

Optimization of formulation components and evaluation of 2-chloroethyl ethyl sulfide (CEES) removal efficiency of a PVA/Fuller's Earth/surfactant-based skin decontamination gel

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ABSTRACT

Vesicants are chemical warfare agents that pose severe risks to human health upon exposure, particularly in military contexts. This study presents an optimized hydrogel comprising Polyvinyl alcohol (PVA), Fuller's earth, and decyl glucoside for the rapid ex vivo decontamination of 2-chloroethyl ethyl sulfide (CEES), a sulfur mustard simulant, from porcine skin. The formulation synergizes the polymeric matrix of PVA, the adsorption capacity of Fuller's earth, and the micellar solubilization of decyl glucoside. Residual CEES was quantified via GC-MS. Using a Box-Behnken design and Response Surface Methodology (RSM), the impact of component ratios on dynamic viscosity and decontamination efficiency (DE) was analyzed. Results revealed that DE is driven by the significant quadratic effects of individual components, rather than linear viscosity trends or pairwise interactions. The optimal formulation (9.0 wt% PVA, 11.7 wt% Fuller's earth, and 2.1 wt% decyl glucoside) achieved a maximum DE of 96.5%. These findings confirm the proposed gel as a highly effective, rapid-response countermeasure for vesicant exposure, validating the role of RSM in formulation design.

Keywords: Vesicants; Sulfur mustard; CEES; Skin decontamination.

1. INTRODUCTION

Despite international regulations, chemical weapons remain a significant threat due to the risk of deployment in military conflicts, terrorist attacks, and industrial accidents [1]. Sulfur mustard (HD), a widely recognized chemical warfare agent, has been extensively used due to its high toxicity, rapid dermal absorption, and severe systemic effects [2]. Among the various routes of exposure, cutaneous contact remains the most common, making rapid and effective skin decontamination crucial to minimize absorption and limit subsequent damage.

Several decontamination products are available for military and commercial use, such as the M291 SDK decontamination kit and Reactive Skin Decontamination Lotion (RSDL). However, these products have notable limitations, including the potential to irritate compromised skin (e.g., RSDL), high costs, limited availability, and their applicability only to specific user groups [3].

In recent years, polymer-based systems like the DDGel formulation have emerged as promising alternatives [4-6]. These systems effectively combine polymers and adsorbents to isolate and trap contaminants within the gel matrix, thereby enhancing decontamination efficiency and minimizing the risk of re-exposure. Furthermore, recent studies have focused on formulas that utilize polyvinyl alcohol (PVA), which combines adsorption, sequestration, and physical removal of contaminants within a single material [7].

In this study, 2-chloroethyl ethyl sulfide (CEES) is utilized as an ideal structural simulant for sulfur mustard (HD) [4]. While sharing the same skin-damaging functional group (-SCH₂CH₂Cl), CEES presents a significantly lower toxicity profile (oral LD₅₀ in rats: 252 mg/kg) compared to HD (oral LD₅₀: 17 mg/kg) [8]. Consequently, CEES was employed to evaluate the decontamination

efficacy of the proposed PVA, Fuller’s earth, and decyl glucoside-based gel on *ex vivo* porcine ear skin. Furthermore, Response Surface Methodology (RSM) was employed to systematically optimize the formulation and analyze the interactions among these components.

2. EXPERIMENTAL

2.1. Materials, chemicals and equipment

2.1.1. Materials

Porcine ears were purchased from a local market, shaved to remove hair, and the subcutaneous fat layer was carefully removed. The skin was then separated to obtain samples with a thickness of approximately 1 mm, followed by washing with 0.9% NaCl solution. The prepared skin samples were stored at -18 °C and used within two weeks.

2.1.2. Chemicals

The chemicals included PVA 217 (Kuraray, Singapore), Fuller’s earth (MilitaryGrade NBC Decontamination, England, BET surface area: 230 m²/g), carboxymethyl cellulose (Duc Giang, Viet Nam), decyl glucoside (DG) (Xilong, China), pure 2-chloroethyl ethyl sulfide solution (CEES) (> 95%, Toronto Research Chemicals, Canada), methanol (Merck).

