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# Sun protective potential and physical stability of herbal sunscreen developed from Afghani medicinal plants

Güneş koruyucu potansiyel ve fiziksel stabiliteye sahip Afgan tıbbi bitkilerinden geliştirilen bitkisel güneş koruyucu

**Short title**

**Sun protective potential and physical stability of herbal sunscreen**

Bitkisel güneş koruyucunun güneş koruma potansiyeli ve fiziksel stabilitesi

Amina Ahmady[1]*, Mohammad Humayoon Amini[2], Aqa Mohammad Zhakfar[1] , Gulalai Babak[1], Mohammad Nasim Sediqi[2],

[1] Department of pharmaceutics, Faculty of pharmacy, Kabul University, Kabul, Afghanistan

[2] Department of pharmacognosy, Faculty of pharmacy, Kabul University, Kabul, Afghanistan

Corresponding author: Amina Ahmady*
Mailing address: Jamal Mina, Faculty of pharmacy, Kabul University, Kabul, Afghanistan
Mobile No.: +93747228758
Email: amina.ahmady@yahoo.com or amina.ahmady@ku.edu.af

**Abstract**

INTRODUCTION: The aim of this study was to develop herbal topical sunscreen formulation based on some fixed oils, in combination with some medicinal plants.
METHODS: The crude and purified extracts were screened for their phytochemical profile and their sun protection potentials. Based on our results, E. angustifolia Purified extract (EAPE), sesame oil and sea buckthorn oil were selected for development of sunscreen formulation. Developed sunscreen formulations containing different concentration of EAPE were evaluated for their different physicochemical properties and stability.
RESULTS: The results of phytochemical analysis revealed presence of phenolic and flavonoids in all tested extracts. EAPE, sesame oil and sea buckthorn oil showed the highest absorption in the ultraviolet (UV) region. Sun protection factor (SPF) value of the developed formulations containing different concentration of EAPE was found to be in the range of 6.37±0.14 to 21.05±0.85. Sunscreen formulation containing 6% EAPE was stable during the period of 8 weeks in oven (40ºC) and refrigerator (4 ºC).
DISCUSSION AND CONCLUSION: Finding of this study revealed the higher sun protection capacity of EAPE than other plant extracts. Sunscreen formulations containing 6% EAPE showed promising SPF value. However, further in – vivo studies are highly emphasized to prove further safety and efficacy of our developed sunscreen formulation.

**Keywords:** Sunscreen, UV filter, Emulgel, Elaeagnus angustifolia

## Introduction

Excessive solar ultraviolet (UV) radiations are responsible for various skin damages such as sunburn, skin pigmentation, premature aging and photo carcinogenesis.[1,2] The main mechanism of skin damage by UV radiations is formation of Reactive Oxygen Species (ROS) that interact with proteins and lipids and subsequently alter them.[3] Solar UV radiation is divided into three categories: ultraviolet C (UVC) (200 – 280 nm), ultraviolet B (UVB) (280 – 320 nm) and ultraviolet A (UVA) (320 – 400 nm). UVB and to a lesser extent UVA are responsible for inducing skin damages.[4,5]

Sunscreens effectively have been used for prevention and treatment of sunburn. But several studies have shown that they are not effective in preventing premature aging and skin carcinoma.[6,7] Since ROS is the main cause of photo aging and skin cancers, an effective sunscreen should contain antioxidant agent in addition to sunblock agent to be effective in prevention of photo aging and skin cancer.[5,8,9]

Herbal extracts and oils have a complex composition which results in exhibition of different effects; such as anti-inflammatory, antioxidant, immunomodulatory, sun blocking, and so on.[10,11] Moreover, their efficacy in treatment of different skin diseases, and improvement of skin appearance is very well understood. Plants due to their

antioxidant potential are known as attractive option to be used in sunscreen formulations for prevention of skin damage due to solar radiation.[5,8,9,12]

Afghanistan is a mountainous country with a rich plant flora encompassing valuable nutritional and medicinal plants. Afghan plant flora is estimated to be comprised of around 3500 species (with 25 – 30% are endemics).[13] Present work was designed in order to evaluate sun protective potential of extracts and fixed oils extracted from some medicinal as well as nutritional plants grow in Afghanistan. In this research, Sea buckthorn (*Hippophae rhamnoides* L.) ripe fruits oil, olive (*Olea europea*) fruits oil, sesame (*Sesamum indicum*) seeds oil were screened for their *in vitro* sun protective potential. Similarly, *Alhagi pseudalhagi* herbs and *Elaeagnus angustifolia* leaf extracts were screened for their phytochemical profile and sun protective potential. Based on the results obtained from the preliminary studies on sun protection potential of above mentioned plants and fixed oils, a topical sunscreen formulation was developed.

