PYRIDINIUM, PIPERIDINIUM AND MORPHOLINIUM CATIONIC SURFACTANTS(1): SYNTHESIS, CHARACTERIZATION, HEMOLYSIS AND ANTIOXIDATIVE EFFICIENCY.

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ABSTRACT

A Series of pyridinium, piperidinium and morpholinium derived cationic surfactants differing in the length of side alkylating chain from C_{10} to C_{18} is described. The compounds were characterized by IR and H^1NMR spectra and an HPLC method used for distintion of all prepared long-chain pyridinium, piperidinium and morpholinium analogues has been successfully developed. The surface properties of these surfactants including critical micelle concentration (CMC), efficiency (Pc_{20}), effectiveness (γ_{cmc}), maximium surface excess (r_{max}) minimum surface area (A_{min}) and free energy of micellization ($\Delta G°_{mic}$) were investigated. The results of studies on hemolytic and antioxidative activities of the prepared compounds differing in alkyl chain length are presented. The hemolytic studies permitted to determine the safe concentration at which the compounds studied did not damage RBC membranes and they may also be used as effective antioxidants.

Keywords: Cationic surfactants, pyridinium, piperidinium, morpholinium, surface properties, HPLC, hemolysis activity, antioxidative activity.

1. INTRODUCTION.

Cationic surfactants have attracted the attention of chemists for a long time, due to their general simple synthesis and their wide application in many fields. Pyridinium surfactants **(Singh, *et al.*, 2009)** in particular, are important as ingredients of cosmetic products **(Martins, *et al.*, 2009)** and are also used as corrosion inhibitors **(Saleh, 2006)**, in emulsion polymerization **(Madaan and Tyagi, 2008)** and textile processing **(Perea-Carpio, 2003)**. Cationic surfactants usually consist of a hydrophilic part represented by quaternary nitrogen moiety and a hydrophobic part represented by a long alkyl chain.

Quite recently, cationic pyridinium surfactants have found applications in the synthesis of TiO_2 nanoparticales **(Ardizzone, *et al.*, 2006)**, in ion liquids synthesis **(Deng, *et al.*, 2007)** or as electrolytes for dye-sensitized solar cells **(Gorolov, *et al.*, 2007).**

Some of the pyridinium salts (C_{12} and C_{16}) were used to solubilize water insoluble compounds in analytical chemistry applications, where these can also serves as a qualitative and quantitative tool **(Akbas and Kartal, 2006)**

It has been known that these compounds are able to form micelles, which play an important role in a decontamination process **(Epstein, *et al.*, 1978; Cabal, *et al.*, 2007; Tiwari, *et al.*, 2010).** These formations are created in water solution, when the critical micellar concentration (cmc) is exceeded. Therefore, many cationic surfactants work as micellar catalysts, i.e. chemical reactions can be accelerated or inihibited by them **(Singh, *et al.*, 2011; Ghosh, *et al.*, 2010, Tiwari, *et al.*, 2009; Tiwari, *et al.*, 2010a and Tiwari, *et al.*,**

2010b), and they are often used to prepare micellar environment for chemical reactions. Dwars et al **(Dwars, *et al.*, 2005)** published a very extensive review on micellar catalysis.

The effects of structural variations in cationic pyridinium surfactants have been widely studied **(Nusselder and Engberts, 1991a; Nusselder, and Engberts, 1991b; Bijma, *et al.*, 1998, Bijma and Engberts, 1997; De Gooijer, *et al.*, 2000)**. These include variation of the tail length and variation of counterion. The change in the logarithm of the CMC as a function of the tail length for surfactants with linear alkyl chains has been described by the Shinoda equation **(Shinoda, 1953)** yielding a linear correlation **(Nusselder and Engberts, 1991b)**. An increase in the length of the alkyl group or the nitrogen of the pyridine leads to a lower CMC value due to the increasing hydrophobocity of the surfactant **(Nusselder and Engberts, 1991a).** When the counterion is changed from iodide to bromide or to chloride, the CMC also has a tendency to increase (**Bijma and Engberts, 1997; De Gooijer, *et al.*, 2000**; **Shinoda, 1953 and Lindman and Wennerstro¨m, 1980).**

Surfactants with different head groups such as, pyridinium chloride, trimethylammonium chloride, and triethanolammonium chloride were prepared and were characterized by spectral (FT-IR and ^{1}H NMR) and physicochemical properties (surface tension, critical micelle concentrations, Kraft point, cloud point, foaming height, wetting power, emulsification power, biodegradablility and antimicrobial activity) **(Ismail, 2013).** The piperidinium, imidazolium, pyridinium, morpholinium, quinolinium were prepared by pairing quaternary ammonium salts **(Andreea, *et al.*, 2013)**

Cationic surfactants offer advantages over other class of surfactants **(Jungerman, 1969; Cross and Singer, 1994; Holland and Rubingh (Eds.), 1991 and Richmond, 1990)**. These substances besides their surface activity do show antibacterial properties, hemolytic and antioxidative efficiency and might used be as cationic softeners lubricants, retarding agents and antistatic agents.

