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Original article

# Improvement of tear ferning patterns of artificial tears using dilute electrolyte solutions

Essam S. Almutleb [1], Basal H. Altoaimi [1], Gamal A. El-Hiti *, Abdullah G. Alotaibi, Mashaaer A. Baashen, Saud A. Alanazi, Ali M. Masmali

*Department of Optometry, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia*

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ABSTRACT

The purpose of the research was to examine how the addition of dilute solutions of sodium chloride (NaCl), potassium chloride (KCl), or a combination of the two would impact the tear ferning (TF) patterns in artificial tears. Artificial tears (1 μL) were mixed with solutions (1–7 μL) of NaCl, KCl, or both (10–30 mg), prepared in double-distilled water (100 mL), to produce homogenous mixtures. A sample (1 μL) of each mixture was dried on a microscopic glass slide at 20 °C and with less than 20% humidity. The TF patterns were inspected using a light microscope, graded, and compared with the corresponding TF grades of pure artificial tears. Significant improvements (Wilcoxon test, $P = 0.001$) in the TF grades of the artificial tears were observed after the addition of solutions of NaCl and KCl or a mixture of both. The TF grades of the artificial tears decreased after adding different volumes and concentrations of electrolyte solutions. Generally, NaCl was more efficient than KCl in improving such grades. The TF grades of Blink Contact Soothing Eye Drops and Refresh Plus Tears were noticeably improved compared to the other grades. For example, Blink Contact Soothing Eye Drops' TF grade improved from 1.7 to 0.6 when 6 μL of NaCl solution (30 mg/100 mL water) was added to 1 μL of the eye drops. When a mixture of NaCl and KCl (1:2; 5–7 μL; 30 mg each in 100 mL water) was added, the TF grade improved to 0.5. In conclusion, the TF test evaluated the effect of adding dilute electrolyte solutions to artificial tears in vitro. Adding dilute solutions of NaCl and KCl, or a mixture of both, significantly improved the TF grades of artificial tears.



## 1. Introduction

Dry eye is a common disorder that affects the ocular surface (Craig et al., 2017). There are various reasons for dry eye, but the most common is the loss of the tear film's homeostasis (Rodriguez-Garcia et al., 2022). The dryness of the eye leads to symptoms of discomfort, visual disturbance, irradiation, inflammation, burning, and redness. Various factors can contribute to the development of dry eyes, such as contact lens wearing, using digital screens for a long duration, disorders such as diabetes, thyroid glands, and refractive errors, smoking, a high body mass index, vitamins A and D deficiency, pregnancy, and many others (Sánchez-González et al., 2022). Dry eye is common among the elderly and women (Farrand et al., 2017) and is a challenge to diagnose since it varies based on signs, symptoms, and definitions (Stapleton et al., 2017). Dry eye questionnaires can detect symptoms (Schiffman et al., 2000). Various tests assess the stability, volume, and quality of the tear film as well as the evaporation rate and osmolarity of tears (Masmali et al., 2014a; Fagehi et al., 2021a; Fagehi et al., 2022a,b). No single test can give a definite diagnosis of dry eye, but several ones should be used.

The tear ferning (TF) test is a repeatable method that can be used in vitro to evaluate the quality of the tear film in animals and humans (Alanazi et al., 2021; Veloso et al., 2020; Masmali et al., 2018a,b). Natural tears from animals or humans and some eye drops when dried at room temperature and low humidity (less than 45%) lead to crystalline patterns called ferns (Horwath et al., 2001). The evaporation of the aqueous contents of tears leads to the precipitation of proteins and electrolytes such as sodium chlo-

* Corresponding author at: Department of Optometry, College of Applied Medical Sciences, King Saud University, PO Box 10219, Riyadh 11433, Saudi Arabia.
*E-mail address:* gelhiti@ksu.edu.sa (G.A. El-Hiti).
[1] These authors contributed equally to this work.

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ride (NaCl) and potassium chloride (KCl) (López Sólis et al., 2013). The ferns of dried tears can be observed using a light microscope and graded. The most common TF grading scales contain four or five grades (Rolando, 1984; Masmali et al., 2014b). The four-point TF grading scale includes grades I and II (healthy eyes) as well as III and IV (dry eyes) (Rolando, 1984), while the five-point TF grading scale includes five grades (0–4) and can be used in 0.1 increments. Dry eye is defined as a TF grade $\geq 2$ based on this scale (Masmali et al., 2014b).

