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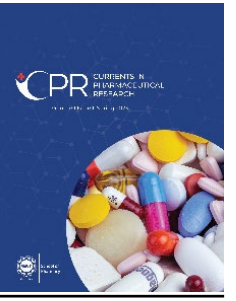

Title:	**Revolutionizing Hyperpigmentation Solutions: Formulation and Characterization of Kojic Acid Gel**
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Revolutionizing Hyperpigmentation Solutions: Formulation and Characterization of Kojic Acid Gel

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ABSTRACT

Kojic acid is a skin-lightening agent that blocks the tyrosinase enzyme and inhibits melanin synthesis. While kojic acid has demonstrated effectiveness in hyperpigmentation treatment, the existing formulations often suffer from issues, such as poor skin penetration, instability, and skin irritation. The current study aimed to overcome these limitations by preparing a kojic acid gel formulation utilizing biocompatible polymers. These polymers included sodium alginate and xanthan gum to enhance therapeutic efficacy for hyperpigmentation treatment. Sodium alginate and xanthan gum were used as polymers, while the excipients included propylene glycol, glycerin, peppermint oil, methylparaben, and propylparaben. Design Expert 11 optimized topical gels' viscosity, spreadability, and permeation responses. The optimized gels were determined for organoleptic properties, pH, drug content, spreadability, viscosity, *in-vitro* drug permeation studies, Fourier Transform Infrared Spectroscopy (FTIR) analysis, anti-oxidant activity, antimicrobial activity, and stability study. Results indicated that the pH of the optimized sodium alginate gel was 6.6 and that of xanthan gum gel was 6.8. The spreadability was 28.5 g.cm/sec and 17 g.cm/sec for sodium alginate and xanthan gum gels, respectively. The viscosity was 5900 mPa.s for sodium alginate gels and 6854 mPa.s for xanthan gum gels. The drug content lied in the range of 90%-110%, which is according to United States Pharmacopeia standards. The permeation study showed an acceptable release profile for both gels. The anti-oxidant assay indicated an optimum anti-oxidant activity, while the antimicrobial activity test showed inhibitory action against bacteria. An accelerated stability study elucidated that the optimized gels had good stability. The results inferred that the prepared gel formulations of kojic acid were stable and reproducible.

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Keywords: biocompatible polymers, hyperpigmentation, kojic acid, sodium alginate, xanthan gum

1. INTRODUCTION

Facial hyperpigmentation or Melasma, also called as the mask of pregnancy, is a disorder commonly characterized by irregular and light or dark brown or ash brown hypomelanosis on neck and facial areas due to increased melanin levels [1]. Melasma is known to affect women more than men. The incidences of Melasma in men comprise only 10% of the total cases. Additionally, post-inflammatory hyperpigmentation and Melasma are the third most common reasons for dermatologist visits [2]. The human skin epidermis comprises keratinocytes, melanocytes, and Langerhans cells. The main determinants of human skin color are two types of melanin pigments: pheomelanin and eumelanin. Melanin is produced by the melanocytes in the epidermis [3]. Facial hyperpigmentation may not only cause cosmetic disfiguration, however, also has an emotional impact on patients. Although, many options exist for the treatment of this skin disorder, all these agents have varying efficacy levels. Overall, most therapeutic agents aim to eliminate the factors provoking hyperpigmentation. Kojic acid is one of the topical agents that can be used to reduce facial pigmentation [4].

Kojic acid is an effective cosmeceutical product for hyperpigmentation. This is because it inhibits the catecholase activity of the enzyme tyrosinase, a rate-limiting step in melanin production. The inhibition of tyrosinase, an essential enzyme in the skin pigment melanin biosynthesis, reduces melanin. Thus, hyperpigmentation is eliminated. Additionally, it is an inhibitor of bacterial, fungal or viral multiplication. It acts as a skin lightener due its well-known anti-oxidant activity [5]. Kojic acid has various advantages which makes it quite an effective compound to be used in the cosmetic industry [6]. For instance, it reduces sun damage by lightening visible pigmentation, protects from UV radiations, and acts as an anti-ageing action (reducing age spots) owing to its anti-oxidant properties.

Despite the wide usage of kojic acid as a skin-lightening agent and other anti-hyperpigmentation remedies, the efficacy of treatment is still questionable due to various factors. The primary reason is the poor penetration of many topical formulations into the skin which limits their ability to target deeper layers of skin where melanin synthesis occurs.

Moreover, traditional preparations tend to be greasy, sticky, or are otherwise uncomfortable to wear. Thus, they are not always used correctly and suboptimal results are likely to occur. These treatments often provide slow and uneven improvements, requiring long periods of application before seeing any visible effects. Additionally, kojic acid itself is chemically unstable, especially when exposed to light, air, or heat, which would slowly affect the efficacy. Many treatments cause skin irritation or sensitization, particularly on sensitive skin, which becomes an issue with long-term use. Keeping in view these gaps, this study focused on the development of a gel formulation of kojic acid using biocompatible polymers, such as sodium alginate and xanthan gum. This allowed better penetration through the skin, stability, and lesser irritation. Thus, better, consistent, and faster results were obtained than a conventional treatment.

Biocompatible polymers, such as sodium alginate and xanthan gum have been of considerable interest in cosmeceutical formulations due to their unique advantages. Sodium alginate, an alga extracted from brown seaweed, is known for its excellent biocompatibility and skin hydration properties. It forms stable and smooth gels when combined with divalent cations, which help to encapsulate active ingredients, such as kojic acid. This ensures their controlled release and improved skin penetration. Furthermore, sodium alginate is a water-retentive ingredient that maintains skin moisture, thus making this formulation even more effective [7].

Xanthan gum, a natural polysaccharide, is an efficient thickening and stabilizing agent that enhances viscosity, spreadability, and overall texture of the gel. Furthermore, it also conditions skin for smoother application [8]. The synergistic interaction between sodium alginate and xanthan gum generates stable and homogeneous gel solutions. This improves the therapeutic value of kojic acid through overcoming some limitations encountered by the existing treatments, such as instability, irritation, and absorption inconsistencies.

2. MATERIALS AND METHODS

2.1. Materials

Kojic acid (Hunan Nutramax, China), sodium alginate (Kimica, Tokyo), xanthan gum (AIE Pharma, Canada), propylene glycol (DaeJung, Korea), peppermint oil (UNI-CHEM, Pakistan), glycerin (UNI-CHEM, Pakistan), methyl paraben and propyl paraben (Sigma Aldrich Chemie GmbH,

U.S.A.), ethanol (Merck, Germany), and distilled water (Research Lab, UCP). All analytical grade chemicals were used for the current study.

2.2. Methods

2.2.1. Linearity Curve of Kojic Acid and FTIR Analysis. The linearity curve of kojic acid was obtained by making a stock solution (1mg/ml) of the drug. Serial dilutions showed a concentration range of 0.1-0.9 mg/ml. A UV spectrophotometer determined the absorbance of each dilution at a wavelength of 220 nm, with distilled water as the blank or baseline control to ensure accurate readings [9]. FTIR was employed to determine the compatibility of drugs with polymer and other excipients [10]. The baseline or control condition was the spectrum of the pure polymer and excipients, which served to identify any possible shifts or interactions between the kojic acid and the excipients.

2.2.2. Formulation Design. The formulations of topical gels for hyperpigmentation were designed and optimized via Design Expert ver. 11 (Table 1). The concentration of sodium alginate and xanthan gum and active ingredient (kojic acid) was constant. The variables selected included the solvent (Propylene Glycol), plasticizer (Glycerin), and an odorant (Peppermint Oil). Propylene glycol was chosen as factor 1 (X_1), glycerin as factor 2 (X_2), and peppermint oil as factor 3 (X_3).

Table 1. Formulation Design by Design Expert ver. 11

S. No.	X_1 (ml)	X_2 (ml)	X_3 (ml)
1	7	2.25	0.1
2	5.25	1.5	0.3
3	3.5	2.25	0.3
4	3.5	2.25	0.1
5	5.25	2.25	0.2
6	7	2.25	0.3
7	7	3	0.2
8	5.25	3	0.3
9	5.25	2.25	0.2
10	3.5	1.5	0.2
11	5.25	1.5	0.1
12	3.5	3	0.2
13	5.25	3	0.1
14	7	1.5	0.2

2.2.3 Preparation of Kojic Acid Gel. The gelling agents, that is, sodium alginate and xanthan gum were soaked in 100 ml distilled water separately and allowed to stand for about three hours until they swelled. A uniform gel was formed by stirring the above-formed mixture using a magnetic stirrer. Methylparaben and propylparaben were dissolved in 20 ml distilled water by heating at 70ºC using a water bath and added to the gels as preservatives. Kojic acid was dissolved in propylene glycol using a homogenizer at 9000 rpm and then incorporated into the gels. This mixture required glycerin and peppermint oil added with continuous stirring.

2.2.4. Numerical Optimization. The responses studied for the gels included viscosity, spreadability and permeability. These responses were added to Design Expert and analyzed by Analysis of Variance (ANOVA) to optimize the data. This resulted in optimized formulations with a desirability of 1.000, and the following composition as shown in Table 2.

