Available online at www.elixirpublishers.com (Elixir International Journal)

Pharmacy

Elixir Pharmacy 42 (2012) 6144-6150

ISSN: 2229-712X

Optimization of disodium edetate topical gel using central composite design and evaluation for external radioactive decontamination

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ARTICLE INFO

Article history:
Received: 2 November] 2011;
Received in revised form:
16 December 2011;
Accepted: 27 December 2011;

Keywords
Topical gel,
Disodium edetate,
External decontamination,
^{99m}Tc-pertechnetate,
Central composite design.

ABSTRACT

In order to develop a superior formulation for skin decontamination of ^{99m}Tc-pertechnetate (a potential radiocontaminant), a topical gel formulation containing disodium edetate was optimized by using 2-factor, 3-level central composite design. Polymer concentration (A) and disodium edetate concentration (B) were selected as the independent variables and the dependent variables were selected as viscosity (Y_1), spreadability (Y_2) and extrudability (Y_3) of the gel. The viscosity of the gel was found to decrease proportionally with spreadability and extrudability, whereas the spreadability was increased proportionally with extrudability. Validation of the optimization study with 13 confirmatory runs indicated a high degree of predictive ability of response surface methodology (RSM). The optimized formulations were evaluated for drug content, *in vitro*, *in vivo*, *ex vivo* and skin irritation studies. The *in vitro* evaluation of the topical gel efficacy study confirmed the good chelation efficacy of disodium edetate molecules with ^{99m}Tc-pertechnetate ions. The *ex vivo* diffusion kinetics study demonstrated that the permeation rate of ^{99m}Tc-pertechnetate through intact skin was decreased to 83.68% after immediate application of disodium edetate gel. *In vivo* studies demonstrated that the application of topical gel is effective in external decontamination of ^{99m}Tc-pertecnetate from male Sprague-Dawley rats. The optimized gels did not produce any dermatological reactions on rats. All the results of the study revealed that disodium edetate loaded topical gel is a promising formulation for the external decontamination of radioactive agents.

Introduction

During radiation exposure, skin, most superficial organ of the body, often receives the highest dose of radiations. Occurrence of external contamination on skin of occupational workers by ^{99m}Tc-pertechnetate is a distinct possibility. Radiation exposure and/or external radionuclide contamination on skin is associated with radiation burns and radiation induced cancer risks [1,2]. Radionuclides may also enter into the systemic circulation by absorption through intact or broken skin, besides ingestion and inhalation routes. External radionuclidic contamination is, therefore, a big concern as it may induce a high external and/or internal radiation exposure in the contaminated individuals [2,3]. An important step in medical management of contaminated victims involves local skin decontamination by rinsing with tepid water and soap solutions, followed by chelation with EDTA (ethylene diamine tetra acidic acid), DTPA (diethylene triamine pentaacidic acid), etc [4,5].

Response surface methodology (RSM), one of the well known approaches for constructing approximation models based on physical experiments and experimented observations aims to optimize a response, which is influenced by several independent variables [6-8]. RSM designs include Box-Behnken design, central composite design (CCD), 3-level factorial design and D-optimal design. Central composite design is expedient in building a second order (quadratic) model for the response variables, without the necessity to use a complete 3-level factorial design [9].

The main objectives of this study are to develop a new topical formulation containing disodium edetate for skin decontamination, characterize its physiochemical properties and evaluate its decontamination efficiency for ^{99m}Tc-pertechnetate, with the accomplishment of *in vivo*, *ex vivo* and *in vitro* studies.

Material and Methods

Materials

^{99m}Tc-pertechnetate was obtained from Regional Centre for Radiopharmaceuticals of the Board of Radiation and Isotope Technology (BRIT), Delhi, India. Disodium edetate (Merck Ltd. Mumbai, India), carbopol 934P (Qualikems, Vododara, India), methyl paraben (Ranbaxy Fine Chemicals Ltd., New Delhi, India), propyl paraben (Ranbaxy Fine Chemicals Ltd., New Delhi, India), triethanolamine (Fisher Scientific, Mumbai, India) and other chemicals /reagents used, were of analytical grade.

Preparation of Gel

The gel was formulated by dissolving the required quantity of disodium edetate in water. Preservatives and carbopol 934P were added to the aqueous media with constant stirring, until the polymer was completely hydrated and formed uniform dispersion.