Artificial tears are the best choice to manage moderate dry eye symptoms (Markoulli et al., 2018; Essa et al., 2018). Lipid-based artificial tears tend to reduce the tear evaporation rate (Kaercher et al., 2014), while those containing macromolecules increase the viscosity and stability of the tear film (Lee et al., 2011). In addition, they tend to reduce inflammation and protect the ocular surface (Song et al., 2014). Approximately 60 million individuals worldwide use artificial tears at a huge cost (Kim et al., 2021). However, artificial tears are not always the answer for managing dry eye symptoms. Therefore, new formulations are still needed to improve the quality and stability of the tear film.

Recently, the TF grades of tears collected from humans and animals have improved after adding several divalent electrolytes (Fagehi et al., 2021b; Fagehi et al., 2022c; Alanazi et al., 2022). Similarly, the TF patterns of Blink Contact Soothing Eye Drops and Refresh Plus Tears improve when mixed with electrolyte solutions (Masmali, 2019). However, the use of monovalent electrolytes such as NaCl and KCl lead to limited or no improvement in the TF grades of natural or artificial tears (Fagehi et al., 2021b; Fagehi et al., 2022c; Alanazi et al., 2022; Masmali, 2019). The reason for this could be the high concentration of the electrolyte solution used (Bachman and Wilson, 1985). In an early report, the artificial tears used were limited to Contact Soothing Eye Drops and Refresh Plus Tears. In addition, no statistical analysis was used to test the significant improvement in TF grades after adding electrolyte solutions. Therefore, this research was performed in an attempt to overcome the limitations of the earlier report. Now we report significant improvements in the TF grades of several artificial tears after adding dilute solutions of NaCl and KCl or a mixture of both.

## 2. Materials and methods

### 2.1. Artificial tears

Blink Contact Soothing Eye Drops, HyFresh Eye Drops, Refresh Liquigel Lubricant Eye Drops, Refresh Plus Tears, and Uni Fresh Lubricant Eye Drops were selected to test the effect of the addition of diluted solutions of NaCl and KCl or a mixture of both electrolytes on their TF grades. Four artificial tears—namely, Blink Contact Soothing Eye Drops, HyFresh Eye Drops, Refresh Liquigel Lubricant Eye Drops, and Refresh Plus Tears (Table 1)—gave clear TF patterns when dried. Therefore, they were mixed with dilute solutions of NaCl and KCl or mixtures of both electrolytes in an attempt to improve their TF patterns. Uni Fresh Lubricant Eye Drops (Table 1) did not exhibit distinctive TF patterns. Consequently, the impact of adding electrolyte solutions remained uncertain and inconclusive. Table 1 shows the five artificial tears used in this research and their ingredients and uses.

### 2.2. Electrolyte solutions

NaCl and KCl were obtained from Avonchem Limited and used as received. To produce homogenous mixtures, electrolytes (10–30 mg) in 100 mL of double-distilled water were stirred for five minutes. Combinations of both electrolytes were prepared by mixing different volumes of both electrolytes.

### 2.3. TF test

The artificial tears (1 μL) were mixed with different volumes (1–7 μL) of several concentrations (10–30 mg in 100 mL of double-distilled water) of NaCl and KCl or both to produce homogenous mixtures. A sample (1 μL) of each mixture was dried on a microscopic glass slide at 20 °C and with a humidity of less than 20% (Alanazi et al., 2019a,b). A light microscope was used to visualize the TF patterns. The grading was based on the five-point grading scale. The TF grades were compared to the ones corresponding to pure artificial tears. Two independent examiners completed the grading to 0.1 increments. The grading scores of both examiners were very similar, and the average to the nearest decimal place was used in cases when different grades were assigned. The second examiner was masked to avoid potential bias.

### 2.4. Statistical analysis

Microsoft Excel (Microsoft Office 2016) was used to record the data. The Statistical Package for the Social Sciences (SPSS) software (IBM Software, version 25) was used to analyze the data, which was not normally distributed (Shapiro-Wilks test; $P$ less than 0.05). After adding electrolyte solutions, the Wilcoxon signed-rank test was used to determine the significant differences in TF grades.

## 3. Results

Table 2 shows that by adding NaCl and KCl or a combination of both, there were significant improvements in the TF grades of the four artificial tears, as confirmed by the Wilcoxon test with a $P$-value of 0.001. The TF grades of the mixtures of each kind of artificial tears (1 μL), as well as different concentrations (10–30 mg in 100 mL of double-distilled water) and volumes (1–7 μL) of the NaCl or KCl solution, are shown in Table 3.

