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Surface, aggregation properties and antimicrobial activity of four novel thiourea-based non-ionic surfactants

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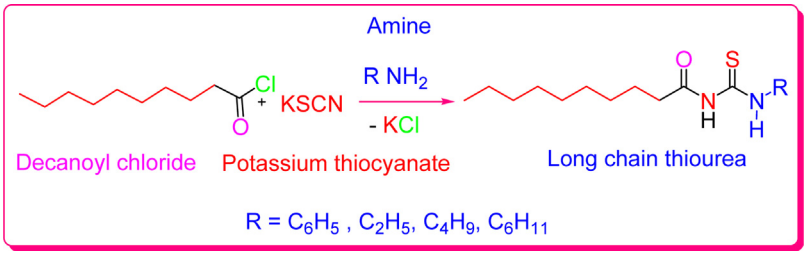
HIGHLIGHTS

- Novel thiourea-based non-ionic surfactants were synthesized and investigated.
- Spectrophtometric and pendant drop techniques were used to evaluate their CMC in ethanol and toluene.
- Besides cleaning agents these surfactants possess good antimicrobial activities.
- The incorporation of thiourea-based group enhances their role in soil fertility.
- These surfactants are economical and environmentally friendly.

GRAPHICAL ABSTRACT



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ABSTRACT

Four novel thiourea-based non-ionic surfactants, 1-decanoyl-3-phenylthiourea, 1-decanoyl-3-ethylthiourea, 1-butyl-3-decanoylthiourea and 1-cyclohexyl-3-decanoylthiourea were synthesized from readily available synthetic building blocks in high yield. The compounds were characterized by ^{1}H NMR, ^{13}C NMR, FTIR and UV–Visible spectrophotometry. These surfactants show very low solubility in water, and interestingly low, but well-defined sub-millimolar critical micelle concentrations (CMCs) in ethanol and toluene, indicating that they are moderately amphiphobic. The antibacterial activity of the synthesized thiourea-based surfactants was tested against five bacterial strains, as well as the antifungal activity against five fungal strains. In all cases, the inhibition of bacterial and fungal growth was significant for all of the synthesized molecules. These molecules are particularly desirable for antimicrobial functionalization of surfaces due to their facile synthesis and their low water solubility, providing robust coatings in aqueous environments.

1. Introduction

As consumer and industrial products become more developed and complex, the demand for bespoke, multi-functional molecules increases. A particularly important subset of such molecules is surfactants – surface active molecules used widely in processing and consumer products as stabilizers, dispersants, solubilizing agents,

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spreading agents, templates, *etc*. [1–3]. By incorporating extra functionality into these molecules, powerful control over chemical and biological systems can be achieved. The added functionality can take the form of biological specificity, antimicrobial activity, on-demand response to changes in pH, salt conditions or external stimuli such as light or magnetic fields, *etc*.

Surfactants are conventionally composed of a polar, water-loving head-group and hydrophobic tail-group. Thus the solution phase behavior of surfactant molecules is controlled by the balance of mutual interactions between their head- and tail-groups, and the relative strength of interactions between these moieties and the solvent. For logical thermodynamic considerations, the properties of surfactant–solvent systems and their propensity for aggregation can therefore be changed by modulating these interactions, as well as by changing solvation via changes in temperature, pressure, *etc*. The investigation of such effects on physiochemical properties of surfactant solution is essential from industrial point of view [4–9]. These changes can be achieved by efficient modification in the molecular structures of surfactants which regulate their physical and chemical characteristics. Extensive research work has been done to elaborate and justify the critical micelle concentration (CMC) and surfactant properties in water [10–13].

Thiourea based amphiphiles represent an exciting new class of surfactants due to a plethora of desirable properties such as: ease of synthesis from readily available building blocks; diverse library of possible molecules; tunable solubility and aggregation properties; unique interactions with metal ions; biodegradability; antimicrobial activity; *etc*. [14,15].

Here, we present four novel thiourea-based surfactants with C10 alkyl chains coupled, via an acyl thiourea linkage, to alkyl or phenyl amines. The unique properties of these molecules are demonstrated using tensiometry and spectroscopy, and their anti-bacterial and anti-fungal activities are explored.

