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Development of hydrophobic surface substrates enabling reproducible drop-and-dry spectroscopic measurements

Jinah Lee [a], Pham Khac Duy [a], Seok Chan Park [b], Hoeil Chung [a,*]

[a] Department of Chemistry and Research Institute for Convergence of Basic Sciences, Hanyang University, Seoul 133-791, Republic of Korea
[b] Nanotechnology Innovation Center of Kansas State, Department of Anatomy and Physiology, Kansas State University, Manhattan, Kansas 66506 USA

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ABSTRACT

We investigated several spectroscopic substrates with hydrophobic surfaces that were able to form reproducible droplets of aqueous samples for reliable high throughput drop-and-dry measurements. An amine-coated substrate, a polytetrafluoroethylene (PTFE) disk, and a perfluorooctyltrichlorosilane (FTS) coated substrate were prepared and initially evaluated for use in the determination of fat concentrations in milks using near-infrared (NIR) spectroscopy. Since the dried milk spots were not compositionally uniform due to the localization of components during sample drying, NIR spectra were collected by fully covering each spot to ensure a correct compositional representation of the sample. The amine-coated substrate yielded more reproducible dried milk patterns because its hydrophobicity was optimal for loading an appropriate amount of milk with decreased component localization after drying. The relative standard deviation (RSD) of the absorbance at 4330 cm^{-1} was 1.0%, thereby resulting in the more accurate determination of fat concentration. In addition, infrared (IR) spectroscopic discrimination between wild and transgenic tobaccos using their extracts was attempted. The extracted metabolites had a low concentration, so an FTS-coated CaF_2 substrate that maximized sample loading was used to improve measurement sensitivity and produce reproducible droplets. The RSD of the absorbance at 1070 cm^{-1} was only 0.8%. Our strategy produced droplets that had consistent sizes and provided reproducible IR spectral features, which enabled the differentiation between wild and transgenic tobacco groups in the principal component (PC) score domain.

1. Introduction

Near-infrared (NIR) spectroscopy is a mature analytical method used for fast non-destructive analysis of diverse samples [1–7]. High throughput NIR analysis [8–10], which measures a large number of samples rapidly, has become popular for routine analysis. When a large number of aqueous samples are subject to analysis, one effective method is the so-called drop-and-dry method, which is the rapid, automated spectral acquisition of multiple dried samples formed on a substrate after solvent evaporation [11–13]. Many samples can be quickly measured using this method and, more importantly, the problem of strong water absorption that limits the available spectral window for NIR analysis and eventually degrades the signal-to-noise ratio of spectra can be eliminated.

Reliable drop-and-dry measurements require the generation of reproducible sample shapes after drying [14–18]. If the shapes of the dried samples are inconsistent, the reproducibility of the observed spectral features and the subsequent accuracy of analysis will be degraded. Typically, a 96-well plate holding a sample within a boundary is used with encompassing walls. However, a loaded sample is attracted to the edge of the well due to surface adhesion, so dissolved components tend to be localized along the wall during solvent evaporation. Component aggregation along the wall makes the acquisition of representative spectra difficult.

In this publication, we have investigated several drop-and-dry substrates that can be used to generate consistent droplet shapes without using any physical partitioning walls for NIR and infrared (IR) spectroscopic analysis. When aqueous samples are dropped on a common substrate such as a glass slide possessing a relatively hydrophilic surface, the droplet spreads out due to the sample-surface interaction, so it is difficult to obtain reproducible droplet shapes. One effective alternative is the use of a surface with a polarity opposite to that of the aqueous sample, which serves to confine the sample within a given area with a regular shape. To evaluate this strategy, three different substrates possessing hydrophobic surfaces (a polytetrafluoroethylene (PTFE) disk,

* Corresponding author.
E-mail address: hoeil@hanyang.ac.kr (H. Chung).

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perfluorooctyltrichlorosilane (FTS) coated glass and amine-coated glass) were prepared and used for drop-and-dry NIR measurements of milk to determine fat concentration. Milk samples were dropped on the substrates, and the corresponding contact angles and reproducibility of droplet formation were compared.

Another important issue for reliable drop-and-dry measurements is the acquisition of spectra representative of the whole dried spot. This is important because the distribution of the dried components in a sample is not always uniform due to the dissimilar interactions between the components and the surface during solvent evaporation. A spectrum covering only a part of the dried sample fails to represent the whole composition of the sample. Therefore, NIR spectra were collected by fully covering each dried milk sample with a proper optical arrangement to ensure representative sampling. The measured spectra were used to determine fat concentrations using a partial least squares (PLS) method.

To extend the applicability of the scheme further, IR spectroscopic discrimination of wild and transgenic tobaccos (genetically modified to have a great chloroplast) was attempted as a simple example of metabolic fingerprinting. Metabolic fingerprinting, which is a sub-field of metabolomics, attempts to classify large numbers of samples based on chemical analysis; it can be used to elucidate different metabolic pathways, and so it requires efficient high throughput analytical methods [19,20]. Unlike the measurements on milk, the concentrations of the extracted metabolites from tobacco samples were low, so an FTS-coated CaF_2 substrate possessing a strong hydrophobic surface was used to maximize sample loading for sensitivity and to provide reproducible sample droplets before drying. The distribution of the principal component (PC) scores obtained from IR spectra of the samples dried on the substrate was examined to identify wild and transgenic tobaccos.