Table.2. Composition of Optimized Gel Formulation

Ingredients	Concentration	
	Sodium Alginate Gel	Xanthan Gum Gel
Sodium Alginate	8g	-
Xanthan Gum	-	1.5g
Kojic Acid	2g	2g
Propylene Glycol	6.910ml	3.550ml
Peppermint Oil	0.297ml	0.226ml
Glycerin	2.726ml	2.990ml
Methyl Paraben	0.1g	0.1g
Propyl Paraben	0.01g	0.01g
Distilled Water	q.s 100ml	q.s 100ml

2.2.5. Organoleptic Evaluation. The formulations of topical gels, including blank preparations (without kojic acid), were visually examined for their physical appearance, odor, consistency, and homogeneity [11].

2.2.6. Rheological Studies. A Brookfield viscometer with spindle no.4 was used to determine the viscosity of the prepared formulations. The speed of the viscometer was set at 12 rpm [12].

2.2.7. pH Determination. A digital pH meter was used to determine the pH of all kojic acid gel formulations [13].

2.2.8. Spreadability. The fixed slide method determined the spreadability of all the prepared formulations [14]. The following formula was used to determine spreadability:

$$S = \frac{M}{L} \times T \quad . \tag{1}$$

2.2.9. Drug Content. Drug content for kojic acid was measured by a spectrophotometer at a wavelength of 220 nm (9) using the formula:

$$\%\ \boldsymbol{Drug\ Content} = \frac{\boldsymbol{Abs\ (Sample)}}{\boldsymbol{Abs(Standard)}} \times \mathbf{100} \tag{2}$$

2.2.10. In-vitro Drug Permeation Studies. The *in-vitro* drug permeation was done using a Franz diffusion (FD) cell. This test was conducted by putting 1 g of gel sample on rabbit skin supported amidst donor and receptor compartments of Franz cell at 37°C. Rabbit skin was used due to its similarity to human skin, particularly in the epidermal thickness and permeability, which makes it a suitable model for the evaluation of skin penetration. A pH 7.4 phosphate buffer was used as media. Five (5) ml of the test sample was drawn at different time intervals for up to 8 hours. Fresh media was used to make up the volume [15] to determine the % drug permeation.

2.2.11. Chemical Compatibility of Gel Formulations. The gel samples were subjected to FTIR analysis in order to determine the compatibility of all ingredients used in their formulation.

2.2.12. Antimicrobial Activity. The antimicrobial activity of gel samples was assessed by the cup plate method [16] using a bacterial culture of *Micrococcus luteus*. The area of the zone of inhibition was measured.

2.2.13. Anti-oxidant Activity. The total anti-oxidant capacity of the gels was estimated using a Diphenyl Picryl Hydrazyl (DPPH) radical scavenging assay. A total of 2.4 mg of DPPH was dissolved in 100 ml of methanol. Test solution (5 µl) was added to 3.995 ml of prepared solution of methanolic DPPH. This mixture was kept at room temperature for 30 mins in the dark. The absorbance of this solution was measured spectrophotometrically at a 515nm wavelength. The absorbance of a blank solution without an anti-oxidant was also noted. DPPH radical scavenging capability was calculated using the following equation:

$$\% \boldsymbol{DPPH\ Scavenged} = \frac{\boldsymbol{AB-AA}}{\boldsymbol{AB}} \times \mathbf{100} \quad (3)$$

Triplicate measurements were performed for each sample to ensure consistent results within a single experiment. A positive control, that is, ascorbic acid was used to validate the results which provided a comparative measure of antioxidant activity.

2.2.14. Stability Studies. The optimized formulations were subjected to stability studies according to the ICH guidelines. This study was conducted by keeping the optimized gel in the stability chamber for six months. The formulations were stored at 40ºC/75% humidity in plastic containers. The formulations were examined initially and then after the six-month intervals for their appearance, texture, color, odor, stage partition, homogeneity, pH, viscosity, and drug content.

3. RESULTS AND DISCUSSION

3.1. Organoleptic Evaluation

The gels made using xanthan gum had a whitish color, while the gels prepared using sodium alginate showed a slightly yellowish color, which complies with the other studies [17, 18]. The gels had a minty smell due to the incorporation of peppermint oil [19]. All the gels had a thick consistency due to glycerin [20].

3.2. pH

The pH of xanthan gum gels ranged from 6.38-7.36 and sodium alginate gels showed a pH range of 6.34-7.21 (Figure 1). Ganz, 2006 suggested that kojic acid remains stable at a pH ranging from 4-9. Thus, it enhances the possibilities to formulate stable cosmeceutical agents [21]. The current study established that the gels had a pH ranging from 6-7, comparable to other studies [22, 23]. Kojic acid, like many active ingredients, is sensitive to pH, with its stability being compromised in more acidic or alkaline environments. At lower pH values, kojic acid may degrade which reduces its efficacy to treat hyperpigmentation [24]. On the other hand, extreme alkaline conditions may alter the gel's texture which compromises its performance and user experience. Furthermore, the pH of normal human skin is around 4.5-6 [25] which indicates that the prepared gels could be effective for dermatological applications.

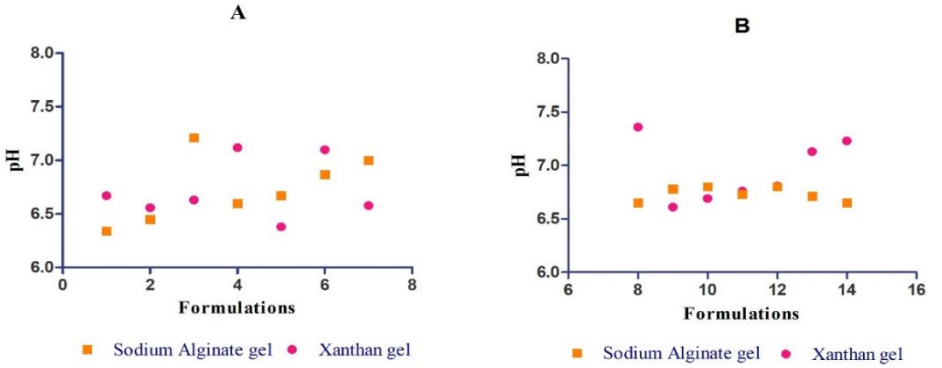


Figure 1. pH of Xanthan and Sodium Alginate Gels (A) Formulation 1-7 (B) Formulation 8-14

3.3. Spreadability

The spreadability of gels with xanthan polymer was 14.06-22.5 g.cm/sec, while that of the gels comprising sodium alginate polymer had a spreadability of 20.45-37.5 g.cm/sec (Figure 2). Studies indicated that sodium alginate had good adhesive properties due to its electrostatic and hydrogel bonding [18]. It was also observed that the gels containing higher glycerin and propylene glycol had lower spreadability values than others. This could be due to both ingredients' thickening properties [26, 27]. As the gel viscosity enhanced, the spreadability decreased in turn. Furthermore, higher spreadability gels often deliver easier and more uniform cover-ups, offering higher cosmetic appeal. The optimized gels of both xanthan gum and sodium alginate had good spreadability.

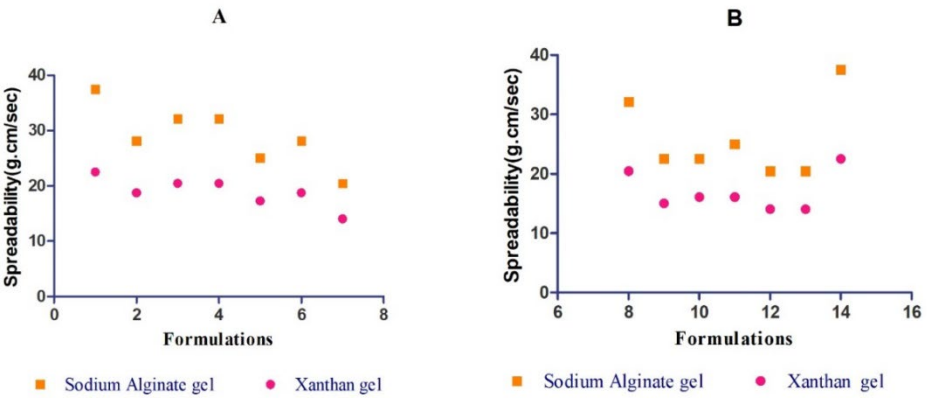


Figure 2. Spreadability of all 14 Formulations of Xanthan and Sodium Alginate Gels (A) Formulation 1-7 (B) Formulation 8-14

3.4. Viscosity

The xanthan gum gel formulations showed a viscosity in the scope of 5432-7453 mPa.s, while sodium alginate gels showed a viscosity range of 4325-6754 mPa.s (Fig 3.3). A higher concentration of polymers resulted in a greater viscosity. The polymeric entanglements enhance gels' viscosity, thereby improving the resistance towards flow and deformation [28]. The viscosity of gels was also influenced by glycerin, which increases hydrogen bonding [29]. Propylene glycol may also enhance the viscosity of gel by expanding the cross-linking between the network [30]. The formulated gels, with balanced viscosity and high spreadability, offered a more pleasant sensory experience, likely encouraging more consistent use.