In the present study, three new series of pyridinium, piperidinium and morpholinium alkyl chlorides with even number of carbon atoms in the hydrocarbon chain (n=10, 12, 14, 16 and 18) were synthesized. The effects of structural variations in the prepared cationic surfactants have been studied. These include variation of the heterocyclic ring and variation of the tail length. Their structures were confirmed by physical –chemical methods and HPLC method of distinguishing members of these whole series was proposed. The investigation of their behaviour was performed by surface tension measurements trying to evaluate the influence of changing the alkyl chain length on the aggregation properties. Hemolytic studies were also performed to determine the safe concentration at which the prepared compounds did not damage RBC membranes. Antioxidative activity allowed to find correlation between it and the hydrophobicity of the compounds.

2. MATERIALS AND METHODS

2.1 Materials

Pyridine, piperidine and morpholine were purchased from Fluka. Thionyl chloride and fatty alcohols (decyl, dodecyl, tetradecyl , hexadecyl and octadecyl alcohols) were purchased from Merck. All solvents were redistilled just before used. The glassware used for synthesis was heated overnight in an oven at 150 ℃ and assembled in the oven, then

cooled before starting the reactions.Triple distilled water from an all pyrex glass apparatus was used for the preparation of solutions for measurements.

FT-IR spectra were recorded in KBr on a Shimadzu FT-IR spectrometer.

^{1}HNMR (400 MHz) spectra were recorded on a Jeol EX400 NMR Spectrometer in DMSO-d_6 using the DMSO signal as a reference.

HPLC system consisted of a P200 gradient pump (Spectra-Physics Analytical Fremont, USA), a 7125 injection valve – 10 µl loop (Rheodyne, Cotati, USA), an UV1000 detector (Spectra-Physics Analytical, Fremont, USA), and a CSW Chromatography Station 1.5 software(DataApex, Prague, Czech Republic).

Surface tension measurements of the prepared surfactants were carried out (25°C) with Du Nouy tensiometer (Kruss type 8451), using distilled water solution of 0.1% weight concentration .

Hemolysis was measured by spectrophotometer with wave length 540 nm.

Antioxidant activity: Absorbance measurements were recorded with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201).

2.2. General procedures

2.2.1. Synthesis of fatty alkyl chlorides:

The preparation of fatty alkyl chlorides(R= C_{10} , $C_{12,}$ C_{14} $C_{16\ and}$ C_{18}) was described by Weil et al **(Weil *et al.*, 1960)**.

Dodecyl derivatives were taken as examples

2.2.2.*Synthesis of fatty pyridinium chlorides* (Pierluigi, *et al.*, 2009):

Pyridine (0.1mol, 0.79 g) and chlorododecane (2mol, 4.09 g) were introduced under nitrogen in round bottom flask. Toluene was added and the reaction was stirred and warmed at 120^0C for 7h. The resulting mixture was cooled to room temperature and washed repeatedly with diethyl ether to remove both toluene and chlorododecane. The product was recrystallized in warm ethanol and cooled in a refrigerator, giving a viscous brownish red oil .Yield (65 %).

FTIR spectra of N-dodecyl pyridinium chloride

IR Spectra showed the following absorption bands at 717 cm^{-1}(CH_2 rocking), 1307.6 cm^{-1} (CH_2 deformation), 2854.5-2923.9 cm^{-1}(CHstretched) and 3359.8 cm^{-1} (C-N+) The FTIR spectra confirmed the expected functional groups in the synthesized cationic surfactants.