2.1.3. Equipment

The equipment employed in this study included heating magnetic stirrer (VELP, Italy), GT-sonic (China), Gas chromatography-mass spectrometry (GC/MS) instrument 6890N/5975C (Agilent, USA, column DB-5MS UI 30 m × 0.25 mm × 0.25 μm), Brookfield viscometer (DV-II+ Pro) (AMETEK Brookfield, USA), pH meter (Mettler Toledo, Switzerland) and other standard laboratory instruments.

2.2. Methods

2.2.1. Preparation of skin decontamination gel (GTD)

Formulations were prepared by dissolving PVA (6–10 wt%) in 45 mL of a water/absolute alcohol solvent mixture (90/10 v/v) at 70–80 °C under continuous stirring until complete dissolution was achieved. Subsequently, 0.1 g of carboxymethyl cellulose (CMC) was added to the mixture, and stirring was maintained until the CMC was fully dissolved. After the addition of a predetermined amount of decyl glucoside (0–3 wt%), the mixture was stirred for an additional 30 min, after which Fuller’s earth (9–15 wt%) was added and the mixture was heated at 80 °C for 30 min. Finally, the formulations were homogenized by stirring for 2 h at room temperature, then ultrasonicated for 30 min to ensure uniform dispersion.

2.2.2. Dynamic viscosity of GTD

The dynamic viscosity of the skin decontamination gels was determined using a Brookfield viscometer (DV-II+ Pro). Measurements were carried out using spindle S64 at a rotational speed of 30 RPM. All measurements were performed at a controlled temperature of 25 °C. The viscosity values reported correspond to the steady-state readings obtained under these conditions.

2.2.3. Identification of CEES

CEES (in methanol) was identified using a gas chromatography-mass spectrometry system (GC-MS 6890N/5975C, Agilent Technologies, USA) equipped with a DB-5MS UI capillary column (30 m × 0.25 mm × 0.25 μm). The operating conditions of the GC–MS analysis are described in detail in table 1.

Table 1. Method of identification of CEES.

GC Program		MS Program
Capillary column: DB-5MS UI (30 m × 0.250 mm × 0.25 μm)	Initial oven temperature: 40 °C, held for 1 min	Electron ionization mode (EI): Relative

Carrier gas flow (He): 20 mL/min	Oven temperature program: 40 - 150 °C at a ramp rate of 10 °C/min Final time: 2 min	Ion source temperature: 230 °C
Injection mode: Splitless	Final oven temperature: 150 °C	Quadrupole temperature: 150 °C
Injector temperature: 200 °C	Injection volume: 1 µL	
Detector temperature: 290 °C	Total run time: 14 min	

2.2.4. Standard curve for CEES

The CEES calibration curve was constructed using a series of standard solutions with concentrations in the mg/L range, prepared by diluting a pure CEES stock solution in methanol (MeOH). A CEES stock solution at a concentration of 10,000 ppm was first prepared and subsequently diluted to obtain standard solutions with concentrations of 10, 20, 40, 60, 80 and 100 ppm. The calibration curve was generated by plotting peak area (y-axis) versus concentration (x-axis). Linear regression analysis was performed using Origin software based on the linear regression equation $Y = A + BX$, where A represents the intercept and B denotes the slope.

2.2.5. Ex vivo decontamination assays

Following the OECD Guideline 428, CEES was topically applied to the surface of porcine skin at a dose of 3 mg/cm² and allowed to penetrate for 5 min. Subsequently, a decontamination gel was applied at a volume of 100 µL/cm² onto the CEES-contaminated area. The gel was evenly spread over the skin surface using a glass rod and left in contact for 30 min. After the reaction period, the gel was completely removed from the skin surface. The entire skin sample was then fully immersed in methanol (MeOH) to extract and quantify the residual contaminant remaining on the skin. The resulting extract was obtained by ultrasonic-assisted extraction for 30 min at room temperature, followed by appropriate dilution and filtration through a syringe filter PTFE 13 mm 0.2 µm prior to CEES quantification by GC/MS analysis.

