Supporting Information for:

A novel biosensor based on Blu-ray disc coating film for determination of total amino acid content in tea leaves

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Experimental details on the characterization of Blu-ray disc (BD) surfaces are described.

1. Contact angle measurements of NaOH-treated BD-R surface

Contact angle measurement is a convenient method for the characterization of solid/liquid interfaces.[1] Water contact angles on activated BD-R surface were measured via an AST Optima system with a horizontal light beam illuminating the liquid droplet under ambient conditions (21-27 °C, 45-60 % relative humidity). The contact angles are equilibrated values of sessile liquid drops of deionized water or buffer solution.

The untreated sample is hydrophobic with a water contact angle of 92 ± 2°. During NaOH solution treatment, the surface became more and more hydrophilic (Figure 1 in the main text) with increasing hydrolyzed time. After 45 min, the angle remained constant at 33 ± 2°. The similar change from hydrophobicity to hydrophilicity was observed when BD-R substrates were micro-patterned with filter paper as masks during base solution treatment.

For contact-angle titrations (Figure 3 in the main text), the activated BD-R samples were immersed in buffer solution for 60 s before the measurement. Buffer solutions were prepared according to Creager et al as follows,[2] pH 0-1, perchloric acid; pH 2-3, phosphoric acid/sodium phosphate monobasic; pH 4-5, acetic acid/sodium acetate;

pH 6-8, sodium phosphate monobasic/sodium phosphate dibasic, pH 9-11, sodium bicarbonate/sodium carbonate; pH 12, sodium phosphate dibasic/sodium phosphate tribasic; and pH 13-14, sodium hydroxide. The ionic strength was kept constant (0.01 M), except at very high and low pH. Exact pH values for buffer solutions were obtained with a pH meter (Oakton, Singapore), and recorded before and after the contact angle measurements. Each point represents an average of at least four measurements. The clear transition of contact angle transition from pH 4 to 9 indicates the ionization of surface carboxylic acid groups. The treated surface becomes more hydrophilic: free energy of solid/liquid interface becomes lower and contact angle decreases, as these carboxylic acid groups are transformed to carboxylate groups upon exposure to a basic aqueous buffer solution.

2. Determination of the surface density of -COOH on activated BD film

To determine the surface density of carboxylic acid groups (-COOH) resulting from hydrolyzed treatment, crystal violet, a cationic dye, was utilized. This method relies on the electrostatic interactions between crystal violet molecules and carboxylate groups. First, the treated substrates were immersed in 1 mM crystal violet solution for 5 min. After rinsing with deionized water, the substrates were incubated first with ethanol aqueous solution (80 %, v/v) and second with 0.10 M HCl (in 20% ethanol aqueous solution) until the dye can't be observed on the surface. Then the two incubation solutions were combined and the absorbance were measured with a UV/Vis spectrometer. So, the concentration of crystal violet released was calculated according to Beer's law ($A = \varepsilon c l$) and utilized to determine the surface density of -COOH groups. The value show in the main text ($6.6\pm0.7\times10^{-9}$ mol/cm^2) is an average over six samples.

3. ATR-IR spectrum of the Hard CoatTM film of Verbatim BD-Rs

We further studied the composition of the Hard Coat™ layer of Verbatim BD-Rs using Infrared spectroscopy. The untreated BD film, treated BD film with hydrolysis, and the supernatant from BD film dissolved with 10 M NaOH solution were dertermined. The spectrum shown in Figure 1S was obtained on a Spectrum Two™ FTIR Spectrometer with an Attenuated Total Reflectance (ATR) accessory (PerkinElmer). From Fig. S1, the spectrums of the untreated and treated sample are the same but very different from dissolved sample, which means some chemical groups changes just on the surface of BD film. There are some distinct peaks in the same spectrums at 2926 cm^{-1} (CH_2 stretching), 1725 cm^{-1} (C=O stretching), 1450-1600 cm^{-1} (C═C stretching from aromatic), 1112-1252

cm^{-1} (C-O stretching from esters), 1057 cm^{-1} (C-O stretching from alcohols), and 700-800 cm^{-1} (C-H stretching from aromatic). Also, There are two distinct peaks in the spectrums of dissolved sample at 3347 cm^{-1} (O–H stretching from alcohols) and 1551 cm^{-1} (C=O stretching from carboxylate salts).

