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The development of a cholesterol biosensor using a liquid crystal/aqueous interface in a SDS-included β-cyclodextrin aqueous solution

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Abstract: Sodium dodecyl sulphate (SDS) including β-cyclodextrin (β-CD) (β-CD_{SDS}) was used to detect cholesterol at the 4-cyano-4'-pentylbiphenyl (5CB)/aqueous interface in transmission electron microscopy (TEM) grid cells. The β-CD acts as a host for SDS (guest). The guest SDS enclosed within the β-CD cavity was replaced with cholesterol by injecting cholesterol solution into the TEM cell at concentrations greater than 3 μM. The replacement of SDS with cholesterol was confirmed by pH measurement and high performance liquid chromatography (HPLC). The SDS excluded from the β-CD altered the planar orientation of the 5CB confined within the TEM grid cell to a homeotropic orientation. This planar-to-homeotropic transition was observed using a polarized optical microscope under crossed polarizers. This convenient TEM grid cell provides a new method for the selective detection of cholesterol without immobilization of the detecting receptors (enzyme, antibody, or aptamer) or the use of sophisticated instruments.

KEYWORDS: Cholesterol, β-cyclodextrin, liquid crystal, biosensor, sodium dodecyl sulphate.

Introduction

Cholesterol analysis has been subjected to considerable scrutiny owing to its importance in clinical diagnosis. Excess cholesterol content in the blood serum results in the development of plaque in the blood vessels, which in turn leads to cardiovascular diseases. Several assays and methods, including chemical, colorimetric, fluorimetric, and polarographic assays, as well as thin layer chromatography, high- performance liquid chromatography (HPLC), gas chromatography, and enzyme-based biosensors have been used to detect cholesterol.[1-4] Among these, enzyme-based biosensors rely on use of cholesterol-selective enzymes, such as cholesterol esterase, cholesterol oxidase, and per oxidase, which are expensive and prone to denaturation. Therefore, alternative, simple, and cost-effective methods for cholesterol detection need to be identified.

Cyclodextrins (CDs) are a series of cyclic oligosaccharides containing 6, 7, or 8 glucose units connected by an α-1, 4 linkage. CDs with 6, 7, and 8 glucose units are called α, β, and γ-CD, respectively. The outer surface of CD is hydrophilic because of the presence of hydroxyl groups; conversely, the inner surface of the molecular cavity is hydrophobic. This structure provides the CDs with a unique ability to form host-guest inclusion complexes with a wide range of suitably sized guest molecules.[5] The guest molecule is held within the CD cavity in these complexes. The complexation largely depends on the dimension of CD and the particular steric arrangement of the functional groups in the guest molecule.[5] The CDs usually favor the unionized guest molecules displaying a higher level of hydrophobicity, compared to the ionized molecules.[5] The host-guest interaction between β-CD and cholesterol has been widely used in the selective extraction of cholesterol from bio-environments, such as food, cell membranes, blood serum, and cultured cell.[6-9]

Nanoparticle-functionalized β-CD have been used in the selective detection and separation of cholesterol.[10-11] Previous studies have suggested that the β-CD can be used as an alternative to enzyme-based biosensors for cholesterol detection. However, the interaction between β-CD and cholesterol does not alter the electrical conductance, pH, or optical appearance. Therefore, this interaction cannot be used to detect cholesterol using electrochemical or potentiometric methods. The fluorescence-based detection of cholesterol has been reported using fluorescence dye-included β-CD hybrid systems. For example, Zhang et al. designed gold nanoparticles functionalized using a fluorescence dye-included β-CD hybrid system. Cholesterol was detected via fluorescence recovery from the released dye, which occurred as a result of the replacement of quenched dyes in the β-CD with cholesterol.[10] Li et al. reported the preparation of superparamagnetic nanoparticles conjugated with β-CD, and their selective binding to cholesterol.[11] Mondal et al. designed a β-CD/graphene hybrid system and incorporated a flourescence dye, rhodamine 6G, for the optical detection of cholesterol; here, the β-CD allowed for selective cholesterol detection, while the graphene transduced the signal emitted by the replacement of rhodamine 6G by cholesterol in the hybrid into an optical signal.[12] This system showed a superior optical response compared to the previously reported nanoparticles/β-CD hybrid systems.[10-11] However, the difficulties in synthesis of nanoparticles and graphene, as well as the use of fluorescent materials, result in the increase in complexity and cost of these methods.

A diverse range of dynamic and equilibrium processes can occur when molecular self-assembly and specific bimolecular recognition events take place at the interfaces formed between nematic liquid crystals (LCs) and immiscible aqueous phases. The orientation of LCs is extraordinarily sensitive to the change in the adjacent (contact) interface. The optical

amplification of LCs make them a unique optical probe for sensing chemical reactions, such as enzymatic reactions,[15–16] ligand-receptor binding,[13],[14-17] and peptide-lipid[18] interactions at the LC/aqueous interface. In this study, a transmission electron microscopy (TEM) grid on an octadecyltrichlorosilane (OTS)-coated glass filled with 4-cyano-4´-pentylbiphenyl (5CB) (a nematic liquid crystal at room temperature) was used to detect cholesterol via host (β-CD)/guest (cholesterol) interactions. Sodium dodecyl sulphate (SDS)-included β-CD (β-CD_{SDS}) was initially introduced to the 5CB/aqueous interface. The SDS present in the TEM grid cell containing β-CD_{SDS} was replaced with cholesterol upon injection of a cholesterol solution. The planar-to-homeotropic (P-H) change in orientation of 5CB due to adsorption of the excluded SDS at the interface was observed through a polarized optical microscope (POM) under crossed polarizers. This cholesterol detection system was observed to be simple, cost-effective, and highly stable. Therefore, it could be applied as a new alternative enzyme-based cholesterol biosensor.

Experimental procedures

Materials: Microscopic glass slides (Duran group, Wertheim, Germany) were cleaned using a piranha solution in order to remove organic contaminants (Caution: Piranha solution is extremely corrosive, necessitating proper care). These were subsequently washed with distilled water, dried under nitrogen, and coated with OTS (Sigma-Aldrich, St. Louis, MO) which aligns the 5CB mesogens perpendicular to the glass surface. Copper TEM specimen grids (G75, grid hole width 285 µm, pitch 340 µm, bar width 55 µm, diameter 3.05 mm, and thickness 18 µm) were purchased from Ted Pella Inc. (Redding, CA). β-CD (Sigma-Aldrich), cholesterol (Sigma-Aldrich), 5CB (TCI Chemicals, Tokyo, Japan), SDS (Sigma-Aldrich), β-D-glucose (Sigma-Aldrich), hemoglobin (Sigma-Aldrich), ascorbic acid (Sigma-Aldrich), and NaCl (Sigma-Aldrich) were used as received, unless otherwise specified.

