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ORIGINAL ARTICLE

Synthesis, Biological Study and Complexation Behavior of Some Anionic Schiff Base Amphiphiles

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Abstract A novel series of anionic Schiff base amphiphiles were synthesized. The chemical structures of these compounds were elucidated using different spectroscopic tools. The surface and thermodynamic properties of the prepared Schiff bases were studied using classical measurements including surface and interfacial tensions. The surface parameters of these compounds, e.g., surface tension, critical micelle concentration, effectiveness, efficiency, maximum surface excess, minimum surface area, and interfacial activity showed their good surface activity. Their thermodynamic parameters of adsorption and micellization including free energy change of micellization and adsorption showed their tendency toward adsorption at the interfaces and also micelle formation at lower concentrations. The complexation behavior of the synthesized Schiff bases were study through the interaction of the Schiff base (IIIa; SBSD) with nickel chloride hexahydrate. All synthesized compounds in addition to $NiCl_2 \cdot 6H_2O$ and Ni Schiff Base complex have been evaluated for their antibacterial activity against Gram-positive and Gram-negative bacteria. The results of the biocidal activities showed high potent action of (Ni-III_a; Ni-SBSD) complex more than Schiff base IIIa; SBSD.

Keywords Schiff base amphiphiles · Nickel complex · Surface · Antibacterial activities

Introduction

Schiff bases are organic compounds with great utility in important fields such as medicine, agriculture, and cosmetic products [1–3]. Some Schiff bases present anticancer, antitumor and antibacterial activity [4–7]. They play a prominent part in the enzymatic or unenzymatic transaminating reactions of the carbonyl compounds with amino acids [8]. In the coordinate chemistry field, a lot of Schiff bases operate as ligands [9]. Coordination compounds exhibit different characteristic properties which depend on the metal ion on to which they are bound, the nature of the metal as well as the type of ligand. These metal complexes have found extensive applications in various fields of human interest [10–15]. Various studies [16–18] have shown a relationship between the metal ions and their metal complexes as antitumor 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 [19].

In the present paper, different self-assembled anionic Schiff bases $III_{a–d}$ in addition to an Ni-SBSD complex were synthesized. The surface and antibacterial activities were discussed.

Experimental

All chemicals used in the present investigation were of Analar or highest purity grade from the BDH, Aldrich and Merck companies and used as received without further purification. FTIR spectra were recorded on a Perkin-Elmer 1420 spectrometer and a biorad FT 57 (KBr). A Bruker model DRX-300 NMR spectrometer with TMS as an internal standard for ^{1}H-NMR spectra was used. Mass

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spectra were recorded on a JEO1 JMS-AX 500 (EI and FAB^+). The elemental analyses of the synthesized materials were performed using a Vario Elementar instrument for elemental analysis.

Synthesis of Potassium Salt of 2-Carboxymethyl Benzaldehyde and 2-Methoxy-3-Carboxymethyl Benzaldehyde ($I_{a,b}$)

An aromatic aldehyde namely, salicyaldehyde and vanillin (0.1 mol) was stirred with an alcoholic potassium hydroxide solution (5 ml, 0.1 mol) and ethanol (20 ml). Potassium chloroacetic solution (0.15 mol) was added drop wise. The pH of the liquid mixture was adjusted with triethylamine, the mixture was refluxed for 6 h under a nitrogen atmosphere.

This reaction mixture was allowed to cool to room temperature, then poured onto crushed ice. The product was extracted with ethyl acetate. The crude $\mathbf{I_{a,b}}$ was recrystallized several times from 60% ethanol solution to give $\mathbf{I_{a,b}}$ (Table 1). IR (KBr pellet): 1,720 (C = O), 1,282 (C–O–C), another band at 832-893 cm^{-1} corresponding to the benzene nucleus for compounds $I_{a,b}$ and characteristic absorption band appears at 2,836 cm^{-1} (OCH_3) for I_b. ^1H NMR ($CDCl_3$) for I_a showed that δ 9.85 (s, 1H, CHO), δ 7.02–7.62 (m, 4H,Ar–H) and δ 4.91 (s,2H,CH_2).