## Material and method

*Chemicals*

Different solvents such as methanol (Merk), ethanol (Merk), ethyl acetate, hexane **(**Sigma-Aldrich**),** petroleum ether (Sigma-Aldrich**)** and diethyl ether (Riedel-deHaen) were used in different steps of extraction processes. Cetostearyl alcohol (CDH), butylated hydroxy toluene (BDH), sodium lauryl sulfate (BDH), propylene glycol (CDH), methyl paraben (BDH), propyl paraben ***(***BDH***),*** xanthan gum (BDH) were used for preparation of emulgel formulation.

*Plant materials*

Olive oil and sesame oil were procured from the local market of Jalalabad and Jowzjan in Afghanistan, respectively. *Elaeagnus angustifolia* leaves were collected from Paghman district of Kabul. *Alhagi pseudalhagi* herb was collected at their blooming time from Kabul University campus. *Hippophae rhamnoides* mature fruits were collected from Kapisa. The collected plant materials were botanically identified and authenticated by Prof. Mohammad Nasim Sediqi, Head of Pharmacognosy department, Faculty of Pharmacy, Kabul University. Collected plants materials were

shade dried, coarsely powdered, passed through sieve No. 1400, and packed in plastic bags for further use.

*Preparation of plant extracts*

In first step, 20 g powder of *E. angustifolia* leaves, and *A. pseudalhagi* herbs were separately extracted by methanol 70% at 70°C, using Soxhlet extractor. The obtained extracts after filteration through Watmann No. 1, were concentrated using Rotatory evaporator, at 40°C. Further concentration was done by water bath, at 70°C. Complete drying was performed in oven (Yamato DX601) at 60°C, until getting a constant weight for each extracts. In case of *H. rhamnoides* fruits Similarly, 20 g of dried powdered drug was extracted by n-hexane at 70°C, using Soxhlet extractor. The hexane extract, was concentrated at 30°C, using Rotatory evaporator, until an orange-colored *H. rhamnoides* fixed oil (HRO) was obtained. The oil was further dried on water-bath, followed by drying in oven at 60°C, to obtain its constant weight.

*Purification of extracts*

For further purification of both *E. angustifolia* crude extract (EACE) and *A. pseudalhagi* crude extract (APCE), the method described by Jarzycka *et al.* was applied with slight modifications.[14] So, the crude extract was first dissolved in 70% methanol, and then was extracted with equal amounts of its volume petroleum ether (4 times). The methanol fraction after being dried, was dissolved in hot distilled water. Ascorbic acid (0.5 mg/g) was added to the mixture. The whole was left in refrigerator for 24 hr. Then the mixture was extracted with diethyl ether (5 fold). The last aqueous fraction was extracted with ethyl acetate (5 fold). In the next step the ethyl acetate was evaporated and the purified dried extracts were used for further studies. The purified extract of *E. angustifolia* and *A. pseudalhagi are* abbreviated as EAPE and APPE respectively.

*Phytochemical screening of the extracts*

Methanolic stock solution of extracts (EACE, EAPE, APCE and APPE) were prepared at final concentration of 2mg/ml. The extracts solutions were tested for the presence of diverse category of phytochemicals such as; alkaloids, phenolic compounds, flavonoids and tannins.