Preparation of SDS included β-CD: SDS inclusion in β-CD was prepared as per a previously reported method.[19] Briefly, β-CD (1 mM) was dissolved in distilled water and stirred for an hour. SDS powder was added to the β-CD aqueous solution at room temperature. The temperature was raised to 60°C in order to increase the solubility of β-CD and SDS; the mixture was continuously stirred for 3 hours. The mixture was then cooled to room temperature and stirred for another 24 hours. In order to determine an optimum concentration for SDS inclusion in β-CD, the aqueous SDS concentrations (C_{SDS}) 10, 50, 100, 170, 250, 300, 340, 680, 1020, 1360, 1700, and 3500 µM were tested with a fixed β-CD concentration of 1mM.

Fabrication of TEM grid cells for cholesterol detection: In order to fabricate a TEM grid cell, a cleaned TEM grid was placed on a small OTS-coated glass (12 mm × 8 mm) glued to another microscopic glass slide, which was walled with silicon sheath (in order to fabricate a chamber

that can hold aqueous solutions). The pores of the TEM grid were filled with 1 µL 5CB, and the excess LC was removed using a capillary tube in order to obtain a uniform 5CB film. Aqueous β-CD_{SDS} (0.5 mL) solution was poured into the TEM grid cell; this was followed by the introduction of a 0.5 mL cholesterol aqueous solution. The cholesterol concentrations (C_0s) 1.3, 3, 5, 13, 26, 52, and 130 µM were tested.

Analysis of cholesterol in the blood-stimulated yeast samples: In order to test the capacity of the TEM grid sensor for cholesterol detection, a complex mixture of yeast extract (YE) (1 mg/mL), hemoglobin (5 mM), ascorbic acid (AA; 0.1 mM), glucose (2 mM), and cholesterol (3, 26, and 52 µM) was prepared. YE denote the processed yeast products, and is acquired by extracting the body cell contents. It is used as a food additive and nutrient, and is comprised of saturated and unsaturated fat, sodium, potassium, magnesium, chromium, carbohydrates (sugar), proteins, and the vitamin B-complex.[20] It can also mimic the contents of a real blood sample, when supplemented with hemoglobin, AA, glucose, and cholesterol.

Instrumentation: The inclusion was characterized using a pH meter (YK-2001 pH, Japan) and by high performance liquid chromatography (HPLC). The HPLC instrument (Model 600E, Waters, Co., Milford, MA) was equipped with a Sugar-Pak I column (6.5 × 300 mm; Waters, Co.) and a refractive index detector (RI, Model 410). Ca-EDTA buffer (50 mg Ca-EDTA/1L dH_2O) was used in the column as the mobile phase with a flow rate of 0.5 mL/min at 90°C. The optical images were captured using a CCD camera (STC-TC83USB, Samwon, Korea) connected to a polarizing optical microscope (POM; ANA-006**,** Leica, Germany). The gray-scale intensities (GIs) of the frames were calculated using Adobe Photoshop CS5 software.

Results and Discussion

Preparation of SDS-included β-CD: In order to determine the optimum concentration of SDS for complete inclusion within β-CD (without any free SDS in water), the pH of the pure SDS and β-CD_{SDS} aqueous solutions was measured at different concentrations. The optimum concentration must be accurately determined as the orientation of the TEM grid cell must be altered by exclusion of SDS via cholesterol inclusion (not by free SDS). Figure 1 shows the pH change in pure SDS and β-CD_{SDS} aqueous solutions as a function of C_{SDS}. The β-CD_{SDS} aqueous solutions were prepared with a fixed β-CD concentration of 1mM. The pH of the SDS aqueous solution linearly increases with the logarithmic increase in C_{SDS} however the pH of the β-CD_{SDS} aqueous solution is maintained at 7 until the C_{SDS} reaches 340 µM; after this level, the pH of β-CD_{SDS} increases with further increase in C_{SDS} as shown in Figure 1. The pure β-CD aqueous solution (1 mM) is neutral at pH = 7 and the SDS aqueous solutions are weak base. The SDS in the β-CD aqueous solution can be included into the cavity of the β-CD through the hydrophobic interactions between its hydrophobic alkyl tail and β-CD. The included SDS at low C_{SDS} (up to 340 µM) did not affect the pH of the β-CD_{SDS} aqueous solution. The increase in pH at C_{SDS} > 340 µM indicates that all SDS cannot be included in the β-CD cavities and some quantities of free SDS (SDS_{free}) remains behind in the β-CD_{SDS} aqueous solution. This SDS_{free} causes an increase in the pH of the aqueous solution. The strength with which the SDS included within the cavity binds to β-CD is not as stable as other hydrophobic materials, such as cholesterol, tween 20, methylene blue, etc.[21] For example, a previous study investigated the effect of different surfactants on rhodamine 6G dye-included β-CD in the β-CD/grapheme hybrid system by measuring the resultant fluorescence intensities.[12] The introduction of SDS (anionic surfactant) into the system provided a lower fluorescence intensity compared to tween 20 (non-

ionic surfactant).[12] This suggested that the hydrophobic cavity of β-CD cannot accommodate higher quantities of SDS than tween 20. Although the pH measurement of the $\beta\text{-CD}_{SDS}$ solution provided us with some information on a possible optimal SDS concentration during preparation of the cholesterol probe in the $\beta\text{-CD}_{SDS}$ solution, the exact quantities of SDS to be included in the $\beta\text{-CD}_{SDS}$ and the SDS_{free} solutions must be analyzed using HPLC.

Figure 2a shows the HPLC chromatograms obtained for SDS (340 µM), β-CD (1 mM), $\beta\text{-CD}_{SDS}$ (C_{SDS} = 340 µM), and $\beta\text{-CD}_{SDS}$ (C_{SDS} = 1020 µM). The SDS and β-CD chromatograms exhibit characteristic peaks at a retention time (RT) of 5.2 and 10.01 min, respectively (Figures 2a(i) and 2a(ii)). The $\beta\text{-CD}_{SDS}$ chromatogram at C_{SDS} = 340 µM shows a characteristic peak at RT = 9.82 min, which shows a slight shift from the peak displayed by β-CD (at 10.01 min) (Figure 2a(iii)). The peak at RT = 5.2 min was almost invisible, indicating the absence of free SDS. This also indicated that nearly all SDS was included within β-CD at C_{SDS} = 340 µM, which was consistent with the pH measurements. However, the $\beta\text{-CD}_{SDS}$ chromatogram at C_{SDS} = 1020 µM shows a peak for free SDS at RT = 5.2 min, as well as a characteristic peak for β-CD at RT= 9.82 min, indicating that the $\beta\text{-CD}_{SDS}$ aqueous solutions prepared with C_{SDS} > 340 µM may possess free SDS. Figure SI 1 demonstrates the calibration plot of the peak area at RT = 5.2 min vs. C_{SDS} obtained from the chromatograms of the pure SDS aqueous solutions in the C_{SDS} range = 3.5 to 340 µM. A linear relationship between the two sets of values with an r^2 value of 0.999. Figure 2b shows the concentration of SDS_{free} ($C_{freeSDS}$) as a function of C_{SDS}. The $C_{freeSDS}$ was calculated from the calibration curve of Figure SI 1. The $C_{freeSDS}$ was observed to be ~ 0 until C_{SDS} = 340 µM; subsequently $C_{freeSDS}$ increased corresponding to the increase in C_{SDS}. Therefore, the C_{SDS} for maximum SDS inclusion in β-CD without any resultant free SDS was determined to be ~ 340 µM, which was consistent with the results of pH measurement. Therefore, the $\beta\text{-CD}_{SDS}$

aqueous solution without free SDS in water was prepared at C_{SDS} = 340 μM; this concentration was used for cholesterol detection.