Synthesis of 4-Amino Alkyl Benzene $II_{a,b}$

Fatty alcohol C_{12}, C_{18} (0.46 mol), aniline (0.46 mol), aniline hydrochloride (0.14 mol) and anhydrous zinc chloride (0.32 mol) were heated in a conical flask equipped with a Dean Stark apparatus to collect the water of the reaction. The reaction mixture was heated for 1 h at 240–250 °C, excess zinc chloride and 4-amino alkyl benzene hydrochloride were removed by adding 40% sodium hydroxide solution (200 ml) to the reaction mixture which was heated at 70 °C for 2 h. The reaction mixture was left to cool and extracted with 60 ml of diethyl ether several times. The collected ether layer was dried over anhydrous sodium sulfate to dryness to yield $\mathbf{II_{a,b}}$, Scheme 1.

IR spectrum (**IIa**): 826 (Ar.nucleus), 2,836 (CH_2), 2,921 (CH_3) and 3,457 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$): 0.88 (t, 3H, CH_3), 1.21–1.27 [m,22H, $(CH_2)_{11}$], 7.52 (d.d, phenyl ring, 4H) and δ 9.53 (s, 2H, NH_2). FAB-MS (m/z): 261.26 (M^+) (41.23%), Fig. 1.

Synthesis of Anionic Schiff Bases Amphiphiles ($III_{a–d}$)

In a conical flask, 0.5 mol of $I_{a,b}$ was condensed with 0.5 mol of $\mathbf{II_{a,b}}$ in presence of 100 ml ethyl alcohol as a solvent. The pH was adjusted from 7 to 8 by triethylamine. The reaction mixture was refluxed for 6 h and left overnight in refrigerator until the product precipitated. The product was washed with hexane and recrystallized from acetone, the final product was dried under a vacuum at 40 °C. The Schiff bases (SB) produced whose chemical formulas are indicated in Table 1, are denoted as SBSD, SBSS, SBVD, SBVS for potassium salts of either (4-alkyl-1-benzyl imino) 2-carboxy methyl benzene or (4-alkyl-1-benzyl imino) 2-methyl-3-carboxy methyl benzene which derived from salicyaldehyde and vanillin with 4-aminoalkyl benzene respectively, and their chemical structures are shown in Scheme 1.

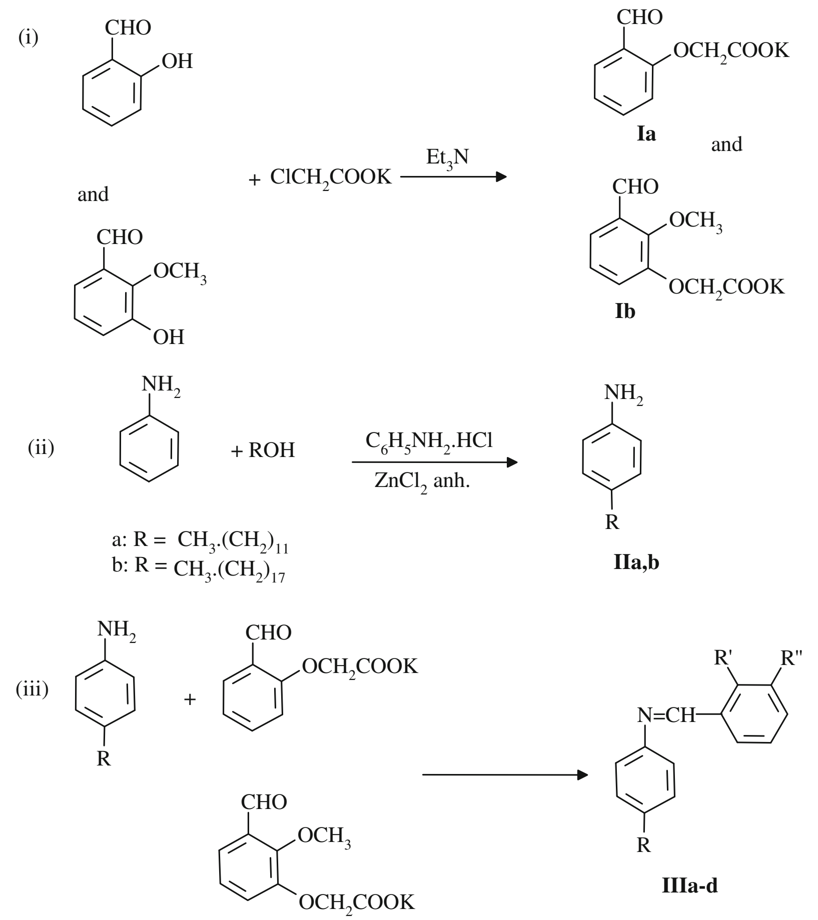
Microelemental analysis of the synthesized Schiff bases showed their purity (Table 1).