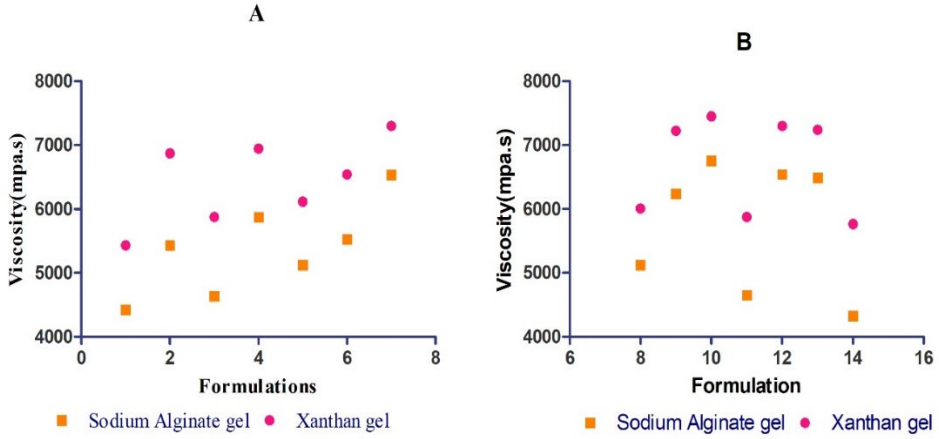


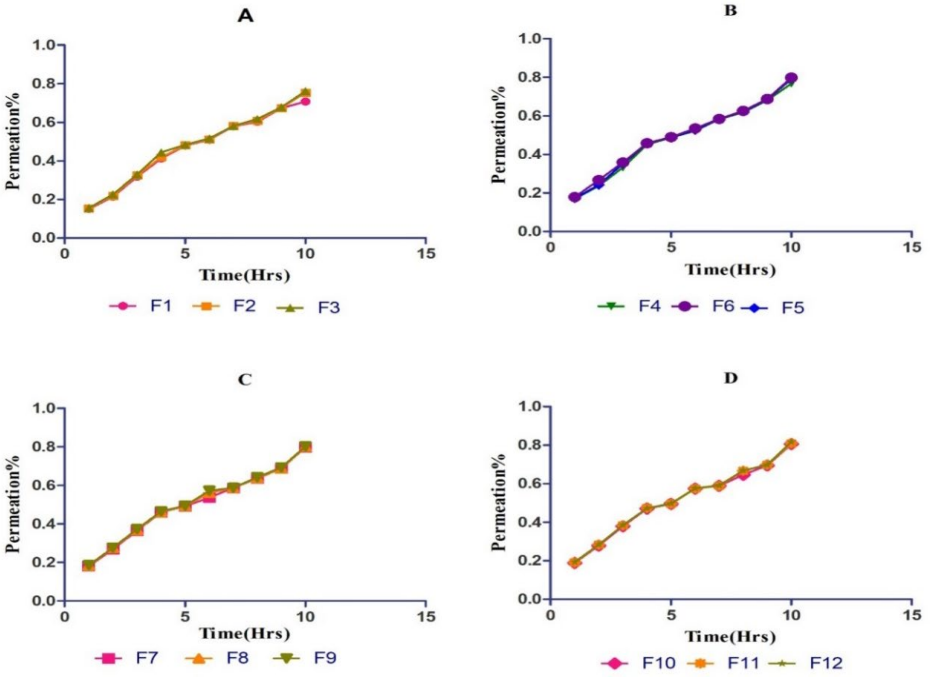
Figure 3. Viscosity of Xanthan and Sodium Alginate Gels (A) Formulation 1-7 (B) Formulation 8-14

3.5. In-vitro Permeation of Kojic Acid

Figure 4 and 5 represent the results of *in-vitro* kojic acid permeation. Approximately 90% of the drug permeated from the gels by 8 hours from both gels. The released profiles of kojic acid topical gels from across the dialysis membrane indicated that the drug release increased over time. Additionally, it was observed that the gels containing peppermint oil had a higher permeation than other formulations. This increase in release could be attributed to peppermint oil's ability to reduce the stratum corneum's barrier resistance [31]. Caliskan *et al*. also studied peppermint oil as a penetration enhancer and reported that this essential oil increased the

permeation of various therapeutic agents from the skin without causing toxicity [32]. It was also observed that the release rate from sodium alginate gels was lower than that from xanthan gum. This could be due to the high viscosity of sodium alginate gels. Studies indicated that viscosity is inversely proportional to the drug release from topical formulations and its permeation through the diffusion barriers [33].

The release profile is consistent with findings in the existing literature, where the gel-based formulations have been shown to enhance the controlled release of active ingredients as compared to other topical formulations, such as creams and lotions. For instance, a study conducted by Yan *et al*, revealed that gel formulations of kojic acid with biopolymer bases (such as xanthan gum) exhibited more efficient and prolonged release than conventional formulations and thus, improved the therapeutic efficacy [34]. The permeation rate observed in this study was comparable to those reported in Saha *et al*. [35] who used similar excipients and noted that the use of biopolymers significantly increased the skin penetration of kojic acid. This comparison highlights the importance to utilize gel formulations in order to increase the efficacy of active ingredients, such as kojic acid which improves both skin penetration and stability over time.



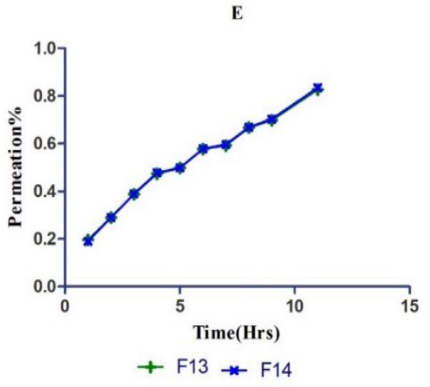
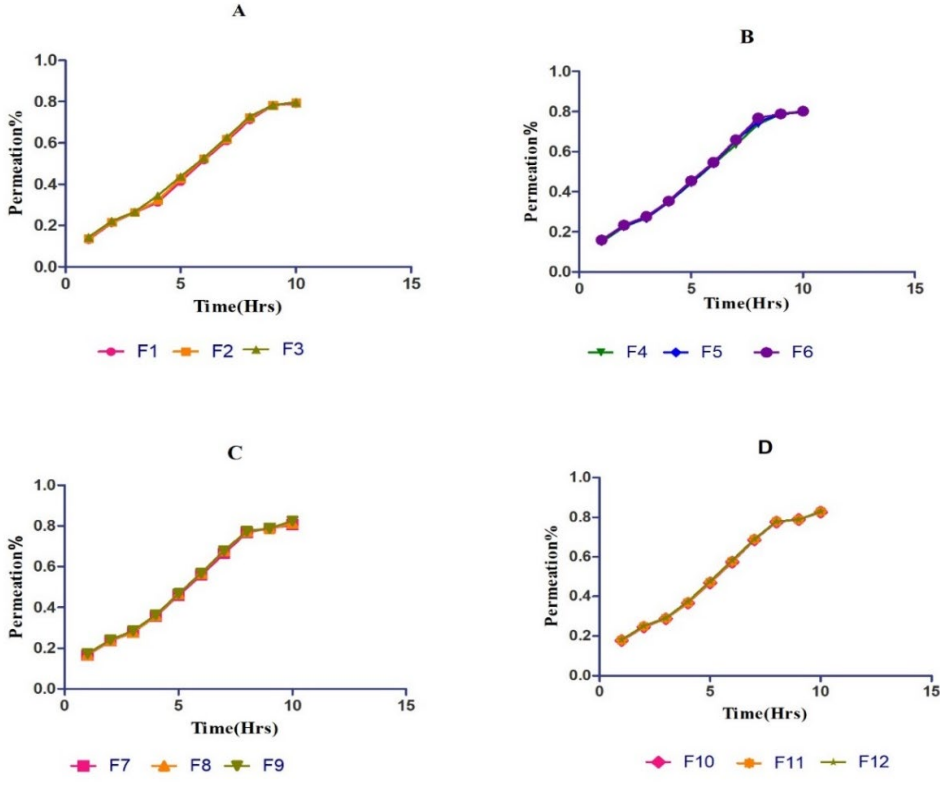


Figure 4. *In-vitro* Permeation of Kojic Acid in Xanthan Gum Gel (A) Formulation 1-3 (B) Formulation 4-6 (C) Formulation 7-9 (D) Formulation 10-12 (E) Formulation 13-14



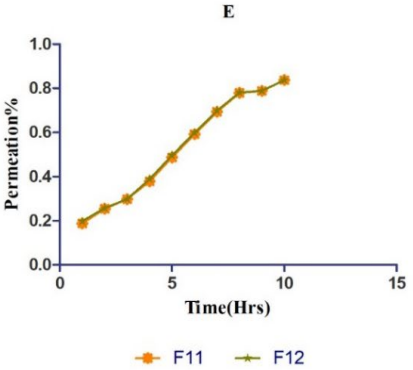


Figure 5. *In-vitro* Permeation of Kojic Acid in Sodium Alginate Gel (A) Formulation 1-3 (B) Formulation 4-6 (C) Formulation 7-9 (D) Formulation 10-12 (E) Formulation 13-14

3.6. Chemical Compatibility of Formulation

Figure 6 represents that the IR spectrum of kojic acid showed peaks at the following locations: at 1775cm-1 showing strong C=O stretching of conjugated anhydride; at 1690 cm-1 due to strong C=O stretching in the primary amide group; at 1465 due to medium C-H bending; at 1205 cm-1 to 1124 indicating C-O stretching.

Figure 7 (A) shows different peaks obtained by IR analysis of the topical gel containing polymer sodium alginate. Peaks were seen: at 3300 and 3400 cm^{-1} due to medium N–H stretching, substantial N–H stretching at 2800 to 3000 cm^{-1}, and between 1650 and 2000 cm^{-1} due to weak C−H bending. While Figure 7 (B) illustrates the spectra obtained from the analysis of gel formulation made from polymer sodium alginate.