**Detection of alkaloids**

**Dragendorff's test:** About 2 ml of test solution was treated with 3 drops of Dragendorff's reagent (potassium Iodide + bismuth nitrate). It was observed for formation of orange red/brown colour precipitate that indicates presence of alkaloids in the test extract.[15]

**Hagers' test:** About 2 ml of test solution was treated with few drops of Hagers' reagent (saturated picric acid solution), and was observed for the formation of yellow precipitate for a positive result.[15]

**Mayer's test:** About 2 ml of test solution was treated with few (2 - 3) drops of Mayer's reagent (solution of Potassium mercuric iodide). It was observed for the formation of a white, pale yellow or cream precipitate.[15]

**Wagner's test:** About 2 ml of test solution was placed in a test-tube followed by addition of 2 – 4 drops of Wagner's reagent (Iodine solution). It was observed for formation of a brown or reddish-brown precipitate.[15]

**Detection of phenols**

**Ferric chloride test:** About 2 ml of extract solution was placed in a clean test-tube followed by the addition of 2 – 3 drops of 1% ferric chloride solution. It was observed for the formation of an intense greenish-black colour which indicates presence of phenolic compounds.[16]

**Lead acetate test:** About 2 ml of the extract solution was placed in a clean test-tube, followed by addition of 1 ml 10% solution of lead acetate. Formation of bulky white precipitates indicates presence of polyphenols.[17]

**Detection of flavonoids**

Flavonoids when treated with alkali solutions (e.g. ammonia and/or NaOH solution) they change their colour. Thus, they are easily detected in plant extracts.[16]

**Alkali reagent test:** About 2 ml of diluted extract solution was placed in a test-tube. A few drops of NaOH 1N solution were added drop-wise in the test-tube, and were observed for increasing intensity of the yellow colour produced in the test-tube

(presence of flavonoids is positive). This intensity of the formed yellow or orange colour will decrease by addition of few drops of dilute HCl.[15,18]

**Ammonia test:** A strip of filter paper after being dipped in the diluted solution, and then it was dried and imposed to ammonia vapors. Appearance of an orange-red or yellow colour on the piece of filter paper indicates the presence of flavonoids in the test sample.[15]

**Shinoda/Pew test:** A small amount of magnesium turnings was added into a test-tube containing about 3 ml of the test extract solution. After addition of 3 drops of concentrated HCl in the test-tube, it was kept aside for the completion of the reaction. After a while it was observed for the formation of a reddish-pink or rose colour that indicates the presence of flavonoids in the sample.[15]

**Detection of tannins**

**Gelatin test:** About 2 ml of extract solution was treated with 2 ml aqueous solution of 1% gelatin and 10% sodium chloride. It was observed for the formation of a white buff colour precipitate (a milky colour) for a positive result.[19]

*UV spectrum of extracts and fixed oil*

For determination of sun protective capacity of the extracts, they were dissolved in methanol, and their absorption spectra of methanol solution at final concentration100 µg/ml were taken using UV spectrophotometer (Shimadzu UVmini 1240) in the range of 290 nm to 400 nm.[20] In the case of the oils, they dissolved in hexane and their absorption spectra at final dilution of 1:100 were taken using UV spectrophotometer (Shimadzu UVmini 1240) in the range of 290 nm to 400 nm.[21]

*Preparation of sunscreen Emulgel*

According to the results were obtained from the previous study, sesame oil, *H. rhamnoides* oil (HR Oil) and EAPE, were selected for development of sunscreen formulation. The emulgel system was prepared by adding different concentrations of the EAPE (where x= 2, 4, 6 and 8%) in to the emulgel formulation, as shown in table 1. For preparation of emulgel, in the first step, gel phase was prepared by dissolving the xanthan gum in a portion of purified hot water (80ºC) containing appropriate amount of polyphenol fraction. Then it was left for 1 hr. to form a homogeneous gel.

The oil and aqueous phases were heated separately to about 60ºC, then the aqueous phase was added to the oil phase with continuous stirring. Afterward, gel phase was added to the mixture and the formulation was mixed vigorously to cool the emulgel to room temperature.

*Physicochemical evaluation of developed sunscreen formulation*

Formulations containing different concentration of EAPE were evaluated in term of emulsion type, colour, spreadability on the skin, precipitate, pH and SPF.

**Determination of pH**

To measure the pH of sunscreen formulation, 1 gm of sample was weighed and diluted with distilled water up to 10 ml. After homogenization, the pH of sample was measured using pH meter (HM-25G).[22] pH of formulations containing different concentration of EAPE are shown in table 4.