IR spectra showed absorption at 850–890 cm^{-1} corresponding to the benzene nucleus and band at 1,210 cm^{-1} indicating the presence of an ether linkage (–C–O–C)–. The appearance of a strong new band at 1,635 cm^{-1} determines the formation of an azomethene. (HC = N) linkage and disappearance of the band at 3,457 due to the (NH_2) group.^1H NMR ($CDCl_3$) for $\mathbf{III_c}$; δ 8.32 (s, 1H, CH = N), δ 7.60 (m, 7H, Ar–H), 4.16–4.19 (s, 2H, OCH_2^-), δ 3.91 (s, 3H, OCH_3), 1.1–1.2 (m, 22H, $(CH_2)_{11}$)

Table 1 Analytical data of the prepared Schiff bases and Ni-SBSD complex

Compound	Molecular formula	m.p. °C	Molecular weight	Calculated/Found			Ni%
				C	H	N	
SBSD	$C_{27}H_{35}NO_3K$	Waxy	460.68	70.40	7.66	3.04	–
				70.19	7.42	2.87	
SBSS	$C_{33}H_{47}NO_3K$	60–62	544.84	72.75	8.69	2.57	–
				72.56	8.42	2.29	
SBVD	$C_{28}H_{38}NO_4K$	Waxy	491.71	68.40	7.79	2.85	–
				68.21	7.62	2.63	
SBVS	$C_{34}H_{50}NO_4K$	72–74	575.87	70.91	8.75	2.43	–
				70.78	8.61	2.32	
Ni-SBSD	$Ni.C_{54}H_{72}N_2O_6{\cdot}2H_2O$	130–32	940.01	68.99	7.66	2.98	6.24
				68.71	7.45	2.74	6.13

Scheme 1 Synthetic route of the synthesized anionic Schiff base amphiphiles



a, R' = OCH_2COOK, R = $CH_3–(CH_2)_{11}$, R'' = H (SBSD)
b, R' = OCH_2COOK, R = $CH_3–(CH_2)_{17}$, R'' = H (SBSS)
c, R' = OCH_3, R = $CH_3–(CH_2)_{11}$, R'' = OCH_2COOK (SBVD)
d, R' = OCH_3, R = $CH_3–(CH_2)_{17}$, R'' = OCH_2COOK (SBVS)

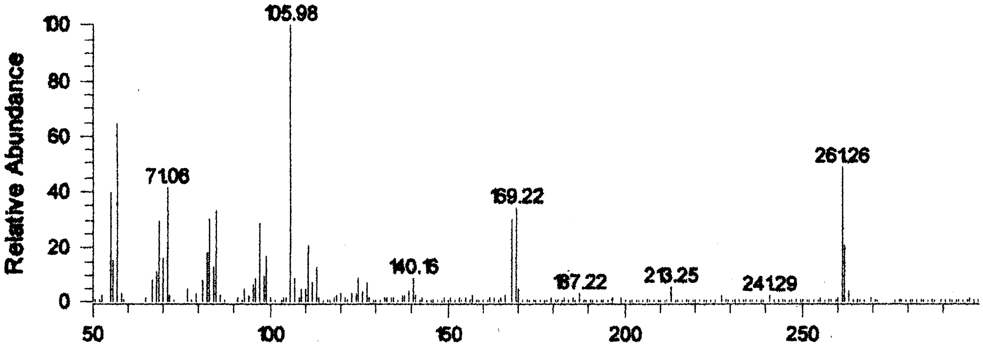


Fig. 1 Mass spectra of II_a

and δ 0.9 (t, 3H, CH_3). EI/FAB$^+$ (*m/z*): 491% (M-1) (14.07%) for **III$_c$**, Fig. 2.

Synthesis of the Metal Complex

All the Schiff base ligands (SBSD, SBSS, SBVD and SBVS) have similar structures, so the Schiff base ligand SBSD was selected to study their behavior towards metal ions. In a typical procedure:

A hot ethanolic solution of $NiCl_2·6H_2O$ (0.01 mol) was added dropwise to a hot ethanolic solution of Schiff base ligand, SBSD (0.02 mol). After complete addition, the reaction mixture was heated under reflux with continuous stirring for 2 h. On cooling at room temperature, the nickel complex was separated and filtered under suction. The crude complex was washed several times with ethanol followed by diethylether and dried in a vacuum desiccator

