Simple Synthesis of Poly(Vinyl Alcohol) Cryogel with Rock Salt Modification for Optical Tissue Phantom

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A B S T R A C T

This study demonstrates the feasibility of using rock salt as a modifier to alter the optical reflectance and morphology of poly(vinyl alcohol) cryogel for optical tissue phantoms. The methodology used simplifies the preparation process and shortens the synthesis time. In certain limit, this simple method of rock-salt modification is effective in controlling the optical reflectance and obtaining a smoother structure of poly(vinyl alcohol) cryogel. Profile analysis showed the sensitivity of several wavelength points of poly(vinyl alcohol) cryogel reflectance spectra but with different responses to the various rock-salt concentrations. Poly(vinyl alcohol) cryogel generated by simple rock-salt modification may be useful for mimicry of certain tissues in optical tissue studies.

INTRODUCTION

Poly(vinyl alcohol) (PVA) is a non-toxic, hydrophilic polymer material and has the ability to efficiently absorb water. It is generally utilized in the form of PVA hydrogel (PVA-H), which can be produced by cross-linking the PVA chains. Cross-linking may be achieved by various methods, including chemical, gamma radiation, UV radiation, and freeze-thawing (Stasko et al., 2009; Mansur et al., 2008; Ahmad et al., 2012). Freeze-thawing is an efficient cross-linking technique, and PVA-H obtained by this cryogenic treatment is termed PVA cryogel (PVA-C). The freeze-thawing process consists of several basic steps: freezing a homogeneous PVA solution at low temperatures, maintaining the solution in the frozen condition for a certain time, and finally defrosting the solution (Gutiérrez et al., 2010). The chemical and mechanical properties of PVA-C have been extensively studied (Pazos et al., 2009). Because of its excellent biocompatibility and mechanical properties, PVA-H/PVA-C has found application in various fields, particularly as artificial tissues in the field of biomedicine (Kobayashi and Hya, 2010).

PVA-C has been widely used as a tissue-mimicking phantom in medical imaging, especially in magnetic resonance imaging (MRI) and ultrasound studies (Surry et al., 2004). However, its application in biomedical optics is relatively new and under-explored. PVA has been used as a matrix or basic medium as an optical tissue phantom in several ways. For example, Hebden et al. (2006) used PVA in the slime form (a viscoelastic fluid) for producing highly compressible phantoms for diffuse optical tomography. Bisaillon et al. (2010; 2011) utilized PVA in the cryogel form for producing an artery phantom for optical coherence tomography. The PVA-C form has also been exploited by Kharine et al. (2003) in the development of a breast phantom to study the applicability of photoacoustic mammography. Devi et al. (2005) then adapted the design of tissue phantoms used for photoacoustic imaging to fabricate phantoms for optical elastography.

At present, PVA-C is listed among the choice candidates for use in the development of versatile optical tissue phantoms, along with fibrin and polydimethylsiloxane (PDMS; commonly known as silicone) (Lamouche et al., 2012). Despite its promising features, there are certain limitations to the use of PVA-C in optical applications. For instance, control of the undesired light scattering caused by its apparent whiteness as well as judicious manipulation to achieve a structure that can mimic certain tissues must be realized. One approach for reducing the whiteness is using dimethyl sulfoxide (DMSO) (Pogue and Patterson, 2006), which, unfortunately, is a volatile and toxic solvent (Paţachia et al., 2009). Increasing the number of freeze-thawing cycles has been reported to be effective for changing the structure, opacity, and translucency of PVA-C (Fray et al., 2007; Chen et al., 2012). However, this approach requires an extended period for the synthesis process. Based on the developments in polymer research, Paţachia et al. (2009) showed that the transparency of PVA-C could also be
tailored by adding different types of salts (Na$_2$SO$_4$, NaCl, and NaNO$_3$) to the PVA solution. By this simple approach, the PVA-C properties can be modified, and cryogels with various degrees of crystallinity and porosity can be obtained (Paţachia et al., 2009). In a recent study by Lozinsky et al. (2011), the addition of alkali metal chloride (LiCl, NaCl, KCl, and CsCl) was reported to affect the physiochemical properties of PVA-C and decrease its macropore size.

In the present work, under the overarching aim to evaluate the challenges, limitations, and possible applications of PVA-C obtained via simple salt modification, we extend the use of rock salt as a modifier to alter the PVA-C for optical tissue phantom. In particular, we assess the extent to which the optical reflectance and morphology of PVA-C can be modified using various concentrations of rock salt. Furthermore, the effects of different PVA to water ratios (without rock salt) on the PVA-C characteristics are also analyzed. The evaluation and discussion are specifically limited to the type of PVA used and the method of PVA-C synthesis considered in this study.