The TF grades of the four artificial tears decreased after adding the NaCl or KCl solution. All the volumes and concentrations of the NaCl and KCl solutions significantly improved the TF grades of the artificial tears. Generally, the NaCl solution was more efficient than the KCl solution in improving the TF grades of the artificial tears. For example, the TF grade of Blink Contact Soothing Eye Drops

**Table 1**
Selected artificial tears use and ingredients.

| Artificial tears | Ingredients | Use |
|---|---|---|
| Blink Contact Soothing Eye Drops (10 mL; Abbott) | Sodium hyaluronate (0.15%) and OcuPure preservative (0.0005%) | Improves tear film stability and replenishes tear film |
| HyFresh Eye Drops (10 mL; Jamjoom Pharma) | Sodium hyaluronate (2.0 mg) | Relieves dry eye symptoms |
| Refresh Liquigel Lubricant Eye Drops (15 mL; Allergan) | Carboxymethylcellulose sodium (1%) | Relieves dry eye symptoms |
| Refresh Plus Tears (4 × 0.6 mL; Allergan) | Carboxymethylcellulose sodium (0.5%), electrolytes, and boric acid | Provides lubrication to reduce dry eye symptoms |
| Uni Fresh Lubricant Eye Drops (30 × 0.4 mL; Jamjoom Pharma) | Carboxymethylcellulose sodium (0.5%) | Relieves burning, irritation, and discomfort of dry eye |

**Table 2**
Significant differences in the TF grades of artificial tears after adding electrolyte solutions.

| Artificial Tears | Electrolyte | *P*-Value* | *Z*-Value* |
|---|---|---|---|
| Blink Contact Soothing Eye Drops | NaCl | 0.001 | −3.43 |
| | KCl | 0.001 | −3.45 |
| | NaCl and KCl | 0.001 | −3.46 |
| HyFresh Eye Drops | NaCl | <0.001 | −3.62 |
| | KCl | <0.001 | −3.53 |
| | NaCl and KCl | 0.001 | −3.47 |
| Refresh Liquigel Lubricant Eye Drops | NaCl | 0.001 | −3.47 |
| | KCl | 0.001 | −3.45 |
| | NaCl and KCl | 0.001 | −3.49 |
| Refresh Plus Tears | NaCl | 0.001 | −3.47 |
| | KCl | 0.001 | −3.45 |
| | NaCl and KCl | 0.001 | −3.44 |

* Wilcoxon signed-rank test.

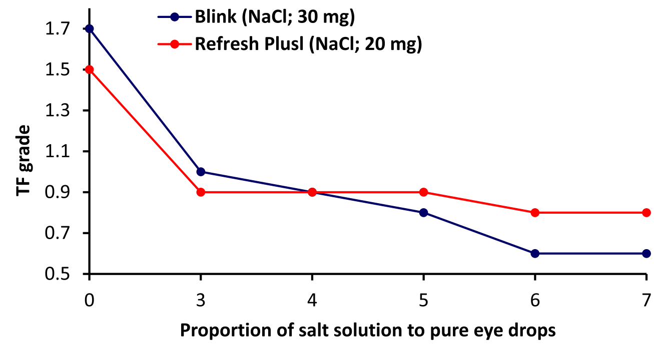


**Fig. 1.** Effect of different volumes of NaCl solutions on the TF grades of Blink Contact Soothing Eye Drops and Refresh Plus Tears.

improved from 1.7 to 0.6 when a NaCl solution (6 µL; 30 mg/100 mL water) was added to the eye drops (1 µL) and to 0.9 when a KCl solution (7 µL; 20 mg/100 mL water) was used. For Refresh Plus Tears, the TF grade improved from 1.5 to 0.8 when a NaCl solution (3 µL; 30 mg/100 mL water) was used and to 0.9 when a KCl solution (4 µL; 30 mg/100 mL water) was added to the pure eye drops. On the other hand, the improvements in the TF grades of HyFresh Eye Drops and Fresh Liquigel Lubricant Eye Drops were significant but limited (from 1.8 to 1.6 to 1.5–1.2) regardless of the concentrations or volumes of either electrolyte solution. Fig. 1 illustrates the impact of different volumes of NaCl solutions on the TF grades of Blink Contact Soothing Eye Drops and Refresh Plus Tears.

Next, our attention turned to using solutions containing mixtures of NaCl and KCl to test their effect on the TF grades of artificial tears. The TF grades of the mixtures of each kind of artificial tears as well as different concentrations and volumes of the mixtures of NaCl and KCl solutions are shown in Table 4.

The mixtures containing both NaCl and KCl solutions led to a significant improvement in the TF grades of the artificial tears. Again, the TF grades of both Blink Contact Soothing Eye Drops and Refresh Plus Tears were the most noticeably improved compared with the others. When a 1:2 solution containing both NaCl and KCl (5–7 µL; 30 mg each in 100 mL water) was added, the TF grade of Blink Contact Soothing Eye Drops improved to 0.5. For Refresh Plus Tears, the TF grade improved to 0.9 when a 1:1 mixture containing NaCl and KCl (5–7 µL; 30 mg each in 100 mL water) was added. Representative images of the TF patterns of the four artificial tears used before and after the addition of selected concentrations and volumes of NaCl and KCl or a mixture of both solutions are shown in Figs. 2-5.