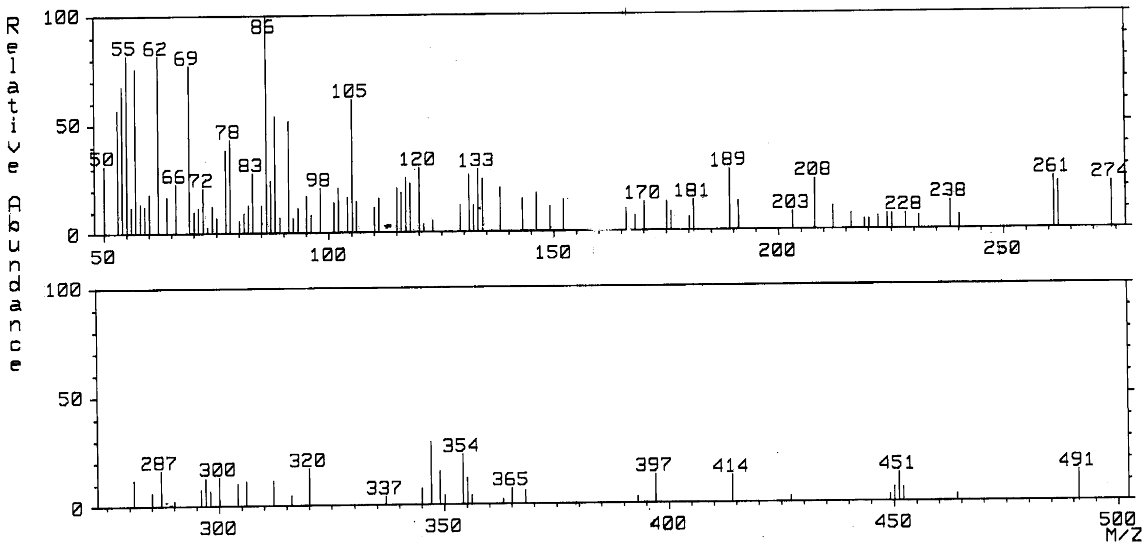


Fig. 2 Mass spectra of III_c

over fused calcium chloride. In order to give conclusive idea about the structure of the nickel complex, the main IR bands was compared with those of the free ligands.

Absorption band at 1,621 cm^{-1} for CH = N which shifted to a lower frequency than Schiff base itself. This indicated that it may be considered as a center of chelation [20]. EI/FAB^{+} (*m/z*) for Ni-SBSD 938 (M^{+}-2) (3.78%) Fig. 3. The nickel content was determined by titration against a standard EDTA solution after complete decomposition of the complex [21].

Surface and Interfacial Tension Measurements

Surface tension (ST) measurements were performed for freshly prepared solutions of surfactants. The concentration ranges from 10^{-1} to 10^{-6} mol/l using a Du-Nouy tensiometer at 25 °C. Also, interfacial tension (IT) measurements were carried out for 0.1 mol/L surfactant solutions against benzene at 25 °C [22].

Antimicrobial Activity

The synthesized anionic Schiff base amphiphiles and the nickel complex were screened for their antimicrobial activity against bacteria using agar well diffusion methods [23]. Bacteria species used in this study were *Staphylococcus aureus* (ATCC 25923), *Streptococcus pyogenes* (ATCC 19615) as Gram-positive and *Pseudomonas phaseolicola* (GSPB 2828), *Pseudomonas fluorescens* (S97) as Gram-negative bacteria.

Thermal Gravimetric Analyses (TGA)

Thermal gravimetric analyses (TGA) of the Ni-SBSD complex were obtained in a nitrogen atmosphere using a

