

Structural and biological behaviors of some nonionic Schiff-base amphiphiles and their Cu(II) and Fe(III) metal complexes

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Abstract

Novel series of nonionic Schiff bases was synthesized and characterized using microelemental analysis, FTIR and ^1H NMR spectra. These Schiff bases and their complexes with Cu and Fe have been evaluated for their antibacterial activity against bacterial species such as *Staphylococcus aureus*, *Pseudomonas aureus*, *Candida albi*, *Bacillus subtilis* and *Escherichia coli* and their fungicidal activity against *Aspergillus niger* and *Aspergillus flavus*. The results of the biocidal activities showed high potent action of the synthesized Schiff bases towards both bacteria and fungi. Furthermore, complexation of these Schiff bases by Cu(II) and Fe(III) show the metal complexes to be more antibacterial and antifungal than the Schiff bases. The results were correlated to the surface activity and the transition metal type. The mode of action of these complexes was discussed. © 2008 Elsevier B.V. All rights reserved.

Keywords: Schiff-base amphiphiles; Metal complex; Gram-negative bacteria; Gram-positive bacteria; Fungi; Potent action

1. Introduction

Co-ordination compounds exhibit different characteristic properties which depend on the metal ion to which they are bound, the nature of the metal as well as the type of ligand, etc. These metal complexes have found extensive applications in various fields of human interest. The nature of a coordination compound depends on the metal ion and the donor atoms, as well as on the structure of the ligand and the metal–ligand interaction. With increasing knowledge of the properties of functional groups, as well as the nature of donor atoms and the central metal ion, ligands with more selective chelating groups, i.e., imines or azomethines, which are more commonly known as Schiff bases, are used for complex formation studies. Schiff bases are characterized by the $-\text{N}=\text{CH}-$ (imine) group which is important in elucidating the mechanism of transamination and racemisation in biological systems [1]. Due to the great flexibility and diverse structural aspects, a wide range of Schiff bases was synthesized and their complexation behaviors were studied [2]. They have been synthesized from a variety of compounds, such

as amino thiazoles, 2-hydroxy-1-naphthalaniline, amino sugars, aromatic aldehydes, isatin, the triazole ring, thiosemicarbazides, amino acids, pyrazolone, etc. [3–7]. Schiff bases have been studied extensively because of their high potential chemical permutation. Literature survey shows that Schiff bases show bacteriostatic and bactericidal activity [8]. Antibacterial, antifungal, antitumour, anticancer activity has been reported and they are also active against a wide range of organisms, e.g., *C. albicans*, *E. coli*, *S. aureus*, *B. polymyxa*, *P. viticola*, etc. [9,10]. Many Schiff bases are known to be medicinally important and used to design medicinal compounds [11–13]. Various studies [14–16] have shown a relationship between the metal ions and their metal complexes as antitumour and antibacterial agents, which is a subject of great interest. It was seen that the biological active compounds become more bacteriostatic and carcinostatic upon chelation with metal ions [17].

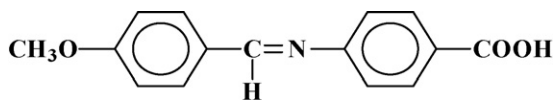
2. Experimental procedures

2.1. Synthesis of Schiff bases

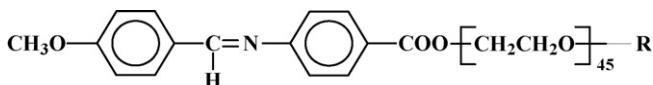
0.5 mol of anisaldehyde was condensed with 0.5 mol of *p*-aminobenzoic acid in presence of 250 mL of ethyl alcohol as a solvent. The reaction mixture was refluxed for 6 h and left overnight until the product precipitated. The product was washed

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Scheme 1. Chemical structures of the synthesized Schiff base (SB).