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Triethanolamine was added to the dispersion to attain the desired consistency. The formulations were formulated and optimized using central composite design. Thirteen confirmatory runs were prepared and evaluated for various individualities like viscosity, extrudability and spreadability.

Experimental Design

For the optimization of topical gel formulation, a central composite design was employed for three dependent and two independent variables to estimate various parameters, interaction and, also to evaluate the quadratic effects of the ingredients on disodium edetate topical gel by constructing polynomial models with Design Expert (version 8.0.0, Stat-Ease Inc., Minneapolis, Minnesota) [11,12]. The polynomial equation generated by this experimental design is given as:

$$Y = d_o + d_1A + d_2B + d_3AB + d_4A^2 + d_5B^2 \quad (1)$$

where Y is the dependent variable; d_o is the intercept; d_1 to d_5 are the regression coefficients computed from the observed experimental values of Y. A and B are the coded levels of independent variables. The terms A, B and A^2, B^2 represent the interaction and quadratic terms, respectively.

pH Measurement

An aqueous dispersion of the gel was prepared and pH of this dispersion was measured using pH meter (Mettler Instruments, Germany), which was calibrated before each use, with buffered solutions at pH 4.0, 7.0 and 9.0 [9].

Rheological Measurements

The viscosity of the formulated gel was measured in triplicate using Brookfield R/S plus cone and plate viscometer (Brookfield Engineering Laboratories Inc., Middleboro, MA, USA) at 25° C and 100 rpm for 60 sec.

Spreadability and Extrudability

The spreadability of the formulated gel was measured by placing 0.5 g of gel within a circle of 1cm diameter pre-marked on a glass plate, over which a second glass plate was placed. Then, a weight of 500 g was allowed to rest on the upper glass plate for 5 min. The increase in diameter due to spreading of gel was observed [10].

Extrudability of formulated gel was determined by measuring the quantity of gel extruded from collapsible tube on application of constant weight. A closed collapsible tube containing 20 g of gel was pressed by applying a constant load of 1 kg at the crimped end. When the cap was removed, gel extruded until pressure dissipated. The extruded gel was collected and weighed.

Evaluation of Disodium Edetate Gel Formulation

The formulated gel was evaluated for *in vitro* chelation efficacy as well *in vivo*; pharmacoscintigraphy and skin irritation studies were performed on two month old male Sprague-Dawley rats, weighing 180-200 g, obtained from the central animal house facility of INMAS (Institute of Nuclear Medicine and Allied Sciences), Delhi, India. Approval for carrying out the animal studies was obtained from the INMAS Animal Ethical Committee, New Delhi and their guidelines were followed throughout the studies.

***In Vitro* chelation efficacy**

A simple method was developed for the measurement of chelation efficacy of disodium edetate gel for ^{99m}Tc-pertechnetate, using ITLC-SG (instant thin layer chromatography with silica gel) chromatography (Gel Man) [11-13]. Three aliquots of 10 ml, each containing placebo gel, disodium edetate gel and aqueous solution of disodium edentate, were prepared respectively, and incubated with ^{99m}Tc-pertechnetate (37MBq) at 37° C for 30 min. The 2 µl of each aliquot were run on ITLC-SG strips at 5, 10, 20 and 30 min interval using 100% acetone as mobile phase. After developing, each ITLC-SG strip was cut into two parts i.e. top and bottom part, and the activity was measured using well-type gamma counter.

***Ex Vivo* Permeation Study of ^{99m}Tc-pertechnetate**

Ex vivo permeation study of ^{99m}Tc-pertechnetate was performed on male Sprague-Dawley rat skin, with and without the application of formulated disodium edetate gel. Rats were sacrificed by cervical dislocation and the shaved skin was removed. Skin explants with a contact area of 1.53 cm^2 were mounted on receptor compartment of the Franz diffusion cell, with dermal face in contact with phosphate buffer (pH = 7.4). Thereafter, ^{99m}Tc-pertechnetate was applied on the outer surface of skin. The contamination was made by depositing 20 µl of 0.37MBq/ml ^{99m}Tc-pertechnetate on the skin sample in the donor compartment of the Franz diffusion cell. Five experimental sets i.e., without application of disodium edetate gel, immediate application, application at 5, 10 and 20 min, after contamination with ^{99m}Tc-pertechnetate were prepared in triplicates, and evaluated by withdrawing 2 ml samples at 0, 15, 30, 45 and 60 min durations. The radioactive counts in each sample were measured using well-type gamma counter, and the calculation was done accordingly, in order to determine the diffusion kinetics of ^{99m}Tc-pertechnetate in the absence and presence of disodium edetate gel [14-15].