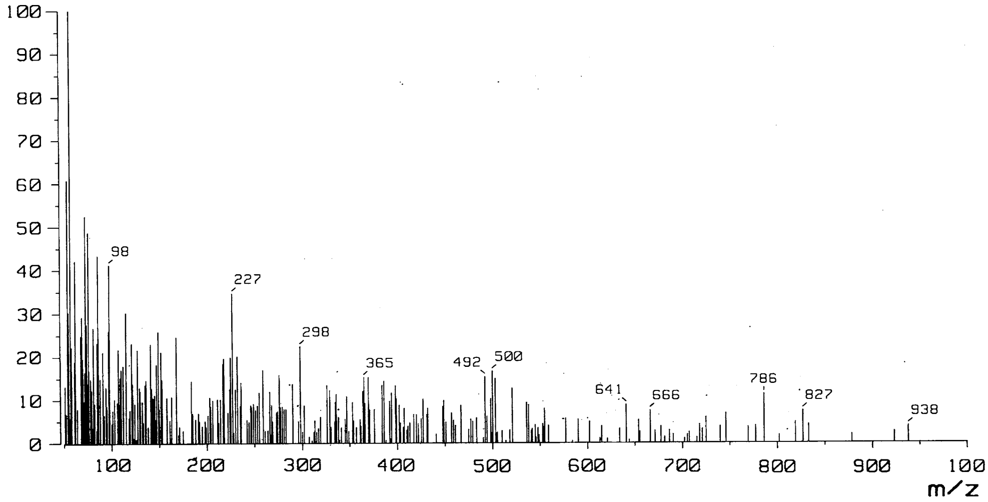


Fig. 3 Mass spectra of the nickel complex for [Ni-(SBSD)$_2$]·2H$_2$O complex

type SDTQ 600 universal V_4-5A thermal analyzer. The heating rates were suitably controlled at 10 °C/min under a nitrogen atmosphere and the weight loss was measured from room temperature up to 1,000 °C.

Results and Discussion

Synthesis

The Schiff base ligands $\mathbf{III_{a\text{-}d}}$; **SBSD, SBSS, SBVD, and SBVS** were prepared by the condensation between 4-amino alkyl benzene $\mathbf{(II_{a,b})}$ with carboxymethyl or methoxyl carboxymethyl aldehyde $\mathbf{(I_{a,b})}$ (1:1 M ratio) in ethanol. The level of the purity of the ligands was checked by thin layer chromatography on silica gel coated plates. All Schiff base ligands were found to having similar structures, so the Schiff base **IIIa**; **SBSD** was selected to study their complexation properties and Ni–SBSD was synthesized. The mass spectrum of the complex $[Ni(SBSD)_2].2H_2O$, Fig. 3, the peak observed at *m*/*z* 461.00 (3.97%) is evidence of the presence of an organic ligand in the complex. The mass spectrum shows the molecular ion peak at *m*/*z* 938 (calculated 940.01) with an abundance of 3.78% confirmed the suggested formula for the prepared complex, moreover, the spectrum shows numerous peaks representing successive degradation of complex molecules corresponding to various fragments, their intensity gives an idea on the stability of the fragments. Scheme 2 demonstrates the proposed paths of the decomposition steps of the nickel-SBSD complex.

Thermogravimetric Study

The thermogravimetric study for $[Ni(SBSD)_2].2H_2O$ complex was carried out within the temperature range from room temperature up to 1,000 °C. The determined temperature ranges and percent losses in mass of the solid complex on heating are given in Table 2. The results obtained are in good agreement with the theoretical formula suggested from the elemental analysis. Figure 4 shows the thermal analysis for the complex under study and reveals the following findings:

The complex under study gives three-stage decomposition pattern. The first stage (28–122 °C) with an experimental mass loss of 11.25% (theoretical 11.08%) represents the loss of two molecules of H_2O (hydrated), one molecule of CH_4 and two molecules of $CH{\equiv}CH$. The second stage within the temperature range 122–380 °C represents the loss of two molecules of $CH \equiv CH$ and two molecules of $C_6H_5–C_6H_5$. The experimental mass loss of this step is 38.45% (theoretical 38.35%). The third stage at temperature range 380–675 °C. may be attributed to the loss of $CH_3(CH_2)_{19}CH_3$ and the experimental mass loss is 31.70% (theoretical 31.56%), leaving behind nickel nitrate as the residue of the decomposition. The overall weight loss 81.40% (theoretical 80.99%). So, the suggested structure of the metal complex is shown in Scheme 2.