**MATERIALS AND METHODS**

**Basic Materials:**

PVA emulsion (E1318, Bostik, Malaysia), double refined rock salt containing 99.3% NaCl (Halogel, Malaysia), and pure water were used in the synthesis of PVA-C. Six cylindrical bottles with height = 5 cm and diameter = 3.1 cm were used as containers.

**Preparation of Rock-Salt Solution:**

Rock-salt solutions with concentrations of 2.5%, 5% and 7.5% were respectively prepared by mixing 1.25, 2.50, and 3.75 g of rock salt with 50 mL pure water.

**Preparation of Poly(Vinyl Alcohol) Solution:**

A 7.5 mL aliquot of PVA emulsion was inserted into each of the six bottles (to achieve a thickness of 1.0 ± 0.1 cm). Subsequently, 7.5 mL of 2.5%, 5%, and 7.5% rock-salt solutions were respectively poured into three of the bottles. The ratio of PVA to rock-salt solution was 1:1. The other three bottles containing 7.5 mL of PVA were respectively filled with 7.5 mL, 15 mL, and 22.5 mL of pure water, giving PVA to water ratios of 1:1, 1:2, and 1:3 respectively. All of the mixed PVA solutions were stirred for ca. 10 minutes and then left for one hour to ensure that the PVA emulsion dissolved completely. Table 1 summarizes the compositions of each of the PVA sample solutions.

**Synthesis of Poly(Vinyl Alcohol) Cryogel:**

All PVA to rock-salt and PVA to water solutions (except one, which was excluded) were frozen at -20 °C for 12 hours and then thawed at room temperature (30 ± 2 °C) for 12 hours. Subsequently, the water in the bottles was removed by slowly compressing the PVA-C obtained until the thickness returned to approximately 1 cm. The morphology of the PVA-C samples were then observed visually using FINEPIX JV250 camera (Fujifilm, Japan).

**Measurement of Optical Reflectance:**

The optical reflectance of the PVA-C samples across the visible wavelength region was measured using a Jaz Spectrometer equipped with an R600-7-SR-125F fiber optics reflection probe, HL 2000 Tungsten Halogen light source, WS-1-SL Labsphere spectralon, and SpectraSuite software (Ocean Optics, USA). The probe consists of a tight bundle of seven optical fibers (six illumination fibers around one read fiber) (Fig. 5.1). The core diameter of each fiber is 600 µm ± 10 µm with a numerical aperture (NA) of 0.22 ± 0.02 (yields an acceptance angle of 24.8° in air). Data sources of channel 0 were selected (200–850 nm) and the acquisition parameters were set as follows: integration time = 4 ms, scans-to-average = 30, and boxcar smoothing = 1.

Prior to the assay of the PVA-C samples, calibration was performed according to the protocol of the SpectraSuite operation manual (SpectraSuite, 2007). This includes the acquisition and storage of standard reference (Spectralon) and dark spectra (with the light path blocked) in the memory. SpectraSuite calculates the
reflectance of a sample as a percentage (% $R_{\lambda}$) relative to the reflectance of a standard reference substance (SpectraSuite, 2007):

$$% R_{\lambda} = \frac{S_{\lambda} - D_{\lambda}}{R_{\lambda} - D_{\lambda}} \times 100\%$$

(1)

where $S_{\lambda}$ = sample intensity at wavelength $\lambda$, $D_{\lambda}$ = dark intensity at wavelength $\lambda$, and $R_{\lambda}$ = reference intensity at wavelength $\lambda$. For the measurements and analysis of the PVA-C samples, the probe was located at 0.5 cm above the sample surface and nine measurement datasets were randomly collected for each sample at different sites. Figure 1 shows the system setup for the measurement of reflectance analysis of the PVA-C samples.

![Fig. 1: System setup for the measurement of reflectance of PVA-C samples.](image)

**RESULTS AND DISCUSSION**

**Poly(Vinyl Alcohol) Cryogel Samples:**

Figure 2 shows the appearance of the PVA solutions prior to initiation of the freezing process and the PVA-C samples obtained after completing the freezing and thawing processes. PVA/PVA-C samples with different rock-salt concentrations are shown in Fig. 2(a), whereas PVA/PVA-C samples with different PVA to water ratios are shown in Fig. 2(b). After the freezing process, the apparent whiteness of the PVA-C samples with different rock-salt concentrations differed based on the amount of rock salt dissolved in the samples. The apparent whiteness of the PVA-C sample with higher rock-salt concentration (rock salt = 5%) was more intense than that of the samples with lower concentrations (rock salt = 2.5%) and without rock salt (rock salt = 0%, PVA to water ratio = 1:1, 1:2, and 1:3). Immediate coagulation occurred during the stirring process for the mixture of PVA emulsion and 7.5% rock salt forming an instant fibrous PVA-H. The irregular structure of this PVA-H caused difficulty in measuring the optical reflectance; thus it was excluded from further measurements.