Preparation of the cholesterol sensor: Figure 3a shows the POM images (under crossed polarizers) of the TEM grid cells in aqueous SDS solutions (varying C_{SDS}). This figure shows the planar orientation at C_{SDS} =0 (without SDS in water) (Figure 3a(i)), which was consistent with the previous reported data.[22-23] The planar orientation observed at C_{SDS} = 68 and 100 μM (Figures 3a(ii) and 3a(iii)) was altered to slightly and completely homeotropic at C_{SDS} = 136 (Figure 2a(iv)) and ≥ 170 μM (Figure 3a(v)), respectively. It has been established that the adsorption of a surfactant (SDS) at the LC/aqueous interface often leads to a P-H transition in the orientation of the LC, as a result of the infiltration of long alkyl tail of SDS into LC when the density of the surfactant exceeds a critical value.[22] Therefore, the critical concentration resulting in homeotropic orientation in the TEM grid cell ($SDS_{crtical}$) was determined to be ~ 150 μM. Figure 3b shows the POM images (under crossed polarizers) of the TEM grid cells in aqueous β-CD_{SDS} solutions (varying C_{SDS}). β-CD_{SDS} displayed planar orientation at C_{SDS} = 170, 340, and 680 μM; this was altered to a slight or complete homeotropic orientation at C_{SDS} = 1020 and 1360 μM, respectively. The concentrations of free SDS were determined to be 2.5, 3.1, 40, 92, and 295 μM in the β-CD_{SDS} aqueous solutions prepared at C_{SDS} = 170, 340, 680, 1020, and 1360 μM, respectively (data from Figure 2b). The aqueous solutions prepared at C_{SDS} = 170, 340, and 680 μM were far below the $SDS_{critical}$ (150 μM); on the other hand, the aqueous solutions prepared at C_{SDS} =1020 μM and 1360 μM were close to and well above $SDS_{critical}$. Therefore, the results observed in Figure 3b could be explained based on the varying concentrations of SDS_{free}. These results were consistent with the data obtained from pH measurement and HPLC analyses.

Therefore, the reference TEM grid cell for cholesterol detection was prepared with β-CD_{SDS} (C_{SDS} = 340 μM), which induces a planar orientation with maximum SDS inclusion in β-CD, with almost no free SDS in water.

Cholesterol detection: Figure 4a shows the POM images (under crossed polarizers) of the TEM grid cells in the aqueous cholesterol solutions (different concentrations; C_0s) without β-CD_{SDS}. All images showed a bright coloring, indicating that the cholesterol itself did not affect the reference planar orientation caused by in-plane birefringence, associated with parallel anchoring of LC along the LC/aqueous interface. Figure 4b shows the POM images (under crossed polarizers) of the TEM grid cells in aqueous β-CD_{SDS} solutions prepared at C_{SDS} = 340 μM ($TEM_{β\text{-}CD/SDS}$ grid cells), after injecting the aqueous cholesterol solutions at different cholesterol concentrations (C_0s). The planar orientation of the 5CB was maintained at C_0 = 1.3 μM (Figure 4b (i)). A slight change in the orientation of 5CB was observed at C_0 = 3 μM (Figure 4b (ii)). Few dark patches appeared at C_0 = 13 μM (Figure 4b (iii)); these patches become more visible at C_0 = 26 and 52 μM (Figures 4b (iv) and (v)), and become completely black upon adoption of a homeotropic orientation at C_0 = 130 μM. From these results, we theorized that the appearance of a homeotropic orientation in β-CD_{SDS} was due to the replacement of cholesterol with SDS. The excluded SDS can be adsorbed on to the 5CB; this adsorption induces the homeotropic orientation. The detailed mechanism of cholesterol detection with the $TEM_{β\text{-}CD/SDS}$ grid cell is shown in Scheme 1. The $TEM_{β\text{-}CD/SDS}$ grid cells detect cholesterol at concentrations as low as 3 μM, a range lower than that detected by many reported enzyme-based cholesterol biosensors. For example, Tan *et al.* reported a detection limit of 240 μM for ChO immobilized on a prussian blue layer of the sol-gel chitosan/silica and MWCNTs.[24] Li *et al.* used modified screen-printed carbon electrodes with immobilized ChO, cholesterol esterase, peroxidase, and potassium

ferrocyanide, and reported a detection limit of 400 µM.[25] Vidal et al. reported a detection limit of 8 µM using a modified platinum (Pt) electrode deposited with a layer of Prussian blue, followed by entrapment of ChO within the electropolymerized polypyrrole layer.[26] However, this latter method requires the application of sophisticated methods, such as the modification of electrode, electropolymerization, and entrapment of ChO. On the contrary, this sensitive $TEM_{\beta\text{-}CD/SDS}$ grid cell, which can be applied as a biosensor for cholesterol detection, has many advantages such as convenient and cost-effective fabrication, and easy detection.

Figure 4c shows the GI of the $TEM_{\beta\text{-}CD/SDS}$ grid cells as a function of C_0; the error bar represents the standard deviation for triple measurements. The linear relationship was found until C_0=52 µM. At $C_0 \geq 52$, the GI values were saturated due to the complete dark homeotropic image. Thus, the dynamic range for cholesterol detection through the GI value is below 52 µM.

Practical application: Several biomaterials are present in human blood samples and the $TEM_{\beta\text{-}CD/SDS}$ grid sensor can be used to interfere with these biomolecules. Figure 5a shows the POM images of $TEM_{\beta\text{-}CD/SDS}$ grid cells in 1 mM aqueous solutions of NaCl, glucose, ascorbic acid, and hemoglobin. No P-H orientational change was observed in the NaCl, glucose, ascorbic acid, and hemoglobin aqueous solutions (Figures 5a(i) to (iv)). Therefore, the $TEM_{\beta\text{-}CD/SDS}$ grid cell detects cholesterol in blood samples without interference from the other components present in the blood.