2. Experimental

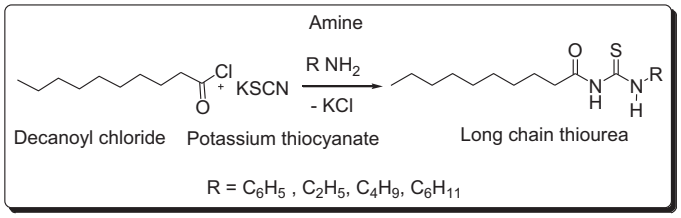
2.1. Materials and methods

Decanoyl chloride, potassium thiocyanate, butylamine, ethylamine, cyclohexylamine and phenylamine (all 99.9% or greater purity) were purchased from Sigma–Aldrich and used as received. Fresh, analytical grade dry acetone was used as the solvent, and dried by distillation before use.

NMR spectra were recorded on a Bruker AC Spectrometer at 300.13 MHz for ^{1}H and 75.47 MHz for ^{13}C. Infra-red characterization was performed using a Thermo Nicolet-6700 FTIR spectrophotometer, and a double-beam Shimadzu UV-1800 UV–visible spectrophotometer provided optical absorbance characterization. Interfacial tensions were measured using the pendant drop technique on a custom-built instrument. Critical micelle concentrations of the synthesized surfactants were determined by UV–Vis spectroscopy and pendant drop technique.

2.2. Antibacterial assay

Antibacterial activities of the surfactants were tested against representative gram negative (*Bordetella bronchiseptica*, *Escherichia coli* and *Bacillus subtilis*) and gram positive (*Staphylococcus aureus* and *Micrococcus luteus*) strains by the disc diffusion method [16]. They were standardized to 0.5 McFarland standards (10^{6} cfu mL^{-1}), after bacterial isolates were cultured for 20 h in a nutrient broth. The nutrient agar medium was prepared by adding 2.3 g nutrient agar (MERCK) to 100 mL distilled water at pH 7.0, which was then autoclaved. The mixture was allowed to cool to 45 °C and then seeded. For this, 75 mL of the seeded nutrient agar was poured and solidified to form triplicate Petri plates. Into these triplicate plates, wells were bored using sterile cork borer of 6 mm diameter. Each well was filled with 0.1 mL of the compound to be tested, and maintained at room temperature for 2 h, then incubated at 37 °C. The respective plates were observed for zones of inhibition after 24 h and the results were compared with that of 1 mg/mL penicillin, which was used as a standard. The relative results were measured by the equation: relative percentage inhibition of the test surfactant = $100(X - Y)/(Z - Y)$, where X represents the total inhibition area of the sample, Y is the solvent (blank) and Z represents the inhibition area of the standard drug used in the test experiment.



Scheme 1. Synthetic method for the preparation of C_{10} series of non-ionic thiourea-based surfactants.

2.3. Antifungal assay

Antifungal activities of surfactants were tested against five fungal strains, namely *F. solani*, *A. niger, M. piriformis, H. solani and A. Flavus*. The antifungal activity of various compounds was determined by the Agar tube dilution technique [17,18]. Samples were prepared by the dissolution of 12 mg of the tested compounds in 1 mL DMSO as solvent. The sabouraud dextrose (6.5 g) agar of Sigma–Aldrich was dissolved in 100 mL double distilled water at pH 5.6 to form a culture media for experimental requirement. 5 mL of sabouraud dextrose agar was shifted into screw capped small test tubes and kept for autoclave at 121 °C for about 20 min. Then, autoclave tubes were kept cool to room temperature and 70 μL of the compound from stock solution was loaded with a sabouraud dextrose agar. The culture media was solidified in tubes by keeping in slanting position at room temperature. The tubes were inoculated containing solidified media and surfactants with 4 mm diameter piece of inoculums taken from 7 days old culture of the fungus. Tubes with DMSO were used as control, while Terbinafine was used as reference drug. The test tubes were incubated for 7 days at 28 °C. During incubation periods, cultures media were examined twice a week. Measurements were made by noting linear length (mm) of fungus in test tubes and growth inhibition was determined with reference to control. The relative inhibition of fungal growth of selected surfactants was calculated by the equation; relative percentage inhibition of the test surfactant = $100 - (A/B) \times 100$, where A is the linear growth (cm) in test sample and B the linear growth (cm) in control.

2.4. General synthetic procedure

The general scheme for the decanoyl-thiourea surfactant synthesis is outlined in Scheme 1. Decanoyl chloride was added to aequimolar amount of potassium thiocyanate dissolved in 50 mL dry acetone. The reaction mixture was heated under stirring at 50 °C for 40 min, and then stirred at room temperature for a further 2 h to obtain decanoyl isothiocyanate. An equimolar amount of the selected amine was then added to the reaction mixture with continuous stirring for 12 h until TLC confirmed that the reaction progress was complete. The reaction mixture was then poured into an ice/water mixture to separate water soluble impurities. The solid product obtained was filtered and washed with doubly