1H NMR (300 MHz, chloroform) ppm, 4.73 (t, J = 7.3 Hz, 2H, -CH_2-),4.99(t, 2H, 1.25 (m, 22H, -$(CH_2)_{11}$-), 0.82 (t, 3H, -CH_3), 1.49 (m , 2H, $CHCHN^+(C_{12}H_{25})_2$ and 7.27 (t,H,Ar-H)

2.2.3. *Synthesis of fatty Piperidinium and Morpholinium chlorides:*

Piperidine (1mol, 0.85 g) or Morpholine (1mol, 0.87g) and chlorododecane (2mol, 4.09g) were refluxed in toluene for 24 h.The resulting mixture was cooled to room temperature and washed repeatedly with diethyl ether to remove both chlorododecane and toluene then evaporated under reduced pressure giving a viscous red oil . The product was recrystallized by using ethanol. **Fig 1**

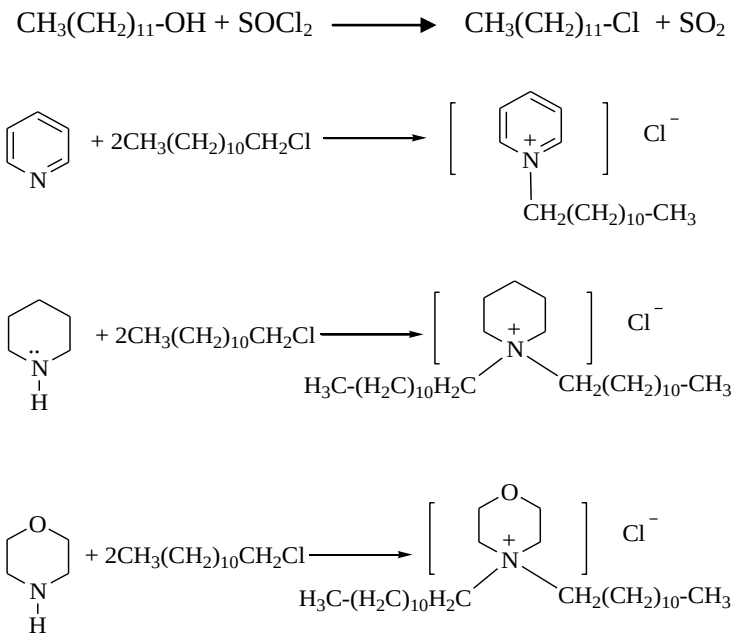

Fig.1. Cationic surfactants based on pyridine, piperidine and morpholine with different alkyl chains (C_{10}, C_{12},C_{14}, C_{16} and C_{18})

FTIR spectra of N-dodecyl piperidinium chloride

IR Spectra showed the following absorption bands at 717.5 cm-1(CH_2 rocking), 1377.1 cm^{-1} (CH_2 deformation) 2854 .5- 2923.9cm^{-1} CH(stretched) and 3402.2 cm^{-1} (C-N+) The FTIR spectra confirmed the expected functional groups in the synthesized cationic surfactants.

^{1}H NMR (300 MHz, chloroform) ppm, 4.73 (t, J = 7.3 Hz, 2H, -CH_2-),4.99(t, 2H,CH_2N+($C_{12}H_{25}$)$_2$) 1.25 (m, 22H, -$(CH_2)_{11}$-), 0.81 (t, 3H, -CH_3) and 1.49 (m , 2H, $CHCHN^+$ ($C_{12}H_{25}$)).

FTIR spectra N-dodecyl morpholinium chloride

IR Spectra showed the following absorption bands at 721 cm^{-1} (CH_2 rocking), 1311.5 cm^{-1} (CH_2 deformation) 2854 cm^{-1} CH(stretched) and 3282.6 cm^{-1} (C-N+) The FTIR spectra confirmed the expected functional groups in the synthesized cationic surfactants.

^{1}H NMR (300 MHz, chloroform) ppm, 4.73 (t, J = 7.3 Hz, 2H, -CH_2-),4.978(t, 2H,CH_2CH_2N+($C_{12}H_{25}$) $_2$, 1.26 (m, 22H, -$(CH_2)_{11}$), 0.86 (t, 3H, -CH_3) and 1.48 (m , 2H, $CHCHN^+$($C_{12}H_{25}$)$_2$)

3.Evaluation Methods of Surface Active Properties

***3.1.Surface tension measurement* (Aiad, *et al.*, 2012)**

The surface tension of the used distilled water was 73 mN/m. Surfactant solutions were aged for 1 h before any measurements were made. Three readings were carried out each sample to determine any change with time and to obtain an average value.

***3.2. Determination of cmc* (Hikota T, and Merguro K. 1970)**

By the surface tension method, cmc of the prepared surfactant was determined. In this method, values of the surface tension obtained for various concentrations of aqueous solutions of the prepared surfactants were plotted vs the corresponding concentrations.