**Determination of precipitation**

Centrifugation test provides very fast information regarding the physical stability of emulsion based system. To perform this test, 1 gm of samples was weighed and centrifuged for 30 min at 3000 rpm. Then the weight of supernatant (separated phase) was measured.[22]

**In-vitro determination of SPF of developed sunscreen formulations**

The sun protective factor (SPF) is the most commonly used parameter for expression of sun protective capacity of sunscreens.[23] Different in-vivo and in-vitro method is used for determination of SPF of sunscreens formulationi. In present work, the SPF of the formulations containing different concentrations of EAPE were determined using in vitro spectrophotometric method, developed by Mansur *et al*. Ethanolic solutions of the sunscreen formulations at the final concentration of 2µl/ml was prepared. The absorption of samples were taken in the range of 290 – 320 nm, every 5 nm using UV-visible spectrophotometer (Shimadzu UVmini1240). SPF of sunscreen formulations were calculated using Mansur equation. Measurements were performed in triplicate and results were shown as Mean ± SD.[24]

$$SPF = CF \sum_{290}^{320} EE\ (\lambda)\ I\ (\lambda)\ ABS\ (\lambda)$$

Where EE (λ) - erythemal effect spectrum; I (λ) - solar intensity spectrum; ABS (λ) - Absorbance of sunscreen product; CF-correction factor (=10). The values of EE x I are constant, predetermined and presented in table 2.[25]

*Physical Stability evaluation of sunscreen formulation*

Pharmaceutical or cosmetic products should be stable during their shelf life. Since the formulation containing 6% extract, had higher SPF and at the same time didn't produce any colour on skin, it selected for conducting physical stability studies. to evaluate the stability of formulation, they were packaged in glass container and stored for 8 weeks in oven at 40 ± 2ºC and in Refrigerator at 4 ± 2ºC. The pH, SPF, precipitation and organoleptic properties of samples were checked after 7, 14, 21, 28, 56 days of preparation. Each test was done in triplicate and the results were recorded as Mean ± SD, as shown in tables 5 and 6 and figures 3 and 4.

*Statistical Analysis*

All experiments were performed in triplicates and values are expressed as mean ± SD. All statistical analysis was performed using Ms. Excel 2016. One-way analysis of variance (ANOVA) and Student t-test was used to assess the differences between different variables. All analysis was performed at the 5% significant level ($p<0.05$).

## Results and discussion

*Phytochemical screening of the plant extracts*

Table 3, represents the results of preliminary phytochemical screening of tested extracts. As shown in Table 3, alkaloids test result is negative. Based on our findings, flavonoids and phenolic compounds were found to be present in all tested extracts and tannins were not present in purified extracts (EAPE and APPE). It may be due to low solubility of tannins in ethyl acetate.

*Sun protective capacity of extracts and fixed oils*

UV spectra of the EACE, EAPE, APCE and APPE are presented in figure 1. The UV Spectra of all plant extracts indicated that, they have sun protective capacity in both UVA and UVB regions. Nearly for all extracts, the absorption was constant in the range of 290 to 370 nm and it decreased after 370 nm. The order of UV absorption of tested

extracts was highest for EAPE > APPE> EACE > APCE, respectively. On the whole, the purified extracts have higher absorption than crude (methanolic) extracts. The procedure which was used for purification of extracts, already developed by Wolski *et al.* for extraction of polyphenolic fraction.[14,26] In many research works polyphenolic and flavonoid compounds have been reported as sun protective agents.[20,27,28,29] So higher amount of polyphenol and flavonoid compounds as well as elimination of other inert chemicals, can be the reasons for increased UV absorption by methanolic solution of EAPE and APPE. UV spectra profile of oil component including olive oil, sesame oil and HR oil, is presented in figure 2. Sesame oil and olive oil have negligible absorption than HR oil. Olive oil has nearly same absorption profile in both UVA and UVB region, but sesame oil showed more absorption in the range of 290 to 310 nm. So sesame oil can provide better protection in the UVB region, than olive oil. Thus sesame oil was selected for use in the formulation. *HR* oil showed very interesting absorption in both UVA and UVB regions. In this research work the oil of full dried fruit (seed and pulp) were extracted using hexane. This oil showed very higher absorption than the oil which was obtained from HR seeds, by other researchers.[30] The obtained oil from the full dried fruit (seed and pulp) had strong orange colour which limited its use in high concentration in topical formulations.