In the preparation of an optical tissue phantom, the ease of manufacturing and the cost-efficiency of the production process must be taken into consideration (Pogue and Patterson, 2006). The use of PVA in the powder form requires a heating process for dilution, which unavoidably increases the preparation time. In the conventional method, 20% PVA by weight in distilled water is heated to 90 °C for 2 hours with continuous stirring for dissolution (Pogue and Patterson, 2006). In another protocol, 12% by weight PVA aqueous solution is heated in distilled water at 80 °C for 3 hours under magnetic stirring for dissolution (Paţachia et al., 2009). Moreover, increasing the number of freeze-thaw cycles in order to change the structure of PVA-C further extends the synthesis time. The use of up to nine freeze-thaw cycles has been reported in previous study, which required about nine days of processing (Fray et al., 2007).

Taking into account this observation, PVA in the emulsion form was used in this study, which readily dissolved in aqueous medium without any heating process. This type of PVA is commonly utilized as a ready-to-use adhesive for home construction and is relatively inexpensive. A single FTC treatment was utilized in the synthesizing of PVA-C, which took only one day to complete. In lieu of additional FT-cycles, salt modification was then used to change the PVA-C structure. The ability and efficacy of rock salt to modify the structure and optical properties of PVA-C were evaluated. Besides being a non-toxic and inexpensive material, rock-salt use also allows for simpler preparation and modification processes.
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A smoother salt (Lozinsky, 2011). In the latter effect, the PVA solution with a lower freezing temperature produces smaller ice and PVA crystals (↓T (Gutiérrez et al., 2010; Lozinsky et al., 2007). Thus, PVA-C with 0% rock salt and PVA-C with 1:1 PVA to water ratio is refer to the same sample; however, for the purpose of comparison, the morphology and reflectance data for this sample was applied to both parts of the study.

The capacity of rock salt to modify the morphology of PVA-C is clearly evident from Fig. 3(a). Different PVA-C structures were achieved by varying the amount of rock salt added to the PVA solution. The ions present in the sample influence the interaction between PVA and the water molecules and affect cross-linking of the PVA-PVA chains (Pazos et al., 2009). The effect of rock salt on the PVA-C morphology can be explained in terms of two effects during the freezing stage: a) the effect of rock-salt concentration on the temperature of the PVA solution (C → T PVA solution) and b) the effect of the temperature of the PVA solution on the size of the ice and PVA crystals (T PVA solution → S crystal).

In the former effect, the addition of rock salt to the PVA solution decreases the freezing temperature of the solution (T PVA solution) (Lozinsky et al., 2011). Note that based on the phase diagram of the salt-solution, (for phase mass of salt ≤ 23.3%) the higher the concentration of salt added, the lower the freezing temperature of the solution (Clark, 2005). In the latter effect, the PVA solution with a lower freezing temperature produces smaller ice and PVA crystals (T PVA solution → S crystal) (Gutiérrez et al., 2010; Lozinsky et al., 2007). Thus, PVA-C with a finer structure is obtained by increasing the rock-salt concentration.

A rock-salt concentration of 7.5% in the PVA solution was sufficiently high to induce immediate coagulation. At this boundary concentration, rock salt in the PVA solution acted as a chemical cross-linking agent. Other such chemical agents used in PVA-H production include glutaraldehyde, acetaldehyde, and formaldehyde. These species react in the presence of sulphuric acid, acetic acid, or methanol, leading to the formation of the hydrogel through covalent bonding (Fray et al., 2007). Stirring the PVA solution further enhances the intermolecular interaction, which then leads to the immediate formation of a fibrous PVA-H. In contrast, variation of the water ratio did not significantly modify the morphology of PVA-C, as shown in Fig. 3(b). This finding supports the postulate that smoother PVA-C structures are obtained because of the effect of the rock-salt (instead of water) content in the PVA solution.