**Table 3**
TF grades of pure artificial tears and their mixtures with NaCl and KCl solutions.

| Electrolyte | Ratio[a] | TF grade | | | |
|---|---|---|---|---|---|
| | | Blink | HyFresh | Refresh Liquigel | Refresh Plus |
| – | – | 1.7 | 1.6 | 1.8 | 1.5 |
| NaCl (10 mg) | 1:1 | 1.3 | 1.5 | 1.5 | 1.1 |
| | 1:2 | 1.3 | 1.5 | 1.5 | 1.0 |
| | 1:3 | 1.3 | 1.5 | 1.5 | 0.9 |
| | 1:4 | 1.2 | 1.5 | 1.5 | 0.9 |
| | 1:5 | 1.2 | 1.5 | 1.5 | 0.9 |
| NaCl (20 mg) | 1:3 | 1.2 | 1.5 | 1.5 | 0.9 |
| | 1:4 | 1.2 | 1.4 | 1.4 | 0.9 |
| | 1:5 | 1.2 | 1.5 | 1.3 | 0.9 |
| | 1:6 | 1.1 | 1.5 | 1.3 | 0.8 |
| | 1:7 | 1.1 | 1.5 | 1.3 | 0.8 |
| NaCl (30 mg) | 1:3 | 1.0 | 1.5 | 1.3 | 0.8 |
| | 1:4 | 0.9 | 1.5 | 1.3 | 0.8 |
| | 1:5 | 0.8 | 1.5 | 1.3 | 0.8 |
| | 1:6 | 0.6 | 1.4 | 1.3 | 0.8 |
| | 1:7 | 0.6 | 1.3 | 1.2 | 0.8 |
| KCl (10 mg) | 1:1 | 1.2 | 1.5 | 1.5 | 1.4 |
| | 1:2 | 1.2 | 1.5 | 1.5 | 1.3 |
| | 1:3 | 1.2 | 1.5 | 1.5 | 1.2 |
| | 1:4 | 1.1 | 1.5 | 1.5 | 1.2 |
| | 1:5 | 1.1 | 1.5 | 1.5 | 1.2 |
| KCl (20 mg) | 1:3 | 1.1 | 1.5 | 1.4 | 1.2 |
| | 1:4 | 1.0 | 1.5 | 1.4 | 1.2 |
| | 1:5 | 1.0 | 1.5 | 1.4 | 1.2 |
| | 1:6 | 1.0 | 1.5 | 1.4 | 1.2 |
| | 1:7 | 0.9 | 1.5 | 1.4 | 1.1 |
| KCl (30 mg) | 1:3 | 1.0 | 1.4 | 1.3 | 1.0 |
| | 1:4 | 1.0 | 1.4 | 1.3 | 0.9 |
| | 1:5 | 1.1 | 1.4 | 1.3 | 0.9 |
| | 1:6 | 1.1 | 1.2 | 1.3 | 0.9 |
| | 1:7 | 1.1 | 1.3 | 1.3 | 0.9 |

[a] The ratio between the volumes (µL) of the artificial tears and the electrolyte solution.