The recovery efficiency of CEES (H_{re}) using ultrasonic extraction with MeOH was determined in a manner analogous to the *ex vivo* experiments, except that no decontamination gel was applied. Recovery was calculated as the ratio between the CEES concentration (ppm) quantified by GC/MS and the corresponding theoretical amount initially applied.

The decontamination efficiency (DE) (%) is calculated using formula (1).

$$\text{Decontamination efficiency (DE)}(\%) = \frac{C_{cal} - \frac{C_{decon}}{H_{re}}}{C_{cal}} \times 100 \quad (1)$$

Where:

C_{cal} represents the theoretical maximum concentration of CEES assuming 100% recovery, calculated from the applied dose (3 mg of CEES extracted in 5 mL of solvent), corresponding to 600 ppm.

C_{decon} represents the concentration of CEES remaining on the skin sample after decontamination, as determined by methanol extraction and subsequent GC-MS analysis, expressed in ppm.

H_{re} denotes the recovery efficiency (%) of the methanol extraction method used for CEES quantification. In this study, the recovery efficiency was experimentally determined to be 80% ($H_{re} = 0.80$), and this value was used to correct the measured CEES concentrations.

2.2.6. Design of experiments

A three-level Box-Behnken design combined with Response Surface Methodology (RSM) was employed using Design-Expert® software to construct the experimental matrix and evaluate the main and interactive effects of the components on decontamination efficiency. Three independent variables were investigated at three levels (-1, 0, and +1): the mass fractions of polyvinyl alcohol

(PVA) (6, 8, and 10 wt%), Fuller's earth (9, 12, and 15 wt%), and the surfactant decyl glucoside (0, 1.5, and 3.0 wt%), respectively.

3. RESULTS AND DISCUSSION

3.1. Identification and standard curve for determining CEES concentration

CEES was successfully identified and quantified using GC-MS. A well-defined chromatographic peak corresponding to CEES was detected at a retention time of 6.4 min, with no significant interference. The compound's identity was conclusively confirmed by its characteristic mass fragmentation pattern, which precisely matched the NIST mass spectral library. The mass spectrum featured a base peak at m/z 75, along with additional diagnostic fragment ions at m/z 61 and 124. The standard curve was constructed based on the relationship between peak area and CEES concentration, yielding the equation $Y = 993.63X - 1885.09$ ($R^2 = 0.9994$).

3.2. Characteristics of skin decontamination gel and relationship between dynamic viscosity and decontamination efficiency

3.2.1. Characteristics of skin decontamination gel

The skin decontamination gel was prepared according to the procedure described in Section 2.2.1. The resulting gel exhibited a homogeneous yellowish-brown appearance and was visually homogeneous, with no observable phase separation. The pH of the gel was measured to be approximately 6.5, which is within the range considered compatible with skin application [9].

Table 2. The dynamic viscosity and decontamination efficiency of formulas.

Sample name	Factor A (%P)	Factor B (% F)	Factor C (%D)	Dynamic viscosity (Pa.s)	Decontamination efficiency, DE (%)
M0	100% water				63.21
M1	6	12	0	1.78	87.37
M2	8	15	0	10.94	86.34
M3	8	12	1.5	8.70	95.14
M4	10	15	1.5	15.12	92.21
M5	10	9	1.5	13.25	92.36
M6	8	12	1.5	8.95	94.99
M7	8	9	0	7.64	89.19
M8	8	12	1.5	9.94	95.99
M9	10	12	0	14.69	93.52
M10	6	9	1.5	1.22	85.16
M11	8	12	1.5	8.74	95.67
M12	6	15	1.5	3.09	84.31
M13	8	9	3	8.41	91.66
M14	10	12	3	14.36	93.62
M15	6	12	3	1.63	88.95
M16	8	15	3	11.65	92.81
M17	8	12	1.5	8.89	95.17

The viscosity of the decontamination gels was determined to assess their suitability for cutaneous application, particularly with respect to spreadability, surface retention, and resistance to uncontrolled flow that could promote contaminant wash-in. Adequate viscosity is a critical parameter to ensure homogeneous coverage of the contaminated skin surface while maintaining sufficient structural stability to retain and sequester the toxic agent prior to removal. Accordingly, the dynamic viscosity of the formulated gels was systematically evaluated under controlled

conditions, and the measured viscosity values for all formulations are summarized in table 2. These data provide the basis for correlating formulation composition with rheological behavior and subsequent decontamination performance.