***In Vivo* Decontamination Efficacy Study of Disodium Edetate Gel on Rats**

Nine adult male Sprague-Dawley rats weighing 180-200 g were equally divided into three groups for optimized gel formulation, placebo gel and disodium edetate solution, respectively. Interscapular area was shaved before 24 h of the study. Rats were anesthetized and externally contaminated with ^{99m}Tc-pertechnetate, by applying to the shaved area (5.30 cm^2), with equal dose (10 µCi/ml). Scintigraphic imaging and counts (static, 60 s) were taken by placing rats under gamma camera (Hawkeye Millennium, Siemens) immediately after contamination (initial counts). Respective formulations were applied on three groups and the static imaging was done before (prewash counts) and after wiping (post-wash counts) the test area with wet cotton swab. The method involves the comparison of optimized gel formulation efficacy, in terms of external decontamination, with placebo gel and disodium edetate solution.

Skin Irritation Studies

The skin irritation studies were also carried out on nine male Sprague-Dawley rats, weighing 180-200 g. The dorsal skin region of the rats was shaved using clipper one day before study. The rats were divided into three groups as group I (for disodium edetate gel), group II (for placebo gel) and group III (for disodium edetate solution). The animals were treated daily for 7 days and the treated skin was examined visually for erythema and edema. The evaluation was made using score method as shown in Table 1.

Accelerated Stability Studies

The optimized gel formulation was subjected to stability testing for three months as per ICH guidelines at 25° ± 2°C, 60% RH and, was analyzed for any change in viscosity, spreadability, extrudability and percentage drug remaining.

Response Analysis for Optimization

Statistical validation of the polynomial equation generated by Design Expert® was established on the basis of ANOVA provision in the software. Total 13 runs with five center points

were generated. Validation of response surface methodology results was performed to find the compositions of optimized formulations over the whole experimental region. Three optimum checkpoint formulations (DC-1 to DC-3) were selected and validated for various response properties and, the experimental values were also quantitatively compared with the predicted values.

Results and Discussion

Characterization of Gel

The prepared gel containing disodium edetate was found to be transparent, uniform in consistency, free from particulates and grittiness, when observed under microscope. The pH values of all the prepared formulations ranged from 5.7 to 6.4, which lies in the normal range of skin pH.

Spreadability plays an important role in uniform application of gel formulation to skin. The spreadability of gel formulations was decreased with an increase in the concentration of carbopol 934P. The values of spreadability (5.5 - 6.7 cm) indicate that the gels were easily spreadable. The extrudability values of all thirteen prepared gels ranged from 1.15 - 2.45 g (Table 4).

Rheological Measurements

Viscosity of all the thirteen formulations was found to be in the range of 337.42 – 717.22 cp. The results observed are summarized in Table 2.

Fitting of Data to the Model

A 2-factor, 3-level central composite experimental design was applied on thirteen confirmatory runs. The independent variables and the responses for all 13 experimental runs are given in Table 2. The ranges of Y1, Y2 and Y3 for all batches were found to be 337.42 - 717.22 cp, 5.5 - 6.7 cm and 1.12 - 2.45 g, respectively. It was observed that the best-fitted model was quadratic and, the comparative values of R^2, SD and % CV observed, are given in Table 3 along with the regression equation generated for each response. All statistically significant ($p< 0.05$) coefficients are included in the equations.

A positive value represents synergistic effect, while a negative sign indicates an antagonistic relationship between the factor and the response. It is evident that both independent variables, namely, the concentration of carbopol 934P (A) and concentration of disodium edetate (B), have interactive effects on all the three responses i.e., Y_1, Y_2 and Y_3.

Contour Plots and Response Surface Analysis

Two dimensional contour plots and three-dimensional response surface plots are shown in Fig. 1(A)-1(B), 2(A)-2(B) and 3(A)-3(B) for responses Y_1, Y_2 and Y_3, respectively. These plots are useful for studying the effect of the factors on the responses.