3.3. Efficiency (PC_{20}) (Bhattacharyya, *et al.*, 1994)

The efficiency (PC_{20}) was determined as the concentration (mol/l) capable of suppressing the surface tension by 20 dyne/cm. The efficiency was determined by extrapolating from ɣ = 52 to the linear portion before CMC of the ɣ vs logC plot at 25˚c.

3.4. Effectiveness (π_{cmc}) (Khaled and Hackerman, 2003)

The surface tension (γ_{cmc}) values at cmc were used to calculate the values of surface pressure (effectiveness), using the following expression:

$$\pi_{cmc} = \gamma_o - \gamma_{cmc}$$

Where γ_o is the surface tension measured for pure water at the appropriate temperature and γ_{cmc} is the surface tension at cmc. The effectiveness of adsorption is an important factor that determines such properties of the surfactant as foaming, wetting, and emulsification, since tightly packed, coherent interfacial films have very different interfacial properties from loosely packed, noncoherent films.

3.5.Maximum surface excess (Γ_{max}) (Zana, 2002)

The surface excess concentration is defined as the surface concentration at surface saturation; the maximum surface excess (Γ_{max}) is a useful measure of the effectiveness of adsorption of the surfactant at the water-air interface, since it is the maximum value to which adsorption can obtained.

$$\Gamma_{max} = \frac{1}{2.303RT}\left(\frac{\delta\gamma}{\delta \log C}\right)_T$$

where R = 8.314 $Jmol^{-1}$ K^{-1} , T is absolute temperature, ($\delta\gamma/\delta$ log C) is the slope of the γ vs Log C plot at 25°C. A substance which lowers the surface tension is thus present in excess at or near the surface, i.e., when the surface tension decreases with increasing the activity of the surfactant, Γ is positive.

3.6.Minimum surface area (A_{min}): (Gamboa, *et al.*, 2006)

A_{min} is the minimum area per molecule of the prepared compounds at the interface and is calculated from the following equation:

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

where N is Avogadro's number and Γ_{max} is the maximum surface excess.

3.7. The standard free energy of micellization ΔG^o_{mic} (Badawi, *et al.*, 2007)

Understanding the process of micellization is important for explaining the effects of structural and environmental factors on the value of the cmc and for predicting the effects on it of new structural and environmental variations. Standard free energy of micellization (ΔG^o_{mic}) plays an important role in facilitating such understanding.

The standard free energy of micellization is given by:

$$\Delta G^o_{mic} = RT \ln cmc$$

4. HPLC Analysis: (Jan Marek, *et al.*, 2012)

After the preparation of the pyridinium, piperidinium and morpholinium salts, an appropriate HPLC method for their distinction in the mixture was developed. The HPLC system consisted of a PLC 1110 gradient pump (Spectra-Physics Analytical Fremont, USA), a 7125 injection valve – 10 µl loop (Rheodyne, Cotati, USA), an UV1000 detector (Spectra-Physics Analytical, Fremont, USA), and a win chrome Chromatograph ver.1.3 Station 1.5 software(DataApex, Prague, Czech Republic). A 250×4.6 mm (5 µm) column was used (Supelco Inc., Bellefonte, USA) for analyses. The mobile phase consisted of 45 % acetonitrile and55 % water. This mixture was prepared as a 0.1 M sodium acetate solution. Finally, the pH was adjusted with acetic acid to 5.0. The samples were delivered isocratically at a flow-rate of 1 ml/min. The absorbance was measured at 257 nm.

5. Hemolytic experiments: (Stock and Dormandy, 1971)

Hemolytic activity of the prepared surfactants was carried out at the Regional Center for Mycology & Biotechnology in Al-Azhar University.

Fresh heparinized blood was used in hemolytic experiments. Blood was centrifuged for 3 min at 3000 x g, the plasma removed and the cells washed twice with isotonic phosphate buffer solution (131 mm NaCl, 1.79 mm KCl, 0.86 mm $MgCl_2$, 11.80 mm $Na_2HPO_4.2H_2O$, 1.80 mM $Na_2HPO_4.H_2O$) of pH 7.4. The erythrocytes were then incubated for half an hour at 37 ℃ in the same solution containing different concentrations of the compounds studied. 2% hematocrits was used. The linear dependence of hematocrit on the concentration enabled, by extrapolation, to calculate the unit hematocrit (1%). After the modification samples were taken, centrifuged and the supernatant was assayed for hemoglobin content using spectrophotometer with wave length 540 nm. The hemoglobin concentration in the supernatant of totally hemolyzed erythrocytes was a measure of the extent of hemolysis. Good mixing of the suspension during all procedure stages was insured.

