MATERIALS & EXPERIMENTAL TECHNIQUES

3- MATERIALS AND EXPERIMENTAL TECHNIQUES

3.1) Materials:

The chemicals used in this study are submitted from Aldrich Chemicals Co. and all of them were in pure grade and used as it is, and the solvents used such as absolute ethyl alcohol, diethyl ether and acetone are pure grade.

A series of quaternary iminium salts namely N-(4-methoxybenzylidene)-N-benzyldodecyliminium chloride (I_a), N -(4-methoxybenzylidene)-N-benzylhexadecyliminium chloride (I_b), N-(4-methoxybenzylidene)-N-benzyloctadecyliminium chloride (I_c), N-benzylidene-N-benzyldodecyliminium chloride (II_a), N-benzylidene-N-benzylhexadecyliminium chloride (II_b), N–benzylidene-*N*-benzyloctadecyliminium chloride (II_c), N-butylidene-N-benzyldodecyliminium chloride (III_a), N-butylidene-N-benzylhexadecyliminium chloride (III_b), and N-butylidene-N-benzyloctadecyliminium chloride (III_c); were prepared through condensation of fatty amines with the aldehydes, then quaternization the product with benzyl chloride.

3.1.1) Synthesis of Schiff Bases:

Different alkyl Schiff bases were synthesized throughout condensation reaction of different alkyl fatty amines, mainly: dodecyl, hexadecyl and octadecyl amine with aldehydes namely: benzaldehyde, anisaldehyde and butyraldehyde to produce the corresponding Schiff base.

Equimolar amounts of desired fatty amines and aldehydes were refluxed in ethanol for 6 hours. The reaction mixture was left to cool and filtered. The products were recrystallized twice from ethanol, and dried. **(B. Erk *et al*, 2000).**

3.1.2) Synthesis of Quaternary Salts:

To a solution of 0.1 mol of Schiff base in absolute ethanol (40 mL), benzyl chloride (12.66 g, 0.1 Mol) was added. The reaction mixture was refluxed for 45 to 90 hours depending on the fatty amine chain length. The reaction mixture was evaporated under reduced pressure; the solid residue was washed with acetone three times to give quaternary iminium salt (I_{a-c}, II_{a-c}, III_{a-c}).

3.2) Structural Confirmations of Prepared Compounds:

The chemical structure of prepared compounds was confirmed by:

FTIR spectra using ATI Mattsonm Infinity series™, Bench top 961 controlled by Win First™ V2.01 software. (Egyptian Petroleum Research Institute), ^{1}H-NMR was measured in DMSO-d_6 by Spect Varian, GEMINI 200 (^{1}H 200 MHz). (Micro Analytical Center, Cairo University) and Mass spectra was measured by GC MS-OP1000EX (Micro Analytical Center, Cairo University).

3.3) Experimental Techniques:

3.3.1) Determination of Surface Active Properties of Prepared Compounds

1) Surface and Interfacial Tension:

Surface and interfacial tension of aqueous solutions of prepared compounds were measured using Du-Nouy tensiometer (Kruss type 8451). Surface tension was measured at different concentrations at different temperatures (15°C, 35°C, 45°C). The interfacial tension measured between 0.1% surfactant solution and light paraffin oil was at 25°C.

2) Determination of Surface Parameters of Prepared Compounds:

Critical micelle concentration CMC, maximum surface excess Γ_{max}, minimum surface area A_{min}, effectiveness π_{cmc}, and efficiency Pc_{20} of prepared compounds were determined as follows:

a) Critical Micelle Concentration (CMC):

The critical micelle concentration values (CMCs) of prepared compounds were determined using surface tension measurements. Where in this method, surface tension values of aqueous solutions of prepared surfactants were plotted versus the corresponding concentrations. The interrupt change in the surface tension-concentrations (SC) curves expresses the critical micelle concentrations.

b) Effectiveness (π_{CMC}):

π_{CMC} is the difference between the surface tension of pure water (γ_o) and the surface tension of surfactant solution (γ) at the critical micelle concentration.

$$\pi_{CMC} = \gamma_o - \gamma$$

c) Efficiency (PC_{20}):

Efficiency (PC_{20}) is determined by the concentration (mol/L) of surfactant solutions capable to suppress the surface tension by 20 dyne/cm.

d) Maximum Surface Excess Γ_{max}:

The values of maximum surface excess Γ_{max} may calculated from surface or interfacial data by the use of Gibbs equation (Shuichi, M. *et al* ; 1991)

$$\Gamma_{max} = (- 1 / 2.303\ RT)\ (\delta\gamma\ /\ \delta \log c)_T$$

Where

Γ_{max} maximum surface excess in mole/cm^2

R universal gas constant 8.31 x 10^7 ergs mole^{-1} K^{-1}

T absolute temperature (273.2 + °C)

$\delta\gamma$ surface pressure in dyne/cm

C surfactant concentration (mol)

$(\delta\gamma/\ \delta \log c)_T$ is the slope of plot surface tension vs. concentration curves below CMC at constant temperature.

e) Minimum Surface Area (A_{min}):