Figure 7 (C) depicts different peaks obtained by IR analysis of the topical gel containing xanthan gum polymer. Peaks were seen at: 3500 and 3700 cm^{-1} showing O–H stretching, a strong N–H stretching leading to peaks at 2800-3000 cm^{-1}. While Figure 7 (D) showed the analysis of gel formulation made of polymer xanthan gum. Both gels showed the peaks of kojic acid, as did the respective polymers. No incompatibility was seen in the FTIR analysis of the either gel.

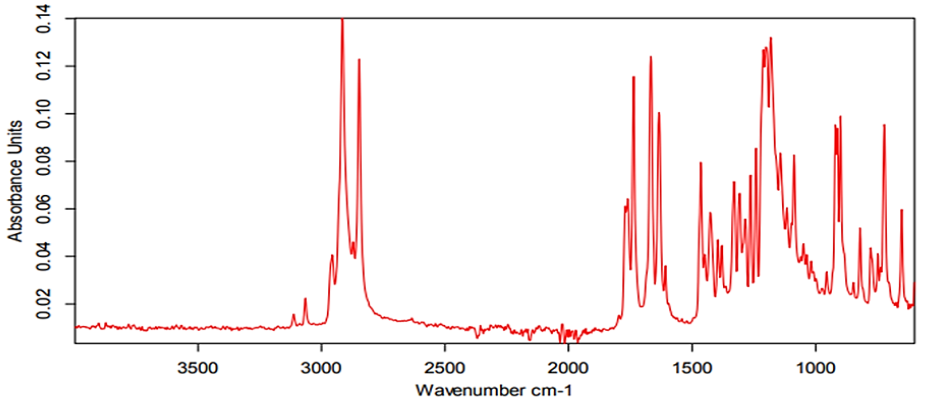


Figure 6. FTIR of Kojic Acid

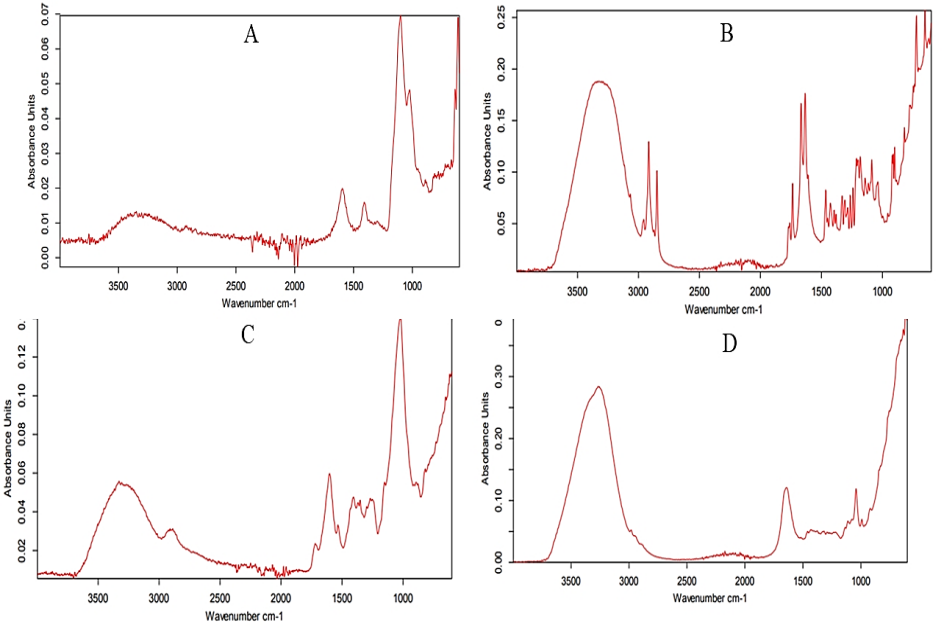


Figure 7. FTIR Analysis A) Sodium Alginate B) Topical Gels with Sodium Alginate C) Xanthan Gum D) Topical Gels with Xanthan Gum

3.7. Characterization of Optimized Gels

The optimized formulations were evaluated for multiple parameters. The results were as follows:

Table 3. Results Obtained from Characterization Tests on the Optimized Formulations

Parameters	Sodium Alginate Gel	Xanthan Gum Gel
Color	Slightly yellow	Whitish
Odor	Minty	Minty
pH	6.6	6.8
Spreadability	28.5 g.cm/sec	17 g.cm/sec
Viscosity	5900 mPa.s	6854 mPa.s
Drug Content	92.7%.	90.5%

3.8. In-vitro Drug Permeation Study

Figures 8 and 9 show the permeation of active ingredients: kojic acid from sodium alginate gels and xanthan gum. The permeation of kojic acid from xanthan gum at 8-hour intervals was 90%, while that of sodium alginate gel was 91%. Kojic acid was released and permeated through the epithelial membrane greatly from both formulations. Studies showed a direct relationship between the drug release rate and the efficacy of topical product [36]. Since the active agent has been sufficiently released from the optimized topical gels, kojic acid should be readily available to act on the skin, thus resulting in an increased permeation and therapeutic effect.

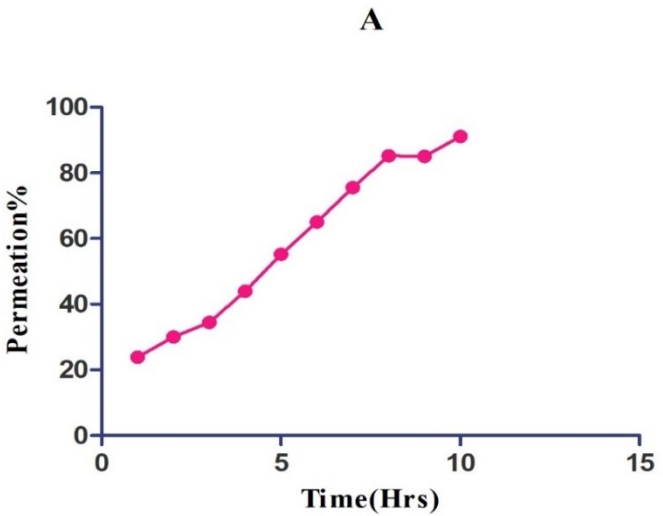


Figure 8. *In-vitro* Kojic acid Permeation of Optimized Sodium Alginate Gel

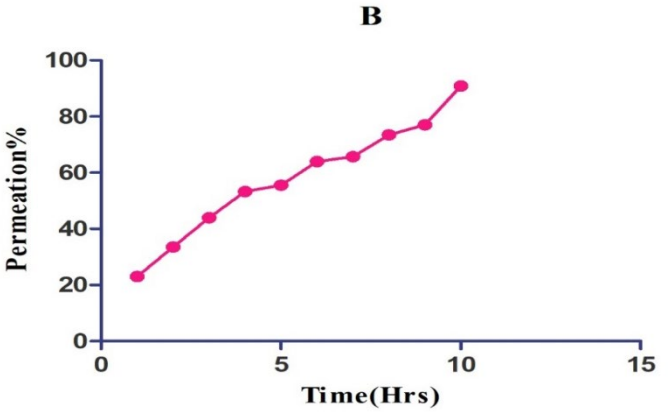


Figure 9. *In-vitro* Kojic Acid Permeation of Optimized Xanthan Gum Gel

3.9. Anti-oxidant Activity

The correlation of total antioxidant activity of ascorbic acid and optimized gels showed that they pursued a comparable direction. The gels had an ideal TAA, concurring the outcomes of this test as shown in Figure 10, 11, and 12. In the current study, kojic acid was used as a skin-lightening agent. Kojic acid lightens the skin through its anti-oxidant activity. Ammar et al. studied its ability to scavenge free radicals by DPPH assay and inferred that kojic acid had intensive anti-oxidant activity [37].

The outcomes could be justified by the results reported by Saraei *et al*., who studied the anti-oxidant potential of kojic acid and received positive results [38]. Another study conducted by Lobato and his coworkers also suggested that kojic acid and its derivatives had a significant ability to scavenge free radicals [39].

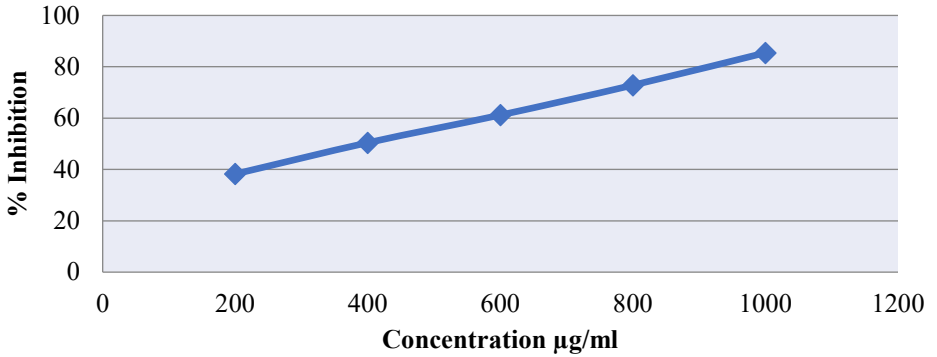


Figure 10. Total Anti-oxidant Activity of Ascorbic Acid (Standard)