Surface Activity

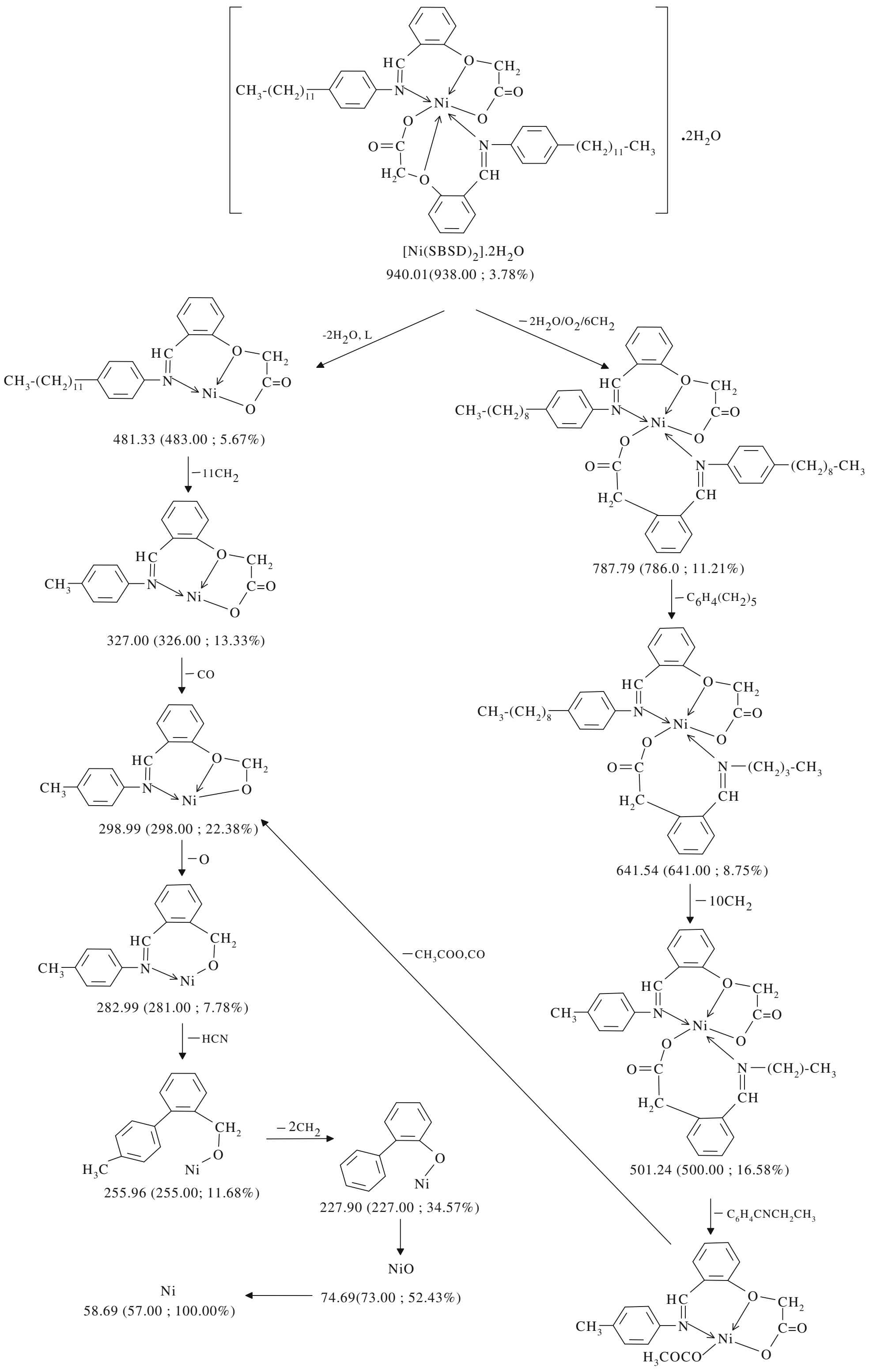
Figures 5 and 6 represent the relation between the surface tension and the logarithm of the concentration (mol/L) of the synthesized anionic Schiff base amphiphiles at 25 °C. It is clear that the surface tension profile has the characteristics of anionic surfactants including the relatively lower surface tension values ranging from 32 to 37 mN/m. Also, it could be observed that increasing the number of methylene group along the hydrophobic chains decreases the critical micelle concentration (CMC) considerably [24]. That effect was explained in a previous work [25–27] due to the repulsion occurred between the hydrophobic chains (non polar 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 same trend was observed in the vanillin series. The highest CMC values were observed for SBVD at 11.22×10^{-3} mol/l, while the lowest value was observed for SBSS at 3.16×10^{-3} mol/l at 25 °C, which referred to the above reasons and also to the presence of $–OCH_3$ group in the benzene nucleus which decrease the repulsion extent. Interfacial tension (IT) values of the synthesized anionic Schiff bases were measured between their aqueous solutions and benzene at 25 °C. Table 3 shows higher interfacial tension (IT) values especially for SBVD (34.5 mN/m). Increasing the hydrophobic chain length decreases the interfacial tension. The efficiency values (PC_{20}) of the targeted amphiphiles were calculated using the data in Figs. 5 and 6, as the concentration of the amphiphile solutions that decrease the surface tension by 20 mN/m [28]. 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 results in a 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 SBSS and SBVS.