*Physicochemical properties and SPF of sunscreen formulation*

The results of physicochemical evaluation and SPF of formulations containing different concentration of EAPE are shown in table 4. Incorporation of different percentage of the extract into the base cream, causes some changes in organoleptic properties of the emulgel formulations. The colour of formulations ranged from light yellow for blank to brown for sunscreen formulation containing 8% EAPE (SUNF8%). Following the administration on the skin, with the exception of SUNF8%, none of them produce any colour on the skin. So sunscreen formulation containing 6% EAPE (SUNF6%), was selected for conducting stability studies. Because it possessed higher SPF and meanwhile, didn't make the skin colourful. All formulations showed suitable viscosity and they easily were spread on the skin. Addition of extract into base cream didn't cause any visible change in the apparent viscosity and spreadability of the formulations. But the formulations containing the extracts seemed to be less greasy. pH of formulations were in the range of 8.03 to 6.39. In the sunscreen formulations as the extract concentration increased, the pH value of the formulations decreased. There

was a negative linear correlation ($R^2$=0.993) between the pH and concentration of extract in the sunscreen formulation. Our result is in agreement with other researchers' work.[20,22] The pH value of the skin is in the range of 5 to 5.5.[31] In an ideal situation, especially in the case of topical formulations which are used frequently, the pH of topical formulation should be slightly acidic in the range of 5 to 5.5. But in practice, pH range of 5 – 7 is acceptable for topical formulations.[32,33] So SUNF6% and SUNF8% have the pH in the range 5 -7 which is acceptable for topical formulations. The SPF of formulations were varied from 0.27 ± 0.08 for base emulgel to 21.05 ± 0.85 for formulation containing 8% EAPE, as it is shown in table 4. The SPF of base emulgel was very negligible (0.27±0.08), but addition of extracts into the base cream, caused considerable increase in the SPF value of emulgel formulations. there was a positive linear correlation ($R^2$=0.999) between the SPF and concentration of the extract in the sunscreen formulations.

*Physical stability of sunscreen formulation*

Table 5 and 6 summarize the physical characteristics of the sunscreen formulations, stored in oven at 40ºC and refrigerator at 4ºC respectively. The evaluation of physical stability, included measurements of SPF, pH, Precipitation, occurrence of phase separation and colour change, during the storage period. These characteristics were observed at 40ºC (oven) and 4ºC (refrigerator) for 8 weeks. The centrifugation test provides fast and reliable information regarding the stability properties of the formulations [22].[16] There was no phase separation in the samples during the storage in both conditions. Even after the centrifugation, no phase separation was observed. There were minor changes in SPF values of formulations during storage at 40ºC (oven) and 4ºC (refrigerator). Figure 3 shows the changes in the SPF of sunscreen formulation containing 6% of *EAPE.* We can say that there were not significant differences between the SPF of formulations during 8 weeks and the SPF values were stable. Formulation's pH is important characteristics which should be compatible with other formulation's component and with the application site to avoid irritation. So measuring the pH of the formulation is necessary to ensure that the pH is stable during the storage condition. pH change in the sunscreen formulations containing 6% of

*EAPE* are presented in figure 4. The pH value changes were in the range of 6.86 ± 0.13 to 6.67 ± 0.09 and from 6.86 ± 0.13 to 6.84 ± 0.08 for samples which were stored in oven and refrigerator, respectively. There were not significant differences in the pH of formulations during 8 weeks. It was observed that the pH of formulations was stable for 8 weeks in the two already mentioned storage condition. It was observed that, during the storage time (oven and refrigerator), the organoleptic properties of formulations, were stable. The only change was related to negligible colour change in the sample which was kept in oven. The colour of formulation seemed to be darker. This change was observed after one week.

## Conclusion

In the present era, sunscreens are extensively used to prevent UV-induced skin damages including sunburn, early aging and skin cancers. Recent researches revealed that, most of the synthetic sunscreens are producing unwanted effects either in the short or long term of their application on the skin. So, there is huge demand worldwide for safe and effective UV-filters, particularly of natural origins. Fortunately, natural or herbal sunscreens are preferred because of being enriched with natural and safe compounds as compared with synthetic products. Based on the findings in the current work, the *E. angustifolia* leaves purified extract (EAPE) which is rich in both flavonoids and polyphenols, exhibited high sun protective capacity. In the current work, the topical herbal sunscreen formulation that was developed based on sesame oil, *H. rhamnoides* fruits oil and enriched with 6% EAPE, indicated SPF value of 16.03 and was stable during 8 weeks' storage in refrigerator 4°C and oven 40°C. However, further in vivo studies are highly emphasized to prove further safety and efficacy of our developed sunscreen formulation.

