CHAPTER II

EXPERIMENTAL

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II.1. Materials and reagents

All the chemicals and reagents used were of the AR grade and double distilled water was used throughout the experiments. *o*-Nitrophenyloctylether (*o*-NPOE) was supplied from Sigma, while dibutylphthalate (DBP), dioctylphthalate (DOP), dioctylsebacate (DOS) and tricresylphosphate (TCP) were purchased from BDH, Sigma, AVOCADO and Fluka, respectively. Polyvinylchloride (PVC relative high molecular weight) and graphite powder (synthetic 1–2 μm) were supplied from Aldrich. Cellulose acetate (CA, Fluka) was also applied as a binding material. In addition, commercial carbon ink was supplied from Gwent (C2010517D4, United Kingdom).

Cetylpyridinium chloride (CPCl, $C_{21}H_{38}NCl$ 339.986), cetyltrimethyl-ammonium bromide (CTABr, $C_{19}H_{42}BrN$ 364.5), dodecyltrimethylammonium bromide (DTABr, $C_{15}H_{34}NBr$ 308.3), didodecyldimethylammonium bromide (DDABr, $C_{26}H_{56}BrN$ 462.6) and sodium dodecylsulfate (SDS, $C_{12}H_{25}SO_4Na$ 288.38) was purchased form Fluka. Septonex (1-(ethoxycarbonyl) pentadecyltrimethyl-ammonium bromide, $C_{21}H_{44}O_2Br$ 288.38) were purchased from Slovakofarma (Hlohovec, CZ). Sodium tetraphenylborate (NaTPB, Fluka) was applied as a titrant for the cationic surfactants.

Phosphotungstic acid (PTA); H_3 [$PW_{12}O_{40}$], phosphomolybdic acid (PMA); $H_3[PMo_{12}O_{40}]$, were purchased from BDH, while silicotungstic acid (STA); $H_4[SiW_{12}O_{40}]$, ammonium reineckate (RN) [$NH_4(Cr(NH_3)_2(SCN)_4)$. H_2O] were purchased from Sigma and Fluka, respectively.

II.2. Solutions

II.2.1. Surfactant solutions

To eliminate the surfactant adsorption effects on the inner surface of vessels, a weighed amount of the surfactant was dissolved in water and filled up to the mark in a volumetric flask. The solution was left standing for at least one day to cover all adsorption centers of the vessel by molecules then the solution was discarded and prepared again in the same manner without intermediate rinsing the flask [257].

A stock solution (10^{-3} M) of CPCl was prepared by dissolving 0.35801 g in doubly distilled water (1L) with continuous stirring. Lower concentrations were prepared by accurate dilutions of the stock solution.

Stock solution (10^{-3} M) of Septonex, CTABr, DDABr and DTABr were prepared by dissolving the accurately weighted amounts in doubly distilled water and the solutions were standardized applying the appropriate recommended method [164, 166. 206]. A stock solution of 10^{-3} M of SDS was prepared by dissolving 0.288 g in distilled water (1L) with continuous stirring.

NaTPB solution (ca.10^{-2} M) was prepared by dissolving a weighed amount of the substance in worm water, adjusted to pH 9 by adding sodium hydroxide and completed to the desired volume with water. The resulting solution was standardized potentiometrically against standard (10^{-2} M) thallium (I) nitrate solution [12].

Aqueous solutions of PTA, PMA, STA and RN were prepared using the analytical grade chemicals and the exact concentrations of these solutions were determined by the appropriate recommended methods [258-260] and lower concentrated solutions were prepared by the appropriate dilutions. The stock solution and the dilutions were kept in dark bottles in the refrigerator at 4 °C.

II.3. Samples

II.3.1. Pharmaceutical samples

Pharmaceutical formulations containing surfactants such as Ezaflour mouth wash solution (Cairo Pharmaceutical and Chemical Industries, for Multipharma, each 100 ml contains 0.05 g CPCl), Citrolin mouth wash and throat disinfectant solution (Pharco Pharmaceuticals Alexandria, each 100 ml contains 0.025 g Cetrimide (cetyltrimethylammonium bromide, CTABr) and Femigin B (vaginal powder) and Antiseptic douche (Pharco Pharmaceuticals Alexandria, each sachet (2.4g) contains 0.063 g SDS) were obtained from local drug stores.