The YE/cholesterol mixture aqueous solution samples containing nearly all components of human blood were tested using the $TEM_{\beta\text{-}CD/SDS}$. Figure 5b shows the POM images (under crossed polarizers) of the $TEM_{\beta\text{-}CD/SDS}$ in the aqueous solutions of the YE (1 mg/mL /cholesterol mixture, at different cholesterol concentrations. The initial planar orientation was maintained

when a pure aqueous YE solution was injected (Figure 5b(i)). Slight homeotropic image appeared when the YE/cholesterol (3 μM) mixture solution were introduced (Figures 5b(ii)).A few dark patches appeared upon introduction of YE/cholesterol (26μM) mixture solution (Figures 5b(iii)). The homeotropic orientation becomes more visible under high cholesterol concentrations in the YE/cholesterol (52 μM) mixture solution (Figure 5b(iv)). These results indicated that the cholesterol content in the YE/cholesterol mixture can be detected at a detection limit of ~ 3 μM. Therefore, the $TEM_{\beta\text{-}CD/SDS}$ provides sufficient specificity for cholesterol detection, without interference from the other components in blood.

Conclusion: A cholesterol biosensor was fabricated using a TEM grid cell containing a SDS-included β-CD aqueous solution ($TEM_{\beta\text{-}CD/SDS}$ grid cell). The maximum concentration of SDS for complete inclusion of SDS within $\beta\text{-}CD_{SDS}$ without any free SDS in the solution was determined at 340 μM; this was confirmed by pH measurement and HPLC analysis. The $TEM_{\beta\text{-}CD/SDS}$ grid cell detected cholesterol through changes in the optical appearance using a POM (at a detection limit of (3 μM) without considerable interference from the major components of blood, such as NaCl, glucose, ascorbic acid, bovine serum albumin, and hemoglobin. This novel, simple, and convenient TEM grid cell can be used as a selective biosensor for cholesterol detection in human blood samples without immobilization of enzyme or antibody during biosensor fabrication.

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References

[1] M.K. Ram, P. Bertoncello, H. Ding, S. Paddeu, C. Nicolini, Cholesterol biosensors prepared by layer-by-layer technique, Biosens. Bioelectron., 16 (2001) 849-856.
[2] J. Wang, Carbon-Nanotube Based Electrochemical Biosensors: A Review, Electroanalysis, 17 (2005) 7-14.
[3] M. Gerard, A. Chaubey, B.D. Malhotra, Application of conducting polymers to biosensors, Biosens. Bioelectron., 17 (2002) 345-359.
[4] J. Wang, Amperometric biosensors for clinical and therapeutic drug monitoring: a review, J. Pharm. Biomed. Anal., 19 (1999) 47-53.
[5] R. Singh, N. Bharti, J. Madan, S.N. Hiremath, Characterization of Cyclodextrin Inclusion Complexes – A Review J. Pharm. Sci. Tech., 2 (2010) 171-183.
[6] P.G. Yancey, W.V. Rodrigueza, E.P.C. Kilsdonk, G.W. Stoudt, W.J. Johnson, M.C. Phillips, G.H. Rothblat, Cellular Cholesterol Efflux Mediated by Cyclodextrins: Demonstration of Kinetic Pools and Mechanism of Efflux, J. Biolog. Chem., 271 (1996) 16026-16034.
[7] A.I. Rosenbaum, G. Zhang, J.D. Warren, F.R. Maxfield, Endocytosis of beta-cyclodextrins is responsible for cholesterol reduction in Niemann-Pick type C mutant cells, Proc. Natl. Acad. Sci., 107 (2010) 5477-5482.
[8] H.S. Kwak, C.S. Jung, S.Y. Shim, J. Ahn, Removal of Cholesterol from Cheddar Cheese by β-Cyclodextrin, J. Agric. Food Chem., 50 (2002) 7293-7298.
[9] C.A. López, A.H. de Vries, S.J. Marrink, Molecular Mechanism of Cyclodextrin Mediated Cholesterol Extraction, PLoS Comput Biol., 7 (2011) e1002020.
[10] N. Zhang, Y. Liu, L. Tong, K. Xu, L. Zhuo, B. Tang, A novel assembly of Au NPs-[small beta]-CDs-FL for the fluorescent probing of cholesterol and its application in blood serum, Analyst, 133 (2008) 1176-1181.
[11] H. Li, M.H. El-Dakdouki, D.C. Zhu, G.S. Abela, X. Huang, Synthesis of [small beta]-cyclodextrin conjugated superparamagnetic iron oxide nanoparticles for selective binding and detection of cholesterol crystals, Chem. Comm., 48 (2012) 3385-3387.
[12] A. Mondal, N.R. Jana, Fluorescent detection of cholesterol using [small beta]-cyclodextrin functionalized graphene, Chem. Comm., 48 (2012) 7316-7318.
[13] V.K. Gupta, J.J. Skaife, T.B. Dubrovsky, N.L. Abbott, Optical Amplification of Ligand-Receptor Binding Using Liquid Crystals, Science, 279 (1998) 2077-2080.
[14] M. Khan, S.-Y. Park, Liquid Crystal-Based Proton Sensitive Glucose Biosensor, Anal. Chem., 86 (2013) 1493-1501.
[15] M. Khan, Y. Kim, J.H. Lee, I.-K. Kang, S.-Y. Park, Real-time liquid crystal-based biosensor for urea detection, Anal. Methods, 6 (2014) 5753-5759.
[16] M. Khan, S.-Y. Park, General Liquid-crystal droplets produced by microfluidics for urea detection, Sens. Actuators, B, 202 (2014) 516-522.
[17] J. Kim, M. Khan, S.-Y. Park, Glucose Sensor using Liquid-Crystal Droplets Made by Microfluidics, ACS Appl. Mater. Inter., 5 (2013) 13135-13139.
[18] J.M. Brake, M.K. Daschner, Y.Y. Luk, N.L. Abbott, Biomolecular Interactions at Phospholipid-Decorated Surfaces of Liquid Crystals, Science, 302 (2003) 2094-2097.
[19] N. Karuppiah, P.B. Kaufman, S.A. Kapustka, A. Sharma, Use of Cyclodextrin-Cholesterol Complex as a Primary Standard in Cholesterol Analysis, Microchem. J., 47 (1993) 325-329.
[20] R. Anderson, Elevated intake of supplemental chromium improved glucose and insulin variables in individuals with type 2 diabetes, Diabetes, 46 (1997) 1786-1791.