**Table 4**
TF grades of pure artificial tears and their mixtures with NaCl and KCl solutions.

| NaCl/KCl (mg each) | Ratio[a] | TF Grade | | | |
|---|---|---|---|---|---|
| | | Blink | HyFresh | Refresh Liquigel | Refresh Plus |
| – | – | 1.7 | 1.6 | 1.8 | 1.5 |
| 1:1 (10 mg) | 1:1 | 1.2 | 1.4 | 1.5 | 1.3 |
| | 1:2 | 1.2 | 1.4 | 1.5 | 1.3 |
| | 1:3 | 1.2 | 1.4 | 1.5 | 1.3 |
| | 1:4 | 1.2 | 1.4 | 1.5 | 1.3 |
| | 1:5 | 1.2 | 1.4 | 1.5 | 1.3 |
| 1:1 (20 mg) | 1:3 | 1.1 | 1.4 | 1.5 | 1.3 |
| | 1:4 | 1.1 | 1.4 | 1.4 | 1.2 |
| | 1:5 | 1.1 | 1.3 | 1.4 | 1.2 |
| | 1:6 | 1.1 | 1.3 | 1.4 | 1.2 |
| | 1:7 | 1.1 | 1.3 | 1.4 | 1.1 |
| 1:1 (30 mg) | 1:3 | 1.1 | 1.3 | 1.4 | 1.1 |
| | 1:4 | 1.1 | 1.3 | 1.4 | 1.1 |
| | 1:5 | 1.0 | 1.3 | 1.4 | 0.9 |
| | 1:6 | 1.0 | 1.2 | 1.4 | 0.9 |
| | 1:7 | 1.0 | 1.2 | 1.3 | 0.9 |
| 1:2 (10 mg) | 1:1 | 1.1 | 1.4 | 1.4 | 1.3 |
| | 1:2 | 1.1 | 1.4 | 1.4 | 1.3 |
| | 1:3 | 1.1 | 1.4 | 1.4 | 1.2 |
| | 1:4 | 1.1 | 1.4 | 1.4 | 1.2 |
| | 1:5 | 1.1 | 1.4 | 1.4 | 1.2 |
| 1:2 (20 mg) | 1:3 | 1.1 | 1.4 | 1.3 | 1.3 |
| | 1:4 | 1.1 | 1.4 | 1.3 | 1.3 |
| | 1:5 | 1.1 | 1.4 | 1.3 | 1.3 |
| | 1:6 | 1.1 | 1.4 | 1.3 | 1.3 |
| | 1:7 | 1.1 | 1.4 | 1.3 | 1.3 |
| 1:2 (30 mg) | 1:3 | 1.0 | 1.4 | 1.3 | 1.3 |
| | 1:4 | 0.9 | 1.4 | 1.4 | 1.3 |
| | 1:5 | 0.5 | 1.3 | 1.4 | 1.2 |
| | 1:6 | 0.5 | 1.3 | 1.4 | 1.2 |
| | 1:7 | 0.5 | 1.3 | 1.5 | 1.2 |

[a] The ratio between the volumes (μL) of the artificial tears and the electrolyte solution.

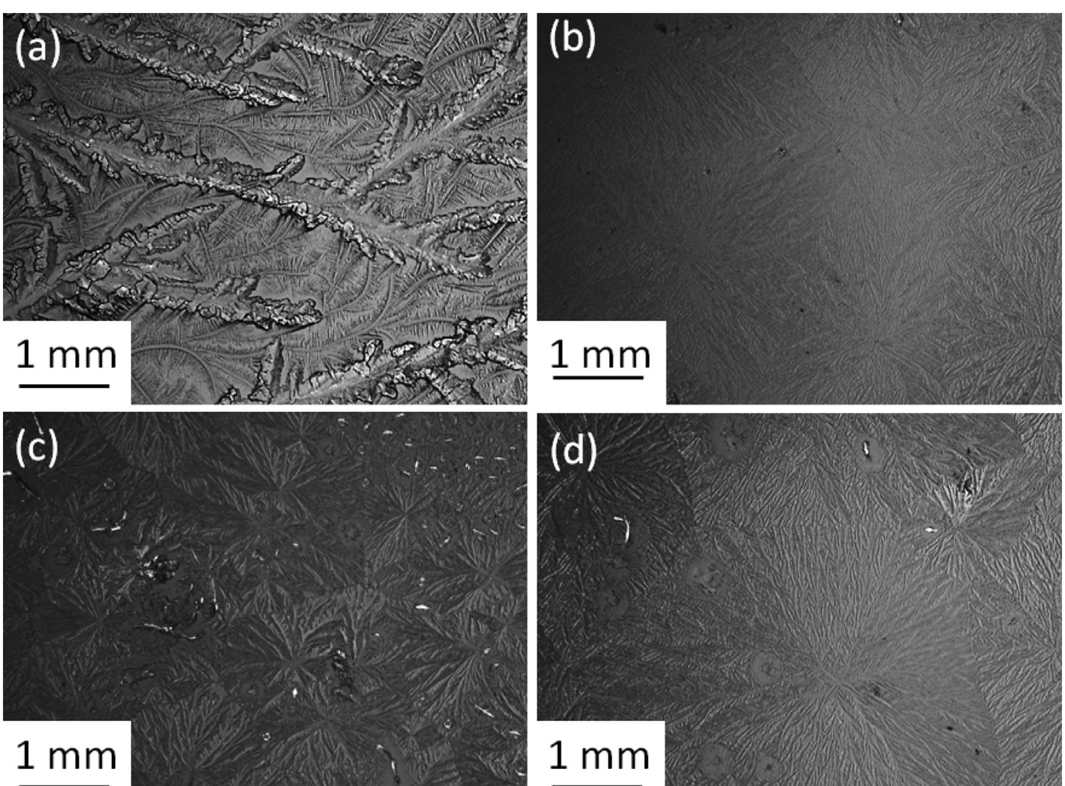


**Fig. 2.** (**a**): Blink, (**b**): Blink + NaCl (30 mg; 1:6), (**c**): Blink + KCl (20 mg; 1:3), and (**d**): Blink + NaCl/KCl (1:1; 30 mg, 1:5).