R = decanoate, decanoate, palmitate, stearate and oleate moieties

Scheme 2. Chemical structure of the synthesized nonionic Schiff-base amphiphiles. R = decanoate, decanoate, palmitate, stearate and oleate moieties.

by petroleum ether and recrystallized from ethanol. The final product was dried under vacuum at 40 °C [18]. The produced Schiff base was denoted as SB and its chemical structure was represented in Scheme 1.

2.2. Synthesis of nonionic Schiff-base amphiphiles

Schiff base (SB) (0.1 mol) was esterified with 0.1 mol of polyethylene glycol-2000-monoalkanoic acid namely: decanoate, dodecanoate, hexadecanoate, octadecanoate and oleate in presence of xylene as a solvent and *p*-toluene sulfonic acid as dehydrating agent. The reaction was completed by the removal of 1.8 mL of water. Then the reaction mixture was distilled under vacuum to complete removal of the unreacted materials and solvent to afford the desired nonionic amphiphiles [19] which were denoted as SBG-10, SBG-12, SBG-16, SBG-18 and SBG-oleate, Scheme 2.

2.3. Synthesis of metal complexes

All the copper(II) complexes were synthesized by following general procedure. The transition metal salts were in chloride form. The corresponding copper(II) salt and the appropriate Schiff-base amphiphiles in 1:2 molar ratio were dissolved, separated in ethanol, and mixed together, while Fe(III) complexes were synthesized in 1:3 molar ratio. The reaction mixture was boiled under refluxing state for 4 h. On cooling at room temperature, nonionic Schiff-base metal complexes were separated. They were filtered under suction, and the crystals were washed with cold ethanol and finally with anhydrous diethyl ether and kept in a desiccator over fused CaCl₂ [2,14–16].

2.4. Structural analysis

The elemental analyses were performed for the synthesized nonionic Schiff-base amphiphiles using Vario Elementar instrument. FTIR spectra were performed using Fourier-transformer infrared spectrophotometer and ¹H NMR spectra were performed using Bruker model DRX-300 NMR spectrometer with TMS as an internal standard. The results were represented in Table 1.

2.5. Antimicrobial studies

The synthesized nonionic Schiff-base amphiphiles were screened for their antimicrobial activity against bacteria and fungi using agar well diffusion method [20].

2.6. Growing of microorganisms

The bacterial strains were cultured on nutrient medium, while the fungi strains were cultured on malt medium. For bacteria, the broth media were incubated for 24 h. As for fungus, the broth media were incubated for approximately 48 h, with subsequent filtering of the culture through a thin layer of sterile Sintered Glass G2 to remove mycelia fragments before the solution containing the spores was used for inoculation.

2.7. Measurements of resistance and susceptibility

For preparation of discs and inoculation, 1.0 mL of inocula were added to 50 mL of agar media (40 °C) and mixed. The agar was poured into 120 mm petri dishes and allowed to cool to room temperature. Wells (6 mm in diameter) were cut in the agar plates using proper sterile tubes. Then, filled up to the surface of agar with 0.1 mL of the nonionic Schiff-base amphiphiles dissolved in DMF (1 mg/mL). The plates were left on a leveled surface, incubated for 24 h at 30 °C for bacteria and 48 h for fungi and then the diameter of the inhibition zones were read. The inhibition zone formed by these compounds against the particular test bacterial strain determined the antibacterial activities of the synthetic compounds. The mean value obtained for three individual replicates was used to calculate the zone of growth inhibition of each sample. The results were compared with a similar run of Cetyl trimethyl ammonium bromide (BRADORAL Trade Mark) [21] as an antibacterial reference and Grisofluvine as an antifun-

Table 1
Elemental analysis of the synthesized Schiff bases nonionic amphiphiles

Compound	Molecular weight	Carbon		Hydrogen		Nitrogen	
		Calc.	Found	Calc.	Found	Calc.	Found
Parent Schiff base	255.27	70.58	70.31	5.13	5.02	5.49	5.24
SBG-10	2391.85	57.74	57.57	8.89	8.80	0.59	0.57
SBG-12	2419.90	58.07	57.34	8.96	8.84	0.58	0.57
SBG-16	2476.32	58.69	58.51	9.08	8.99	0.57	0.54
SBG-18	2504.06	58.99	58.81	9.14	9.05	0.56	0.53
SBG-oleate	2502.04	58.04	58.86	9.02	8.93	0.56	0.51

gal reference. Both antimicrobial activities were calculated as a mean of three replicates.