Response Analysis through Polynomial Equations

Response 1(Y_1): Effect on Viscosity of Gel

The model proposed the following polynomial equation for viscosity of gel:

$$Y_1 = 351.49 + 51.87A - 33.93B - 98.95AB + 134.58A^2 + 36.86B^2 \quad (2)$$

where Y_1 is the viscosity of topical gel, A is the amount of carbopol 934P used and B is the amount of disodium edetate used.

The model was found to be significant (F-value = 219.48; $p < 0.0001$), while the lack of fit was found to be not significant (F-value = 2.92; p = 0.1637). The predicted (0.9660) and adjusted (0.9891) R-square values were in reasonable agreement. The signal-to-noise ratio was also found to be satisfactory, as the observed adequate precision ratio of 38.981 is above 4.

Factor A appeared to have more profound effect on viscosity than factor B. As the polymer level increased, the viscosity also increased dramatically. Fig. 1(A) and 1(B) show the effect of polymer and drug levels on viscosity. Factor B which is the concentration of the disodium edetate used, affected the viscosity in opposite direction to that observed with factor A. The negative coefficient value of factor B indicated the decrease in viscosity with an increase in factor B. It was observed that, when higher concentration of disodium edetate was used, lower viscosity was obtained. This might be due to the fact that higher amount of disodium edetate stabilized the formulated topical gel and, prevented it from aggregation.

Response 2 (Y_2): Effect on Spreadability of Gels

The following polynomial equation prevailed from the model for spreadability of gel:

$$Y_2 = 6.52 - 0.055A + 0.073B + 0.18AB - 0.46A^2 - 0.18B^2 \quad (3)$$

where Y_2 is the spreadability of topical gel, A is the amount of carbopol 934P used and B is the amount of disodium edetate used.

The model was found to be significant (F-value = 16.41; $p < 0.0002$), while the lack of fit was found to be not significant (F-value = 0.24; p = 0.8637). The predicted (0.8103) and adjusted (0.8652) R-square values were in reasonable agreement. The signal-to-noise ratio was also found to be satisfactory, as the observed adequate precision ratio of 9.996 is above 4. The effect of polymer and drug levels on spreadability is shown in Fig. 2(A) and 2(B). The results revealed that spreadability of the gel decreased with an increase in the concentration of carbopol 934P.

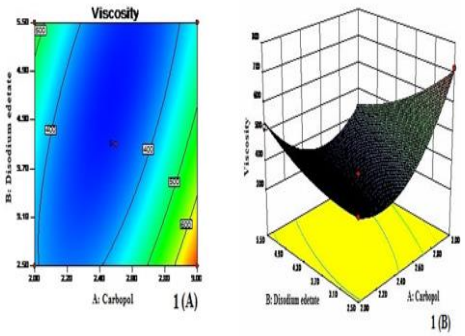

Figure 1. Contour plot showing effect of polymer concentration (A) and disodium edetate concentration (B) on response Y_1

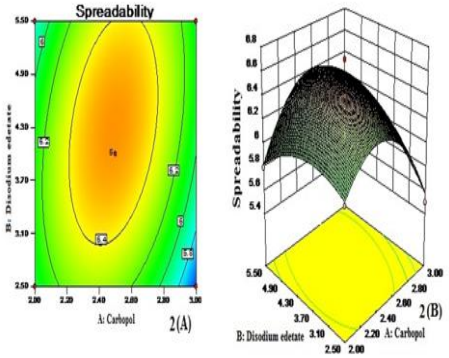

Figure 2. Contour plot and response surface plot showing effect of polymer concentration (A) and disodium edetate concentration (B) on response Y_2

Response 3 (Y_3): Effect on Extrudability of Gels

The model proposed the following polynomial equation for extrudability of gel:

$$Y_3 = 2.36 - 0.097A + 0.027B + 0.048AB - 0.56A^2 - 0.46B^2 \quad (4)$$

where Y_3 is the extrudability of topical gel, A is the amount of carbopol 934P used and B is the amount of disodium edetate used.

The model was found to be significant (F-value = 25.67; $p < 0.0001$) while the lack of fit was found to be not significant (F-value = 1.96; $p = 0.2615$). The predicted (0.7483) and adjusted (0.9114) R-square values were in reasonable agreement. The signal to noise ratio was also found to be satisfactory, as the observed adequate precision ratio of 11.449 is above 4. The effect of different independent variables on extrudability of gel (Y_3) is shown in Fig 3(A) and 3(B). It was revealed that extrudability of gel decreased with increase in concentration of the carbopol 934P.