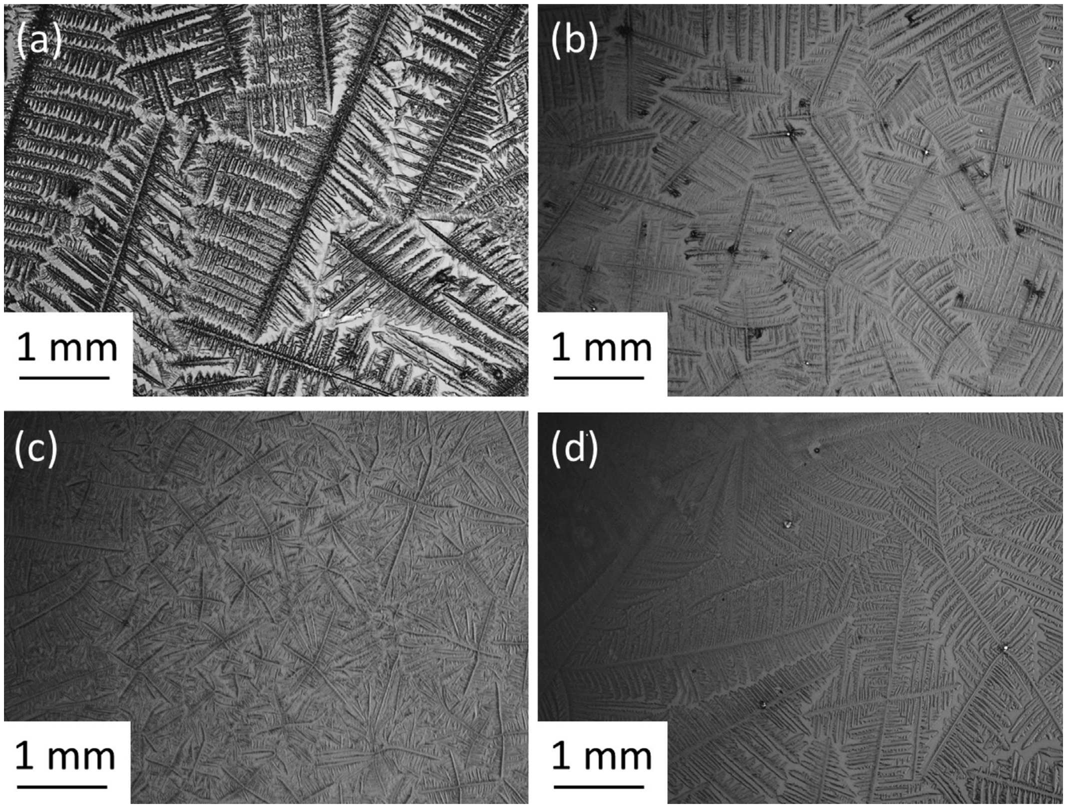


**Fig. 3.** (**a**): HyFresh, (**b**): HyFresh + NaCl (30 mg; 1:4), (**c**): HyFresh + KCl (30 mg; 1:6), and (**d**): HyFresh + NaCl/KCl (1:1; 30 mg, 1:6).

