Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

HYBRID SURFACTANTS DECORATED WITH COPPER ION: Aggregation behavior, antimicrobial activity and anti-proliferative effect

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Biological activity

Hemolytic Potential: Human red blood cells (RBC) were obtained from a volunteer, and EDTA was added as an anticoagulant. Human erythrocytes were separated from the heparinized blood, before preparing the RBC suspension for the hemolysis test. In this process, blood was centrifuged at 5,000 rpm for 10 min. After discarding the supernatant, the erythrocytes were resuspended in isotonic phosphate buffer saline (PBS) (pH=7.4) to remove white cells and other debris followed by centrifugation at >5000 rpm for 5 min. The above washing step was repeated three times. An erythrocyte stock dispersion (packed RBC) was resuspended in PBS in such a manner that it yielded an optical density of approximately 2 at λ = 575 nm after total hemolysis in the assay (control, 100% hemolysis).[23] Briefly, 500 µL of erythrocyte stock dispersion was fixed in a stoppered test tube into which varied amount of samples (pre micellar, micellar and post micellar concentrations) were added. Each tube volume was then adjusted to 2 mL total volume with PBS and the tubes were shaken for 5 min until the formulation-RBC mixture was completely dispersed. Each sample was then left for equilibration at room temperature for 15 min. Finally, the tubes were centrifuged at a constant speed >5000 rpm for 10 min. Thereafter, the supernatant was extracted and its absorbance was measured spectrophotometrically at 414 nm with a double beam spectrophotometer (Thermo Scientific Evolution 160 UV-Vis Spectrophotometer) using quartz cells. The percentage of hemolysis was calculated by comparing the absorbance of the sample (supernatant) with that of a control sample, totally hemolyzed with double distilled water (during hemolysis assay, the hemoglobin releases into the solution, which makes the resultant solution visually red).

The percentage of hemolysis was calculated by the following equation:

$$\text{Hemolysis (\%)} = (A/A_{100}) \times 100 \quad (1)$$

where, A is the absorbance value of the sample (supernatant); A_{100} is the absorbance value of the positive control. All experiments were performed in duplicate.

Cytotoxic studies:

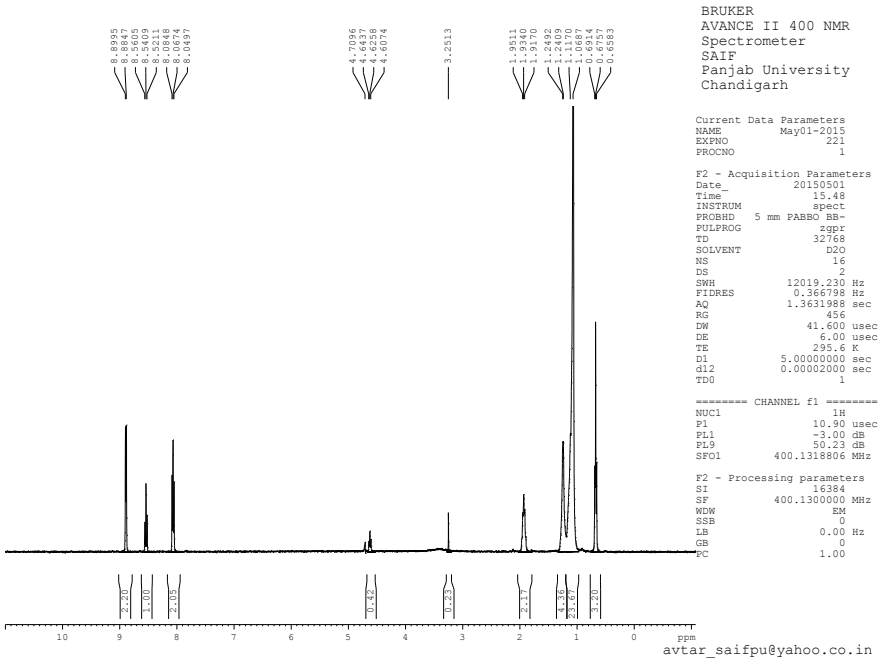
Cell culture and treatment*:* The cells were grown in RPMI-1640, DMEM and MEM medium containing 10% FCS, 100 μg/ml kanamycin and streptomycin. Cells were grown in a CO_2 incubator (Thermocon Electron Corporation, USA) at 37˚C with 5% CO_2 gas environment and 95% humidity. Cells grown in monolayer cultures were trypsinised with trypsin (0.1% w/v)/EDTA (1 mM) solution. Soon after cells were ready to detach, the trypsin/EDTA solution was removed. Cells were dispersed gently by pipetting in complete growth medium, centrifuged at 200xg, for 5 min. Cells were dispersed in a complete medium in culture flasks and incubated in the CO_2 incubator. Cells grown in semi-confluent stage (approx. 70% confluent) were treated with copper complexes dissolved in DMSO while the untreated control cultures received only the vehicle (DMSO, < 0.2%).