6. Antioxidant assay: (Dodge, *et al.*, 1963)

DPPH Radical Scavening Activity

The antioxidant activity of extract was determined at the Regional Center for Mycology and Biotechnology (RCMB) at Al-Azhar University Cairo, Egypt by the DPPH free radical scavenging assay in triplicate and average values were considered,. Freshly prepared (0.004% w/v) methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was prepared and stored at 10℃ in the dark. A methanol solution of the test compound was prepared. A 40 uL aliquot of the methanol solution was added to 3 ml of DPPH solution. Absorbance measurements were recorded immediately with a UV-visible spectrophotometer (Milton Roy, Spectronic 1201). The decrease in absorbance at 515 nm was determined continuously, with data being recorded at 1 min intervals until the absorbance stabilized (16 min). The absorbance of the DPPH radical without antioxidant (control) and the reference compound ascorbic acid were also measured. All the determinations were performed in three replicates and averaged. The percentage inhibition (PI) of the DPPH radical was calculated according to the formula:

$$PI = [\{(AC-AT) / AC\} X 100] \quad (1)$$

Where AC = Absorbance of the control at t= 0 min and AT= absorbance of the sample +DPPH at t = 16 min.

7. Results & Discussion

A series of cationic surfactants based on pyridine, piperidine and morpholine with different alkyl chains (C_{10}, C_{12},C_{14}, C_{16} and C_{18}) were synthesized according to the procedure outlined in Fig.1. The results obtained with the prepared compounds are shown in table 1 (yields, melting point and HPLC retention times). The satisfactory purity was usually reached after one or two crystallizations from the proper solvent. It was observed that yields increased with the increasing of alkyl chain length and the compounds with the chain length of (C_{10} *and* C_{12}) were difficult to convert into crystals due to their low melting points.

Table 1. Yields, melting points and retention times of pyridinium, piperidinium and morpholinium alkyl chlorides:

Comp. No	Side alkylating chain (R)	yield (%)	m.p (c)	HPLC. Rt(min)
2a	C_{10}	60	Oily	3.15
2b	C_{12}	65	Oily	4.97
2c	C_{14}	70	33-35	8.20
2d	C_{16}	75	42-44	9.03
2e	C_{18}	80	51-53	13.94
3a	C_{10}	70	Oily	3.20
3b	C_{12}	75	Oily	3.29
3c	C_{14}	85	50-52	4.05
3d	C_{16}	90	60-62	5.43
3e	C_{18}	92	65-67	8.54
4a	C_{10}	55	Oily	4.30
4b	C_{12}	60	Oily	6.42
4c	C_{14}	70	55-57	8.56
4d	C_{16}	80	65 -67	10.01
4e	C_{18}	82	70-72	11.56

Additionally, the HPLC method for products obtained in this study was developed. It allows distinguishing all prepared cationic surfactants (pyridinium, piperidinium and morpholinium alkyl chlorides) . HPLC data show that the shortest retention time was formed for the C_{10} pyridinium, piperidinium and morpholinium alkyl chlorides. It is supposed that this novel HPLC assessment could be easily used for characterization of mixtures of related pyridinium, piperidinium and morpholinium compounds.

The surface tension (γ) of surfactants was measured for a range of concentrations above and below the critical micelle concentration (CMC). Representative plots of surface tension versus -logC for pyridinium, piperidinium and morpholinium alkyl chlorides are shown in Figs.2, 3 and 4.