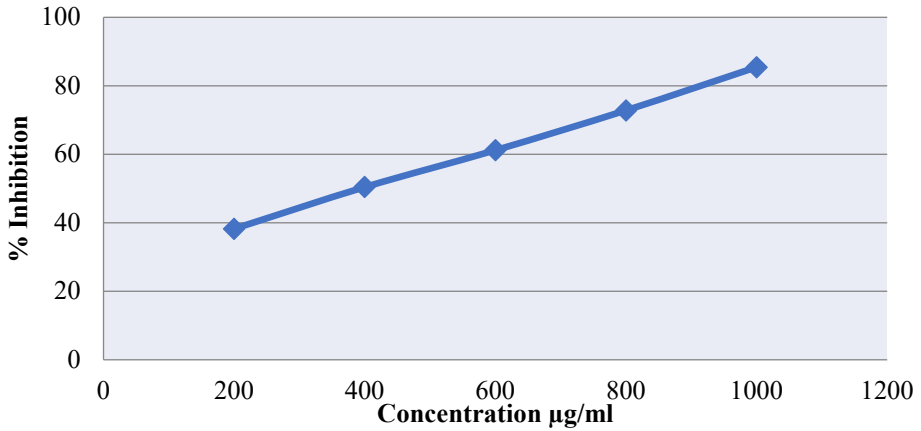


Figure 11. Total Anti-oxidant Activity of Xanthan Gum Gel

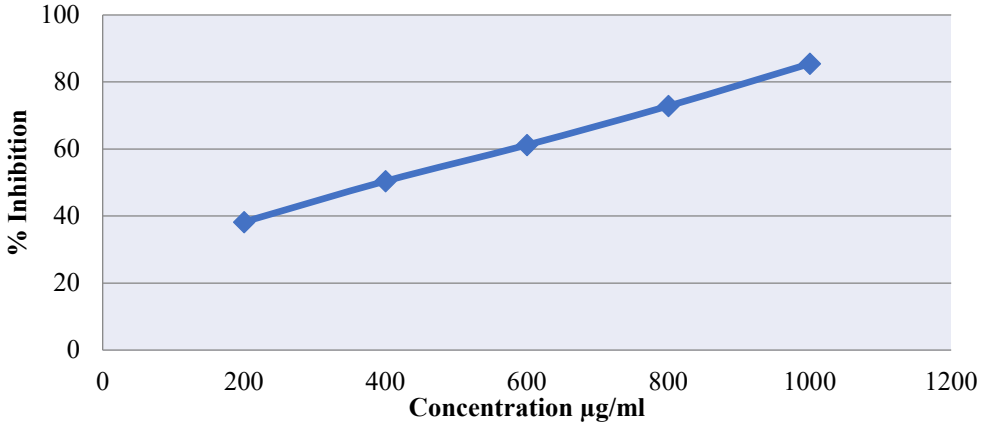


Figure 12. Total Anti-oxidant Activity of Sodium Alginate Gel

3.10. Antimicrobial Activity

The zones of inhibitions acquired by this test are noted in Table 4. The antimicrobial activity of the gel was tested containing kojic acid. Results showed that its inhibitory impact was equivalent to that of marketed kojic acid (kojic acid whitening cream). Likewise, the inhibitory effects of gels containing kojic acid as an active ingredient were higher than the marketed formulation of kojic acid (Fig 3.13). Literature review showed that kojic acid and its derivatives have a chelating effect, having a catechol-like function that contributes to its antibacterial action [40]. Yu Wu and his coworkers determined the antimicrobial potential of kojic acid and reported

that it damaged the integrity of bacterial cell's cell membrane, leading to its inactivation [41].

The results indicated that the prepared gels had an antimicrobial action comparable to the standard kojic acid formulation.

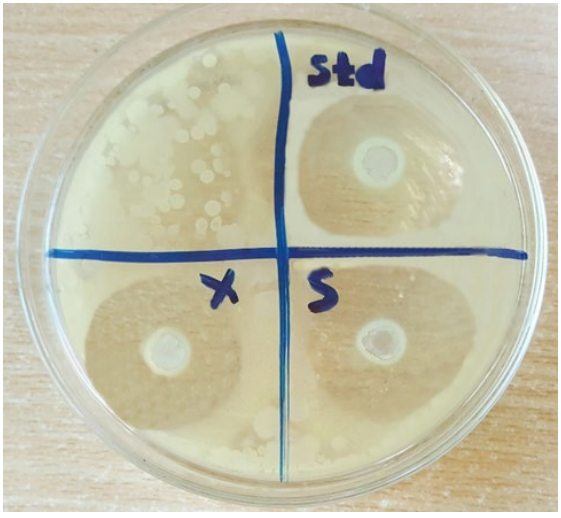


Figure 13. Antimicrobial Study of Gels with Kojic Acid
Note. Std = standard, X =Xanthan gum, S = Sodium alginate

Table 4. Antimicrobial Action of Kojic Acid Formulations

Sample	Zone of Inhibition in cm
Standard Formulation (Kojic Acid Whitening Cream)	2.2
Sodium Alginate	2.7
Xanthan Gum	2.8

3.11. Stability Study

Table 5 shows the physicochemical assessment for both topical formulations. It has been shown that sodium alginate disrupted the bacterial cellular surface, leading towards the leakage of intracellular components. Sodium alginate chelation property could modulate the production of toxins and inhibit the microbial growth. Its bacteriostatic activity was proven against various bacterial species including Proteus, *Pseudomonas, Escherichia,* and *Acinetobacter* [42]. So, it could be inferred that sodium alginate gels remained stable owing to the antimicrobial properties of the

polymer itself. Adding paraben preservatives in the xanthan gum gel leads towards its good stability. Feizabadi and his coworkers reported that parabens inhibited microbial growth and increased the product's shelf life [43].

Table 5. Results of Stability Study after 6 Months

Parameters	Xanthan gum Gel	Sodium alginate Gel
Colour	White	Slightly yellow
Odour	Minty	Minty
pH	6.8	6.7
Spreadability (g.cm/sec)	16.8	28
Viscosity (mPa.s)	6850	5954
Drug Content (%)	89.4	91.3

3.12. Response Surface Methodology

Responses, such as the penetration of active ingredients, gel viscosity, and gel spreadability were analyzed using response surface methodology. Design Expert was utilized to create contour and 3D graphs depicting various variables' influence including oleic acid, eucalyptus oil, and glycerin.

3.12.1. In-Vitro Permeation of Kojic Acid. Figures 14 and 15 show the graphs representing the effects of variables on kojic acid permeation from sodium alginate gels and xanthan gum gels, respectively. Tables 6 and 7 show the values of analysis of variance of Kojic acid permeation from sodium alginate gels and xanthan gum gels, respectively. These graphs by Design Expert showed that the permeation of kojic acid was positive for both gels. The overall response was constructive. All combinations were positive for the permeation of kojic acid from sodium alginate gels. A similar case was seen in the xanthan gum gels, where all three variables enhanced the permeation of the active ingredient.

Studies showed that peppermint oil is a permeation enhancer when incorporated in gels [44]. Other factors, glycerin and propylene glycol, also significantly increased the permeation. Propylene glycol increased the permeation; however, the effect was low as compared to other two factors.

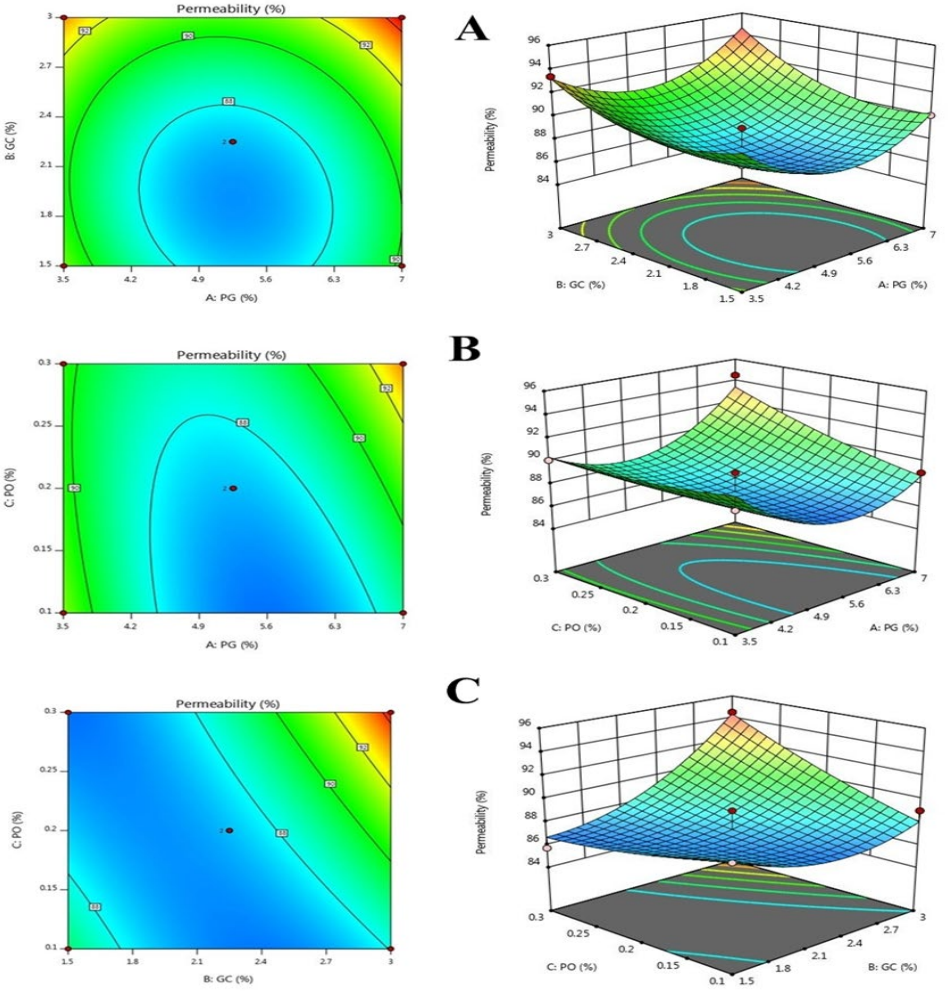


Figure 14. Effect of Variables on Kojic Acid Permeation from Sodium Alginate Gel. A) GC and PG B) PO and PG C) PO and GC