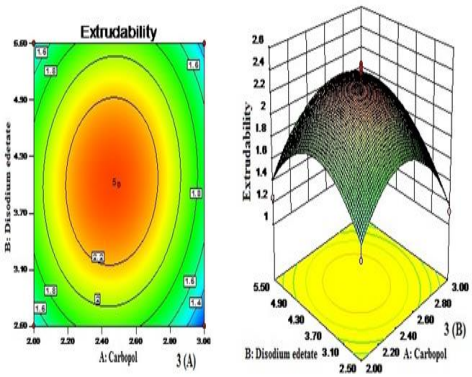

Figure 3. Contour plot and response surface plot showing effect of polymer concentration (A) and disodium edetate concentration (B) on response Y_3Data Analysis and Optimization

The optimum formulation was selected by applying following constraints on the response Y_1 (525-575 cp), Y_2 (5.8-6.2 cm), and Y_3 (1.5-1.8 g). Point prediction of the design expert software was used to agree on the optimized composition, which is DC-1. It predicted the optimized process parameters to be 529.18 cp viscosity, 5.76 cm spreadability and 1.53 g extrudability of the gel formulation, with 1.99% polymer and 5.34% disodium edetate, respectively. Based upon the prediction, three formulae were randomly selected and the responses of viscosity, spreadability and extrudability were evaluated. The validation for RSM, which involves all the three checkpoint formulations, was found to be within limits (Table 4). The percentage prediction error assures the validity of generated equations, and thus, depicts the domain of applicability of RSM model. From this analysis, the optimized formulation (DC-1) was subjected to *in vitro* chelation studies, *in vivo*, *ex vivo*, skin irritation and stability studies.

Evaluation of *In Vitro* Chelation Efficacy

The chelation efficacy of disodium edetate gel for ^{99m}Tc-pertechnetate was evaluated and compared with placebo and disodium edetate solution (control). Data is shown in Table 5 and Fig. 4. The results revealed that formulated disodium edetate gel is able to chelate 71.85% of ^{99m}Tc-pertechnetate within 5 min of contamination, whereas placebo gel and disodium edetate solution showed only 15.32% and 17.39% chelation, respectively, after 30 min of contamination, which demonstrates that the formulated disodium edetate gel is much more effective in decontamination of ^{99m}Tc-pertechnetate.

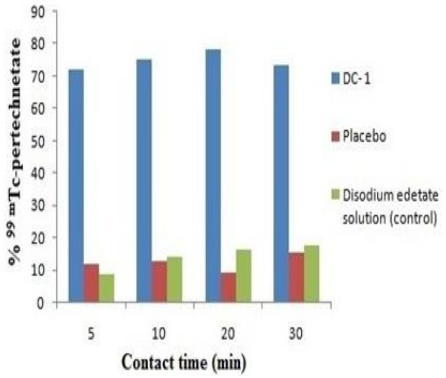

Figure 4. Extraction ability of disodium edetate topical gel for ^{99m}Tc-pertechnetate

***Ex Vivo* Permeation Study of ^{99m}Tc-pertechnetate**

Ex vivo permeation study of ^{99m}Tc-pertechnetate through rat skin was performed with and without the application of formulated disodium edetate gel. The results (Fig. 5) demonstrated that the immediate application of disodium edetate gel after contamination, reduced 83.68% of ^{99m}Tc-pertechnetate

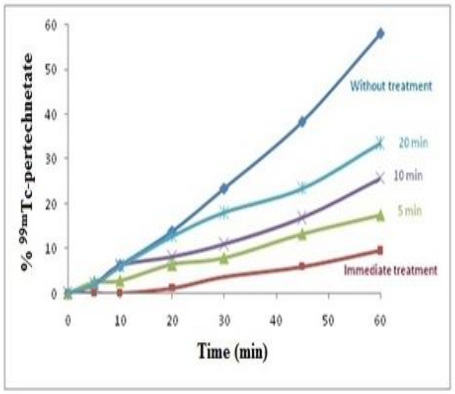

Figure 5. Diffusion kinetics of ^{99m}Tc-pertechnetate through intact skin without treatment, treatment given immediately and after 5, 10, 20 min with disodium edetate topical gel

permeation through skin as compared to control (without disodium edetate gel).