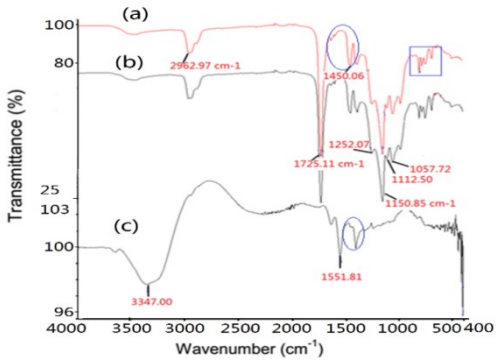

Fig. 1S ATR-IR spectrum of the Hard Coat™ film of Verbatim BD-Rs. (a) untreated sample, (b) treated sample, and (c) dissolved sample

4. ^{13}C-NMR spectrum of hard coating layer of Verbatim BD-Rs

We further studied the composition of BD film using ^{13}C-NMR spectrum as Fig. S2 shown. The peaks at 175 ppm and 43 ppm probably indicate a kind of lactone containing six-membered ring; the peaks at 130 ppm and 136 ppm indicate the group of benzene ring; the peaks at 76 ppm and 65 ppm indicate the group of ester (CH_2OOC or CHOOC); the peaks at 160 ppm indicates the group of esters (COOR) or ketones (COX).

Fig. 2S. ^{13}C-NMR spectrum of the Hard Coat™ film of Verbatim BD-Rs.

5. Possible hydrolysis reactions

In this study, the challenging question is unknown material composition and what exact reactions occurred on the surface upon hydrolysis. Based on above analysis of spectrums and some references, we listed three possible main compositions and hydrolysis reaction. One composition is a (meth)acrylate-based polymer having lactone ring unit ,[3] another polymer Having Glutaric Anhydride Unit and a diluent having two (meth)acryloyl groups within one molecule .[4]

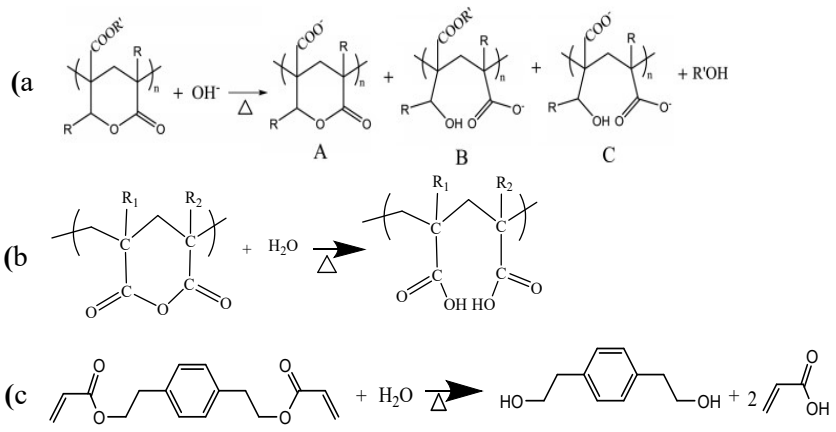

Fig. 3S Possible hydrolysis reactions on the BD-R surface. (a) polymer having lactone ring unit; (b) Polymer Having Glutaric Anhydride Unit; (c) A diluent having two (meth)acryloyl groups within one molecule

Reference

1. Y. C. Ko, B.D. Ratner and A.S. Hoffman, *J. Colloid Interf. Sci.*, 1981. **82**, 25-37.

2. S.E. Creager and J. Clarke, *Langmuir*, 1994. **10**, 3675-3683.

3. S. Weng, X. C. Li, M. Niu, et al., *Anal.Chem.*, 2016, **88**, 6889–6896.

4. S. Kondo and H. Yoneyama, Hardcoat laminate: *U.S. Patent,* 2013, **8**,726.