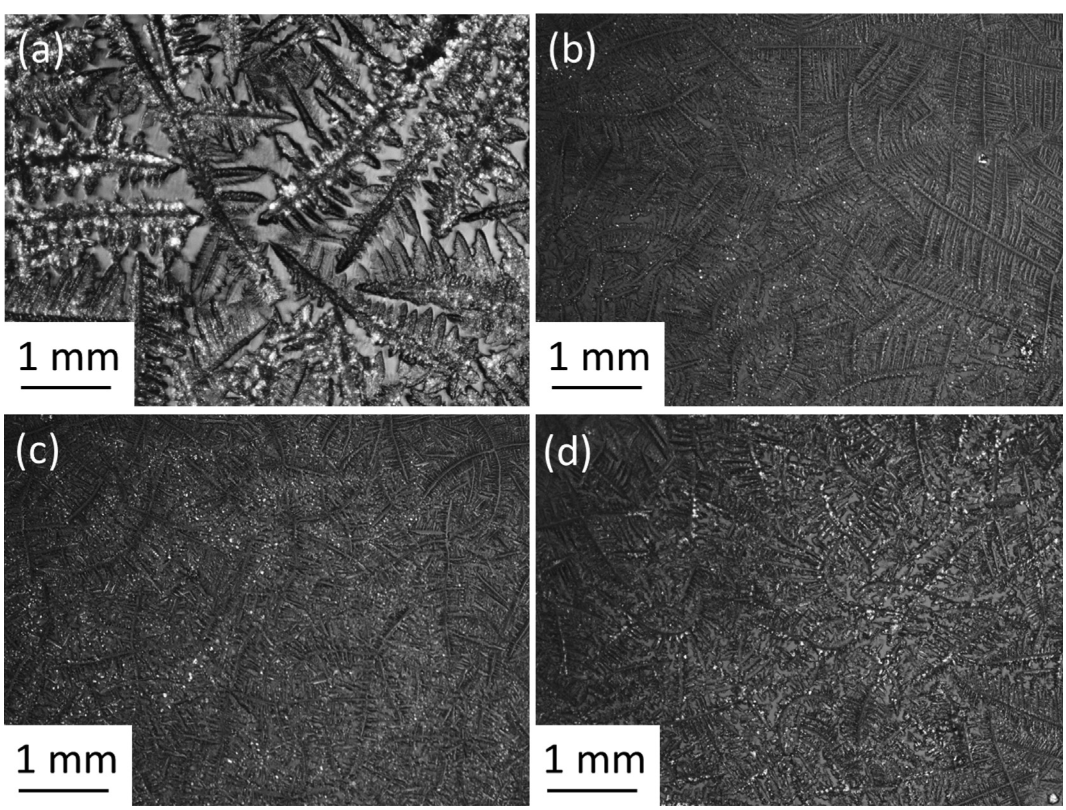


**Fig. 4.** (**a**): Refresh Liquigel, (**b**): Refresh Liquigel + NaCl (30 mg; 1:7), (**c**): Refresh Liquigel + KCl (30 mg; 1:6), and (**d**): Refresh Liquigel + NaCl/KCl (1:1; 20 mg, 1:7).

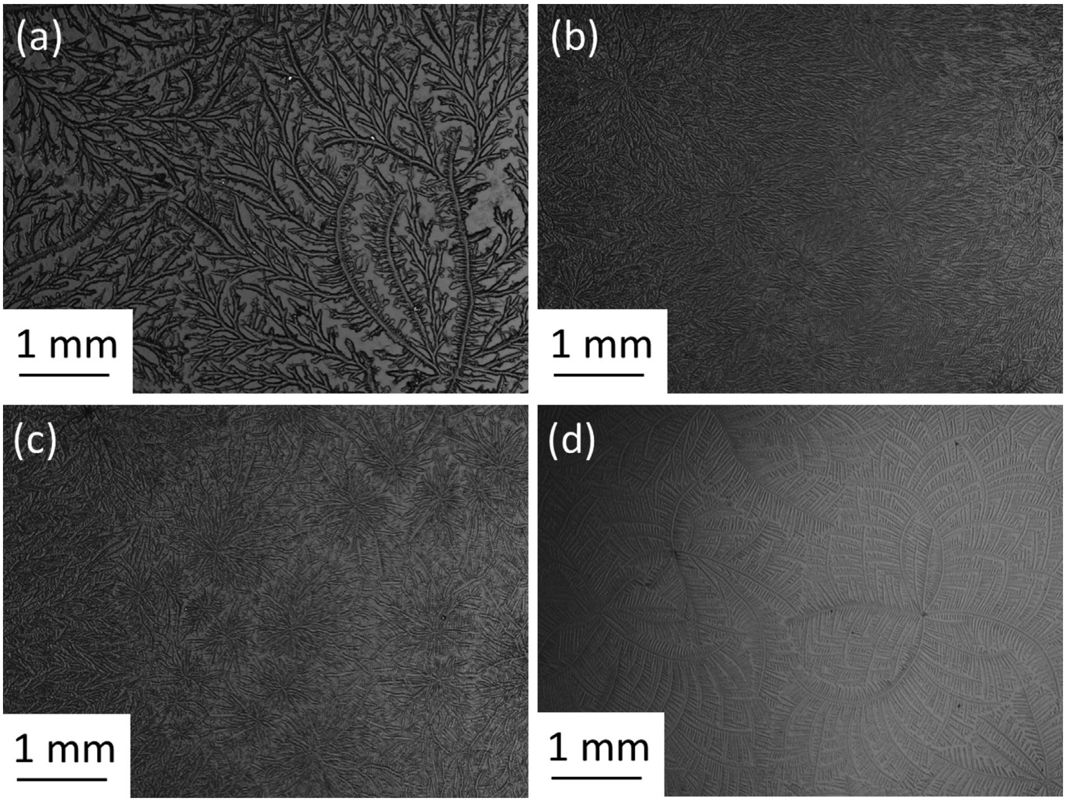


**Fig. 5.** (**a**): Refresh Plus, (**b**) Refresh Plus + NaCl (10 mg; 1:5), (**c**): Refresh Plus + KCl (10 mg; 1:2), and (**d**): Refresh Plus + NaCl/KCl (1:1; 30 mg, 1:6).

