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Using phenolphthalein as a promising indicator to monitor the vacuum freeze-drying process

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ABSTRACT

To improve the quality of the product, an important subject in vacuum freeze-drying process is to accurately monitor the state of water at its freezing stage and that of the residual water at its drying stage. In this paper, an easy-to-perform method is shown to monitor the state of water during the whole freeze-drying process (i.e. the state of water in the freezing stage and that of the residual water in drying stage) based on colour changes of phenolphthalein. A sample prepared with phenolphthalein was sensitive to structural changes of water hydrogen bonding network and exhibited colour-changing effect. This makes it possible to accurately trace the dynamic freeze-drying process, thus providing a valuable tool for preparing high-quality products.

1. Introduction

Vacuum freeze-drying is a drying process in which the solvent contained inside the products is removed from a frozen solution by sublimation [1–3]. This drying method has been widely applied to diverse fields such as biological products, pharmaceutical and food industries because it can maximally preserve the original properties such as hydration, flavour, activity and the shape wanted.

Products to be dried were frozen first and then dried at a low vacuum. The freezing and drying processes respectively determine the final morphology and quality of the product. Therefore, monitoring the whole freeze-drying process is crucially important. Over the past decades, a number of methods have been employed to monitor the freeze-drying process [3–9], most of which tend to establish a large number of mathematical models and algorithms to estimate the residual water content [2,4–7,10–13]. That means an increasing cost will be needed for measuring the related parameters. Nevertheless, the freeze-drying process remains unable to be precisely monitored, especially the transition stage from free water drying to bound water drying.

In this paper, a promising method is reported to monitor the freeze-drying process based on the colour changes of phenolphthalein. Phenolphthalein is commonly used as an acid–base indicator and generally considered to be ionized in alkaline solution giving red colour [14,15]. Actually, its degree of ionization is not only related to the pH value of solution, but closely interrelated with the structure state of water. For example, the alkaline solution of phenolphthalein is red at room temperature, but the red will fade when the solution is frozen into ice. This is because the degree of ionization of phenolphthalein is much weaker in ice than that in solution. In this paper, phenolphthalein was used in combination with Na_2CO_3 to monitor the whole freezing-dry process, which exhibited a sensitive colour changes at different stages. Na_2CO_3 was chosen to offer an alkaline condition because of its applicable pH value.

2. Materials and methods

Pre-experiment: Phenolphthalein and Na_2CO_3 powders were first placed in a centrifuge tube and dried in an oven at 323 K for over 300 min; then the tube was sealed and taken out, shaken to make the powder fully mixed. Subsequently, the powder was exposed at a high relatively humidity (RH) at room temperature for over 600 min and fully mixed again. Finally, some water was added to the powder until it was dissolved.

During the above-mentioned process, either the powder mixture itself or its solution was examined with ELISA (Enzyme Linked Immunosorbent Assay, PowerWave XS2, BioTek) to record the absorption peaks in the range 400–800 nm.

Preparing and testing the indicator: A total of 0.5 g phenolphthalein and 3 g Na_2CO_3 were added to 100 mL sterile water. Then a

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drop of mixed solution (200 μL) was added onto a layer of ice (3 mm in thickness) which was placed at 193 K for over 30 min. Thereafter the plate was transferred into the freeze-dryer (FD5-10, SIM), and after that, the freezing and drying processes were traced with a camera. Finally, the absorption of dried mixture was measured.

3. Results and discussion

Pre-experiment: The aim of the pre-experiment is to confirm whether phenolphthalein is sensitive to the structural change of residual water. It was found that phenolphthalein–Na_2CO_3 powder seemed colourless after being dried in the oven for over 300 min and showed quite a weak absorption peak at 555 nm (Fig. 1, picture a, curve a). The powder was then exposed to a high humidity condition (RH ~40%), which presented light red and a little stronger absorption peak at 555 nm (Fig. 1 picture b and curve b). This is because every particle surface within the powder absorbed water vapor from ambient atmosphere and covered with a water nanofilm (i.e. bound water). Previous studies have demonstrated that the water nanofilm is ubiquitous [16–18]. This film is considered to be liquid-like (a state between liquid and solid), where molecules can ionize and diffuse in a low degree. Based on this logic, phenolphthalein molecules and inorganic ions (Na^+ and CO_3^{2-}) are able to diffuse into the film, leading to the ionization of phenolphthalein molecules. It is worth mentioning that the red became much deeper and showed much stronger maximum absorption at 555 nm when some water was added to the powder (Fig. 1, picture c and curve c).