MTT Cell Proliferation Assay:

This assay is a quantitative colorimetric method for the determination of cell survival, proliferationand is a colorimetric assay for assessing cell viability. NAD(P)H-dependent cellular oxidoreductase enzymes may, under defined conditions, reflect the number of viable cells present. These enzymes are capable of reducing the tetrazolium dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide to its insoluble formazan, which has a purple color. Tetrazolium dye assays can also be used to measure cytotoxicity (loss of viable cells) or cytostatic activity (shift from proliferation to quiescence) of potential medicinal agents and toxic materials. The assessed parameter is the metabolic activity of viable cells. Metabolically active cells reduce pale yellow tetrazolium salt (MTT) to a dark blue water-

insoluble formazan, which can be, after solubilization with DMSO, directly quantified. The absorbance of the formazan directly correlates to the number of viable cells. The cells were seeded at a density of 15,000(HL-60), 6000(PC-3) and 5000(MIA-Pa-Ca-2) in 96-well plates. Cultures were incubated with 1, 3, 5, 7 and 10 μM concentrations of test materials and incubated for 48 h. After 44 h, 20 μL of MTT dye was added at a concentration of 2.5 mg/mL for 4h. The supernatant was aspirated and MTT-formazon crystals dissolved in 150 μL of DMSO; OD was measured at λ 540 (reference wavelength, λ 620) on an ELISA reader (Thermo Laboratories, U.S.A). Cell growth was calculated by comparing the absorbance of treated versus untreated cells. All experiments were performed in duplicate.

CPC



BRUKER
AVANCE II 400 NMR
Spectrometer
SAIF
Panjab University
Chandigarh

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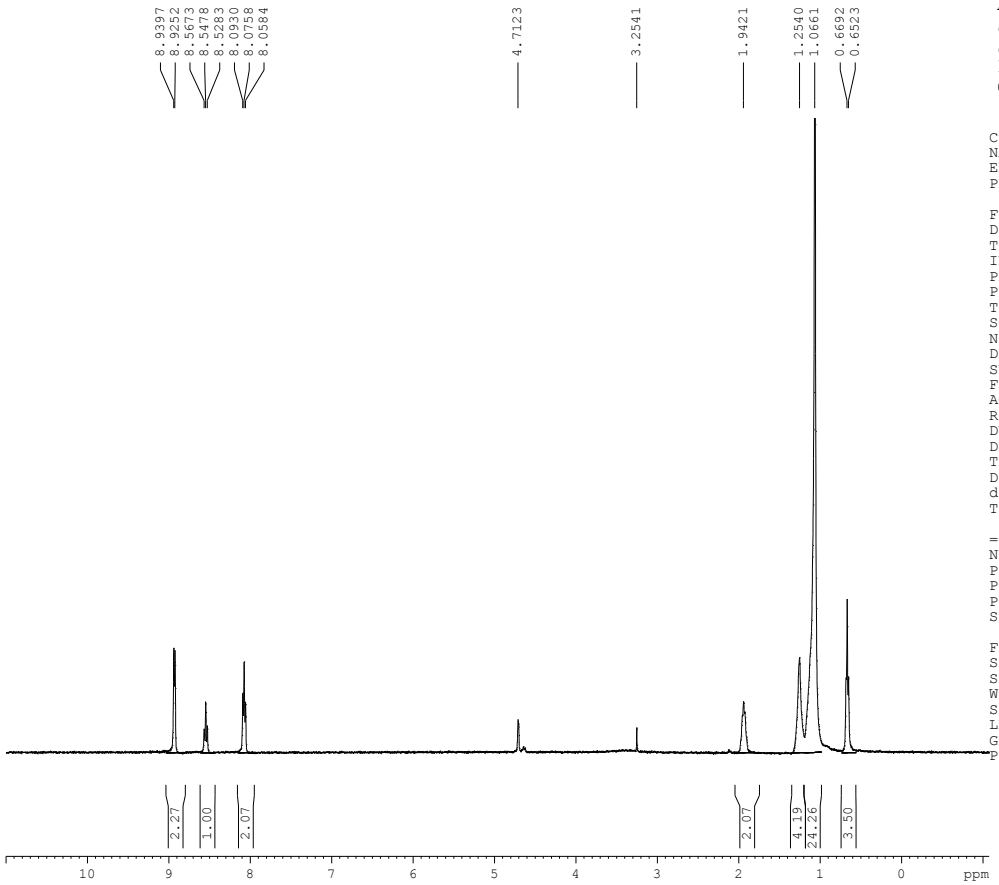
Cucpc I