II.3.2. Detergent samples

Commercial detergents, Persil and OMO (Port Said for Detergents and Chemical Industries, Egypt), Arial and Tide (Procter and Gamble, Egypt) were obtained from local markets.

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II.3.3. Water samples

The anionic surfactants in waste water samples (Dakahlyia, Egypt) and the sea water sample (Port Said and Suez in Suez Canal area, Egypt) were also analyzed.

II.4. Instrumentation

Laboratory potential measurements were performed using a 692-pH meter (Metrohm) equipped with a double junction silver-silver chloride reference electrode (Metrohm 6.0726.100) in conjugation with different surfactant ISE. Commercial surfactant electrode (Cationic Surfactant Electrode, Metrohm 6.0507.150) was used as a second sensing electrode for comparing the results. The electrode thicknesses were measured by Coating Thickness Gauge MiniTest 600 Electrophysik, Germany. A portable system (Fig. 1) consisted of the bielectrode strip (containing both the reference and working electrode), Digital Multimeter (46-Range Digital Multimeter, Radioshack) connected to a portable PC and Brand digital burette was used for the field measurement of surfactants.

II.5. Preparation of the screen printed electrodes

A manual screen printer was used to produce disposal SPEs. An array of 12 electrodes was printed on a flexible X-ray film by forcing the prepared conductive ink to penetrate through the mesh of a screen stencil. The screen printing electrodes involved several stages which were described in details below [261]:

II.5.1. Selection of the screen template:

A screen consisting of a heavy duty polyester fabric (I 003 M Sefar Pet 1000 with mesh count of 36) was pre-tensioned to ca 30×40 cm wooden frame. For the stainless steel template, steel sheets with 50, 100 and 200 micrometer thickness were pre-tensioned to a steel frame and contain grooves with the same electrode dimensions.

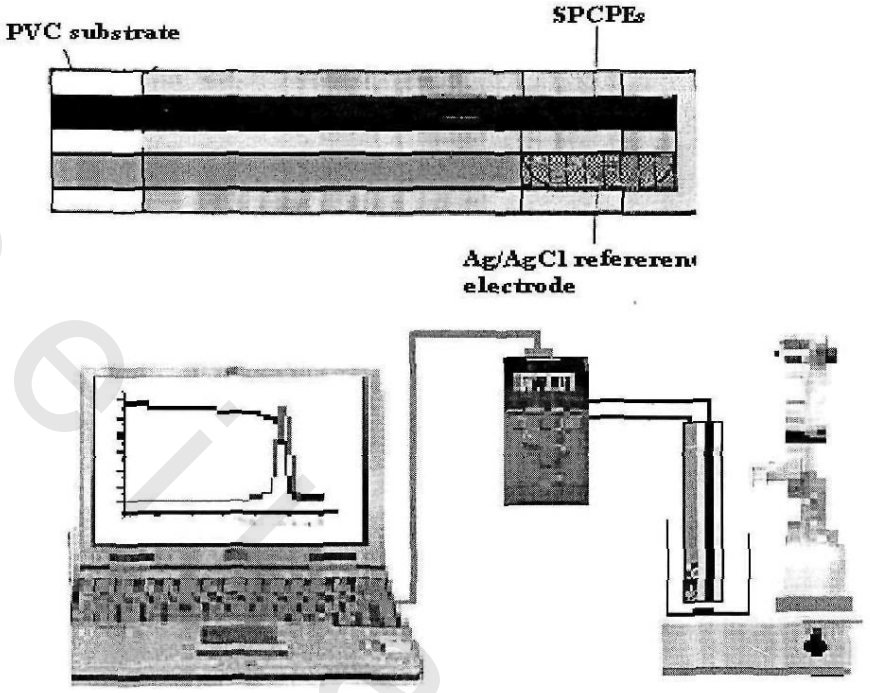

Figure 1: Schematic diagram of the portable system used for the potentiometric titration of surfactants, consisted of the bielectrode strip (containing both the reference and working electrode), digital multimeter connected to a portable PC and Brand digital burette.