***In Vivo* Studies**

In vivo studies performed on Sprague-Dawley rats demonstrated that the optimized formulation (DC-1) decontaminated 74.50% of ^{99m}Tc-petechnetate effectively from the skin, as compared to 16.75% by placebo and, 08.97% by disodium edetate solution. The results of *in vivo* studies are shown in Table 6 and Fig. 6.

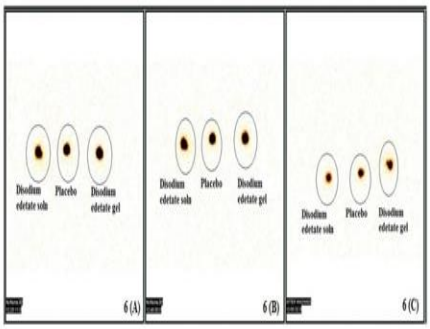

Figure 6. Gamma scintigraphy images: immediately after contamination 6 (A), prewash 6 (B) and post-wash 6 (C)

Skin Irritation Studies

Skin irritation studies were performed on Sprague-Dwaley rats. It was found that the optimized gel was unable to produce any dermatological reaction, which indicated its safety and acceptability for topical application.

Accelerated Stability Studies

Accelerated stability studies were carried out using the optimized topical gel, as per ICH guidelines, for a period of three months. The samples were evaluated for any change in viscosity, spreadability, extrudability and drug content. The data is presented in table 7. A sigma plot was constructed using Sigmaplot^{TM} 10 software (Cranes Software International, Bangalore, India), and is depicted in Fig. 7. Further, the shelf life of the optimized formulation (DC-1) was found to be 23 months and 19 days.

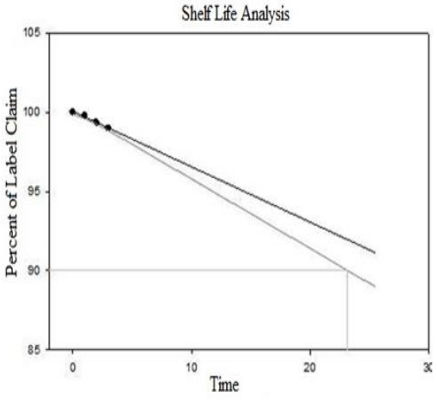

Figure7. Sigma plot showing shelf life analysis of optimized disodium edetate topical gel

Conclusion

The present study demonstrates that the formulated disodium edetate topical gel using 2 factors, 3 levels central composite design could be successfully applied for external decontamination of ^{99m}Tc-pertechnetate. The quantitative responses: viscosity, spreadability and extrudability of gels, for different combination of independent variables: polymer concentration and disodium edetate concentration, were obtained experimentally and the results were found to fit the designed model. The experimental responses of the optimized formulation were found to be close to the predicted responses, which confirmed that the experimental design methodology is a very useful tool in pharmaceutical pre-formulation studies. It requires fewer experimental runs and is less time consuming. Various factorial designs have been used largely for optimization of different transdermal dosage forms. The results demonstrate that the developed topical gel formulation is a viable alternative for conventional methods by virtue of its ability of decontaminate the external contamination of ^{99m}Tc-pertechnetate, ease of application, resulting in better patient compliance.

Acknowledgements

The authors wish to thank the Director of INMAS for his kind cooperation in providing the necessary facilities for carrying this research work.

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Table 1. Score Method for Skin Irritation Study

CONDITIONS	SCORE
Erythema and eschar formation	
No Erythema	0
Very slight erythema (barely perceptible)	1
Well-defined clear Erythema	2
Moderate to severe Erythema	3
Severe Erythema (deep redness) or eschar formation	4
Edema formation	
No edema.	0
Very slight edema (barely perceptible	1
Slight edema (edges of area well defined be definite raising)	2
Moderate edema (swelling of approximately 1 mm)	3
Severe edema (swelling $\geq$1 mm, and extending beyond the exposed area)	4
Maximum Score	4