The experimental results demonstrated that the gel formulations (M1 - M17) achieved significantly higher decontamination efficiency compared to the water-only control (M0), which achieved 63.21% DE. This disparity is primarily attributed to the hydrophobic nature of CEES, which resists simple aqueous rinsing but is effectively emulsified and sequestered by skin decontamination gel.

3.2.2. Relationship between dynamic viscosity and decontamination efficiency

The relationship between dynamic viscosity and decontamination efficiency (DE) was analyzed in the context of the functional roles of the individual components in the decontamination gel. As shown in table 2, the dynamic viscosity of the formulations was primarily governed by the PVA content, which controls the formation of a continuous polymeric matrix [10]. This matrix plays a critical role in confining both the adsorptive Fuller's earth particles and the contaminant within the gel phase, thereby contributing to contaminant isolation during the decontamination process. Formulations with similar viscosities exhibited markedly different decontamination efficiencies, indicating that viscosity alone does not directly dictate decontamination performance.

Nevertheless, viscosity strongly influences the effectiveness of the decontamination mechanism by defining an operational window for gel application and surface retention [11]. Low-viscosity formulations, such as M1 (1.78 Pa.s) and M10 (1.22 Pa.s), exhibited relatively low DE values (~85–87%). This reflects insufficient resistance to flow and a limited ability of the PVA network to confine active components, leading to uncontrolled spreading and reduced local Fuller's earth concentration for adsorption. In contrast, excessively high viscosity formulations, such as M4 (15.12 Pa.s) and M9 (14.69 Pa.s), did not consistently achieve higher DE values compared to intermediate viscosities. Such highly viscous gels can hinder homogeneous spreading and restrict effective interfacial contact with the skin, thereby limiting contaminant mobility toward Fuller's earth adsorption sites [11]. Within an appropriate viscosity range, decyl glucoside plays a complementary role by reducing interfacial tension, facilitating contaminant transfer into the gel phase where adsorption and PVA confinement proceed efficiently. Overall, these results demonstrate that dynamic viscosity functions as an enabling parameter supporting the combined action of the components, rather than a direct controlling factor for decontamination efficiency.

3.3. Response surface methodology for decontamination efficiency

Response surface methodology (RSM) was employed to optimize the decontamination efficiency as a function of three core independent variables: the mass fractions of poly(vinyl alcohol) (PVA), Fuller's earth, and decyl glucoside. This formulation's decontamination mechanism operates through a highly synergistic three-step process: First, decyl glucoside (a nonionic surfactant) acts as the primary mobilizing agent. By reducing surface tension, it improves skin wetting and drives micellar solubilization via a "roll-up" mechanism, efficiently detaching hydrophobic CEES droplets from the skin into the aqueous gel phase [12]. Second, Fuller's earth functions as the adsorptive core. Its layered aluminosilicate structure and high surface area rapidly adsorb the surfactant-mobilized CEES, securely locking the simulant within its porous matrix via physical entrapment. Furthermore, utilizing Fuller's earth in a gel dispersion rather than a loose powder mitigates dust generation and secondary contamination [13-15]. Finally, PVA serves as the film-forming polymeric matrix. It provides a robust, continuous network that encapsulates the Fuller's earth particles and confines the contaminant at the application site. This structural confinement effectively prevents the lateral spread of the vesicant and its re-absorption into the skin [10].

A Box-Behnken experimental design (RSM) was employed to systematically evaluate the individual and interactive effects of three independent variables - mass fractions of PVA (% P), Fuller's earth (% F), and decyl glucoside (% D) - on decontamination efficiency (DE). Based on the

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experimental data, the resulting second-order polynomial model correlating DE with the coded variables is expressed as follows:

$$Y = 95.39 + 3.24 \times A - 0.3375 \times B + 1.33 \times C + 0.175 \times A \times B - 0.370 \times A \times C - 1.0 \times B \times C - 3.01 \times A^2 - 3.87 \times B^2 - 1.52 \times C^2$$

Table 3. Results of the multivariate ANOVA.