II.5.2. Preparation of the silk screen stencil containing the electrode template:

The required final electrode shape contained 3×4 electrodes (each electrode of 5×35 mm) was printed twice on a polyester sheet and this positive sheet was used to produce the electrode template. Photographic emulsion (S22 Sericol) was spread on the polyester fabric using a rubber squeeze and the emulsion-coated screen was placed in dark incubator to dry at about 50 °C for 15 min, then the photographic positive containing the electrode shapes was taped to the emulsion-coated screen and exposed to UV light from 70-cm above the screen for 13–15 min afterwards. The UV light served to fix the emulsion to the screen which permanently blocked the mesh pores; while the photographic positive prevented the light reaching the screen, the emulsion remained "soft" and was washed out with water leaving the screen containing the electrode images. The prepared screen was held vertically and dried with a hair drier and once it was dry, the stencil was ready for use.

II.5.3. Preparation of the graphite ink suspension

The working electrode (type A) was printed using home made ink which was prepared by mixing 1.8 g *o*-NPOE, 5 g PVC solution (8% in cyclohexanone-acetone mixture 1:1) and 3 g carbon powder. After thoroughly mixing of the ink components with a magnetic stirrer, the prepared ink was sonicated for 15 min to improve the dispersion of ink components and remove air bubbles.

II.5.4. Printing of the screen-printed carbon paste electrodes (SPCPEs) and reference electrodes.

A polyacetate sheet (sheet for X ray cleaned with conc. HNO_3 and washed several times with water and then cleaned with commercial thinner) was used as a substrate which was not affected by the curing temperature or the ink solvent and easily cut by scissors. The well mixed graphite ink was poured onto the mesh and forced into the mesh with the aid of a 6 inch squeegee (Sericol SE –C52 medium hardness) held at angle of approximately 60 ^{0}C and mesh was held away from the polyacetate sheet. The squeegee was then pulled back across the template; this ensured the electrode templates were fully loaded with the ink. The wooden frame was pushed down onto the polyacetate sheet and the squeegee drawn across the

template in a single swift action, which forced the ink through the mesh and onto the polyacetate sheet. The stencil frame was then released, revealing the electrodes printed onto the polyacetate sheet. The electrodes were cured at 60 ^{0}C for 2 hr and then cut out from the substrate. A pseudo silver/silver chloride electrode was firstly printed using a homemade PVC ink containing silver/silver chloride (65:35%) and cured at 60 ^{0}C for 30 min. The resistance value was determined with a two point prop and expressed in terms of Ω for the whole electrode. After finishing the printing process the stencil was cleaned with commercial thinner solution in order to remove the excess of ink within the template.

II.5.5. Preparation of double layers screen printed electrodes types B, C

These types of the working electrodes were prepared in two steps printing processes. Conducting pads (carbon based ink for type B consisting of 3 g carbon powder + 5 g PVC solution 12% and silver based ink for type C consisting of 0.9 g sliver + 1.2 g PVC solution 8%) were firstly printed on the substrate and cured at 60 ^{0}C for 2h, then the electrode cocktail (consisting of 240 mg plasticizer and 240 mg PVC powder dissolved in 6 ml of cyclohexanone-acetone mixture) was printed on the conducting pad surface. The printed electrodes were cured and insulated typically as in the electrode of type A.