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AVANCE II 400 NMR
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Cucpc II

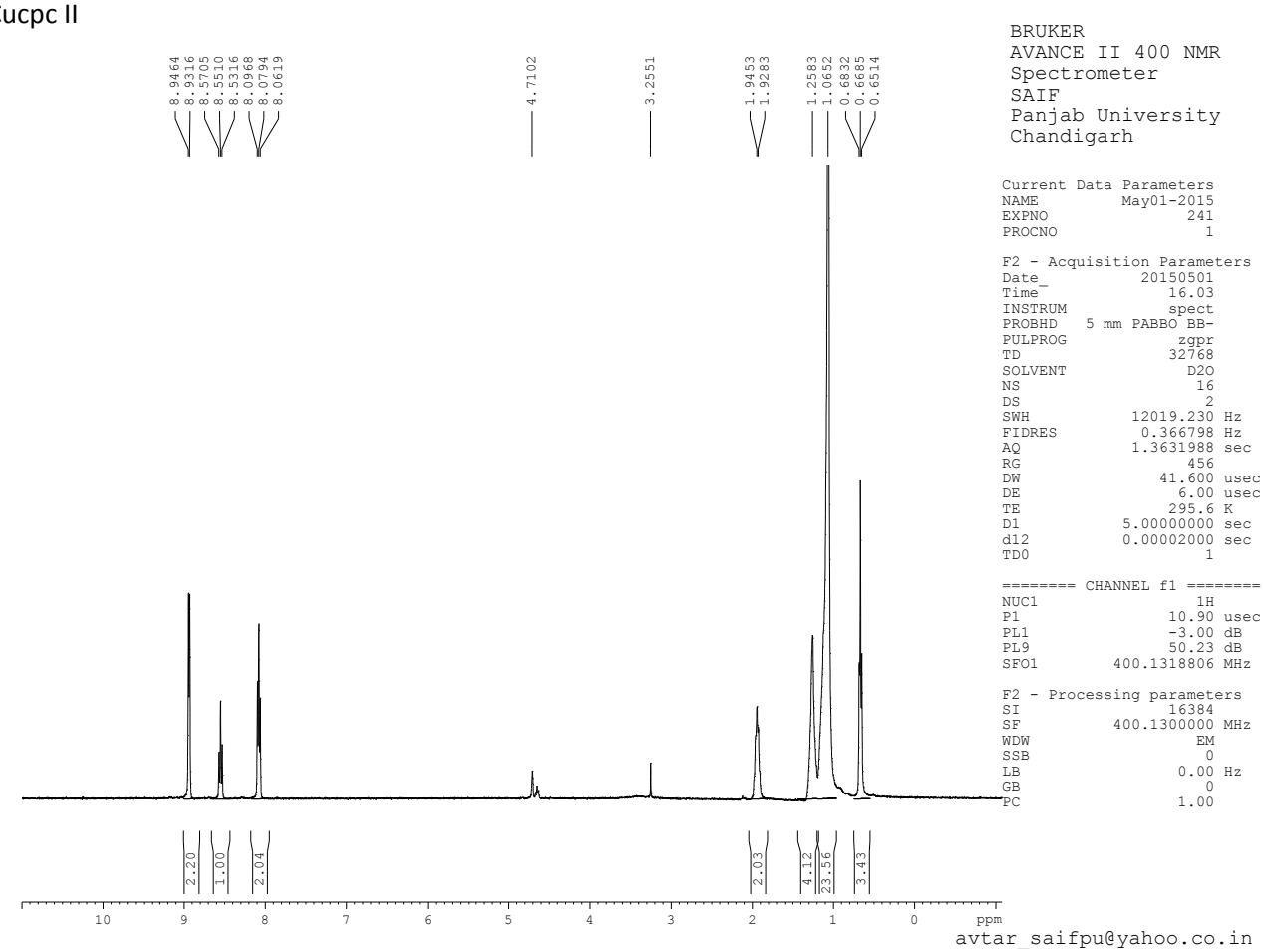

Figure FS1: IHNMR of CPC, Cucpc 1 and Cucpc II

Thermogravimetric analysis

For solid state reaction, rate constant (k) is calculated by kinetic eqn.

$$\frac{d\alpha}{dt} = kf(\alpha) = Ae^{-\frac{E}{RT}}f(\alpha) \qquad (S1)$$

where, A (pre-exponential factor) and E (activation energy) are the Arrhenius parameters; α is the fractional reaction; t is time; R is the gas constant; T is temperature in Kelvin, and f (α) is the kinetic function.

In isothermal kinetic studies, the rate constant is given by:

$$g(\alpha) = kt \quad (S2)$$

where, $g(\alpha)=\int_0^{\infty}\frac{d\alpha}{f(\alpha)}$ can be calculated by the integration of f(α).

Under non-isothermal conditions, in which a sample is heated at a constant rateβ=d*T*/dt, the explicit temporal in equation (3) is eliminated through the trivial transformation, so that $T=T_0+\beta$, where T_0 is the starting temperature and T is the temperature at any time *t*. Upon integration,eq. (1) may be written as:

$$g(\alpha)=\frac{A}{\beta}\int_{T_O}^{T}e^{-E/RT}dt \quad \text{(S3)}$$

Doyle's equation leads by Integration of equation (3) by considering that reaction rate is negligible at low temperatures

$$g(\alpha)=\frac{AE}{R\beta}\int_{T_O}^{T}\frac{e^{-x}}{x}-\int_0^{\infty}\frac{e^{-u}}{u}du=\frac{AE}{R\beta}P(x) \quad \text{(S4)}$$