Source	Sum of Squares	df	Mean square	F-Value	p-Value
Model	225.71	9	25.08	18.19	0.0005
A-% PVA	83.98	1	83.98	60.93	0.0001
B-% Fuller	0.9112	1	0.9112	0.6611	0.4429
C-% decyl glucoside	14.10	1	14.10	10.23	0.0151
AB	0.1225	1	0.1225	0.0889	0.7743
AC	0.5476	1	0.5476	0.3973	0.5485
BC	4.0	1	4.0	2.90	0.1323
A ²	38.11	1	38.11	27.65	0.0012
B ²	63.17	1	63.17	45.83	0.0003
C ²	9.71	1	9.71	7.04	0.0328
Residual	9.65	7	1.38		
Lack of Fit	8.94	3	2.98	16.80	0.0099
Pure Error	0.7093	4	0.1773		
Cor Total	235.36	16			

The adequacy of the developed quadratic model was evaluated by ANOVA (table 3). The model exhibited high statistical significance ($p = 0.0005$, $F = 18.19$), and the high coefficients of determination ($R^2 = 0.9590$, adjusted $R^2 = 0.9063$) indicate that it explains most of the variability in decontamination efficiency (DE). These results confirm a valid and meaningful relationship between the selected formulation variables and DE. Analysis of the linear terms revealed that PVA content (Factor A) exerted the most dominant effect on DE ($p = 0.0001$), controlling gel structure and confinement. Decyl glucoside (Factor C) also showed a significant linear contribution ($p = 0.0151$), confirming its role in enhancing interfacial contaminant transfer. In contrast, Fuller’s earth content (Factor B) was not linearly significant ($p = 0.4429$). Furthermore, all interaction terms (AB, AC, BC) were not statistically significant ($p > 0.05$). This suggests that DE is primarily governed by the individual components’ distinct functional roles and their nonlinear effects, rather than strong pairwise binary interactions. The quadratic terms (A^2 , B^2 and C^2) were all statistically significant ($p < 0.05$). Their negative coefficients indicate pronounced curvature in the response surface, implying each factor has an optimal range. Exceeding these limits diminishes decontamination performance - reflecting practical constraints where excessive PVA hinders spreading, overly high Fuller’s earth loading reduces effective contact, and excessive surfactant disrupts gel integrity. Although the “lack-of-fit” test was significant ($p = 0.0099$) - reflecting the inability of a standard quadratic model to perfectly capture the system’s highly complex, coupled multi-phase dynamics (simultaneous spreading, solubilization, and adsorption) - the very low pure error confirms excellent experimental reproducibility. Furthermore, an adequate precision value of 8.94 (> 4) ensures a robust signal-to-noise ratio. Therefore, while serving as an empirical approximation, the model remains highly reliable for navigating the design space and identifying the optimal formulation.

Three-dimensional response surface plots (Figure 1) revealed pronounced curvature, confirming a nonlinear relationship between formulation composition and DE. All components exhibited optimal ranges, where DE initially increased but declined at extreme levels. This highlights that maximal DE strictly requires a precise balance between adsorption capacity and interfacial transport. Ultimately, numerical optimization identified an ideal formulation window:

9.0 wt% PVA, 11.7 wt% Fuller's earth, and 2.1 wt% decyl glucoside - yielding a maximum DE of 96.5%. The optimized formulation achieved a highly competitive 96.5% decontamination efficiency (DE) against CEES within just 30 minutes.