[21] A.J.M. Valente, O. Söderman, The formation of host–guest complexes between surfactants and cyclodextrins, Adv. Colloid Interface Sci., 205 (2014) 156-176.
[22] J.M. Brake, A.D. Mezera, N.L. Abbott, Effect of Surfactant Structure on the Orientation of Liquid Crystals at Aqueous-Liquid Crystal Interfaces, Langmuir, 19 (2003) 6436-6442.
[23] N.A. Lockwood, J.J. de Pablo, N.L. Abbott, Influence of Surfactant Tail Branching and Organization on the Orientation of Liquid Crystals at Aqueous−Liquid Crystal Interfaces, Langmuir, 21 (2005) 6805-6814.
[24] X. Tan, M. Li, P. Cai, L. Luo, X. Zou, An amperometric cholesterol biosensor based on multiwalled carbon nanotubes and organically modified sol-gel/chitosan hybrid composite film, Anal. Biochem., 337 (2005) 111-120.
[25] G. Li, J.M. Liao, G.Q. Hu, N.Z. Ma, P.J. Wu, Study of carbon nanotube modified biosensor for monitoring total cholesterol in blood, Biosens. Bioelectron., 20 (2005) 2140-2144.
[26] J.-C. Vidal, J. Espuelas, E. Garcia-Ruiz, J.-R. Castillo, Amperometric cholesterol biosensors based on the electropolymerization of pyrrole and the electrocatalytic effect of Prussian-Blue layers helped with self-assembled monolayers, Talanta, 64 (2004) 655-664.

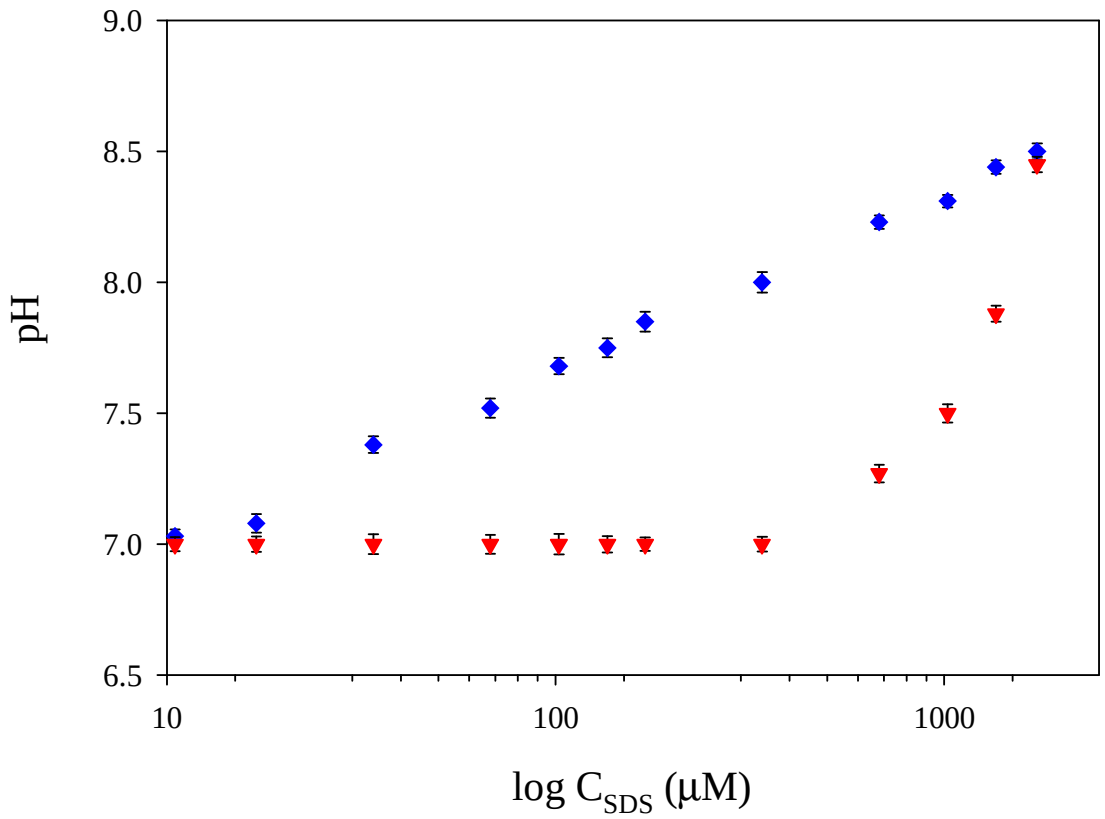
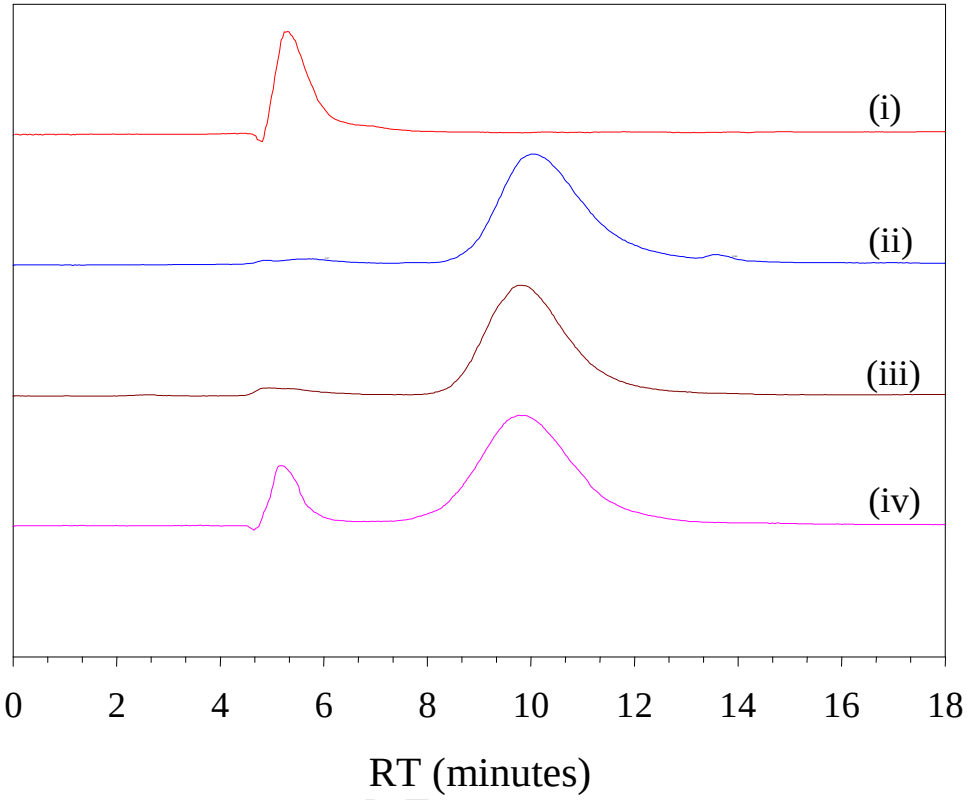
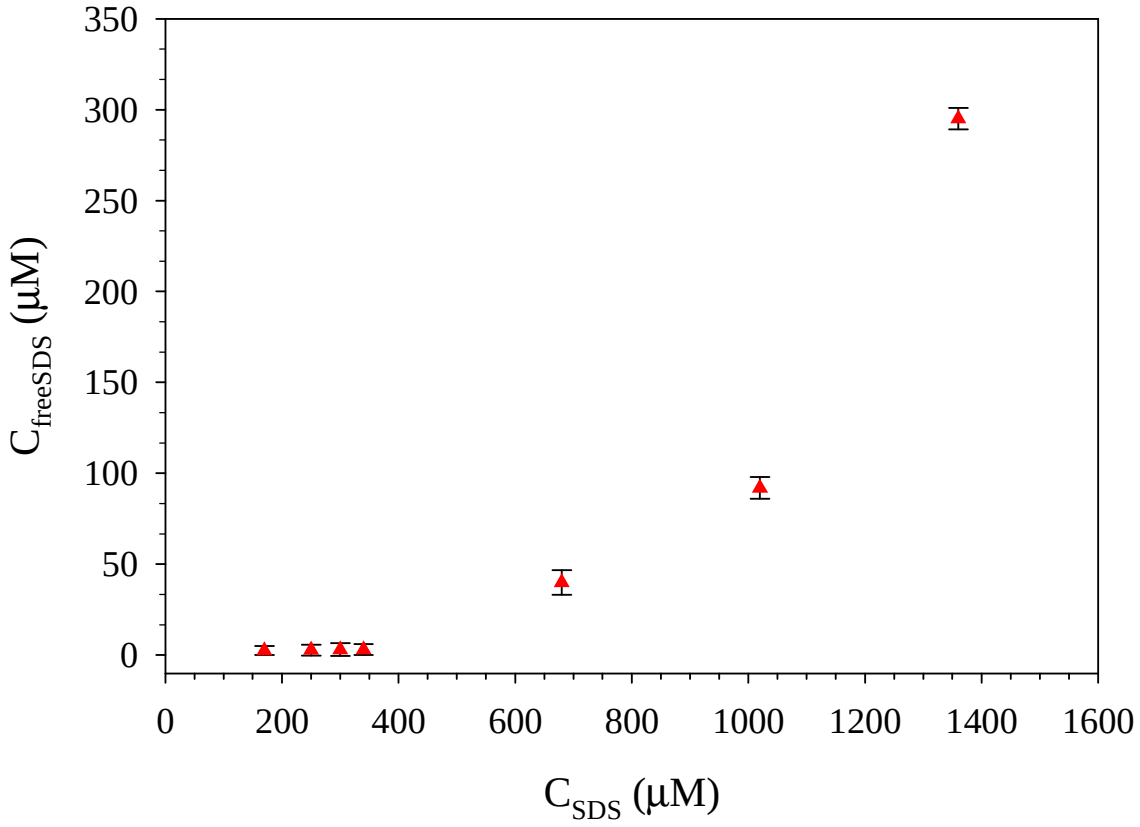


