a pre-marked 1 cm diameter circle on a glass plate, followed by placement of another glass plate and a 50 g weight. The increase in diameter and time required for separation of the slides were recorded. The spreadability '*S*' was calculated using the formula: S=M*L/t (where *M* is the applied weight, *L* is the distance moved, and *t* is the time taken.)

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Drug content

The drug content of the selected batch F13 was 84.81±0.25%; the formulations were within the acceptable range, indicating uniform distribution of the drug within the excipients.

Extrudability

Formulations were filled into metal tubes that were collapsible and pressed to extrude a 0.5 cm ribbon within 10 s. The ease of extrusion was used to assess formulation consistency and applicability.[26]

In vitro drug release

An *in vitro* drug release study was performed using a dialysis membrane.[27,28] A quantity of gel equivalent to 10 mg of the drug was placed in a dialysis bag, which was subsequently immersed in a phosphate buffer (pH 6.8) serving as the receptor medium. The system was maintained at 37±1°C while stirring at 100 rpm. Samples were withdrawn at predetermined time periods over 24 h and replaced with fresh medium. Drug content was analysed spectrophotometrically at 240 nm after suitable dilution.[29]

Drug Release Kinetics

The drug release data were analysed using various kinetic models, including zero-order, first-order, Higuchi, and Korsmeyer-Peppas models, to elucidate the underlying release mechanism.[30]

In vitro Drug Diffusion

In vitro diffusion studies were performed using a Franz diffusion cell, in which a suitable membrane was positioned between the donor and receptor compartments. The temperature of the system was maintained at 37±0.5°C, and samples were collected at predetermined time intervals and analysed by UV spectrophotometry.[31]

Stability study

Stability studies were performed over a period of three months in accordance with ICH guidelines. The formulations were periodically assessed for drug content to evaluate their stability and detect any potential drug leakage.

RESULTS

Drug - excipient compatibility study

The FTIR spectra of pure ALS and mixture of drug with all excipients are shown in Figure 2.

Vesicle Size and Morphology

Scanning electron microscopy for the selected formulation was carried out. The results are shown in the following SEM photograph Figures 3 and 4. Zeta potential of Proniosomes suspension was measured by Malvern zeta-sizer (Zetasizer ver 7.13; Serial Number: MAL1227437) and zeta potential was recorded which was found as of -37.9 mV.

Physical appearance and homogeneity

The Proniosomes gel formulations have a turbid white appearance.

Viscosity

Viscosity was found in the range of 2675 centipoises to 14250 centipoises of all formulations.

pH Determination

The pH value for the optimised drug loaded F13 formulation was recorded 6.2±0.2, which lies in the normal pH range (5.5 to 7.0) of the skin.

Spreadability

Optimised Proniosomes gel formulation displayed excellent spreadability i.e. gel is easily spreadable. Spreadability of proniosomal gel was found to be 24.42±0.14 g.cm/sec.

Drug content

The drug content of the selected formulation, F13, was found to be 84.81±0.25%, which was within the acceptable range, indicating uniform mixing of the drug with the excipients.

Extrudability

The formulations exhibited good extrudability, with the gels being easily dispensed from the collapsible tubes.

In vitro diffusion studies

The *in vitro* drug diffusion study was performed using the dialysis membrane method to assess the release profiles of the selected formulations. Among the tested formulations, F13 demonstrated the highest cumulative drug release, reaching 94.46% over 12 h, indicating an efficient and sustained release pattern. Incorporation of proniosomal vesicles into the gel matrix effectively extended drug release compared with the plain drug gel, suggesting its potential for improved transdermal drug delivery.

Drug release kinetics

The drug release profiles were evaluated by fitting the data to various kinetic models, Among the models examined, the Higuchi model showed the best fit, with the highest regression coefficient