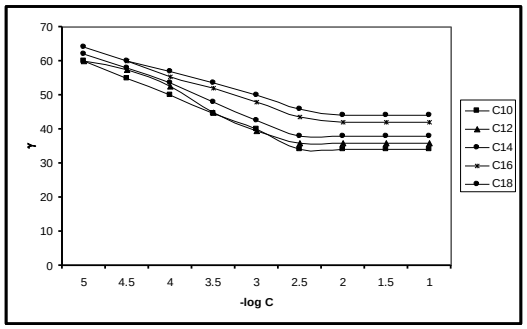

Fig. (2): Ssurface tension(ɣ)vs –logc for pyridinium alkyl chlorides

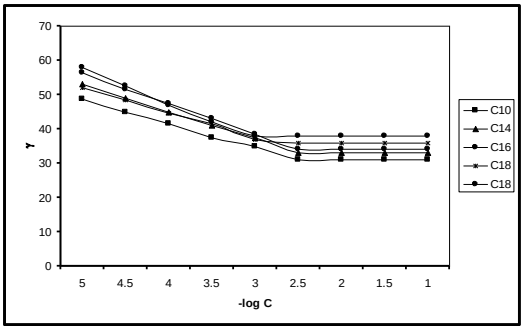

Fig. (3): Surface tension(ɣ)vs –logc for piperidinium alkyl chlorides

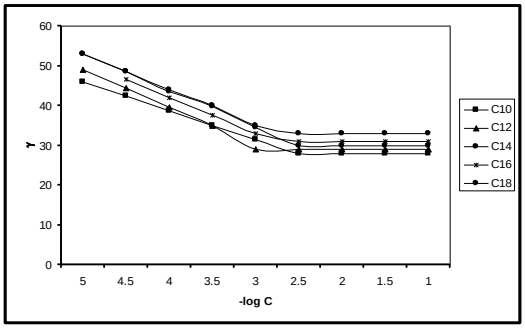

Fig. (4): Surface tension(ɣ)vs –logc for morpholinium alkyl chlorides

It is also observed that when the logarithms of concentration (C) of surfactant series at constant temperature are plotted against the number of carbon atoms in the alkyl chain, a linear plot is often obtained. Such plots are illustrated in Fig 5 and the data show a linear dependance.

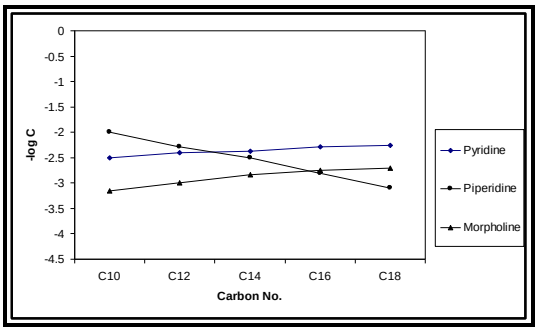

Fig. (5): -log(C) VS Carbon No. of pyridinium, piperidinium and morpholinium alkyl chlorides

Surfactants form aggregate of molecules or ions called micelles, which are formed when the concentration of the surfactant solute in the bulk of the solution exceeds a limiting value, the so-called critical micelle concentration (CMC), which is a fundamental characterization of each solute-solvent system.

A linear decrease in surface tension was observed with increase in concentrations for all surfactants up to the CMC, beyond which no considerable change was noticed. The CMC data obtained from the break point in the ɣ-log concentration plots are shown in table 2. The

CMC of a surfactant is regarded as a measure of the stability of its micellar form relative to its monomeric form. In the charged pseudo-phase model of micelle formation, the standard free energy of micelle formation per mole of surfactant is given by the equation shown in the experimental part.

A comparison of CMC for homologous series of surfactant demonstrates that increasing the length of the hydrocarbon chain has the tendency of lowering the concentration at which aggregation is initiated, owing to enhanced hydrophobic interaction between the counter ion and micellar core. Increasing the length of the hydrocarbon chain increases the average micellar aggregation number **(Small, 1986)**. Beside the evaluation of the CMC, several parameters can be obtained from surface tension measurements by using Gibbs adsorption equation shown in the experimental part and table 2.

Table 2. Surface tension characterization of pyridinium, piperidinium and morpholinium alkyl chlorides:

Comp No	Side alkylating chain (R)	$\gamma_{(cmc)}$ (mN/m)	Cmc $x10^{-3}$	C_{20} (Mm) $x10^{-3}$	Cmc/C_{20}	Pc_{20} (mol/cm²)	Π_{cmc}	Γmax (mol/A²)	A_{min} (nm^2)	ΔG_{mic}
2a	C_{10}	32	3.16	3.38	0.93	2.40	41.0	2.96	56.00	-14.40
2b	C_{12}	35	3.98	3.16	1.26	2.50	37.2	3.00	55.33	-13.40
2c	C_{14}	37	4.80	2.40	1.13	2.60	35.0	3.09	53.70	-13.00
2d	C_{16}	42	5.10	2.30	1.22	2.70	31.0	3.19	52.00	-12.80
2e	C_{18}	44	5.60	2.28	3.15	2.80	29.0	3.32	50.00	-12.58
3a	C_{10}	31	3.16	1.00	3.16	2.00	42.0	2.77	60.00	-13.90
3b	C_{12}	33	4.21	2.30	1.83	2.63	39.0	2.80	59.30	-13.29
3c	C_{14}	34	4.90	2.00	2.45	2.69	38.0	2.90	57.20	-13.00
3d	C_{16}	36	5.00	1.99	2.51	2.70	37.0	3.00	55.00	-12.90
3e	C_{18}	38		1.125	4.36	2.90	35.0	3.07	54.00	-12.88
4a	C_{10}	28	2.70	3.07	0.34	2.05	45.0	3.02	54.90	-14.40
4b	C_{12}	29	2.80	2.92	0.13	2.15	44.0	3.13	53.00	-16.94
4c	C_{14}	30	2.90	2.78	0.48	2.25	43.0	3.20	51.90	-14.18
4d	C_{16}	31	3.00	2.72	0.30	2.30	42.0	3.30	50.30	-14.10
4e	C_{18}	33	3.10	2.66	0.39	2.35	40.0	3.60	46.10	-14.00