2. Experimental section

2.1. Milk and tobacco samples

A total of 90 commercially available milks from 10 different manufactures were purchased at local markets. The milk fat concentration was 0.0%, 2.0% or 4.0%. To prepare samples with different fat concentrations, two samples were mixed to generate concentrations in the 0.0–4.0% range at a concentration interval of approximately 0.09%. A total of 45 milk samples were prepared.

Wild and transgenic tobacco (*Nicotiana tabacum* cv. Xanthi) plants were grown in constant environment conditions at 25 °C on a 16-h photoperiod in a growth chamber (Conviron, Plant Growth Chamber CG108). The transgenic great chloroplast tobaccos were generated by introduction of the *NTFtsZ* gene [21,22]. The relative humidity was not controlled, but was maintained within the range of 60-80%. The transgenic tobaccos showed similar growth rates in moderate light conditions compared to the wild tobaccos; however, their growth was greatly retarded under high or low light intensity. Approximately eight-week-old plant leaves were collected and washed with distilled water. After removal of the remaining moisture with filter paper (3 M), the leaves were immediately frozen with liquid nitrogen and then freeze-dried. After being ground to a fine powder, leaf samples were stored at −70 °C. Finally, 13 wild and 13 transgenic tobacco leaves were prepared. Thirty milligrams of a powder sample was added to 600 mL of an extraction solvent (water (80%):methanol (20%)) in an Eppendorf tube. Then, the tube was sonicated for 90 min at 40 °C and was centrifuged for 5 min at 13,000 rpm. The supernatant solution was used for measurement.

2.2. Preparation of substrates

Amine-coated glass, originally designed for attachment of negatively charged phosphate groups in a DNA backbone for microarray analysis, was purchased from LumiNano (Seongdong-Gu, Seoul, Korea). A polytetrafluoroethylene (PTFE) disk (thickness: 0.5 mm) was purchased at a local store. For the preparation of the FTS-coated glass substrate, a clean glass slide was activated with UV–O_3 to generate hydroxyl groups on the surface for the coating, and the slide was kept in a vacuum chamber under 20 mtorr with FTS overnight at room temperature. An FTS-coated CaF_2 substrate was prepared by the same procedure for the IR spectroscopic measurement of the tobacco extracts. Contact angles of the samples on these substrates were measured using an automatic contact angle measuring system (Easydrop DSA20S, KRÜSS, Germany).

2.3. Acquisition of NIR and IR spectra

To acquire representative sample spectra by fully covering a substrate with a dried sample, the sampling part of a spectrometer (ABB Bomem Inc., Canada) was modified for collimating radiation using a concave lens to form a large illumination area up to 100 mm^2 (10 mm × 10 mm). An Al plate with a hole matching the size of a dried spot was positioned for measurement (128 scans). Dried milk and tobacco extract spots were 10 and 6 mm in diameter, respectively. The resolutions of NIR and IR spectra were 8 and 4 cm^{-1}, respectively.

NIR images of the dried milk were acquired using a Hyperion 3000 FT-UV–vis-IR microscope attached to a Bruker Vertex 80 spectrometer equipped with a MCT detector. The mapping interval was 580 μm in both the *x* and *y* directions (aperture size: 100 μm), and a total of 400 spectra were collected in each mapping. IR line mapping of a dried spot of tobacco extract was performed using an IR microscope (aperture size: 50 μm) equipped with an MCT detector (SensIR Technologies, Danbury, CT, USA). All the spectral pretreatments and multivariate analyses, including baseline corrections, normalizations, PLS and principal component analysis (PCA), were accomplished using Matlab version 7.0 (The MathWorks Inc., MA, USA). NIR images of the dried milks were also constructed using the same software.

3. Results and discussion

3.1. Examination of droplet shapes formed on substrates

The surface hydrophobicity influences the droplet shape and volume formed on a substrate. For an aqueous sample, a more hydrophobic surface results in a droplet that is more condensed at the surface, which could help to provide more consistency in droplet shape. To examine the formation of droplets on a glass slide as well as the tested substrates, contact angles of milk droplets (fat concentration: 4.0%) were measured. The volume of each droplet was 150 μL. The FTS surface produced the highest contact angle of 102.1°, and the contact angle decreased to 84.4° for the PTFE disk. The strong hydrophobicity of these substrates was responsible for the high contact angles. As expected, the amine surface further decreased the contact angle to 50.2°, and the contact angle was smallest on the bare glass surface (17.2°).

When the same sample volume was loaded, the contact area of the droplet decreased with increasing surface hydrophobicity. Once a target spot size was determined, a larger sample volume can be loaded on a more hydrophobic surface, which improves the measurement sensitivity. The size of the droplet contact area for analysis of milks was set to 10 mm in diameter, and the loading volumes of milk required to achieve this diameter were