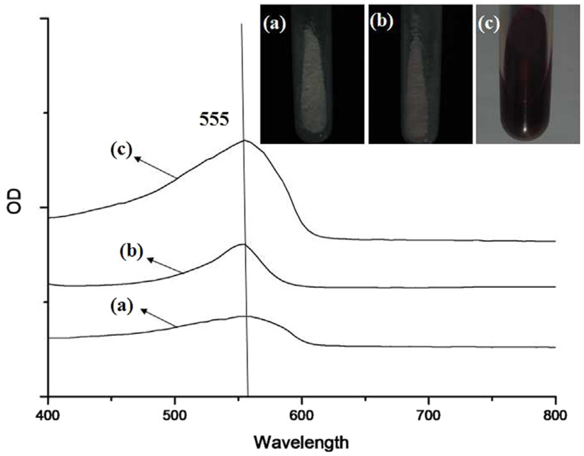

Fig. 1. (a) The mixture of phenolphthalein and Na_2CO_3 (1/100, w/w) dried for 300 min in an oven, then (b) exposed to the 40% RH for 300 min and (c) finally mixed with 2 mL water.

The above-mentioned suggests that the color changes of phenolphthalein is sensitive to different forms of water, i.e. liquid, liquid-like and solid forms of water. Therefore, it is promising to use phenolphthalein as a color indicator to monitor the whole freezing-dry process.

Preparing and testing the indicator: Fig. 2 showed the freezing process monitored by phenolphthalein before drying. About 30 min later, the red completely faded with the phase transition from liquid to ice (Fig. 2, a-f). The phase transition sharply decreased the degree of ionization of phenolphthalein, leading to the disappearance of the red. The color changes can be used to estimate what time it is appropriate to end the freezing process depending on different experimental needs.

It is necessary to point out that the droplet in Fig. 3 was deposited on a thin layer of pure ice (~3–4 mm, marked in Fig. 3). With the sublimation of the pure ice layer, the droplet will form a mushroom shape and fall down when the mushroom stalk is thinner. The formation of mushroom will transform the bottom surface from an “external face” to an “internal face” (Fig. 3, c) and the colour changes of its bottom surface can be seen clearly.

At the beginning of the drying process, the colourless sample gradually turned red (Fig. 4, a–b). This was due to the fact that some residual water was firmly confined to internal surface and difficult to be sublimated. The sample turned red during the sublimation drying process. With further sublimation, the ice layer underneath the droplet became thinner, making the whole sample a mushroom shape (Fig. 4, c). As the mushroom stalk turned thinner, it became unsteady and finally fell down, exposing its “internal face” (Fig. 4, d). It was clear that the “internal face” did not completely turn red, although the whole outside of the droplet did, which explained that the sublimation drying occurred from

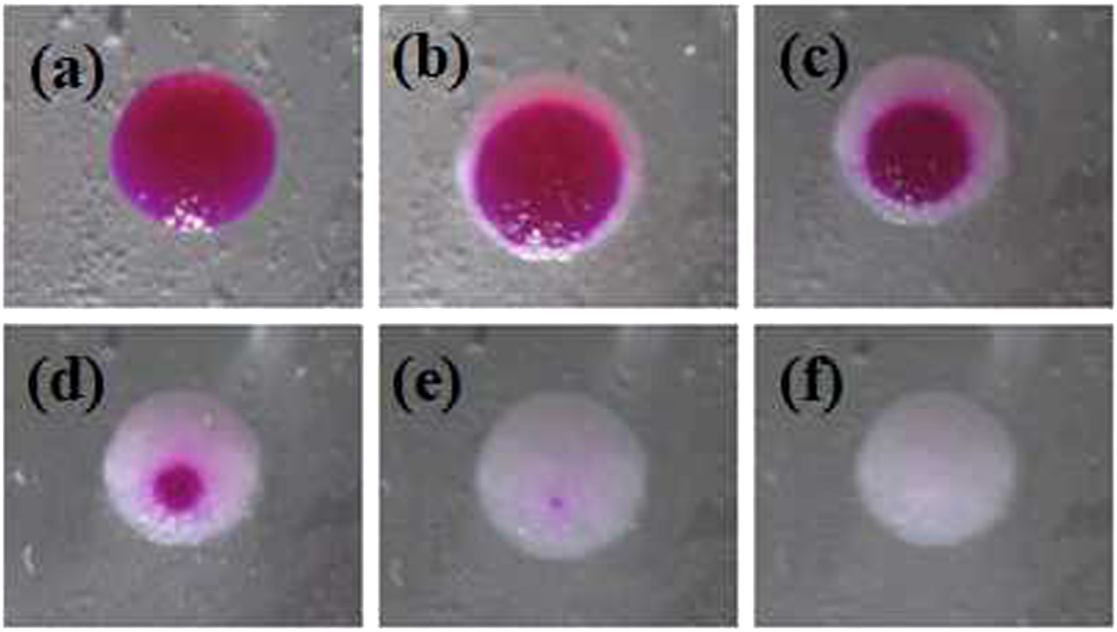

Fig. 2. The sample color changes with freeze time at (a) 0 min, (b) 5 min, (c) 10 min, (d) 20 min (e) 25 min and (f) 30 min.