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Application of Three-Dimensionally Printed Probe and Reservoir to Critical Micelle Concentration Determination by Microvolume Surface Tension Measurement

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ABSTRACT

It is important to determine a critical micelle concentration (CMC) of a surfactant in a protein formulation for stabilizing the protein at maximum by preventing it from interfacial denaturation. There are several techniques for CMC determination. Among them, surface tensiometry is the most common approach because this has a long history and much data at many research fields. However, large amount of sample solution is usually required for the measurement (e.g., more than 1 mL is necessary when a standard reservoir like a glass petri dish is used). This is one of the hurdles for protein formulators because only a small amount of protein could be used at the early-stage development. In this research, we tried to minimize the required amount of sample solution for surface tension measurement by developing appropriate probe and reservoir using a three-dimensional printer (3D printer). The advantages and capabilities of 3D printer are (1) to control the shape and size of the printed material precisely, (2) to change the figure freely, and (3) to prepare the prototype quickly. After the experiments and thereby the refinement of probe as well as reservoir, we found that CMCs of polysorbate 20, polysorbate 80, and poloxamer 188 in water and protein formulations could be precisely detected using a probe 0.5 mm in diameter and small reservoir with a pocket of 7.5 mm in diameter/0.25 mm in depth which were made by a 3D printer. Furthermore, the required sample solution per each measurement could be reduced to 80 μL, which means more than 90% reduction against a standard reservoir.

Introduction

Many injectable products contain surfactants for the purpose of solubilizing active pharmaceutical ingredients and suppressing the absorption of hydrophobic components onto the container surface and so on.[1-6] Polysorbate 20 (PS20) and polysorbate 80 (PS80) are commonly used for protein formulations, and show the significant stabilization of protein to prevent from interfacial denaturation, aggregation, and precipitation.[7] The stabilizing effect is usually increased when the concentration of surfactant gets close to a critical micelle concentration (CMC), which is a value unique to each formulation and is affected by the surrounding circumstance of surfactant.[8,9] This means it is mandatory to confirm a CMC in the course of each protein formulation development, not to check a literature value obtained with an aqueous solution without proteins.

Currently, there are many methods for CMC determination using surface tension, dynamic light scattering, electrical conductivity, and ultrasonic resonance.[9] Among them, the surface tension measurement is the most common approach and has a long history and much data in many research fields.[10-12] For dynamic light scattering and electrical conductivity, it is known that CMC detection in a protein formulation is almost impossible because the signals caught by these methods at micelle formation is so small that it will be hidden behind the background noise from the protein and other excipients in the formulation. Also, the ultrasonic resonance technology is not widely used for CMC determination. Therefore, most of protein formulators use the surface tension measurement for obtaining CMC, but one difficulty is the amount of sample solution required for the measurement, which is normally 1 mL or more. In an early-stage development in pharmaceutical industry, only