The γ - log concentration plots provides information about area per molecule at air-water interface, effectiveness π_{cmc} and efficiency Pc_{20} of the surfactants.

Table 3 indicates the CMC/C_{20} ratio which estimates the tendency of the surfactant to form micelles relative to the tendency to adsorb at the air/water interface.

Table 3. Concentration of compounds inducing 50% (c_{50}) and 100% (c_{100}) hemolysis of erythrocytes (RBC) at hematocrit 2%, and causing 50% inhibition of peroxidation of erythrocyte membrane lipids (I_{50}) and (I_{20})are values of percentage inhibition of peroxidation of membrane lipids subjected to 20 Mm concentration of compounds studied.

parameters	Compounds														
	Pyridinium alkyl chlorides					Piperidinium alkyl chlorides					Morpholinium alkyl chlorides				
	2a	2b	2c	2d	2e	3a	3b	3c	3d	3e	4a	4b	4c	4d	4e
C_{50}[µg/ml]	13.7	17.93	20.01	22.91	24.69	6,83	7.96	10.56	11.06	12.27	3.36	5.64	6.96	7.69	8.99
C_{100}[µg/ml]	28.81	35.61	31.17	39.85	40.65	13.61	14.94	19.85	21.69	23.58	7.01	10.81	13.56	15.61	18.01
I_{50}[µg/ml]	8.1	9.5	10.2	18.1	21.0	7.5	8.8	13.6	17.5	19.3	6.8	7.9	10.5	12.9	19.5
I_{20}%	54	59	64	79	83	25	39	45	76	80	31	43	56	74	79

Antioxidation value of ascorbic acid (reference) = 11.2µg/ml

Where

2a Decyl pyridinium chloride. 3a Decyl piperidinium chloride. 4a Decyl morpholinium chloride.
2b Dodecyl pyridinium chloride. 3b Dodecyl piperidinium chloride. 4b Dodecyl morpholinium chloride
2c Tetradecyl pyridinium chloride 3c Tetradecyl piperidinium chloride. 4c Tetradecyl morpholinium chloride
2d Hexadecyl pyridinium chloride. 3d Hexadecyl piperidinium chloride. 4d Hexadecyl morpholinium chloride
2e Octadecyl pyridinium chloride. 3e Octadecyl piperidinium chloride. 4e Octadecyl morpholinium chloride

Values of efficiencies Pc_{20} of the prepared surfactants are shown in table 2. The efficiency increases with increasing molar ratio of methylene units. This is due to the fact that the efficiency of adsorption at interfaces increases linearly with increase in carbon atoms in the hydrophobic groups.

The effectiveness π_{cmc} is determined by the difference between surface tension values at CMC (γ_{CMC}) and the surface tension measured for pure water at the appropriate temperature γ_o . The most efficient one is that which gives the greatest lowering of surface tension for a given CMC. Pyridinium alkyl chloride was found the most efficient one because it achieved the maximum reduction of the surface tension at CMC. (Fig 2 and table 2).

The number of surfactant molecules at the air-water interface at the CMC at 25˚c is expressed as Γ_{max}. A substance lowering the surface energy is present in excess at or near the surface. It is clear that increasing the number of methylene units increases Γ_{max}.

The minimuim surface area A_{min} is defined as the area occupied by surfactant molecules at the air-water interface when the solution is at equilibrium. Data in table 2 show that the consequent increase of Γ_{max} leads to crowding at the interface. This is due to the decrease in the minimium surface area with the increase in the hydrophobic chain length of the prepared surfactants. Values of standard free energy of micellization ΔG^{o}_{mic} are always negative, indicating the spontaneousness of this process. Data in table 2 indicate that ΔG^{o}_{mic} increases slighty with increasing chain length.