2.8. Microorganisms

The antimicrobial activities of the synthesized surfactants were tested against *S. aureus*, *S. typhi*, *B. subtilis* and *E. coli* while, the fungicidal activities were tested against *A. niger* and *A. flavus*.

3. Results and discussion

3.1. Analyses

Microelemental analyses of the synthesized nonionic Schiff bases showed their purity at about 98.5%. IR spectra of the synthesized nonionic Schiff-base ligands showed absorption band at 850–890 cm^{-1} corresponding to the benzene nucleus and another band at 1200 cm^{-1} indicating the presence of ether linkage. The presence of absorption band at 1718 cm^{-1} indicates the formation of C=O ester group. The disappearance of the two bands at 1735 and 3315 cm^{-1} due to the carbonyl $\nu_{\text{C=O}}$ and ν_{NH_2} stretching vibrations and the appearance of a strong new band at 1630 cm^{-1} determines the formation of azomethene $\nu_{\text{HC=N}}$ linkage. The absorption bands at 2833 and 2942 cm^{-1} are corresponded to the stretching of the two groups CH_2 and CH_3 in the fatty chains. The band appearing at 1630 cm^{-1} due to azomethene was shifted to a lower frequency by 1–15 cm^{-1} in Fe and Cu complexes, indicating participation of azomethene nitrogen in the interaction with the metal ion. ^1H NMR spectra: 0.9 ppm (s, 6H, CH_3); 1.3 ppm (m, nH, CH_2) (where $n = 16\text{H}$ decanoate, 28H hexadecanoate, 32H octadecanoate) and 3.6 ppm (t, 196H, OCH_2).

3.2. Surface activity

Fig. 1 represents the relation between the surface tension and $-\log$ concentration of the synthesized nonionic Schiff-base amphiphiles at 25 °C. It is clear that the surface tension

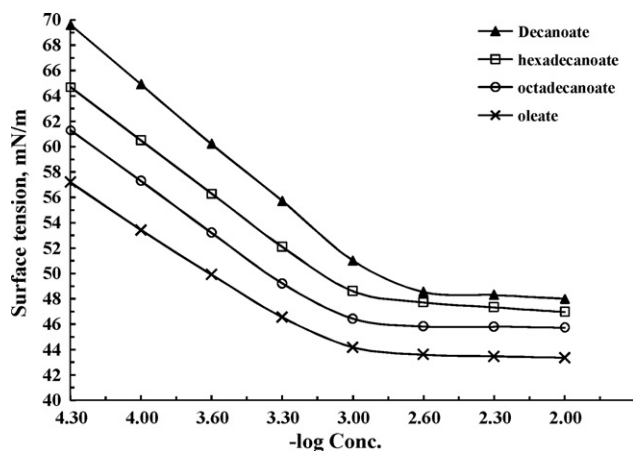


Fig. 1. Effect of the hydrophobic chain length on the surface tension of the synthesized Schiff-base amphiphiles.

profile has the characteristics of the nonionic surfactants including the relatively higher surface tension values. Also, it could be observed that increasing the number of methylene groups along the hydrophobic chains from 10 to 18 units decreases the critical micelle concentrations considerably [22]. That effect was explained in previous works [23,24] due to the repulsion occurred between the hydrophobic chains (nonpolar phase) and the water phase (polar phase), which forced the molecules to adsorb at the air–water interface and to micellize in the bulk of their solutions in order to decrease that repulsion. The highest CMC values was observed for SBG-10 at 1.73 mM/L, while the lowest value was observed for SBG-oleate at 0.15 mM/L at 25 °C, which referred to the above reasons and also to the unsaturation in oleate chain which increase the repulsion extent. The efficiency values (Pc_{20}) of the targeted amphiphiles were calculated using the data in Fig. 1, as the concentration of the amphiphiles solutions that decreases the surface tension to 51 mN m^{-1} . The Pc_{20} values describe the ability of these molecules to adsorb at the interface and also indicate the accumulation extent of the surfactant molecules at the interface. Increasing the hydrophobic chain length of the studied amphiphiles result in fast decrease in the surface tension of the surfactant solution indicating the high tendency of the longer hydrophobic molecules to adsorb at the interface. The lowest Pc_{20} value was observed to SBG-oleate and SBG-18.