Figure 1. pH change in (♦) pure SDS and (▼) β-CD_{SDS} aqueous solutions as a function of C_{SDS}. The values are presented as the average of triplicate experiments.



(a)



(b)

Figure 2. (a) HPLC chromatograms of (i) SDS (340 μM), (ii) β-CD (1 mM), (iii) β-CD_{SDS} (C_{SDS} = 340 μM), and β-CD_{SDS} (C_{SDS} = 1020 μM). (b) $C_{freeSDS}$ as a function of C_{SDS}. The error bars represents the standard deviation.

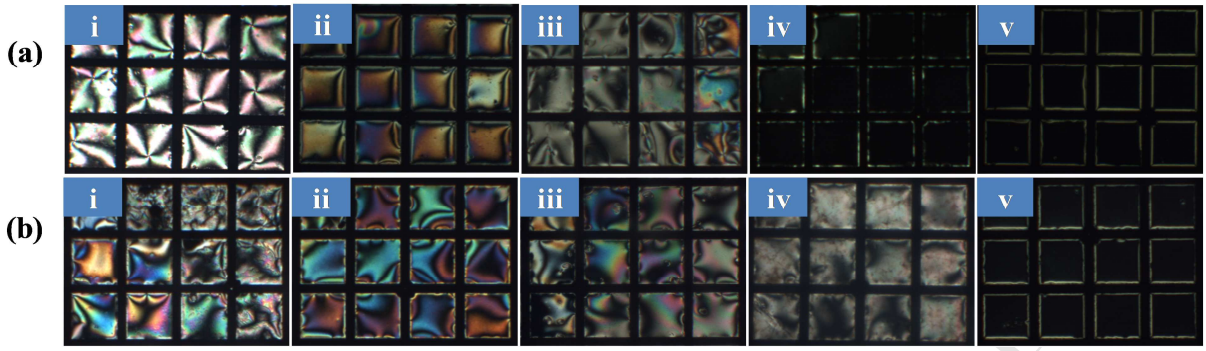
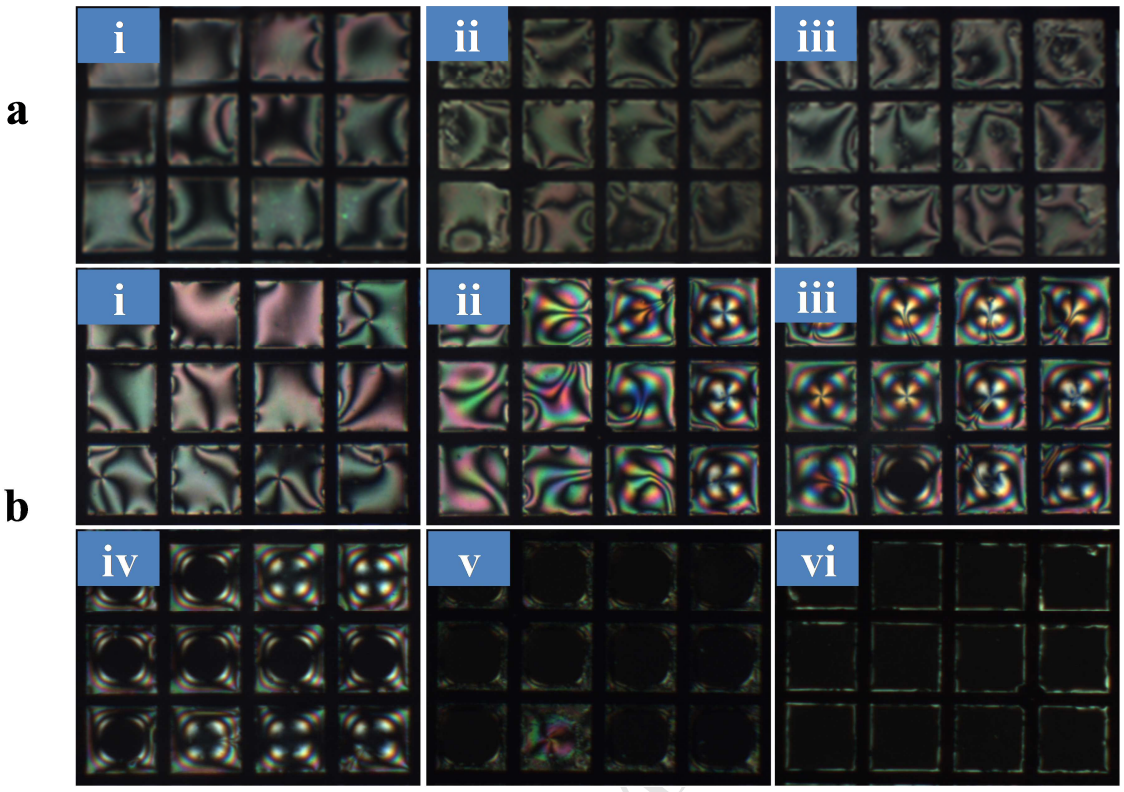