where, $u=E/RT$ and *x* is the corresponding value of *u* at which a fraction of material has been decomposed. The above equation can be reformulated as:

$$\ln g(\alpha)-\ln P(x)=\frac{AE}{R\beta}=B \quad \text{(S5)}$$

where, Bis a constant for a particular reaction at a constant heating rate. The integral function P(x) has no analytical solution; it may be written in an expanded form and estimated by using a procedure of trial-and-error type involving iteration.

(i) *Coats-Redfern method*

$$g(\alpha)=\frac{ART^2}{\beta E}\left[1-\frac{2RT}{E}\right]e^{-E/RT} \quad \text{(S6)}$$

So the equation takes the form:

$$-\ln\frac{g(\alpha)}{T^2} = -\ln\frac{AR}{\beta E}\left[1-\frac{2RT}{E}\right]+\frac{E}{RT} \quad \text{(S7)}$$

The fraction mass loss (α) and corresponding $(1-\alpha)^n$ are calculated from TG curves,where *n* depends upon the reaction model.

$$-\log\frac{1-(1-\alpha)^{1-n}}{T^2(1-n)} = \log\frac{AR}{\beta E}\left[1-\frac{2RT}{E}\right]-\frac{E}{2.303RT} \text{ for n} \neq 1 \quad \text{(S8)}$$

$$-\log\frac{-\log(1-\alpha)}{T^2} = \log\frac{AR}{\beta E}\left[1-\frac{2RT}{E}\right]-\frac{E}{2.303RT} \text{ for n=1} \quad \text{(S9)}$$

In general, *2RT/E<<1* exhibits a small variation with T. So, it is assumed that the term (1–2RT/E) is approximately constant and equal to unity. Therefore, plotting the left hand side of the above equations against 1/*T* gives the slope -2.303*E/R* which yields the value of activation energy and the intercept gives value of *A* with excellent correlation coefficients, which indicates a good fit of the linear function.