Values of the minimum surface area occupied by the nonionic Schiff-base amphiphiles at the interface (A_{min}) were calculated according to the equation:

$$A_{\text{min}} = \frac{10^{16}}{N_{\text{AV}} \cdot \Gamma_{\text{max}}}$$

where Γ_{max} and N_{AV} are the maximum surface excess and Avogadro's number, respectively.

Increasing the maximum surface excess values indicates the increasing of adsorbed molecules at the interface, hence the area available for each molecule will decrease. That causes the compacting of surfactant molecules at the interface to form denser layer. The values of critical micelle concentration, efficiency and minimum surface area of the Schiff-base nonionic amphiphiles were listed in Table 2.

3.3. Thermodynamics of adsorption and micellization

The micellization and adsorption processes of the amphiphile molecules are occurred instantly, but commonly one process

Table 2
Surface parameters of the synthesized Schiff-base amphiphiles

Compound	CMC (mM/L)	Pc_{20} (mM/L)	A_{min} (nm^2)	ΔG_{mic}	ΔG_{ads}
SBG-10	2.13	1.37	0.82	−7.66	−8.43
SBG-12	1.99	1.00	0.89	−7.75	−8.52
SBG-16	1.00	0.60	0.93	−8.61	−9.47
SBG-18	0.89	0.52	1.10	−8.75	−9.63
SBG-oleate	0.79	0.21	1.34	−8.90	−9.79

$$\Gamma_{\text{max}} = (-d\gamma/d \log C)/(8.3 \times 10^7 \times \text{RT});$$

$$\Delta G_{\text{mic}} = [2.303 \text{ RT } \log(\text{CMC})] - [\log(55.5)];$$

$$\Delta G_{\text{ads}} = \Delta G_{\text{mic}} - (0.6023 \times 10^{-2} \times \pi \text{ CMC} \times A_{\text{min}}).$$

Table 3
Biological activity of the synthesized Schiff bases

Compounds	Tested microorganisms						
	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Candida albi</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aureus</i>	<i>Escherichia coli</i>
SBG-10	13	15	13	15	16	22	22
SBG-12	13	14	13	16	16	22	17
SBG-16	16	14	14	15	16	13	18
SBG-18	16	15	14	18	16	13	15
SBG-oleate	24	16	14	20	17	15	19
Reference	11.6	11.6	12.3	12.3	12.3	12.3	12.3

may be predominating than the other. The predominance of any of the two processes is governed by the thermodynamic variables of this process. In the investigated amphiphiles, both adsorption and micellization thermodynamic functions were calculated based on the methodology of Rosen [25] and using the surface activity data in Table 2. The free energy changes of micellization and adsorption showed negative sign indicating the spontaneously of the two processes at 25 °C. Also, ΔG_{mic} decreased gradually by increasing the hydrophobic chain lengths. But, ΔG_{ads} has slight increase in negativity than ΔG_{mic} . The maximum depression in ΔG_{mic} and ΔG_{ads} was observed at -13.10 and -14.29 kJ/mol for SBG-oleate, respectively. That showed the higher tendency of these amphiphiles towards adsorption rather than micellization. The tendency towards adsorption is referred to the interaction between the aqueous phase and the hydrophobic chains which pumps the amphiphile molecules to the interface. The presence of these amphiphiles at the interface decreases the phases interaction.