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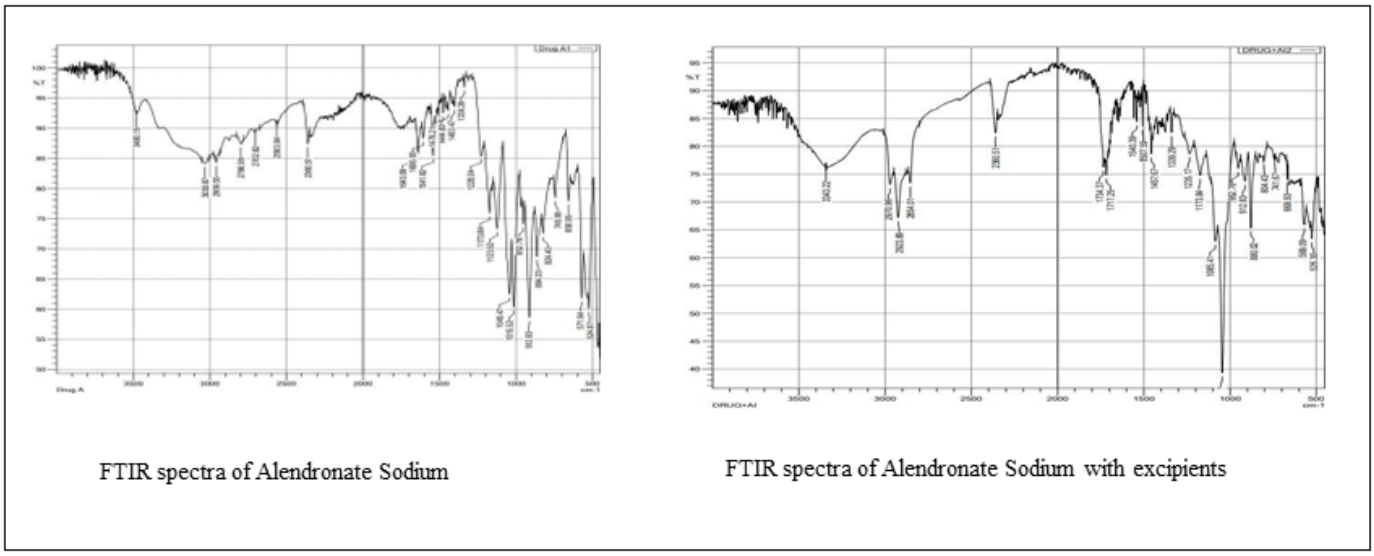


Figure 2: FTIR spectra of Alendronate Sodium and Alendronate Sodium with Excipients.

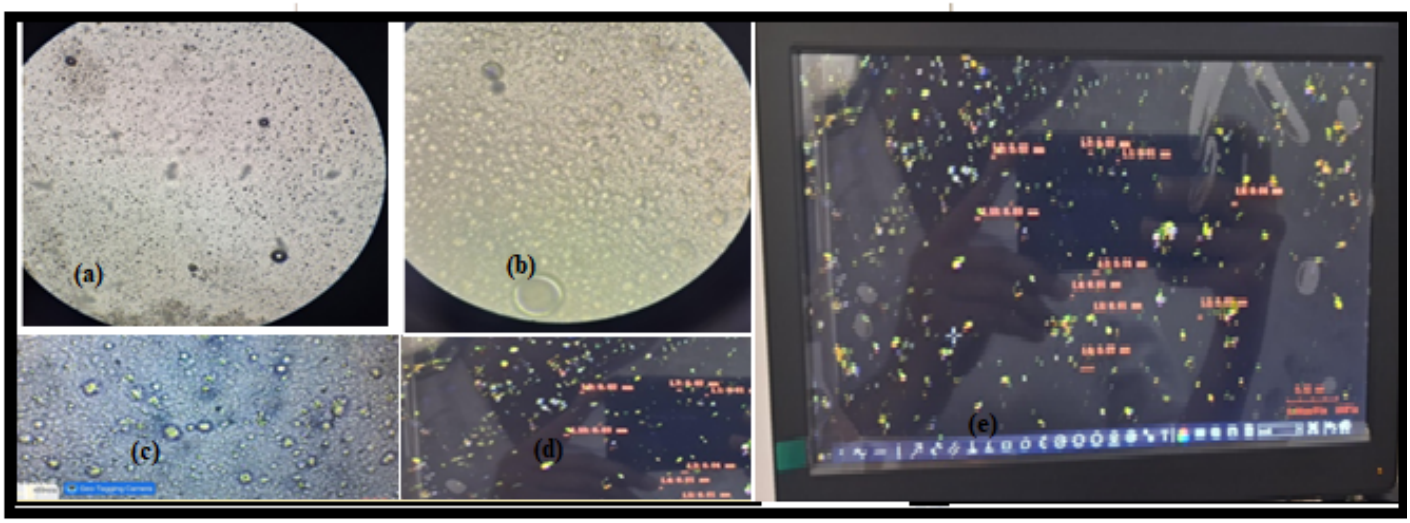


Figure 3: Proniosomes vesicles under optical microscope 10X (a) and 45X (b); under Digital optical microscope (c, d); vesicles size determination under Digital optical microscope (e).