Table 6. Analysis of Variance of Kojic Acid Permeation from Sodium Alginate Gels

Term	Degree of Freedom	*F* Value	*p* Value	Significance
Model Name	9	3.41	0.1247	No
X_1	1	4.06	0.1143	No
X_2	1	5.57	0.0777	No
X_3	1	0.2377	0.6514	No
X_1X_2	1	5.26	0.0836	No

Term	Degree of Freedom	*F* Value	*p* Value	Significance
X_1X_3	1	4.94	0.0904	No
X_2X_3	1	4.14	0.1116	No
X_1^2	1	0.0076	0.9349	No
X_2^2	1	1.28	0.3206	No
X_3^2	1	4.01	0.1159	No

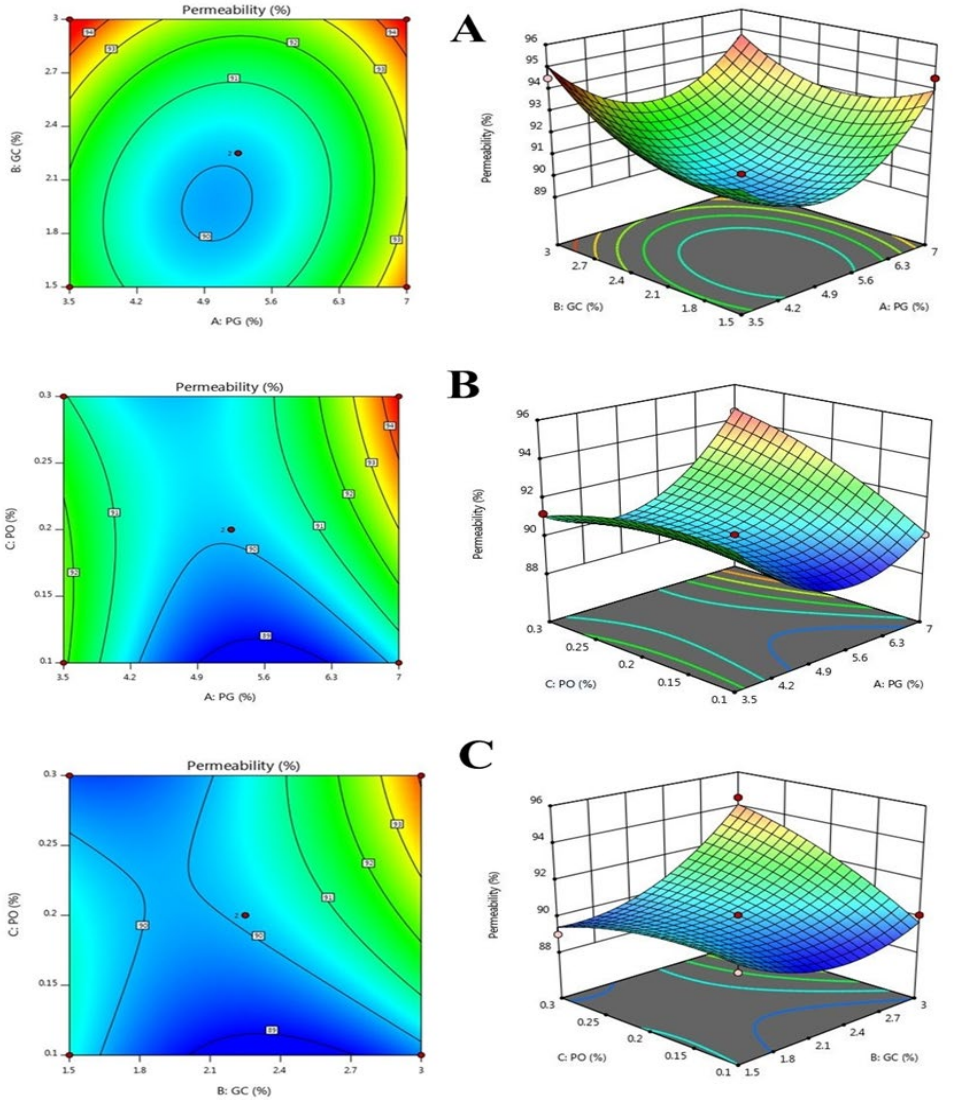
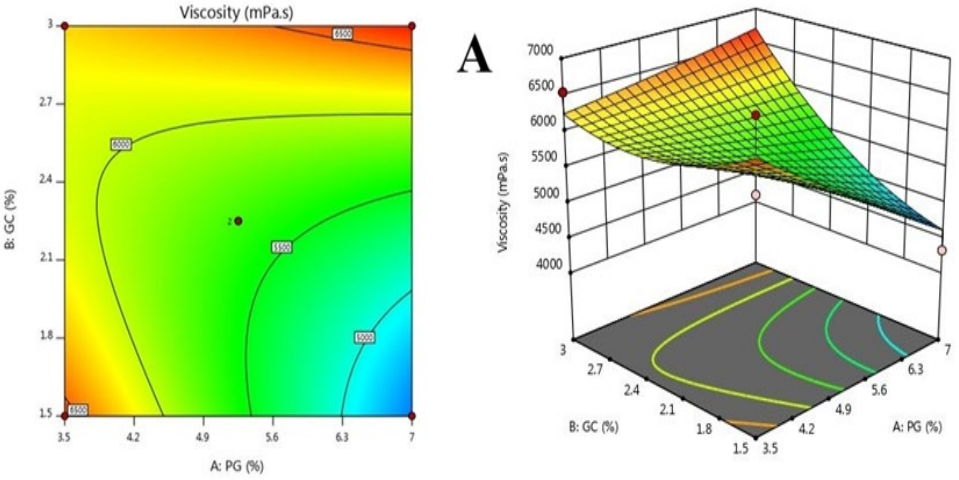


Figure 15. Effect of Variables on Kojic Acid Permeation from Xanthan Gum Gel. A) GC and PG B) PO and PG C) PO and GC

Table 7. Analysis of Variance of Kojic Acid Permeation from Xanthan Gum Gels

Term	Degree of Freedom	*F* Value	*p* Value	Significance
Model Name	9	17.01	0.0076	Yes
X_1	1	3.62	0.1299	No
X_2	1	19.68	0.0114	Yes
X_3	1	14.58	0.0188	Yes
X_1X_2	1	3.23	0.1468	No
X_1X_3	1	20.06	0.0110	Yes
X_2X_3	1	20.04	0.0110	Yes
X_1^2	1	52.12	0.0020	Yes
X_2^2	1	16.11	0.0159	Yes
X_3^2	1	2.56	0.1852	No

3.12.2. Viscosity of Gels. Figure 16 and 17 illustrate the graphs representing the effects of variables on the viscosity of sodium alginate and xanthan gum gels, respectively. Tables 8 and 9 show the values of analysis of variance of viscosity of gels, respectively. The polynomial equation and graphs indicated that glycerin increased gels' viscosity. Glycerin is a thickening agent which increases the viscosity of gels. It was observed that propylene glycol and peppermint oil lowered the viscosity of prepared gels.



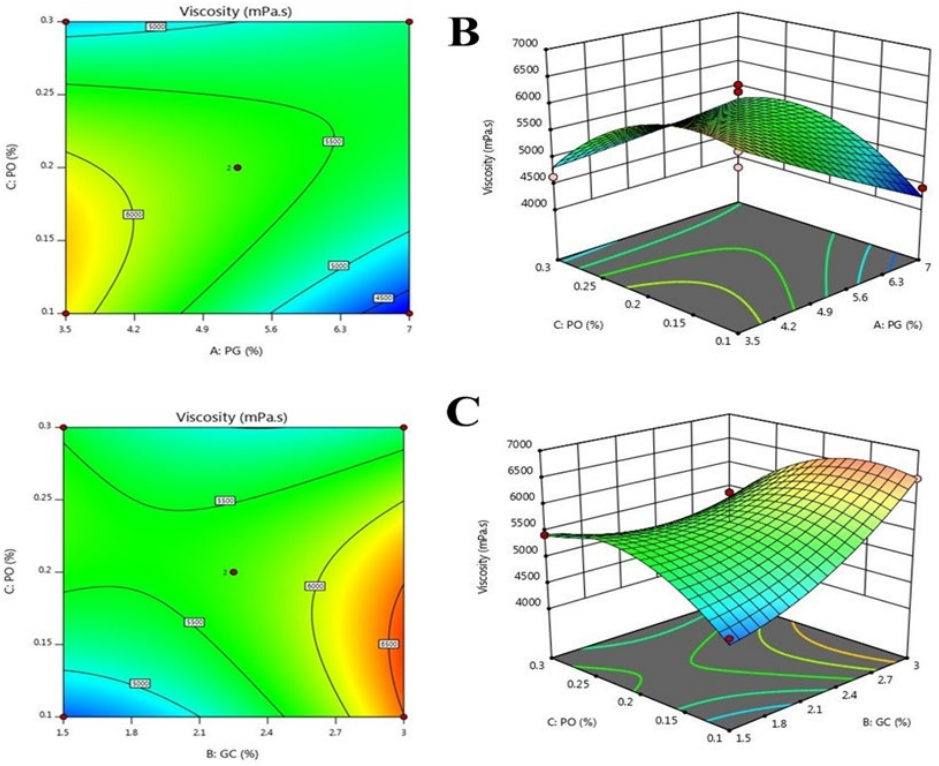


Figure 16. Effect of Factors on Viscosity of Sodium Alginate Gels. A) GC and PG B) PO and PG C) PO and GC