The maximum surface excess concentration $\mathbf{\Gamma_{max}}$ was calculated from the relationship [29]:

$$\Gamma_{\max} = -1/RT(\partial\gamma/\partial \ln c)_{\mathrm{cmc}}$$

where $(\partial\gamma/\partial \ln c)_{\mathrm{cmc}}$ is the maximum slope of the plot γ versus log c at CMC at temperature $T = 298$ K and $R = 8.314\ \mathrm{mol^{-1}K^{-1}}$. The $\mathbf{\Gamma_{max}}$ values are directly used to calculate the minimum surface area occupied by the

Scheme 2 Proposed fragmentation route of $[Ni(SBSD)_2]\cdot 2H_2O$ complex



anionic Schiff base amphiphiles at the interface $\mathbf{A_{min}}$ according to the equation:

$$A_{min} = \frac{10^{16}}{N_{Av}\Gamma_{max}}$$

where $\mathbf{N_{Av}}$ is the Avogadro number. Increasing the maximum surface excess values indicates the increasing of adsorbed molecules at the interface, hence the area available for each molecule will decrease. This causes the

Table 2 Thermoanalytical results of $[Ni(SBSD)_2]\cdot 2H_2O$ complex

Complex compound	Stage	Temp. range (°C)	Mass loss%		Evolved moiety
			Calculated	Found	
$[NiC_{54}H_{72}N_2O_6].2H_2O$	I	28–122	11.08	11.25	2 H_2O (hydrated), CH_4 and 2 $CH \equiv CH$
	II	122–380	38.35	38.45	2 $CH \equiv CH$ and 2 $C_6H_5–C_6H_5$
	III	380–675	31.56	31.70	$CH_3(CH_2)_{19}CH_3$

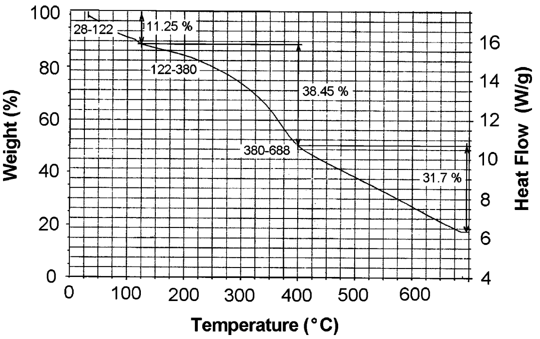


Fig. 4 TG thermogram for metal complex, $[Ni\text{-}(SBSD)_2]\cdot 2H_2O$ complex

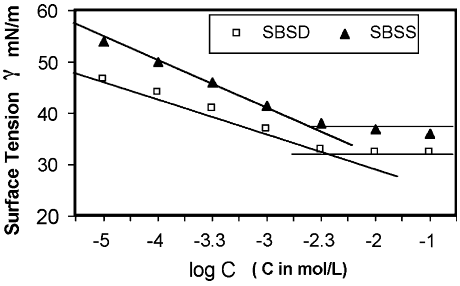


Fig. 5 Variation of the surface tension with the surfactant concentration for $III_{a,b}$

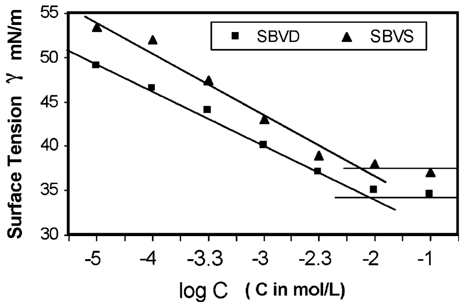


Fig. 6 Variation of the surface tension with the surfactant concentration for $III_{c,d}$

compacting of surfactant molecules at the interface to form a denser layer (Table 3).

The difference between the surface tension of the prepared compounds at their CMC and that of the pure water is called their effectiveness ($\pi_{\mathbf{cmc}}$):

$$\pi_{\text{cmc}} = \gamma_{\text{o}} - \gamma_{\text{cmc}}$$

where γ_{o} is the surface tension of pure water and γ_{cmc} is the surface tension of the surfactant solution at CMC. The most efficient one results in the greatest lowering in surface tension at critical micelle concentration. SBSD and SBVD were found to be the most efficient compounds, giving the largest reduction in surface tension at CMC because they achieved a lower surface tension at CMC, Table 3.