The area per molecule at the interface provides information on the degree of packing and the orientation of adsorbed surfactant molecule. The average area (in square angstrom) occupied by each molecule adsorbed on the interface (Shuichi, M. *et al.*, 1991) is given by:

$$A_{min} = 10^{16} / \Gamma_{max} N$$

Γ_{max} maximum surface excess in mole / cm^2

N Avogadro's number 6.023×10^{23}

3) Determination Thermodynamic Parameter of Micellisation and Adsorption:

The thermodynamic parameters of adsorption and micellization of synthesized cationic surfactants were calculated according to Gibb's adsorption equations as follows:

$$\Delta G^{o}_{mic} = RT \ln (CMC)$$

$$\Delta G^{o}_{ads} = \Delta G^{o}_{mic} - 6.023*10^{-2} * \pi_{CMC} * A_{min}$$

$$\Delta S_{mic} = - d (\Delta G^{o}_{mic} / \Delta T)$$

$$\Delta S_{ads} = - d (\Delta G^{o}_{ads} / \Delta T)$$

$$\Delta H_{mic} = \Delta G^{o}_{mic} + T \Delta S_{mic}$$

$$\Delta H_{ads} = \Delta G^{o}_{ads} + T \Delta S_{ads}$$

4) Determination Foaming Power:

Foam height of 0.1 % solutions of prepared surfactants were measured in bi distilled water using Ross-Miles, the test was carried out at 25°C and 35°C; the half life time was measured **(Ross *et al*, 1941).**

5) Determination Corrosion Inhibition of Prepared Compounds:

The weight loss technique [ASTM G31-72 Approved 1990] was used to measure the corrosion inhibiting effect of prepared quaternary compounds for mild steel specimens having a composition (wt %): 0.21 C, 0.035 Si, 0.51 Mn, 0.82 P, and the remainder is Fe. Each specimen was sequentially was machined into regular shapes of 54.006 cm^2 cross-sectional area. The specimens were sequentially abraded with different emery paper, degreased with acetone, washed with distilled water and dried. Corrosive solutions of 1M HCl in absence and in presence of inhibitors at concentration between $1*10^{-5}$ to$1*10^{-3}$ M which prepared from bi distilled water.

The inhibition efficiency (E %) of an inhibitor was calculated from the following equation:

$$\mathbf{E\% = \{(CR - CR')/ CR\} *100}$$

Where CR and CR' are the corrosion rates of carbon steel in absence and presence of inhibitor at given inhibitor concentration and temperature, which calculated from the following equation:

$$\mathbf{CR = K W / ATD}$$

Where

W is the weight loss in g.

A is the Area of mild steel species in Cm^2.

T is the time of immersion in hours.

D is the density of species (g/cm^3)

K Constant ($3.45*10^6$)

The density of mild steel is 7.88 g/cm^3

6) Determination of Antimicrobial Activity of Prepared Compounds:

The antimicrobial activity of synthesized products was measured against a wide range of test-organisms comprising: (bacteria and fungi)

1-Source of microorganisms:

The different species of tested organisms were obtained from the unit of Micro Analytical Center, Cairo University, Cairo, Egypt.

2-The media

The following media used in the antimicrobial activity of synthesized products, the bacterial species grow on nutrient agar, while fungi mould grow on Czapek's dox agar.

a) Nutrient agar

Nutrient agar consists of:

Beef extract	3.0 g/l
Peptone	5.0 g/l
Sodium chloride	3.0 g/l
Agar	20.0 g/l

Then, complete the volume to one liter, heated the mixture until the boiling, and sterilize the media by autoclave.

b) Czapek's Dox agar

Czapek's Dox agar consists of:

Sucrose	20.0 g/l
Sodium nitrate	2.0 g/l
Magnesium sulfate	0.5 g/l
Potassium Chloride	0.5 g/l

Ferrous sulfate	0.01 g/l
Agar	20.0 g/l

Then, complete the volume to one liter, heated the mixture until the boiling, and sterilize the media by autoclave.

c) Microorganisms

Bacteria

Gram-positive: *Staphylococcus albus*

Gram-positive: *Streptococcus faecalis*

Gram-positive: *Bacillus subtilis*

Gram-negative: *Escherichia coli*

Fungi

Candida albicans

Asperigllus flavus

An assay is made to determine the ability of an antibiotic to kill or inhibit the growth of living microorganisms, the technique which used is:

Filter-paper disc-agar diffusion (Kirby-Bauer) (D. N. Muanza *et al*,1994).

1. Inoculate flask of melted agar medium with the organism to be tested.
2. Pour this inoculated medium into a Petri dish.
3. After the agar has solidified, a multilobed disc that impregnated with different antibiotics laid on top of agar.
4. The antibiotic in each lobe of disc diffuses into medium and if the organism is sensitive to a particular antibiotic, no growth occur in a large zone surrounding that lobe (clear zone).

5. The diameters of inhibition zones were measured after 24-48 hours at 35-37°C (for bacteria) and 3-4 days at 25-27°C (for yeast and fungi) of incubation at 28°C

Measure each clear zone and compare between them to determine the antibiotic which is more inhibitor.