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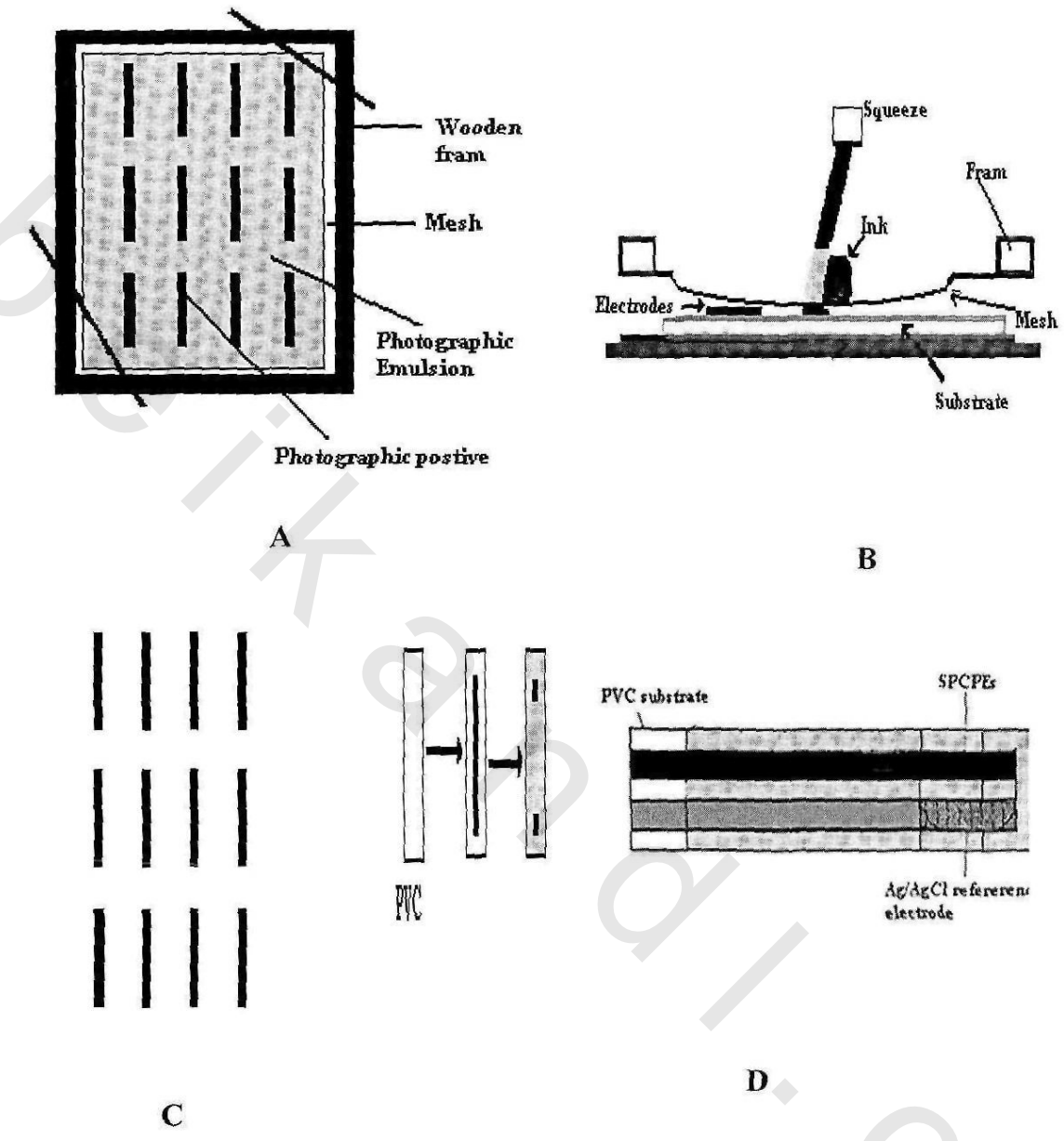

Figure 2: Schematic diagram for the preparation of the screen printed electrodes. a) preparation of the screen template, b) printing of the electrodes, c) image of the printed electrodes and d) insulating of the electrode and the bielectrode strip.

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II.6. Factors affecting the ink performance

II.6.1. Effect of plasticizer content

PVC solution (8% in cyclohexanone-acetone mixture 1:1) was mixed with different amount of DOP as a plasticizer ranging between 0-2g then 3 g of the carbon powder was added. After stirring for 15 min, the ink was sonicated and applied for printing of the electrodes.

II.6.2. Effect of plasticizer type

At the optimum plasticizer content, DOP was replaced by DBP, DOS, TCP or *o*-NPOE and the printed ink was prepared typically as in II.6.1.

II.6.3. Effect of the binding material

PVC and CA were dissolved in cyclohexanone-acetone mixture 1:1 and mixed with 1.8 g of *o*-NPOE then 3 g of the carbon powder was added and after stirring for 15 min, the ink was sonicated and applied for printing of the electrodes.