Table 8. Variance of Viscosity of Sodium Alginate Gels

Term	Degree of Freedom	*F* Value	*p* Value	Significance
Model Name	9	3.41	0.1247	No
X_1	1	4.06	0.1143	No
X_2	1	5.57	0.0777	No
X_3	1	0.2377	0.6514	No
X_1X_2	1	5.26	0.0836	No
X_1X_3	1	4.94	0.0904	No
X_2X_3	1	4.14	0.1116	No
X_1^2	1	0.0076	0.9349	No
X_2^2	1	1.28	0.3206	No
X_3^2	1	4.01	0.1159	No

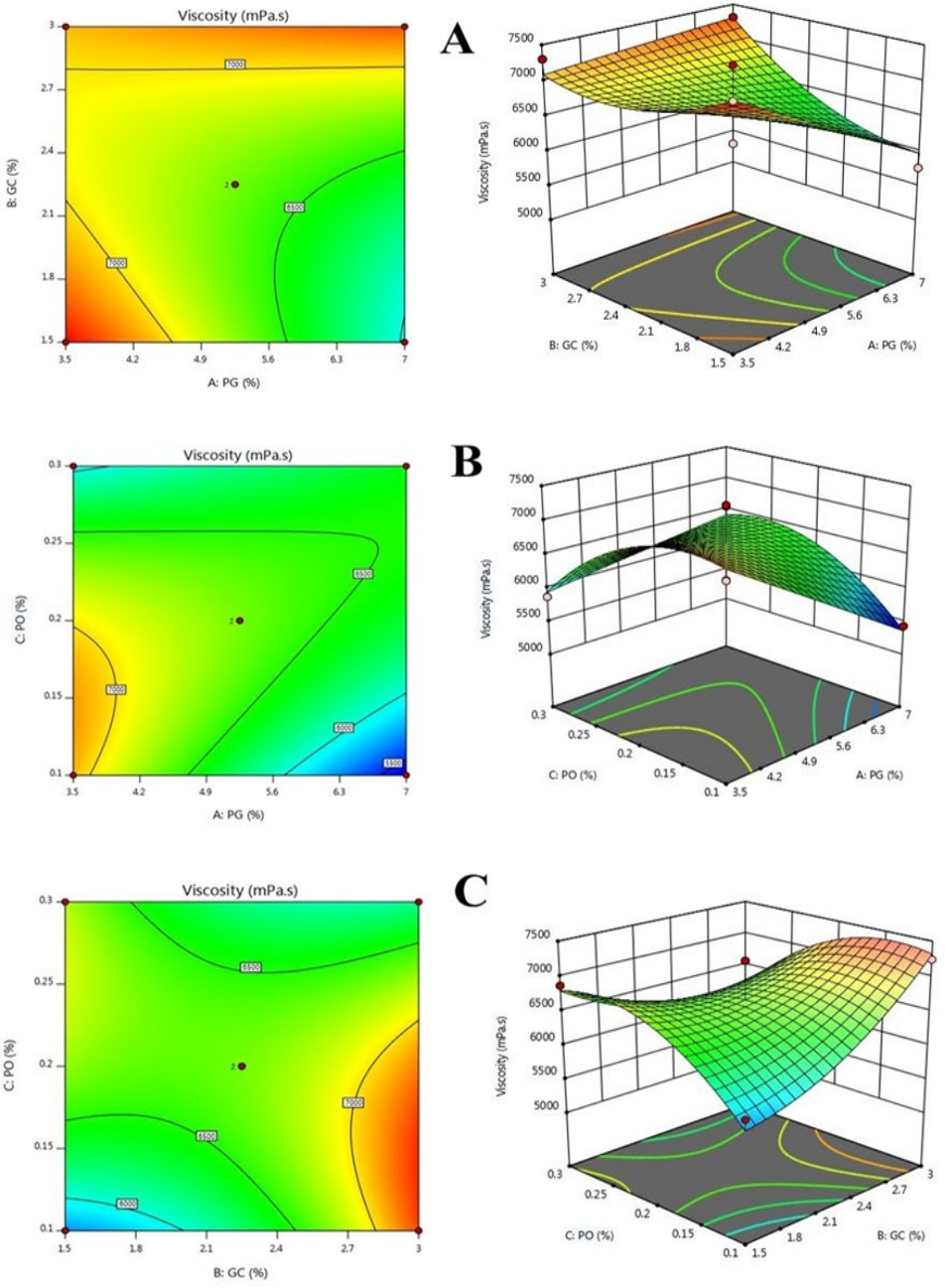


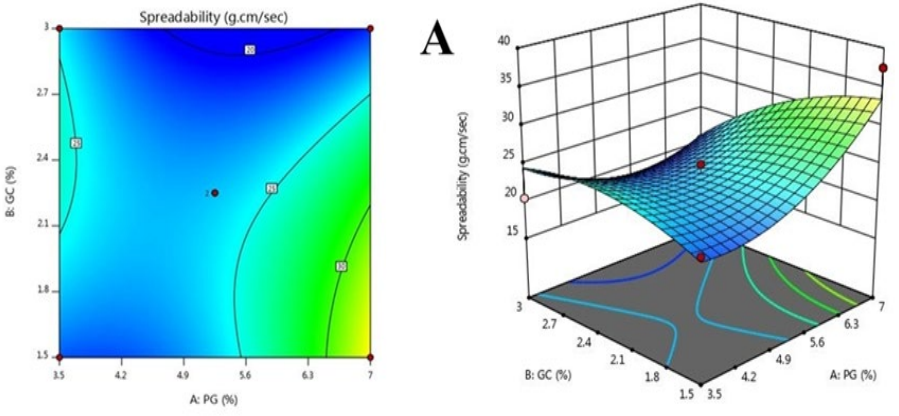
Figure 17. Effect of Factors on Viscosity of Xanthan Gum Gels. A) GC and PG B) PO and PG C) PO and GC

Table 9. Analysis of Variance of Viscosity of Xanthan Gum Gels

Term	Degree of Freedom	*F* Value	*p* Value	Significance
Model Name	9	3.06	0.1470	No
X_1	1	3.95	0.1177	No
X_2	1	2.20	0.2120	No
X_3	1	0.0234	0.8858	No
X_1X_2	1	3.51	0.1342	No
X_1X_3	1	5.85	0.0729	No
X_2X_3	1	6.13	0.0685	No
X_1^2	1	0.0009	0.9777	No
X_2^2	1	1.36	0.3083	No
X_3^2	1	3.38	0.1398	No

3.12.3. Spreadability of Gels. Figure 18. and 19 illustrate the graphs showing the effects of three variables on the spreadability of both gels. Tables 3.8 and 3.9 show the analysis of variance for spreadability of sodium alginate and xanthan gum gels, respectively. The response in general was constructive. Propylene glycol and peppermint oil both increased the spreadability.

In comparison, glycerin lowered the spreadability. As spreadability and viscosity have an inverse relation so, the factors decreasing the viscosity were responsible for the increase in spreadability.



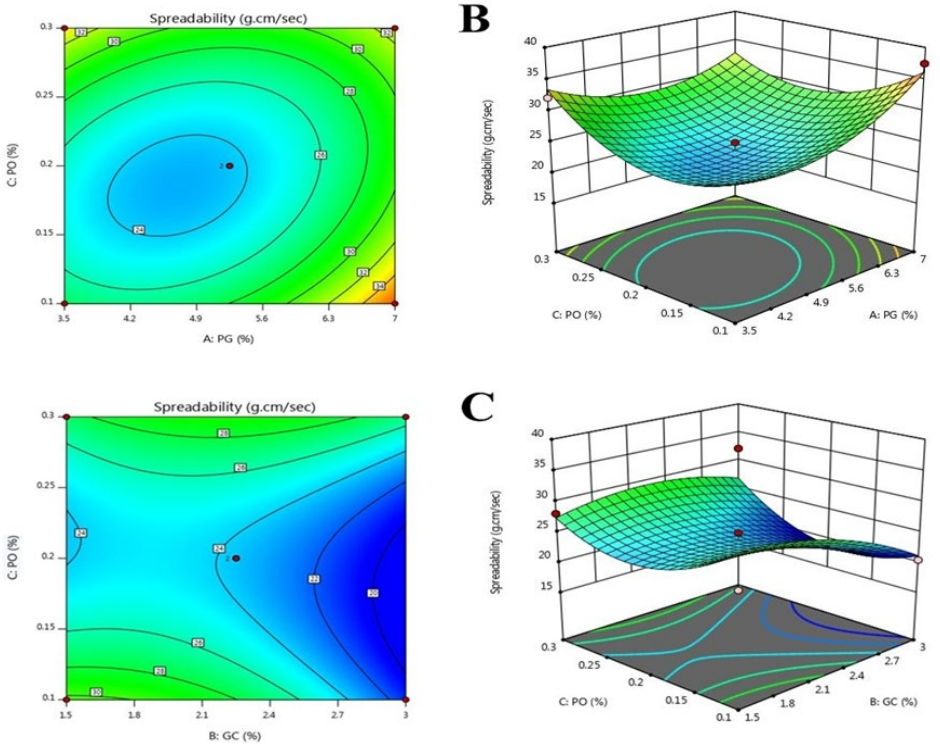


Figure 18. Effect of Factors on Spreadability of Sodium Alginate Gels. A) GC and PG B) PO and PG C) PO and GC