(ii)*Madhusudanan-Krishnan-Ninanmethod*

$$-\ln\frac{g(\alpha)}{T^{1.9206}} = -\ln\frac{AR}{\beta E}+3.7678-1.9206\ln E-0.12040\frac{E}{RT} \quad \text{(S10)}$$

(iii) *Wanjun–Yuwen–Hen–Cunxin method*

$$-\ln\frac{g(\alpha)}{T^{1.8946}} = -\ln\frac{AR}{\beta E}+3.6350-1.8946\ln E-1.0014\frac{E}{RT} \quad \text{(S11)}$$

(iv) *Van Krevelen method*

$$\ln g(\alpha) = \ln\left(\frac{A(0.368/T_m)^{\frac{E_a}{RT_m}}}{\beta(\frac{E_a}{RT_m}+1)}\right)+\left(\frac{E_a}{RT_m}+1\right)\ln T \quad \text{(S12)}$$

(v) *Horowitz–Metzger method*, a new parameter $T = T_m + \theta$ has been introduced. If the order of reaction is 1, T_m is defined as the temperatureat which $(1 - \alpha)_m = 1/e = 0.368$ and the final expression is:

$$\ln\ln(\alpha) = \frac{E\theta}{RT_m^2} \quad \text{(S13)}$$

In the above equations; α, g(α), β, T_m, *E, A, R*are the degree of reaction, integral function of conversion, heating rate, DTG peak temperature, activation energy (kJ mol^{-1}), pre-exponential factor (min^{-1}) and gas constant (8.314 $Jmol^{-1}K^{-1}$), respectively.

Thermodynamics of micellization

Thermodynamic parameters such as ΔG_m^o (Gibbs free energy), ΔH_m^o (enthalpy) and ΔS_m^o (entropy) of micelle formation have been estimated using following equations

$$\Delta G_m^o = (2-\beta)RT\ln X_{cmc} \quad \text{(S14)}$$

$$\Delta H_m^o = -RT^2(2-\beta)d\ln X_{cmc}/dt \quad \text{(S15)}$$

$$\Delta S_m^o = (\Delta H_m^o - \Delta G_m^o)/T \quad \text{(S16)}$$

where, R, T, β and X_{cmc}represents gas constant, absolute temperature, degree of ionization and cmc in terms of mole fraction, respectively.