II.6.4. Effect of the binding material content

PVC and CA solutions in cyclohexanone-acetone mixture 1:1 were prepared by dissolving different amounts of the over mentioned binder in cyclohexanone used for the preparation of the printing inks.

II.6.5. Effect of ink solvent

The cyclohexanone-acetone mixture 1:1, as a solvent for the binding material, was replaced by THF, acetone, thinner or other high boiling point solvent and the resulting binder solution was mixed with 1.8 g of *o*-NPOE then 3 g of the carbon powder was added and after stirring for 15 min, the ink was sonicated and applied for printing of the electrodes.

II.6.6. Effect of carbon content

PVC solution in cyclohexanone-acetone mixture 1:1 was mixed with 1.8g *o*-NPOE and different amounts of the carbon powder ranging between 0.5-4 g, after complete mixing of the ink components, the result ink was used for fabrication of the working electrode type A.

II.7. Preparation of carbon paste electrodes (CPEs)

The sensing electrode was prepared by intimate mixing of accurate weights (500 mg) of highly pure graphite powder and plasticizer (0.2 ml of DOP, TCP, DBP

or *o*-NPOE). This matrix was thoroughly mixed in the mortar and the resulted paste was used to fill the electrode body (Figure 3) [262]. A fresh surface was obtained by gently pushing the stainless-steel screw forward and polishing the new carbon-paste surface with filter paper to obtain a shiny new surface.

II.8. Preparation of coated wire electrode and coated graphite electrode

For the preparation of sliver CWEs, the metal wire 1.5 mm diameter was sealed into the end of PVC tube. The wire was polished, carefully cleaned in strong ammonia solution, rinsed, carefully dipped in 50% nitric acid for 1 min, rinsed with distilled water but don't dry. The wire was cathodized [263-264] against a sliver anode at 5mA/cm^2 in 0.1 M HCl for 30 s, the bubble was allowed to disperse from the wire and the electrode was washed and left to dry. The electrode was immersed in the cocktail consisting of (240 mg *o*-NPOE + 240 mg PVC + 6 ml THF) 20 times and after each the solvent evaporated using air gun (Figure 4).

Consequently, a commercially available TK Pencil fine Japanese type was used as a graphite-based material for the MG ion-sensitive electrode. The rod was immersed in chloroform for 10 min, formed to the required size and then ignited in a colorless flame for 1 min. After cooling, the rod was mounted into a Teflon tube. The open end of the tube was then connected to a slight vacuum and the other end containing carbon rod was immersed into the cocktail 20 times after each the solvent evaporated using air gun. The electrode was kept dry at room temperature for 24 h and preconditioned by soaking in suspended surfactant ion pair solution for 24 hr.

II.9. Preparation of PVC membrane electrode with an internal reference solution

For PVC electrode, the cocktail (consisting of 240 mg *o*-NPOE, 240mg PVC and 6 mL THF) was stirred for 5 min and poured into Petri dish "5 cm" diameter. After 24 h of slow evaporation of solvent, a master membrane with 0.11 mm thickness was obtained which was mounted on the softened end of the PVC tubing with the help of adhesive solution prepared by dissolving PVC in THF. The PVC Closed tube with the membrane was filled with 0.01 KCl and 0.001 M surfactant solution under investigation using Ag /AgCl as internal reference electrode

(Figure 5). The fabricated electrodes were soaked in suspended surfactant ion pair solution for 24 hr.