3.4. Biological activity

Table 3 shows the antibacterial and antifungal activities of the prepared nonionic Schiff bases, which were evaluated by a well diffusion method against Gram-positive, Gram-negative bacteria and fungi. Cetyl trimethyl ammonium bromide (BRADORAL Trade Mark) and Grisoflavin were taken as the reference drugs for antibacterial and antifungal activities. The biological activities of the reference compounds gave 11.6 and 12.3 mm well diameter for fungi and bacteria, respectively. The synthesized nonionic Schiff-base amphiphiles showed higher antifungal and antibacterial activities. The hydrophobic chain length has a remarkable influence on the biological activities of the targeted compounds. Increasing the hydrophobic chain

length increases the antifungal activity to great extent. While, the antibacterial activities were increased gradually. Some exceptions were appeared in case of *P. aureus* and *E. coli*. These results are in consistence with the adsorption tendency of these amphiphiles at the interfaces, Table 2. The highest antibacterial and antifungal activities were observed for SBG-18 and SBG-oleate.

Tables 4 and 5 show the antibacterial and antifungal activities of the synthesized copper and ferric complexes of the nonionic Schiff bases. It is clear that the complexation of the Schiff bases by copper and/or ferric cations increases their antibacterial and antifungal activities. Metal chelates generally have higher antibacterial and antifungal activities than the free ligand. This is because of an increase in cell permeability. The lipid membrane, which surrounds the cell, favors the passage of only lipid soluble materials and it is known that liposolubility is an important factor controlling antimicrobial activity. By complexation, the ionic groups and the electron delocalization over the Schiff-base molecules participate in the complex molecules which in turn increases the hydrophobic character of the metal chelate and increasing the liposolubility thus favoring its permeation through lipid layer of microorganism. It therefore, confirms that chelation of the nonionic Schiff bases with transition metals tend to make the ligands acting more powerful and potent bactericidal and fungicidal. The biological activities of the synthesized metal complexes were generally decreased by increasing the hydrocarbon chain length. That could be referred to the adsorption ability of the complexes containing long chains at the interfaces. Increasing the hydrocarbon content in these metal complex molecules increases their hydrophobicity, hence the molecules will tend to form micelles. That will decrease their biological activities than the more adsorbed metal complex molecules (with shorter hydrocarbon chain lengths).

Table 4
Biological activity of the synthesized Schiff-bases copper complexes

Compounds	Tested microorganisms						
	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Candida albi</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aureus</i>	<i>Escherichia coli</i>
SBG-10	24	16	15	28	23	27	24
SBG-12	30	15	14	22	16	22	19
SBG-16	28	15	15	18	17	16	18
SBG-18	20	17	15	21	18	17	19
SBG-oleate	26	18	16	21	19	19	21
Reference	11.6	11.6	12.3	12.3	12.3	12.3	12.3

Table 5
Biological activity of the synthesized Schiff-bases ferric complexes

Compounds	Tested microorganisms						
	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>	<i>Candida albi</i>	<i>Bacillus subtilis</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aureus</i>	<i>Escherichia coli</i>
SBG-10	28	14	15	24	20	21	19
SBG-12	19	13	13	16	15	17	14
SBG-16	28	17	15	18	16	18	16
SBG-18	24	19	14	14	17	18	15
SBG-oleate	18	–	14	16	19	20	15
Reference	11.6	11.6	12.3	12.3	12.3	12.3	12.3

The copper complexes of the targeted compounds showed higher biological activities than the ferric complexes. The higher copper activity towards bacteria and fungi than the ferric complexes could be referred to the electronic configuration of the tridentate complex. This confirms that antibacterial and antifungal activities are dependent on the molecular structure of the ligands and the type of the complex formed either bi- or tridentate [26,27]. It appears from the above results that Cu(II) and Fe(III) nonionic Schiff-base complex may be able to maintain a good antibacterial and antifungal activities and be an effective antibacterial broad-spectrum drug that may be able to solve some problem of antibacterial resistance. Especially SBG-18 and SBG-oleate Schiff bases and the metal complexes of the shorter hydrocarbon chain Schiff bases (SBG-10, SBG-12), which may be able to solve some problems of antifungal and antibacterial resistance.

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