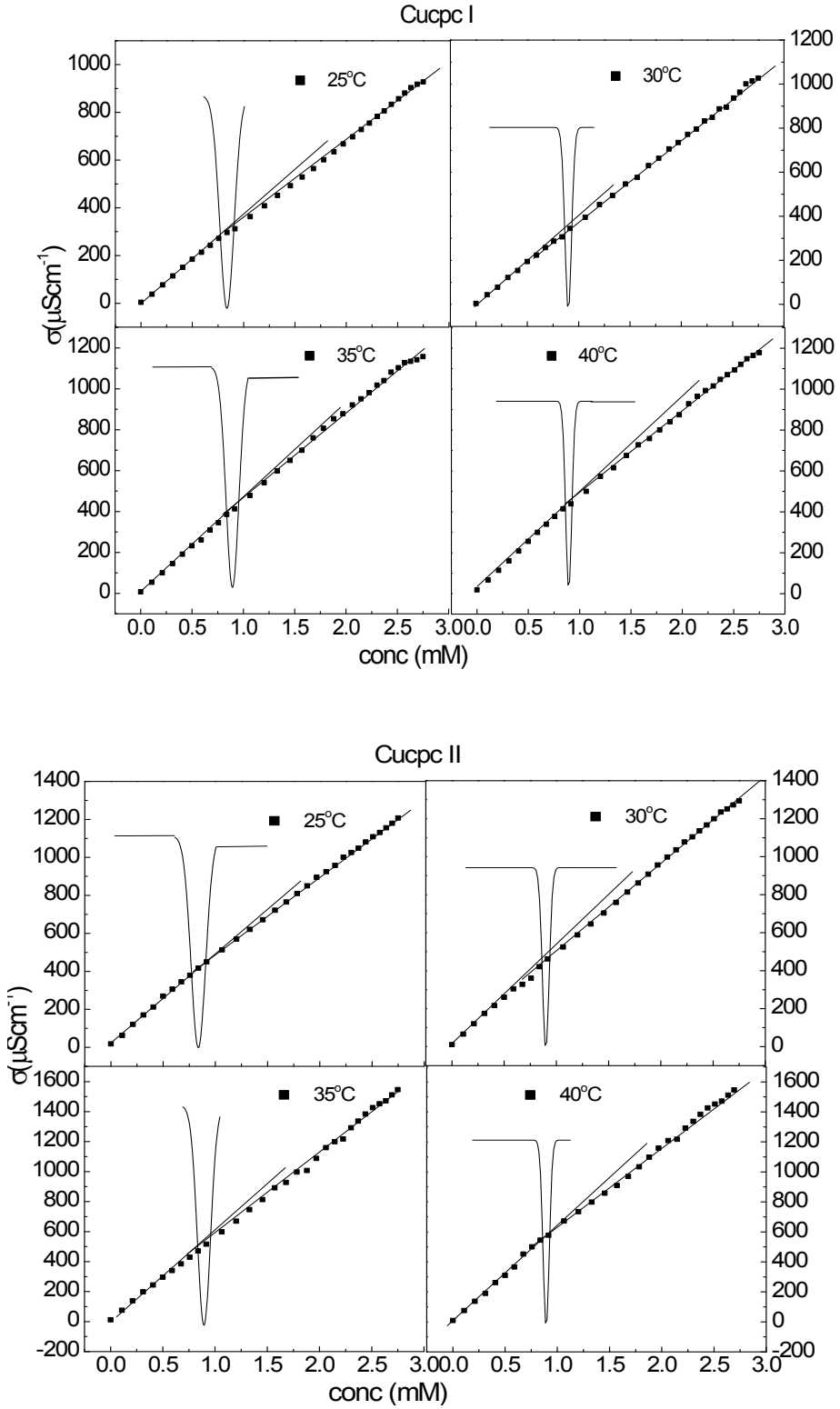

Figure S2. Variation of specific conductance for (a) Cucpc I (b) Cucpc II at different temperatures and (c)

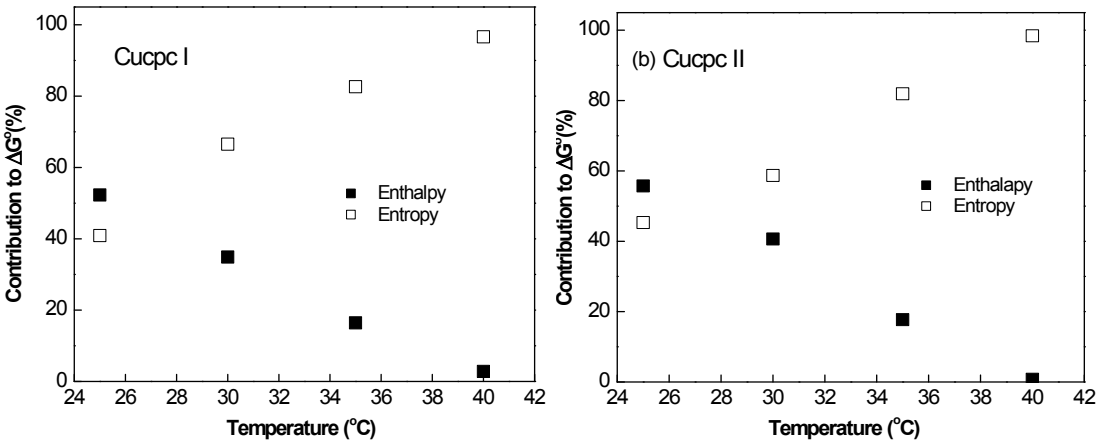

Figure ES3: Enthalpic and entropic contributions to ΔG^{o}_{m}