Figure 3. POM images (under crossed polarizers) of the TEM grid cells in (a) aqueous SDS solutions at C_{SDS} = (i) 0, (ii) 68, (iii) 136, (iv) 170, and (v) 340 μM, and (b) β-CD_{SDS} solutions prepared at C_{SDS}= (i) 170, (ii) 340, (iii) 680, (iv) 1020, and (v) 1360 μM. . C_{SDS} is concentration of SDS and β-CD_{SDS} is SDS-included β-CD.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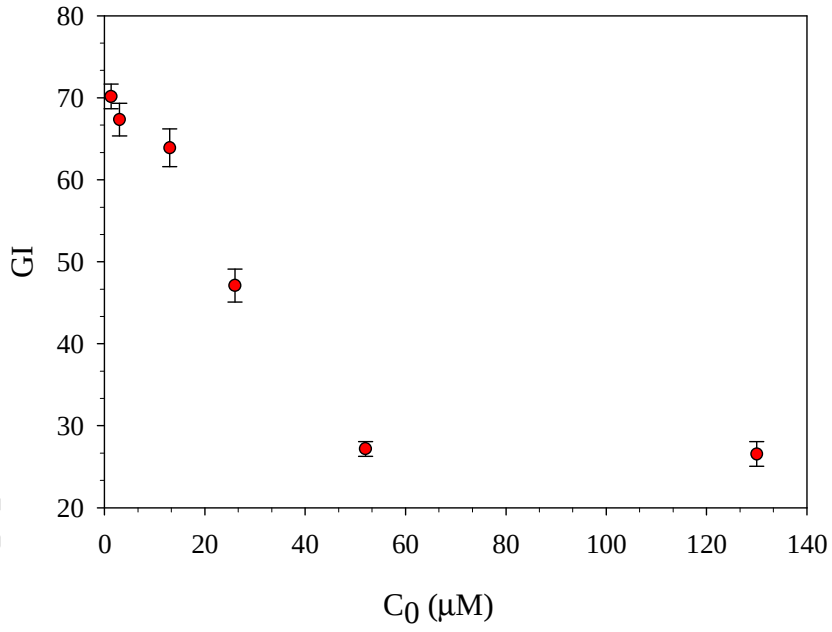

GI
80
70
60
50
40
30
20
0
20
40
60
80
100
120
140
C0 (μM)

(C)

Figure 4. POM images (under crossed polarizers) of the TEM grid cells (a) in the aqueous cholesterol solutions at different concentrations (C_0s) (i) 13, (ii) 52, and (iii) 130 μM without β-CD_{SDS}, and POM images of the grid cells in the (b) aqueous β-CD_{SDS} solutions prepared at C_{SDS} = 340 μM ($TEM_{β\text{-}CD/SDS}$ grid cells) after injecting the aqueous cholesterol solutions at C_0 = (i) 1.3, (ii) 3, (iii) 13, (iv) 26, (v) 52, and (vi) 130 μM, (c) the GI of the $TEM_{β\text{-}CD/SDS}$ grid cells as a function of C_0; the error bar represents the standard deviation for triple measurements. C_{SDS} is concentration of SDS, β-CD_{SDS} is SDS-included β-CD, and $TEM_{β\text{-}CD/SDS}$ grid cell is TEM grid cell in the aqueous β-CD_{SDS} solution prepared at C_{SDS} = 340 μM.

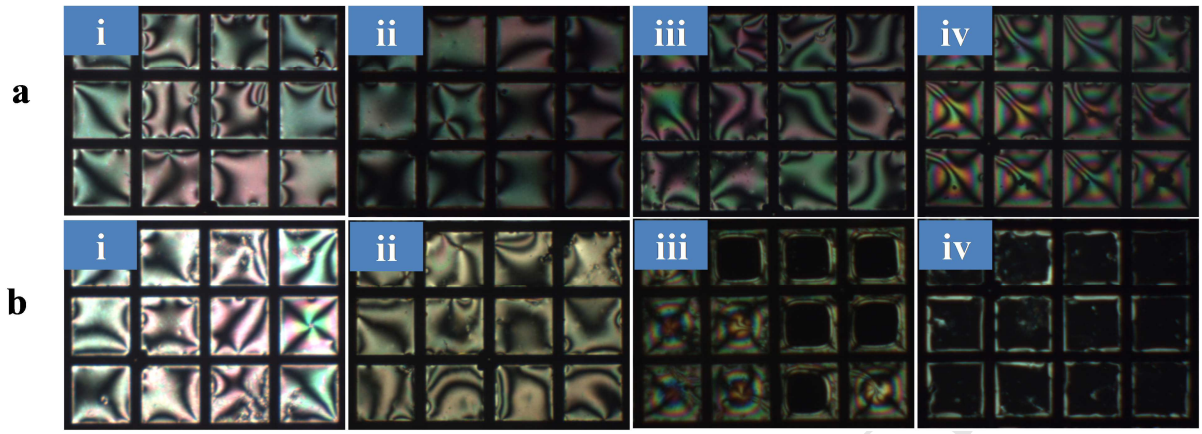
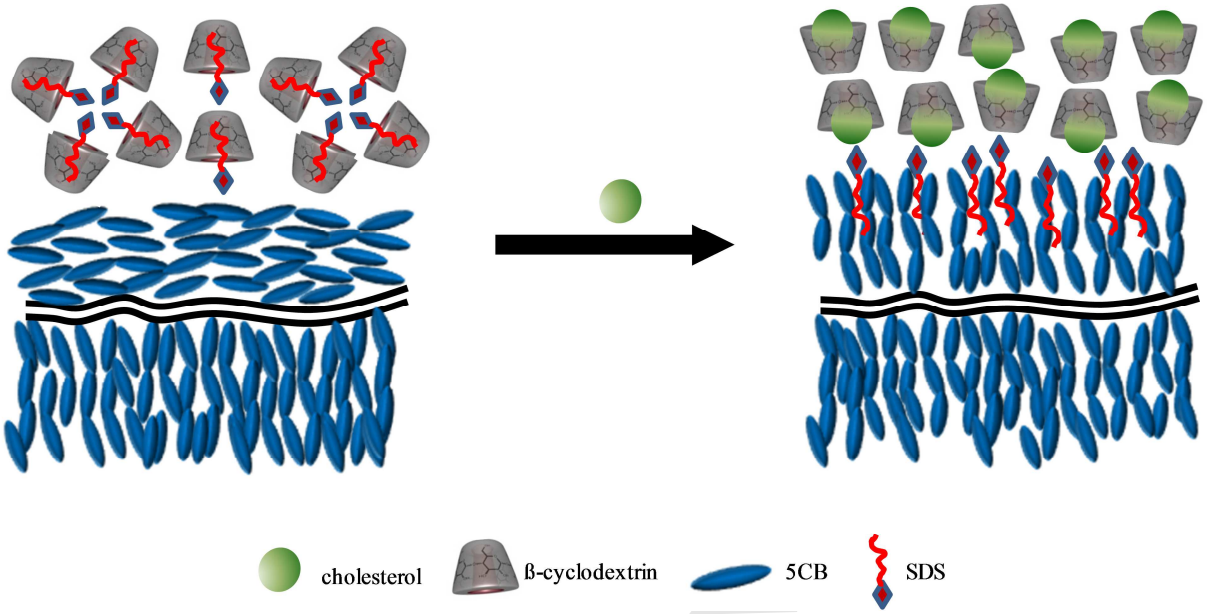