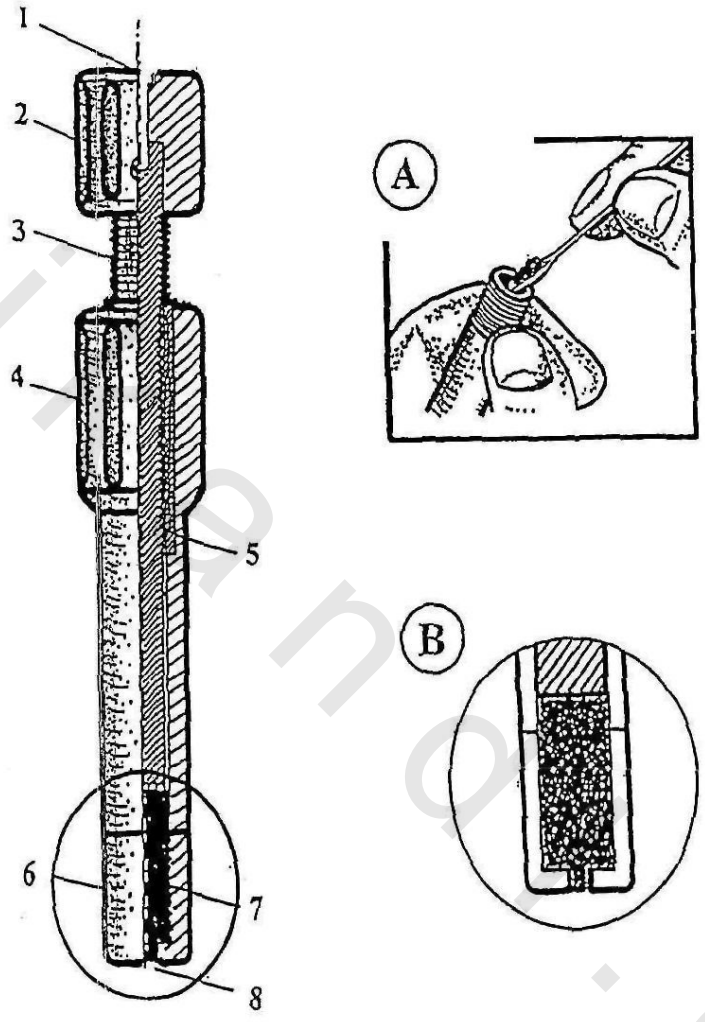

Figure 3: A Typical carbon paste holder: 1- socket; 2- turning head; 3- screw head; 4- electrode body; 5- steel piston; 6- exchangeable Teflon electrode end; 7- carbon paste; 8- hole.