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| Table 1 ingredients included in emulgel formulation | |
|---|---|
| Ingredients | Weight% |
| Phase A (Oil phase) | |
| Sesame oil | 14.5 |
| Hippophae rhamnoides Oil | 0.5 |
| Cetostearyl alcohol | 5 |
| Butylated hydroxy toluene | 0.05 |
| Phase B (Aqueous phase) | |
| Sodium lauryl sulfate | 0.55 |
| Propylene glycol | 5 |
| Methyl paraben | 0.2 |
| Propyl paraben | 0.1 |
| Purified water | qsp |
| Phase C (gel phase) | |
| Xanthan gum | 0.5 |
| EAPE | x |
| Purified water | qsp |

| Table 2 Value of EE×I, used in the calculation of SPF[26] | |
|---|---|
| EE×I | Wavelength (nm) |
| 0.015 | 290 |
| 0.0817 | 295 |
| 0.2874 | 300 |
| 0.3278 | 305 |
| 0.1864 | 310 |
| 0.0839 | 315 |
| 0.018 | 320 |

| Table 3 Results of phytochemical screening of plants extracts | | | | | |
|---|---|---|---|---|---|
| S. No. | Phytochemicals | EACE | EAPE | APCE | APPE |
| 1 | Alkaloid | - | - | - | - |
| 2 | Phenols | | | | |
| | FeCl3 Test | + | + | + | + |
| | Lead acetate test | + | + | + | + |
| 3 | Flavonoids | | | | |
| | Alkali R. Test | + | + | + | + |
| | Ammonia test | + | + | + | + |
| | Shinoda Test | + | + | + | + |
| 4 | Tannin | | | | |
| | Gelatin test | + | - | + | - |

| Table 4 physical characteristics and SPF of emulgel formulation containing different concentration of EAPE | | | | | |
|---|---|---|---|---|---|
| | Blank | SUNF 2% | SUNF 4% | SUNF 6% | SUNF 8% |
| Colour | Light Yellow | Yellow | Yellowish brown | Brown | Brown |
| Emulsion type | O/W | O/W | O/W | O/W | O/W |
| Spreadability on the skin | Suitable | Suitable | Suitable | Suitable | Suitable |
| pH | 8.04±0.16 | 7.75±0.12 | 7.32±0.12 | 6.86±0.09 | 6.39±0.06 |
| Precipitation | – | – | – | – | – |
| SPF | 0.27±0.08 | 6.37±0.14 | 11.59±0.11 | 16.03±0.12 | 21.05±0.85 |

Table 5 SPF and physical characteristics of emulgel formulation during 8 weeks storage in oven (40°C)

| | Oven (40ºC) | | | | |
|---|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week4 | Week 8 |
| SPF | 15.92 ± 0.34 | 15.53 ± 0.48 | 15.60 ± 0.25 | 15.77 ± 0.51 | 15.26 ± 0.84 |
| pH | 6.77 ± 0.13 | 6.78 ± 0.07 | 6.77 ± 0.11 | 6.72 ± 0.03 | 6.67 ± 0.09 |
| Precipitation | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| Phase separation | - | - | - | - | - |
| colour change | SD | SD | SD | SD | SD |
| SD: Slightly darker than the sample at the time of preparation | | | | | |

Table 6 SPF and physical characteristics of emulgel formulation during 8 weeks storage in Refrigerator (4°C)

| | Refrigerator (4°C) | | | | |
|---|---|---|---|---|---|
| | Week 1 | Week 2 | Week 3 | Week4 | Week 8 |
| SPF | 16.17 ± 0.15 | 15.60 ± 0.21 | 15.77 ± 0.26 | 15.95 ± 0.11 | 15.54 ± 0.26 |
| pH | 6.82 ± 0.15 | 6.82 ± 0.2 | 6.81 ± 0.15 | 6.81 ± 0.19 | 6.84 ± 0.08 |
| Precipitation | 0±0 | 0±0 | 0±0 | 0±0 | 0±0 |
| Phase separation | - | - | - | - | - |
| Colour change | - | - | - | - | - |