Figure 5. POM images (under crossed polarizers) of $TEM_{\beta\text{-}CD/SDS}$ grid cells in (a) 1 mM aqueous solutions of (i) NaCl, (ii) glucose, (iii) ascorbic acid, (iv) hemoglobin, and (b) (i) yeast extract (1 mg/mL) solution and aqueous solution of the YE/cholesterol mixture at different cholesterol concentrations of (ii) 3, (iii) 26, and (iv) 52 μM in the mixture. $TEM_{\beta\text{-}CD/SDS}$ grid cells is TEM grid cells in the aqueous $\beta\text{-}CD_{SDS}$ solutions prepared at C_{SDS} = 340 μM.



Scheme 1. Model of the $TEM_{\beta\text{-}CD/SDS}$ grid cell, and its cholesterol detection mechanism.

Supporting information

The development of a cholesterol biosensor using a liquid crystal/aqueous interface in a SDS-included β-cyclodextrin aqueous solution

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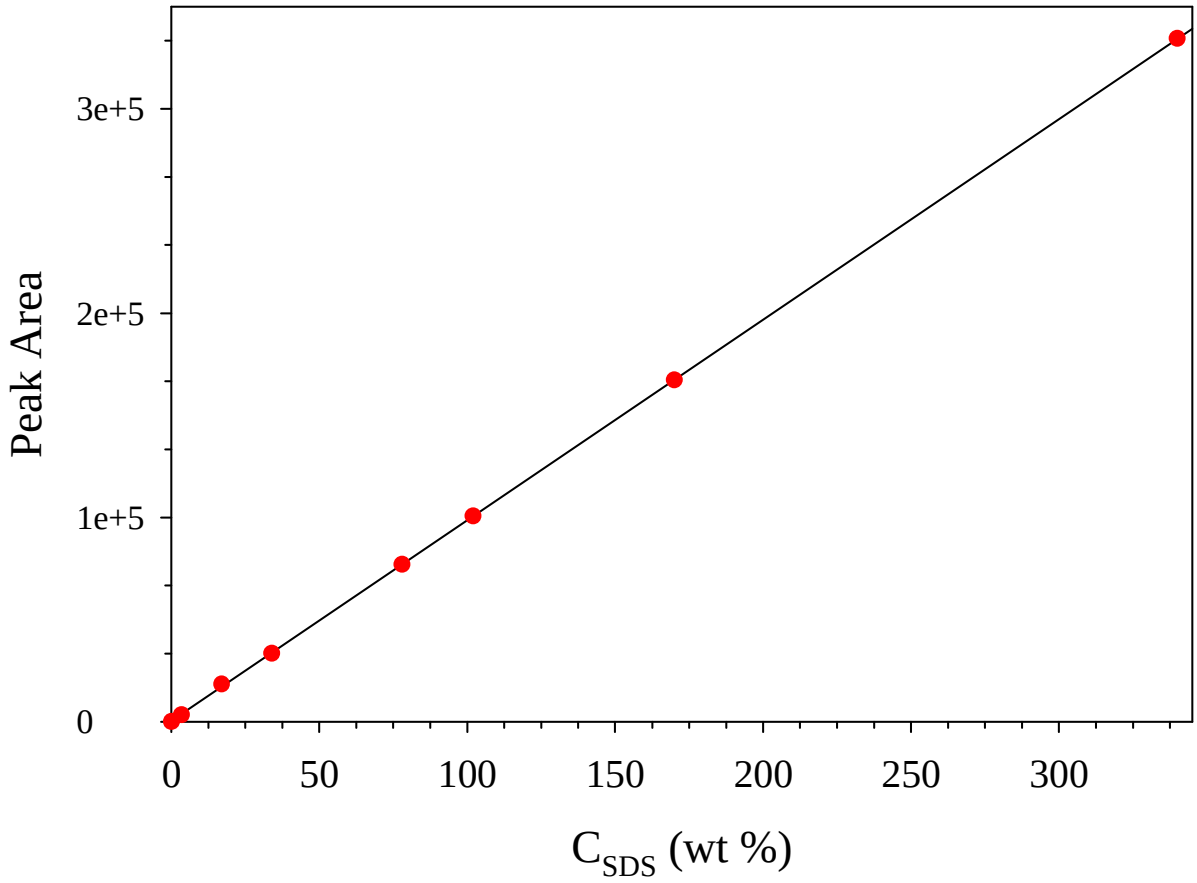

Figure SI 1. Plot depicting the relationship between the peak area at RT = 5.2 min and C_{SDS}, obtained from the HPLC chromatograms of the pure SDS aqueous solutions (C_{SDS} range = 3.5 to 340 µM).

Highlights

1. β-CD-SDS inclusion was used for the detection of cholesterol at 5CB/aqueous interface.
2. The 5CB exhibit planar orientation in β-CD-SDS solution.
3. The β-CD act as host for SDS guest.
4. The SDS enclosed within the β-CD cavity was replaced by cholesterol.
5. The released SDS from the β-CD caused homeotropic orientation of 5CB.
6. The cholesterol was detected from planar-to-homeotropic transition of 5CB.
7. This convenient TEM grid cell provides a new method for the selective detection of cholesterol.