**Optical Reflectance of Poly(Vinyl Alcohol) Cryogel:**

Figure 4 shows the effect of different concentrations of rock salt on the optical reflectance of PVA-C. In general, it was found that adding rock salt to the PVA solution intensified the optical reflectance of PVA-C across the visible wavelength region [Fig. 4(a)]. This finding is in accordance with the FTIR reflectance spectra of PVA-C with and without salt addition reported in a previous study (Paţachia et al., 2009). Compared to the PVA-C without rock salt (0%), addition of 2.5% rock salt increased the reflectance by as much as 6−17% depending on the wavelength. Raising the rock-salt concentration to 5% increased the reflectance by as much as 21−38% across the various wavelengths.

The increase in the reflectance values was linearly correlated with the concentration of rock salt, i.e., the higher the concentration of rock salt added to the PVA-C, the higher the reflectance values measured. This correlation is shown in Fig. 4(b) for the cases of green (532 nm) and red (633 nm) light. The increasing reflectance values of these PVA-C samples were attributed to the crystallinity and porosity of the respective samples. PVA-C with a higher rock-salt concentration has finer PVA crystals and smaller pores than PVA-C with lower salt concentration, and is expected to reflect more light.

**Morphology of Poly(Vinyl Alcohol) Cryogel:**

Figure 3 exhibits the morphology of the PVA-C samples with different rock-salt concentrations and PVA to water ratios. From visual observation, it was found that PVA-C prepared with 5% rock salt had a smoother structure than PVA-C with 2.5% rock salt and 0% rock salt (PVA-C that contains PVA and water only in a ratio of 1:1) [Fig. 3(a)]. Furthermore, visual observation of the morphology of PVA-C prepared with different PVA to water ratios showed little variation of the structure [Fig. 3(b)]. Note that PVA-C with 0% rock salt and PVA-C with 1:1 PVA to water ratio is refer to the same sample; however, for the purpose of comparison, the morphology and reflectance data for this sample was applied to both parts of the study.

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Fig. 3: Morphology of PVA-C modified using various rock-salt concentrations (a) and PVA to water ratios (b).

Fig. 4: (a) Optical reflectance of PVA-C with 0%, 2.5%, and 5% rock-salt concentrations across visible wavelength region. (b) Correlation between optical reflectance of PVA-C and rock-salt concentration at 532 nm and 633 nm.

The effect of increasing the water ratio on the optical reflectance of PVA-C is shown in Fig. 5. The reflectance values of the PVA-C samples with higher water ratio were higher across the visible wavelength range. The reflectance of PVA-C with a PVA to water ratio of 1:2 was 8–11% higher than that of PVA-C with a PVA to water ratio of 1:1, whereas the reflectance of PVA-C with a PVA to water ratio of 1:3 was 24–26% higher compared to the PVA-C with PVA to water ratio of 1:1 [Fig. 5(a)]. Again, the increase in the reflectance values was also linearly correlated with the water ratio. The higher the ratio of water in the PVA solution, the higher the reflectance values of the respective PVA-C. Figure 5(b) shows the correlation between the reflectance values of PVA-C and the water ratio using green (532 nm) and red (633 nm) light.

Because the structures of the PVA-C samples are quite similar even when different water ratios are used, it is expected that the increase in the reflectance values is due to water molecules trapped inside the PVA-C. Note that during the freezing process of the PVA solution, the crystallization of water and PVA occurs at different freezing points. At zero temperature (0 °C), the water inside the PVA solution is crystallized and then the PVA solute is subsequently crystallized at sub-zero temperatures (< 0 °C) (Gutiérrez et al., 2010). PVA solution with a higher water ratio is expected to produce PVA-C with more ice crystals trapped between the PVA crystals. After thawing, the water molecules remain trapped within the PVA matrix, even though some of the water was...
removed by compressing the PVA-C surface. The trapped water molecules are expected to act as a glossy medium which reflects light entering the PVA-C.

Fig. 5: (a) Optical reflectance of PVA-C with 1:1, 1:2, and 1:3 PVA to water ratios across visible wavelength region. (b) Correlation between optical reflectance of PVA-C and water ratio at 532 nm and 633 nm.

Sensitivity and Behaviour of Poly(Vinyl Alcohol) Cryogel Reflectance Spectra:

Figure 6 shows the normalized reflectance spectra of the PVA-C samples. It gives better resolution than that in Fig. 4(a) and Fig. 5(a) for analysis of spectral profiles. The spectral analysis was performed in order to detect any changes in the profiles caused by the addition of rock salt and water to PVA-C. Changes in the spectral profile may furnish details on the sensitivity and behaviour of that point of wavelengths to the concentration of rock salt and water.

