Experimental Section

Materials

Shellac (AQUAGOLD) (25% w/w) based on SSB 57, a dewaxed and decolorized shellac (Acid no.: 65-80, Saponification no.: 190-230 and hydroxyl no. 230-280) was received as a gift sample from SSB Stroever GmbH & Co. KG (Bremen, Germany). Xanthan gum (acetate content = 3.53% and pyruvate content = 0.9%) and Nile red dye was purchased from Sigma Aldrich Inc., USA. Sunflower oil (food grade) was purchased from local supermarket. 0.1 N HCl and 0.1 N NaOH were purchased from VWR BDH Chemicals, UK. Fluorescently labelled xanthan gum (with Rhodamine B) was synthesized in-house. Water purified by the MilliQ system was used for all the experiments.

Preparation of emulsions

Emulsions were prepared by following a two step procedure by first emulsifying sunflower oil in xanthan gum solution using T 25 basic ULTRA-TURRAX® (IKA®-Werke GmbH & Co. KG, Germany) at a speed of 6500 rpm for 60 seconds. Accurately weighed aqueous shellac solution was then added to the formed emulsions and the mixture was further homogenized for 60 seconds. Emulsions were prepared by varying the oil volume fraction (Φoil= 0.1 to 0.6) and xanthan gum:shellac ratios (10:1 and 1:10 wt/wt).

For confocal microscopy, emulsions were prepared by replacing xanthan gum with fluorescently labelled xanthan gum and incorporating oil soluble dye (nile red) in sunflower oil, rest of the procedure was followed as above.

Analysis of droplet size, surface charge and morphology

The particle size (volume weighted mean, D (4,3) and of emulsions was measured using Mastersizer (Malvern Instruments Ltd, UK). Surface charge was measured through electrophoretic mobility on using a Zetasizer Nano ZS (Malvern Instruments Ltd, UK) after appropriate dilution. All measurements were carried out at 25°C and the results reported are averages of three readings.

The morphology of emulsions was studying using optical microscopy-Morphologi 3, (Malvern Instruments, UK). Imaging of fluorescent samples was done using a confocal microscope (Leica TCS-SP5) in combination with a Leica DMI6000 inverted microscope. Excitation wavelengths of 488 nm and 496-566 nm were used for Nile Red and Rhodamine B respectively.

For studying the pH responsive behaviour, the pH of emulsion was changed from neutral to acidic (pH 1.2) and back to neutral using 0.1 N HCl and 0.1 N NaOH respectively.

Molecular interactions

The thermodynamics of the binding was assessed using an isothermal titration calorimeter (VP-ITC Microcal, Northampton, MA). The solutions were degassed by using a Microcal Thermo Vac degassing unit. The reference cell was filled with degassed milliQ water. The sample cell of volume 1.4422 ml was filled with 0.033 mM xanthan gum and thermostated at 20 °C and 37 °C. The syringe of volume 250 μL was filled with the titrant (6.25 mM shellac), and the rotating speed of the syringe was set at 307 rpm. The titrant solutions were added in 10 μL aliquots (24 injections with 10 second duration each) at 180 seconds intervals. The heat released or absorbed upon each injection was measured as function of time. The heat of dilution from the blank titration of titrants into pure milliQ water was subtracted from the raw data. Data acquisition and analysis were performed with Microcal Origin software (version 7 SR4), and the single set of binding sites was applied to fit binding isotherms. Thermodynamic parameters, including the binding constant (K_a), observed binding enthalpy (*ΔH*) and binding stoichiometry (*n*) were calculated by iterative curve fitting of the binding isotherms using following equation.

$$Q = \frac{\left(1+[M]_t.n.K_a + K_a.[L]_t\right) - \sqrt{\left[\left(1+[M]_t.n.K_a + K_a.[L]_t\right)^2 - 4.[M]_t.n.K_a{}^2.[L]_t\right]}}{2.K_a \big/ V.\Delta H}$$

Where Q is the cumulative heat, $[M]_t$ the total concentration of reactant in the sample cell, $[L]_t$ the total concentration of titrant added, and V the volume of the sample cell.

Gibbs free energy (ΔG) was determined from the binding constant using equation: $\Delta G = - RT \ln Ka$, Where R is the gas constant ($8.314\ J.mol^{-1}K^{-1}$) and T is the absolute temperature (in Kelvin) and the entropy (ΔS), from the second law of thermodynamics ($\Delta G = \Delta H - T\Delta S$).