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0022-3549/

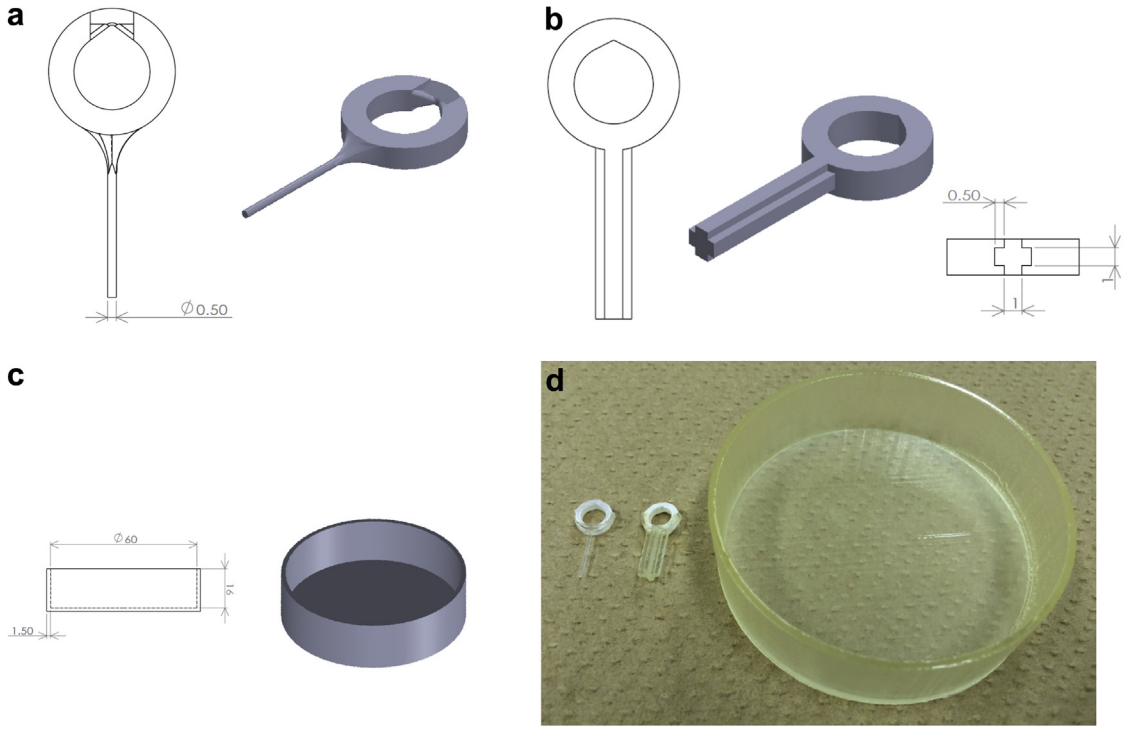

Figure 1. Drawings of (a) 0.5-mm rod-shaped probe, (b) 2.0-mm cross-shaped probe, (c) 60-mm reservoir in 3D CAD software, and (d) actual printed models.

a tiny amount of protein solution is usually generated and a small part of the amount could be used for formulation development. As a microvolume surface tensiometer, Delta 8 is supplied by Kibron (Helsinki, Finland) and requires only 50 μL per measurement. The main purpose of this instrument is to screen the candidates of cosmetics, ink, detergent, and so on. However, the availability of the instrument seems limited in the field of pharmaceutical research, especially at drug product division.

The development of probe and reservoir for a conventional surface tensiometer was conducted in this study with the aim of minimizing the necessary amount of sample solution. Wilhelmy method was applied as a measurement principle because this method can continuously monitor the surface tension for a long time, whereas the other major principle, Du Noüy method, cannot do so.[13] For surface tension measurement of surfactant-containing solution, the continuous monitoring is important because the air:liquid and liquid:solid interface dynamically assemble for seconds or minutes to the most stable state via a random association. Therefore, it is mandatory to wait for the stabilization of interfacial assembly for the precise measurement. In Wilhelmy method, the surface tension is calculated according to Equation 1, where F is the capillary force on the probe and L is the wetted perimeter of the probe:

$$F = L\,\gamma\cos\theta \tag{1}$$

The contact angle θ depends on the wettability of the probe, and the complete wetting ($\theta = 0^\circ$) is normally assumed when it is made of a hydrophilic material like platinum. In that case, the surface tension is simply obtained by Equation 2:

$$\gamma = F/L \tag{2}$$

F is precisely measured by an automatic scale installed in a surface tensiometer. Given that L is also accurately determined despite the scale down of probe size, the surface tension can be appropriately obtained. If this is the case, the reservoir can be also minimized accordingly. This indicates that the accuracy of probe modeling is the most important point for improving the measurement system, and a three-dimensional printer (3D printer) is applied for preparing prototypes of probes as well as reservoirs. In recent years, the 3D printer is innovatively developed and widely used in the fields of hobby, car design, organ models, and space industry, for example.[14-17] There are several techniques for 3D modeling, and here we used a buildup printing method in which an acrylate resin is spotted from a nozzle of printer onto the stage according to the figure drawn by a 3D computer-aided design (3D CAD) software and is almost simultaneously hardened by ultraviolet (UV) irradiation. The advantages and capabilities of 3D printer are summarized below, and the application of 3D printer seems best for the optimization of surface tensiometer in view of these benefits:

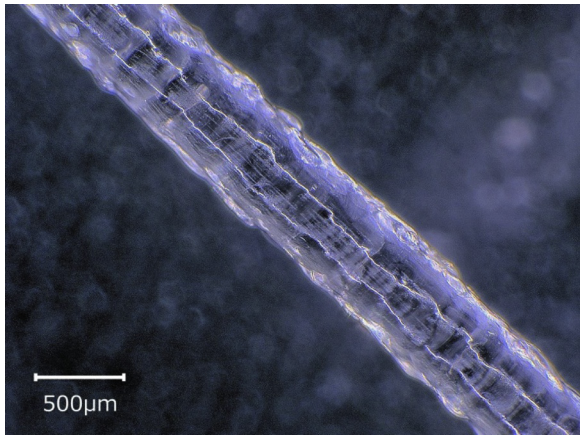

Figure 2. Digital microscope image taken through 100 power lens (rod-shaped probe of 0.5 mm in diameter).