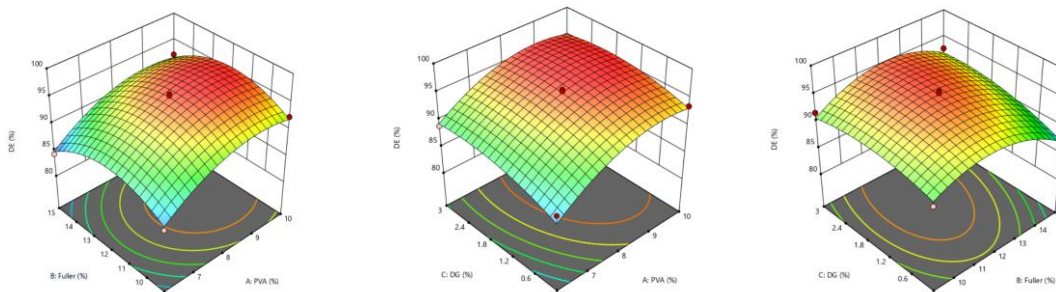


Figure 1. Response surface plots of interactions:
a) % PVA - % Fuller; b) % PVA - % DG; c) % Fuller - % DG.

For comparison, the DCBRN-01VN liquid decontaminant required 60 minutes to approach complete removal, achieving only 82.9% at 30 minutes (Vu et al. [8]). Furthermore, while polymer matrices like DDGel provide superior physical containment over liquid lotions such as RSDL (Cao et al. [4, 5]), and PVA-based films effectively entrap simulants, the latter require impractical drying times of 20–24 hours (Toader et al. [7]). By contrast, our proposed system overcomes these limitations by synergizing rapid micellar sequestration and high-capacity physical entrapment. Reaching 96.5% efficiency in a brief 30-minute window confirms its promising potential as an effective, rapid-response alternative for vesicant skin decontamination.

To revalidate the optimized conditions, three gel samples were prepared using the optimized formulation established above and evaluated for their decontamination performance. The average Decontamination Efficiency (DE) was determined to be $96.15 \pm 0.3\%$. This result is in close agreement with the predicted value of 96.5%, confirming the validity of the optimization model.

4. CONCLUSIONS

A skin decontamination gel composed of PVA, Fuller's earth, and decyl glucoside was developed and evaluated for CEES removal on *ex vivo* porcine skin. The study demonstrated that decontamination efficiency relies on film formation, adsorption, and surfactant effects, governed by the non-linear effects of the components and dynamic viscosity. Response surface methodology identified an optimal efficiency of ~96.5% at 9.0 wt% PVA, 11.7 wt% Fuller's earth, and 2.1 wt% decyl glucoside. While promising for emergency and military applications, further research is required to validate the gel against broader chemical agents, assess *in vivo* compatibility, and scale up for practical deployment.

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TÓM TẮT

Tối ưu hóa thành phần công thức và đánh giá hiệu quả loại bỏ 2-chloroethyl ethyl sulfide (CEES) của gel tiêu tẩy dùng cho da trên cơ sở PVA/Fuller’s Earth/chất hoạt động bề mặt

Chất độc loét da là một tác nhân hóa học có khả năng gây ra các mối nguy hiểm nghiêm trọng trong các tình huống chiến tranh sử dụng vũ khí hóa học. Với một hướng tiếp cận mới, nghiên cứu này phát triển và tối ưu hóa một hệ gel tiêu độc cho da đơn giản (PVA/đất sét Fuller/decyl glucoside) để loại bỏ chất mô phỏng chất độc mù tạt - CEES. Công thức được xây dựng dựa trên sự hiệp đồng giữa mạng lưới polymer của PVA, khả năng hấp phụ của đất sét Fuller và sự hòa tan micelle của decyl glucoside. Kết quả thực nghiệm trên da lợn *ex vivo*, sử dụng phương pháp bề mặt đáp ứng (RSM) cho thấy hiệu suất tiêu tẩy phụ DE bị chi phối bởi hiệu ứng phi tuyến rõ rệt của từng thành phần, thay vì chỉ phụ thuộc tuyến tính vào độ nhớt hay các cặp tương tác. Công thức tối ưu được xác định gồm 9,0% PVA, 11,7% đất sét Fuller và 2,1% decyl glucoside, đạt hiệu suất tiêu tẩy 96,5%, chứng minh tiềm năng ứng dụng của hệ gel như một giải pháp đơn giản để tiêu tẩy chất độc loét da, đồng thời khẳng định giá trị của việc ứng dụng mô hình RSM trong xây dựng công thức của gel.

Từ khoá: Chất độc loét da; Mù tạt; CEES; Tiêu tẩy cho da.