The interfacial activity (I_{activ}) which expressed physicochemical parameter $\mathbf{\Delta G_{ads}/A_{min}}$ where $\mathbf{\Delta G_{ads}}$ is a standard free energy of adsorption of the surfactant at the air/solution interface and $\mathbf{A_{min}}$ is the minimum surface area of the surfactant as shown in Table 3. It is clear from Table 3 that $\mathbf{I_{act.}}$ decreased by increasing the alkyl chain and the high depression were observed for SBSS.

Thermodynamic of Adsorption and Micellization

The micellization and adsorption processes of the amphiphile molecules occurred instantly, but commonly one process may be predominate over the other. The predominance of any of the two processes is governed by the thermodynamic variables of this process.

From Table 3 values of $\mathbf{\Delta G_{mic}}$ and $\mathbf{\Delta G_{ads}}$ are always negative indicating these two processes are spontaneous; however, there is a slight increase in the negative values of $\mathbf{\Delta G_{ads}}$ compared to those of micellization. This suggests the tendency of the molecules to be micellized at the interface and the presence of these amphiphiles at the interface to decrease the phases interaction.

Biological Activity

Table 4 shows the antibacterial activity of the prepared anionic Schiff bases (IIIa–d), nickel SBSD complex and $NiCl_2.6H_2O$. Chloramphenicol and cephalothin were taken as the reference drugs for antibacterial activity. The difference in activity depends on the length of hydrophobic chains and the interfacial properties of the prepared surfactants. Increasing the hydrophobic chain length increase the antibacterial activity, these results are in consistence

Table 3 Surface parameters of the synthesized anionic Schiff base amphiphiles

Surfactant	IT mN/m	CMC 10^{-3} mol/L	π_{cmc} mN/m	$C_{20} \times 10^{-3}$ mmol/L	PC_{20} mmol/L	A_{min} nm^2	ΔG_{mic} kcal/mol	ΔG_{ads} kal/mol	$I \times 10^2$ activity
SBSD	33.0	5.01	39.0	2.82	2.55	205.32	−14.87	−15.35	−7.48
SBSS	30.5	3.16	34.5	4.47	2.35	155.29	−16.01	−16.33	−10.52
SBVD	34.5	11.22	37.5	2.51	2.60	164.68	−12.87	−13.24	−8.04
SBVS	31.5	5.62	33.5	3.16	2.50	143.72	−14.58	−14.87	−10.35

$\Delta G_{mic} = [2.303\ RT \log CMC] - [\log 55.5]$, $\Delta G_{ads} = \Delta G_{mic} - (0.6023\ 10^{-2})\ \Pi_{cmc}\ A_{min}$

Table 4 Biological activity of the synthesized Schiff bases and $[Ni\text{–}(SBSD)_2]\cdot 2H_2O$ complex

Compounds	Tested microorganism							
	Staphylococcus aureus (G^+)		*Streptococcus pyogenes* (G^+)		*Pseudomonas phaseolicola* (G^-)		*Pseudomonas fluorescens* (G^-)	
Concentration	2	1	2	1	2	1	2	1
SBSD	5	3	7	3	10	7	8	4
SBSS	18	16	22	17	16	11	15	10
SBVD	20	14	17	15	16	10	18	14
SBVS	22	18	20	14	22	15	21	14
$NiCl_2\cdot 6H_2O$	15	13	18	16	14	11	17	14
Ni-SBSD	32	28	30	26	32	21	28	24
Control	42	28	38	30	36	25	38	30

Control Chloramphenicol was used as the standard for the Gram positive and cephalothin for the Gram negative bacteria

with the adsorption tendency of these amphiphiles at the interface.

The relationship between interfacial activity of the surfactants and their biological activity for some strains of microorganisms was studied by Stephen and Donald [30]; in each case the interfacial activity is expressed by $\Delta G_{ads}/A_{min}$, Tables 3, 4 and Figs. 5, 6 indicate that there is an excellent linear relationship between interfacial activity and biological activity. Also, Table 4 shows the antibacterial activity of the synthesized nickel–SBSD complex and clear that the complexation of the Schiff bases by nickel cation increases their antibacterial activity.

Metal chelates generally have higher antibacterial activity than the free ligands due to an increase in cell permeability [31]. 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 raises the liposolubility thus favoring its permeation through the lipid layer of a microorganism. Therefore it confirms that chelation of the anionic Schiff bases with a transition metal tends to make the ligands act more powerfully against bacteria.

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