(R^2=0.9559), indicating that drug release predominantly occurred through a diffusion-controlled mechanism.

Stability study

Stability studies of the optimised formulation F13 were performed in accordance with WHO and ICH guidelines. Accelerated stability testing was conducted as per ICH recommendations, with ALS content in the samples determined using UV spectrophotometry. The formulation was stored under two conditions: 25±2°C /60%±5% Relative Humidity (RH) and 40±2°C /75%±5% RH, representing long-term and accelerated storage conditions, respectively, for three months. No noticeable changes in the color or viscosity of the proniosomal gel were observed throughout the study, indicating satisfactory formulation stability under both storage conditions.

DISCUSSION

The Alendronate Sodium Proniosomes gel was successfully prepared and optimised using a modified coacervation-phase separation method. The method enabled entrapment of hydrophilic Alendronate Sodium using Span 60, Span 40, and soya lecithin, thereby supporting vesicle stability and drug encapsulation through bilayer formation. The compatibility of the drug with all excipients was proven through Fourier transform infrared spectroscopy, showing no interactions (Figure 2). The preliminary batches produced desirable results; therefore, Design of Experiments (DOE) was subsequently applied for further

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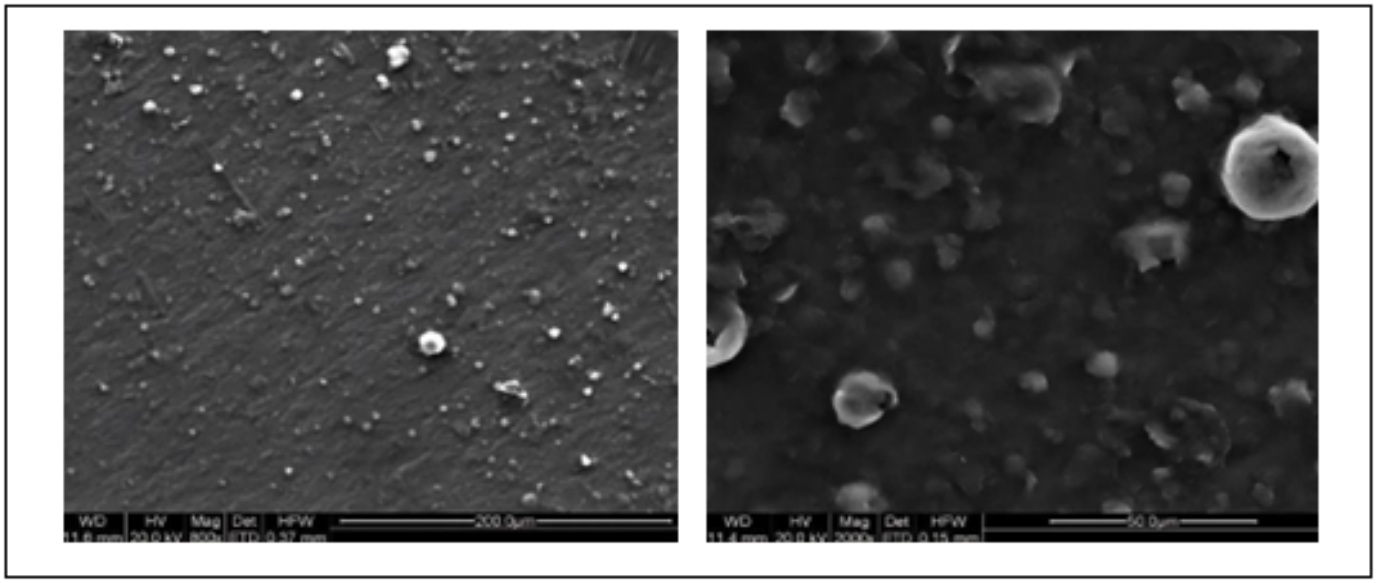


Figure 4: Scanning electron micrographs of Proniosomes suspension.