The results obtained of hemolytic activity are summarized in table 3. Both the C_{50} and C_{100} parameters indicate that the hemolysing potency of the quats of alkyl pyridinium**,** piperidinium and morpholinium series increased with lipophilic chain length. Quats of longest chains (2e, 3e and 4e) (C_{18} pyridinium**,** C_{18} piperidinium and C_{18} morpholinium alkyl chlorides) were over twice more effective in hemolysing RBC than those of shortest chains (2a, 3a and 4a) (C_{10} pyridinium**,** C_{10} piperidinium and C_{10} morpholinium alkyl chlorides) because of their higher lipophilicity, meaning greater membrane structure disorder.

The antioxidative effect was measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical with UV light at 1 min intervals until the absorbance stabilized (16 min.) and used conc. of (50 μg/ml). Measurements of the antioxidative activities of the quats (table 3) showed that they followed the hemolytic sequence and increased with quats lipophilicity and was about twice greater for quats of longest alkyl chain compared with those of shortest alkyl chain. It can be seen that percentage inhibition of lipid peroxidation by I_{20} concentration increased with an increase of their hydrocarbon chain length. Thus, all the studied derivatives incorporated into erythrocyte membranes without evident damage, which is an essential condition for using them as an antioxidant (from 54% for C_{10} pyridinium derivative to 83% for C_{18} pyridinium derivative) except those of C_{10}, C_{12} piperidinium and morpholinium derivatives as well as C_{14} piperidinium derivatives were less than 50%.

We have also determined concentrations of compounds inhibiting peroxidation by 50% (I_{50}) (table 3). It can be seen that the longest alkyl chain compounds protected RBCs about 2 to 3 times better than the shortest alkyl chain ones for pyridinium (2c, 2d & 2e), piperidinium (3c, 3d & 3e) and morpholinium (4c, 4d & 4e) alkyl chlorides series. The reason for the found difference in the antioxidative activity must be due to different lipophilicity of the molecules. It was already shown that lipophilicity belongs to factors deciding of the depth of incorporation of a compound in the lipid phase of membrane **(Kleszczynska*, et al.*, 2000a and Kleszczynska*, et al.*, 2000b)**

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المركبات الكاتيونية ذات النشاط السطحى المشتقة من البيريدينيوم ، البيبيريدينيوم و المورفيلينيوم (١): التحضير، الخواص، دراسة النشاط الخاص بمضادات تكسير كرات الدم الحمراء و مضادات الأكسدة .

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لقد اوضحت هذه الدراسة مجموعة من المركبات الكاتيونية ذات النشاط السطحى المشتقة من البيريدينيوم ، البيبيريدينيوم و المورفيلينيوم والتى تختلف فى طول السلسلة الكربونية من كربون (١٠- ١٨) . ولقد تم اثبات المركبات المحضرة بواسطة طيف الأشعة تحت الحمراء و كذلك طيف الرنين المغناطيسى و كذلك بطريقة HPLC و ذلك للتفرقة بين كلا من مركبات البيريدينيوم ، البيبيريدينيوم و المورفيلينيوم طويلة السلسلة التى تم تحضيرها و تمت التفرقة بين هذه المركبات بهذه الطريقة بنجاح. وكذلك تم دراسة الخواص ذات النشاط السطحى لهذه المركبات متضمنة على القدرة على احداث الميسل عند التركيز الحرج (CMC) و كفاءة هذه المركبات لخفض التوتر السطحى (Pc_{20}) و كذلك التوتر السطحى عند التركيز الحرج (γ_{cmc}) وكذلك قيم اكبر عدد من المركبات الممتزة على السطح (r_{max}) و قيم مساحة السطح (A_{min}) و الطاقة الحرة لعمل الميسل ($\Delta G°_{mic}$) . و تضمنت الدراسة على نتائج النشاط الخاص بمضادات تكسير كرات الدم الحمراء و كذلك مضادات الأكسدة لهذه المركبات المحضرة و التى تختلف تبعا لطول السلسلة الهيدروكرنية و تهدف الدراسة الخاصة بمضادات تكسير كرات الدم الحمراء على تحديد التركيز الآمن التى لا يتم عنده تكسير كرات الدم الحمراء بواسطة هذه المركبات و كذلك فان المركبات المحضرة يمكن استخدامها ايضا كمضادات الاكسدة عالية النشاط.