## 4. Discussion

This study showed that using dilute solutions of NaCl and KCl or a mixture of both significantly improved the TF grades of artificial tears. The most significant improvement in TF grades was noticeable for Blink Contact Soothing Eye Drops and Refresh Plus Tears. A previous report indicated that using a high concentration of different volumes of NaCl (680 mg in 100 mL) does not improve the TF grades of both Blink Contact Soothing Eye Drops and Refresh Plus Tears. Using a high concentration of NaCl leads to an increase in the TF grade of both artificial tears (Masmali, 2019). In addition, using a high concentration (140 mg in 100 mL) of KCl did not improve the TF grade of Refresh Plus Tears (Masmali, 2019). Similar observations have been made when a high concentration of either the NaCl or KCl solution is added to tears collected from humans (Alanazi et al., 2022b), sheep (Fagehi et al., 2022c), camels, and goats (Fagehi et al., 2022c). The high concentration of either electrolyte seems to disturb the balance between anions and cations within tears, reducing their qualities.

The reason why NaCl resulted in a more noticeable improvement in tear ferning patterns of artificial tears compared to KCl is unclear. Different factors, including the concentration and type of electrolyte present, can influence the pattern of tear ferning. The interaction between electrolytes, specifically sodium cation ($Na^+$) and chloride anion ($Cl^-$), with macromolecules such as mucins and proteins in the tear film or artificial tears leads to the production of ferns (Golding et al., 1989). The ferns have well-defined crystallization patterns and provide information about the quality of the tear film or artificial tears. When the branches within the ferns come together with no spaces, this indicates normal tears. The ratios between monovalent (Na and K) and divalent cations (calcium and manganese) play an important role in fern formation (Kogbe et al., 1991). The ratio could vary between artificial tears and natural ones. For example, $K^+$ and $Cl^-$ are less prevalent in Refresh Plus Eye Drops compared with the tears of humans and camels (Masmali et al., 2018b). These cations and anions are responsible for forming TF patterns, and their presence is important to keep the tear film healthy (Masmali et al., 2018b). In addition, the balance among Na, K, and Cl ions affects fern formation more than their concentrations. Moreover, mucins and proteins facilitate the formation of TF patterns, but they are not part of the fern structure (Kogbe et al., 1991; Pearce and Tomlinson, 2000). Indeed, adding large molecules and divalent electrolyte solutions improves the TF grades of artificial tears (Masmali, 2019). This study provides an opportunity to improve the quality of known artificial tears. In addition, it is possible to produce new artificial tears with better formulations and efficiently improve the tear film quality in dry eye subjects.

Artificial tears containing macromolecules such as sodium carboxymethylcellulose and sodium hyaluronate can be used to relieve the symptoms of dry eye. In addition, they can improve the tear film volume, stability, and quality (Masmali et al., 2018; Okanobo et al., 2012; Lee et al., 2015; Ayaki et al., 2012). Various studies have covered the correlation between TF grades and other tear film parameters that were assessed by questionnaires or tests (e.g., Schirmer, phenol red thread, noninvasive tear breakup time) (Masmali et al., 2015a; Masmali et al., 2016). The correlations between TF grades and other tear parameters were generally poor. It should be noted that each test assesses a different tear film parameter The TF test is a reliable and consistent in vitro method for identifying dry eye and other common clinical tests (Masmali et al., 2015a,b; Masmali et al., 2016). The mean difference of the TF grades of human tears recorded at different times on the same day was approximately 0.1 ± 0.4 on the five-point grading scale (Masmali et al., 2015a). In some cases, the changes in the TF grades were relatively small and within the 95% limit of agreement for the repeatability of human tears. However, the tear ferns obtained from artificial tears in this study were graded as almost identical between two independent observers, and the mean difference was less than ± 0.1.

This research has some limitations that should be addressed in a related future study. First, the number of artificial tears that produced TF patterns was limited ($N$ = 4). Second, the effect of low concentrations of other electrolytes containing sodium and potassium should be tested. Third, the test can only be applied to artificial tears capable of producing ferns when dried. Further research is required to determine the optimal dilution level of electrolyte solutions (e.g., NaCl and KCl) that result in the most significant enhancement of tear ferning patterns in artificial tears.

## 5. Conclusions

The TF test evaluated the effect of adding dilute electrolyte solutions to artificial tears in vitro. The TF grades of artificial tears such as Blink Contact Soothing Eye Drops and Refresh Plus Tears improved significantly after adding dilute solutions of NaCl and KCl or a mixture of both.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgments

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jksus.2023.102860.

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