Table 2. Observed Response in CCD for Topical Gel

Run	Independent factors		Dependent factors		
	Carbopol (A)	Disodium edetate (B)	Viscosity (Y_1) (cp)	Spreadability (Y_2) (cm)	Extrudability (Y_3) (g)
1	2.50	1.88	463.424	6.1	1.48
2	2.00	5.50	520	5.8	1.25
3	3.21	4.00	692.206	5.6	1.15
4	2.50	6.12	393.55	6.3	1.6
5	3.00	2.50	717.226	5.5	1.12
6	1.79	4.00	555.613	5.7	1.55
7	3.00	5.50	432.995	6	1.24
8	2.00	2.50	408.427	6	1.32
9	2.50	4.00	354.781	6.5	2.42
10	2.50	4.00	337.425	6.7	2.45
11	2.50	4.00	358.162	6.3	2.38
12	2.50	4.00	361.769	6.4	2.12
13	2.50	4.00	345.314	6.7	2.42

Table 3. Summary of Results of Quadratic Model for Regression Analysis of Responses Y_1, Y_2, and Y_3

Quadratic model	R^2	Adjusted R^2	Predicted R^2	SD	% CV
ResponseY_1	0.9937	0.9891	0.9660	13.45	2.94
ResponseY_2	0.9214	0.8652	0.8103	0.15	2.40
ResponseY_3	0.9483	0.9114	0.7483	0.16	9.30

Table 4. Composition of Checkpoint Formulations, Predicted and Experimental Values of Response Variables and Percentage Prediction Error

Code	Optimized formulation composition	Response variables	Experimental values	Predicted values	Percentage prediction error
DC-1	1.99 : 5.34	Y_1 (cp)	529.18	525.00	0.78
		Y_2 (cm)	5.76	5.86	-1.73
		Y_3 (g)	1.53	1.5	1.96
DC-2	1.99 : 5.31	Y_1 (cp)	538.71	527.08	2.14
		Y_2 (cm)	5.74	5.85	-2.09
		Y_3 (g)	1.48	1.5	-1.35
DC-3	1.98 : 5.26	Y_1 (cp)	533.84	530.19	0.68
		Y_2 (cm)	5.7	5.84	-2.45
		Y_3 (g)	1.45	1.5	-3.44

Table 5. Data Showing Comparison of Extraction Ability of Disodium Edetate Loaded Topical Gel (DC-1), Placebo and Disodium Edetate Solution (Control)

Contact time	DC-1	Placebo	Disodium edetate solution
5 min	71.85% ± 0.01	11.70% ± 0.02	8.55% ± 0.02
10 min	75.0% ± 0.03	12.44% ± 0.03	13.8% ± 0.05
20 min	78.48% ± 0.03	9.15% ± 0.05	16.17% ± 0.03
30 min	73.63% ± 0.02	15.32% ± 0.04	17.39% ± 0.03

Table 6. *In Vivo* Data Showing Comparison of Optimized Formulation Effectiveness for External Decontamination with Disodium Edetate Solution and Placebo

Formulations	Initial counts (cps) ^{99m}Tc-pertechnetate	Prewash counts (cps) ^{99m}Tc-pertechnetate	Post wash counts (cps) ^{99m}Tc-pertechnetate	% ^{99m}Tc-pertechnetate removed	% ^{99m}Tc-pertechnetate left
Disodium edetate solution (control)	4259 ±1.29	4024 ±0.96	3877 ±1.02	8.97% ±0.75	91.03% ±0.75
Placebo	4776 ±0.77	4529 ±1.01	3976 ±1.61	16.75% ±0.6	83.25% ±0.6
DC-1	4833 ±0.53	4587 ±1.02	1232 ±1.46	74.51% ±0.3	25.49% ±0.3

Table 7. Results of Stability Study Conducted on the Optimized Topical Gel for 90 Days at 25 ± 2°C and 60 ± 5% RH

Time (Days)	Viscosity[a] ± S.D. (cp)	Spreadability[a] ± S.D. (cm)	Extrudability[a] ± S.D. (g)	% Drug remaining
0	529.85 ± 1.17	5.74 ± 0.15	1.78 ± 0.11	100
30	528.25 ± 1.35	5.61 ± 0.33	1.74 ± 0.15	99.78
60	531.81 ± 0.84	5.72 ± 0.11	1.75 ± 0.085	99.34
90	531.52 ± 1.03	5.67 ± 0.16	1.76 ± 0.11	98.97

[a] Not significant ($p > 0.05$)