Addition of rock salt changed the spectral profile at several wavelength points. Fig. 6(a) shows the reflectance spectra of PVA-C with and without rock-salt addition. Three main peaks were observed respectively at 493 nm, 514 nm, and 626 nm. For PVA-C sample without rock salt addition (blue line), the peaks at shorter wavelengths (493 nm and 514 nm) were found to be more intense than that at longer wavelength (626 nm). The addition of 2.5% rock salt to the PVA-C changed the spectral profile. The peaks at 493 nm and 514 nm lost intensity, whereas the peak of 626 nm was enhanced (red line). By further increasing the rock salt concentration to 5%, these changes in the spectral profile became more pronounced. In contrast with PVA-C without rock salt, the peaks at shorter wavelengths (493 nm and 514 nm) were less intense than that at longer wavelength (626 nm) (green line).

The use of a PVA to water ratio greater than 1:1 did not produce any significant change in the profiles of the reflectance spectra. As shown in Fig. 6(b), the intensity of the peaks at 493 nm, 514 nm, and 626 nm remained similar for all PVA-C samples with different PVA to water ratios. As was noted above [Fig. 4(a) and 5(a)], both rock salt and water can increase the reflectance values across the wavelengths. However, changes in the spectral profile were brought about only for PVA-C with rock-salt addition, as observed in Fig. 6. This change is attributed to the chemical properties of the rock salt. The analysis demonstrates the sensitivity of the 493 nm, 514 nm, and 626 nm wavelength points to the rock-salt addition. The shorter wavelengths (493 nm and 514 nm), however, exhibit an opposite response to the rock-salt concentration compared to the longer wavelength (626 nm).

Challenges, Limitations, and Applications:

The use of rock salt in modifying the optical reflectance and morphology of PVA-C tissue phantoms is deemed feasible, based on the demonstrated results. However, several challenges were faced in this study, particularly in the analysis of the PVA-C samples using a fiber optics spectrometer. The major challenges relate to the wet condition and uneven surface of PVA-C. It is necessary to store PVA-C samples wet or with water in
a sealed container in order to sustain their durability (Lamouche et al., 2012). However, the wetness of PVA-C samples prevents contact measurement between the fiber optics probe and the sample. Furthermore, the use of rock salt as a modifier for PVA-C tissue phantoms is limited to a certain concentration. The immediate coagulation that occurred for the PVA solution with 7.5% rock salt limits the usable concentration range to that boundary.

The PVA-C/PVA-H obtained through either the freeze-thawing process or immediate coagulation can mimic human tissues under several conditions. For example, the variation in the PVA-C morphology by varying the rock-salt concentration may be exploited in the mimicry of skin tissue for different anatomic sites and ages. The surfaces of skin tissues at different anatomic sites and of different ages are not similar (Gambichler et al., 2006). Moreover, the immediate coagulation of PVA solution is seen useful for producing PVA-H to simulate fibrous tissue. However, a special technique is required to form PVA-H fibers in a highly salty medium. Additionally, the sticky nature of PVA-C/PVA-H may also be utilized to resemble tissue that envelopes bone, especially in osteoporosis studies using optical methods.

All of the prospective applications mentioned above make PVA-C/PVA-H a potential matrix medium for a tissue phantom. Apart from these applications, the possibility of using PVA as a scatterer in the manufacturing of optical tissue phantoms can also be explored. Apart from having the appropriate mechanical properties to be used as a matrix medium, PVA also has internal scattering properties resulting from its apparent whiteness (Pogue and Patterson, 2006). The feasibility and practicality of PVA as a scatterer depends primarily on the combination of materials used in the fabrication of the optical tissue phantom.

![Fig. 6: Profile of PVA-C reflectance spectra showing the sensitivity and behaviour of 493 nm, 514 nm, and 626 nm to the rock-salt concentration (a). PVA-C with different PVA to water ratios, however, did not show any significant change on that wavelength points (b).](image)

**Conclusion:**

By judicious choice of the type of PVA, a method for PVA-C production based on modification of the PVA-C properties by rock-salt addition that could simplify the production process and shorten the fabrication time of optical tissue phantoms was presented herein. The use of rock salt with different concentrations was effective and feasible for achieving control of the optical reflectance of PVA-C and to produce PVA-C tissue phantoms with smoother morphology. At the limiting concentration of rock salt, PVA-H was spontaneously generated. Furthermore, the optical reflectance of the PVA-C tissue phantom could be manipulated by varying the PVA to water ratio with no effect on the PVA-C morphology. The spectral profile of the PVA-C optical reflectance was sensitive to the addition of rock salt with the variation of the reflectance intensity at several wavelength points in response to the rock-salt concentration. Such behaviour did not occur with the variation of the water ratio. Overall, simple rock-salt modification may be effective for altering the properties of PVA-C, which may be useful for the mimicry of certain tissues in optical studies.
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