Table 10. Variance of Spreadability of Sodium Alginate Gels

Term	Degree of Freedom	*F* Value	*p* Value	Significance
Model Name	9	1.04	0.5261	No
X_1	1	0.9364	0.3880	No
X_2	1	1.35	0.3097	No
X_3	1	0.1037	0.7635	No
X_1X_2	1	1.58	0.2775	No
X_1X_3	1	0.6161	0.4764	No
X_2X_3	1	0.5142	0.5130	No
X_1^2	1	1.27	0.3229	No
X_2^2	1	0.4690	0.5311	No
X_3^2	1	2.21	0.2112	No

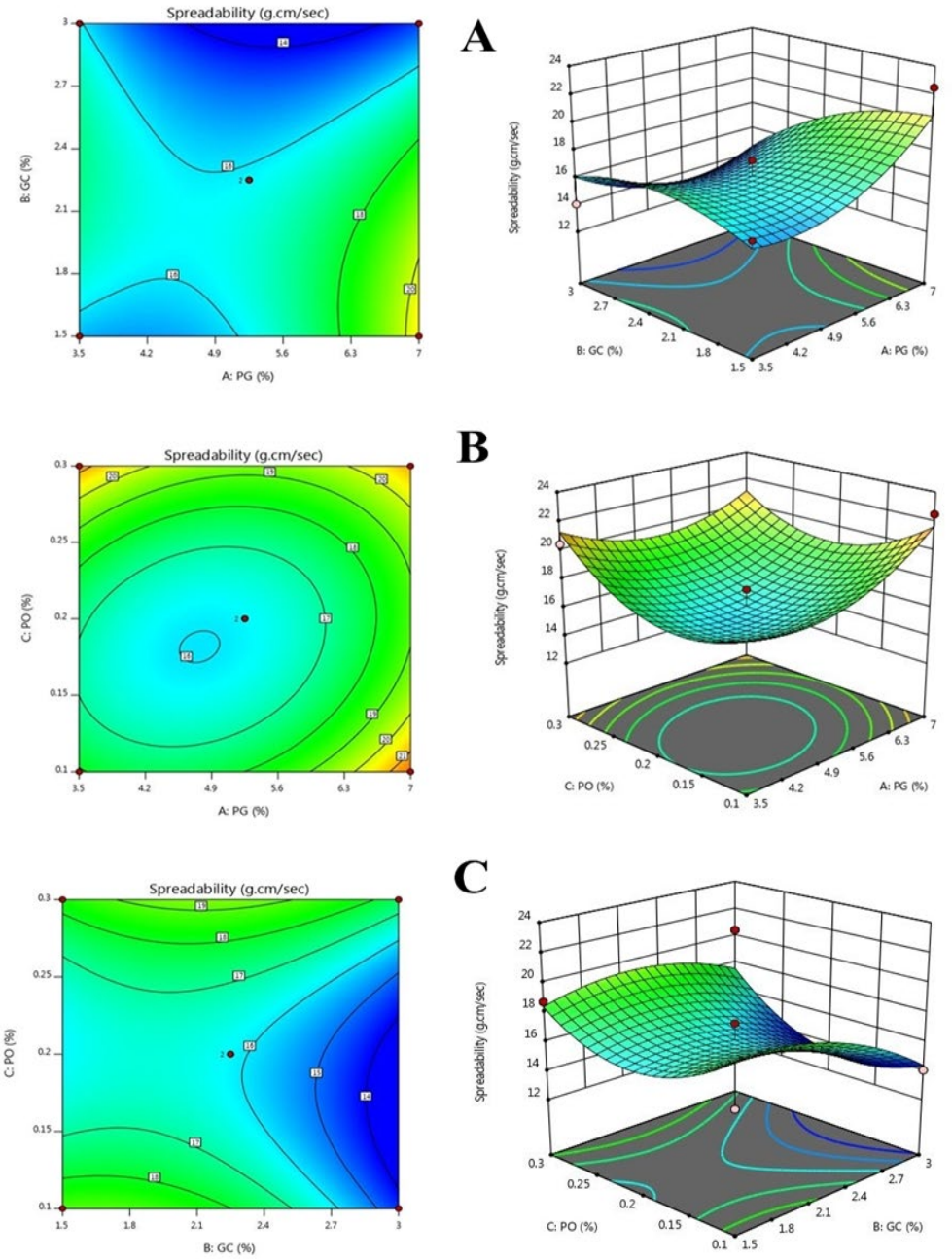


Figure 19. Effect of Factors on Spreadability of Xanthan Gum Gels. A) GC and PG B) PO and PG C) PO and GC

Table 11. Variance of Spreadability of Xanthan Gum Gels

Term	Degree of Freedom	*F* Value	*p* Value	Significance
Model Name	9	0.8997	0.5914	No
X_1	1	0.5652	0.4940	No
X_2	1	1.42	0.2987	No
X_3	1	0.3480	0.5870	No
X_1X_2	1	1.02	0.3703	No
X_1X_3	1	0.3458	0.5881	No
X_2X_3	1	0.3385	0.5919	No
X_1^2	1	1.09	0.3547	No
X_2^2	1	0.5663	0.4936	No
X_3^2	1	2.00	0.2297	No

3.13. Mathematical Modeling

Quadratic model was employed for mathematical modeling of variables and response calculation.

$$Y= X_0 + X_1 + X_2+ X_3 + X_1X_2+ X_1X_3 + X_2X_3 + X_1^2 + X_2^2 + X_3^2 \quad (4)$$

X_1 indicates Propylene glycol as factor 1, X_2 indicates glycerin as factor 2, and X_3 indicates peppermint oil as factor 3.

Permeation of Kojic Acid from Sodium Alginate Gels = + 87.36 + 0.1394 + 1.65 + 0.9612 + 0.55 + 1.37 + 2.20 + 3.16 + 1.79 + 0.4137 (5)

Viscosity of Sodium Alginate Gels = + 5679 - 375.375 + 439.75 - 90.87 + 604.5 + 585.75 - 536.5

Spreadability of Sodium Alginate Gels = + 23.75 + 2.04 - 2.45 + 0.68 - 3.75 - 2.34 + 2.14 + 3.76 - 2.29 + 4.96 (9)

Permeation of Kojic Acid from Xanthan Gum Gel = + 90.1+ 0.4127 + 0.9625 + 0.8285 - 0.5512 + 1.37 + 1.37 + 2.48 + 1.38 - 0.5484 (6)

Viscosity of Xanthan Gum Gels = + 6669.5 - 316.75 + 236.37 - 24.38 + 422.25 + 544.75 – 558 - 7.5 + 293.75 - 463.25 (8)

Spreadability of Xanthan Gum Gels = + 16.15 + 0.8475 - 1.35 + 0.665 - 1.61 - 0.9375 + 0.9275 + 1.86 - 1.34 + 2.52 (10)

From equation 4, it can be concluded that the gel viscosity was elevated by glycerin. Glycerin is a thickening agent which increases the viscosity of

gels. The value of X_0 was positive which showed that the overall response was influential. It was observed that propylene glycol and peppermint oil decreased the viscosity of prepared gels.

The effect of all three variables on gel spreadability was found to be constructive. Propylene glycol and peppermint oil increased spreadability. Contrary to that, glycerin reduced the spreadability. The mathematical models showed that the permeation of kojic acid was positive for both types of gels. All combinations were positive for the permeation of kojic acid from sodium alginate gels. A similar case was seen in the xanthan gum gels, where all three factors improved the permeation of the active ingredient.

4. CONCLUSION

In the current study, kojic acid topical gels were successfully prepared and characterized for hyperpigmentation. The evaluation tests performed on the prepared gels showed good physicochemical characteristics. Furthermore, the current study indicated that kojic acid gels have high anti-oxidant and antimicrobial activities. The percentage drug content was between 90-110% that complied with the USP limits. The characterization tests including the organoleptic evaluation, viscosity, spreadability, pH, *in-vitro* permeation study, accelerated stability study, and skin irritation test, proved that the formulated gels were stable.

The current study advanced the field of dermatology since it provided a better topical formulation for the treatment of hyperpigmentation. The utilization of biopolymers, such as sodium alginate and xanthan gum, also enhances the stability and delivery of kojic acid. While the favorable sensory properties of the formulation help increase patient compliance. The gel-based delivery system is a promising approach for the treatment of hyperpigmentation, especially with its prolonged efficacy and fewer side effects. This allows better patient adherence than that of conventional formulation. Future studies would look into the long-term efficacy and safety of such gels in clinical settings, further cementing their role in dermatological practice.

CONFLICT OF INTEREST

The authors of the manuscript have no financial or non-financial conflict of interest in the subject matter or materials discussed in this manuscript.

DATA AVAILABILITY STATEMENT

Data will be provided by corresponding author on reasonable request.

FUNDING DETAILS

No funding has been received for this research.

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