optimisation. The Alendronate Sodium Proniosomes suspension was optimised using Box-Behnken Design (BBD), resulting in improved drug entrapment efficiency and mean particle size. The prepared Proniosomes suspension exhibited high drug entrapment efficiency, stability, and retention properties, showing the best entrapment efficiency (82.81%) and mean particle size (19.55 nm). Formulation F13 displayed the highest drug release (94.46%) after 12 h. The optimised formulation showed enhanced drug release and diffusion relative to the plain drug gel formulation. *In vitro* release kinetics were best described by the Higuchi model, consistent with sustained drug release. The Proniosomes gel formulation demonstrated prolonged drug release, maintaining a constant drug concentration of over 12 h. The optimised formulation's reduced dosing frequency can minimise the risk of developing resistance to Alendronate Sodium. The stability study confirmed good formulation stability over the period of three months. The optimised formulation appears amenable to scale-up and manufacture, supporting its potential suitability for larger-scale production. Digital optical microscopy images showed Proniosomes in the nanometre range (Figure 3). Formulation F13 exhibited a zeta potential of -37.9 mV, representing the net surface charge of the Proniosomes. The relatively high surface charge provides electrostatic repulsion between vesicles, which may reduce aggregation and contribute to physical stability. Negative charge results in rapid blood clearance. The Proniosomes gel was tested for physical appearance, pH, spread ability, viscosity, drug content and extrudability.

CONCLUSION

The Alendronate Sodium-loaded Proniosomes gel was successfully developed using the coacervation-phase separation method and optimised according to the Quality by Design (QbD) approach. The formulation strategy began with the identification of the Quality Target Product Profile (QTPP), followed by the selection of Critical Quality Attributes (CQAs), including vesicle size and drug entrapment efficiency. Subsequently, the Critical Material Attributes (CMAs) and Critical Process Parameters (CPPs) were determined and screened. Optimisation was carried out through the Box-Behnken Design, resulting in an ideal formulation composed of 151.541 mg cholesterol, 364.295 mg Span 40, and 303.313 mg Span 60. Formulation F13 contained Span 60, Span 40, cholesterol, and soya lecithin and achieved an entrapment efficiency of 82.81% with a vesicle size of 19.55 nm. The optimised gel demonstrated favourable physicochemical characteristics, including appropriate pH, spread ability, viscosity, and extrudability, indicating compatibility with the skin. Pharmacokinetic studies *in vitro* were conducted. *In vitro* drug release followed Higuchi diffusion kinetics, with 94.46% of the drug released over 12 h. The formulation was physically and chemically stable for three months under various storage conditions. This optimised Proniosomes gel is an effective transdermal delivery system. The optimised Proniosomes gel represents a potential alternative to conventional oral Alendronate Sodium therapy and may offer improved bioavailability for osteoporosis management.

Several animal models can be considered for preclinical evaluation of the Alendronate sodium-loaded Proniosomes gel. Recognised models include ovariectomized rats, orchiectomized

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male rats, ovariectomized mice, and rabbit models. The ovariectomized rat model is widely regarded as a useful model for evaluating anti-osteoporotic formulations because it closely mimics postmenopausal bone loss and provides a platform for assessing therapeutic effects. The Alendronate sodium-loaded Proniosomes gel was found to be effective when tested for efficacy. However, the present study is limited by financial constraints and the technical complexities associated with surgical induction of ovariectomy, which restricted the use of this model. These limitations may affect the comprehensive *in vivo* evaluation and translational applicability of the findings.

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ABBREVIATIONS

ALS: Alendronate Sodium; **SEM:** Scanning Electron Microscopy; **PBS:** Phosphate-Buffered Saline; **FTIR:** Fourier Transform Infrared Spectroscopy; **ANOVA:** Analysis of Variance; **HLB:** Hydrophilic-Lipophilic Balance; **BCS:** Biopharmaceutics Classification System; **CQA:** Critical Quality Attributes; **QTPP:** Quality Target Product Profile; **CMA:** Critical Material Attributes; **CPP:** Critical Process Parameters; **EE:** Encapsulation Efficiency; **DoE:** Design of Experiments; **QbD:** Quality by Design; **BBD:** Box-Behnken Design; **% EE:** Percentage Entrapment Efficiency; **ZP:** Zeta Potential; **TDDS:** Transdermal Drug Delivery Systems; **RH:** Relative Humidity; **WHO:** World Health Organization; **ICH:** International Council for Harmonization.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

FINANCIAL SUPPORT AND SPONSORSHIP

No funding was received for this study (self-funded).

AUTHOR CONTRIBUTION

Dr. Jaya Agnihotri: Conceptualization, supervision, methodology, and critical revision of the manuscript. **Pratibha Patil:** Experimental work, data collection, analysis, and manuscript preparation. **Naina Prajapati:** Assistance in manuscript preparation. **Maitri Parab:** Assistance in manuscript preparation.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and analysed during the current study are not publicly available; however, they may be obtained from the corresponding author upon reasonable request.

ETHICS STATEMENT

The study did not involve human participants, animals, or plant materials. Therefore, ethical approval and informed consent were not required.

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