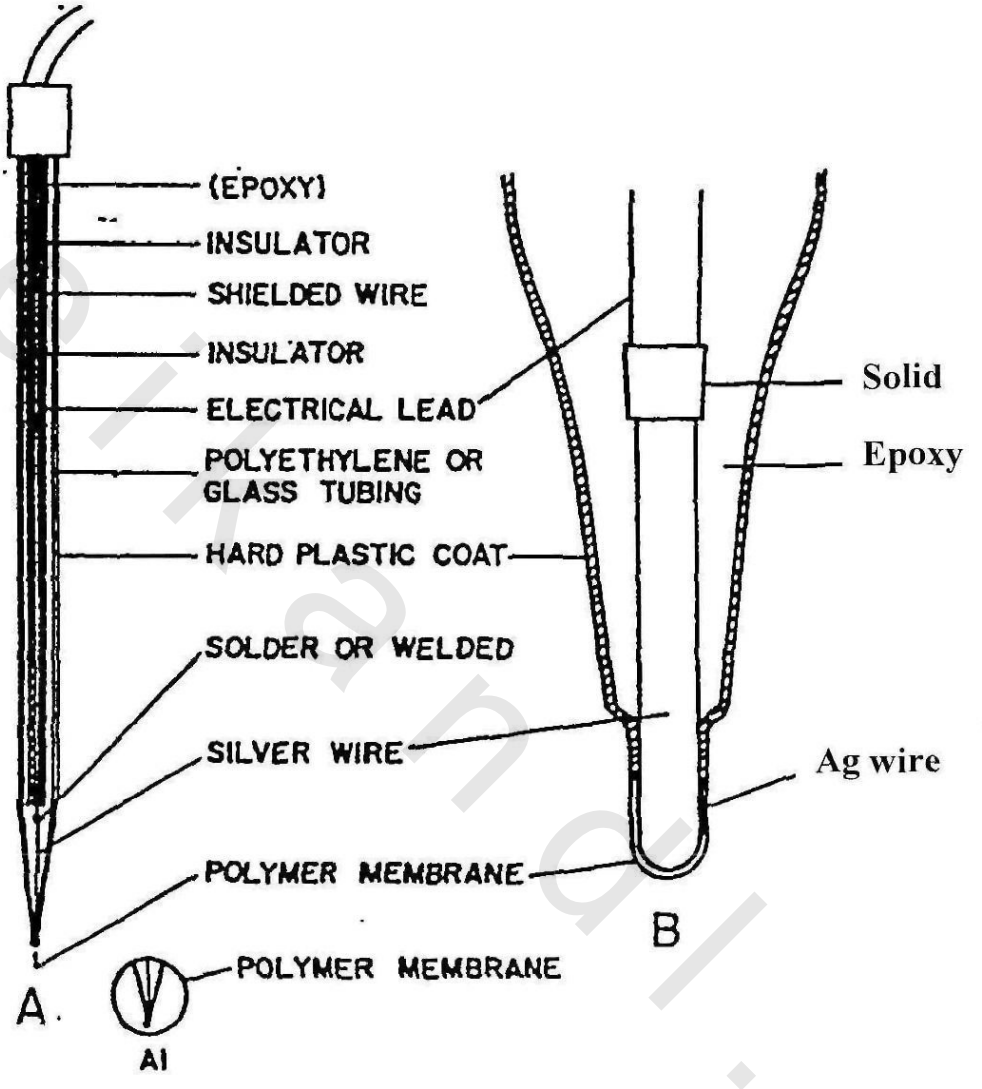

Figure 4: A Typical coated wires ISE

(A) Miniaturized electrode body

(B) Magnified view of the tip of a CWE

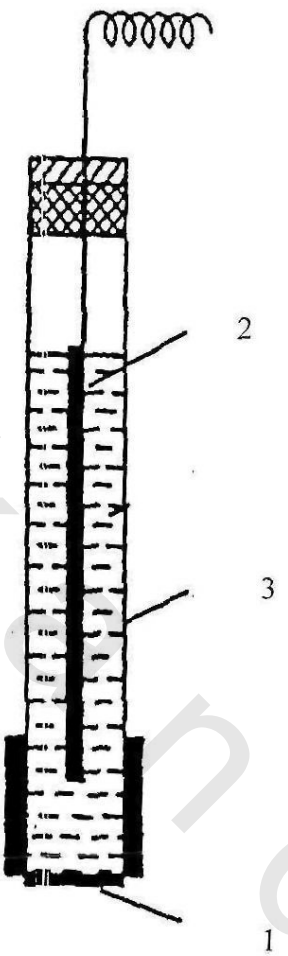

Figure 5: A Typical PVC-membrane electrode with internal reference solution (1) TPB-PVC sensing membrane; (2) inner reference electrode with inner electrolyte; (3) electrode body.

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II. 10. Procedures

II.10.1. Potentiometric titration of surfactant in pure solutions.

Aliquots of the surfactant solution containing 0.09-15.4 mg were pipetted into a 10 ml beaker and the volume was completed to 5 ml with water. NaTPB was used as a titrant in the potentiometric titration of cationic surfactant and CPCl was used as titrant with anionic surfactants. The titration process was monitored potentiometrically using the different fabricated sensors where the potential readings were plotted against the volume added of the titrant to estimate the equivalence points. The first and second derivatives of the titration curves were treated with Origin plotting program.

II.10.2. Effect of pH

The effect of pH on the performance of the potentiometric titration of CPCl with NaTPB was evaluated at different pH values (2-8) by addition of small volumes of HCl and/or NaOH solution (0.1−1M of each) to the titration medium. The total potential change and the potential break at the end point at each pH value were calculated.

II.10.3. Effect of titrant

2 ml 10^{-2} M Surfactant solution (CPCl) were transferred to a 10 ml volumetric flask and diluted up to the mark with bidistilled water. The contents were transferred quantitatively to a beaker and the solution was then potentiometrically titrated against different titrants including NaTPB, RN, PTA, PMA or STA using SPCPEs as a sensing electrode where the total potential change and the potential break for each titrant were calculated.

II.11. Sample analysis

II.11.1. Potentiometric titration of surfactants in the pharmaceutical samples

A known volume of Ezafluor or Citrolin solutions was made up to 25 ml with water in a volumetric flask. 1 ml Aliquot of the dilute solution was transferred to a 10 ml beaker containing a 2 ml citrate buffer of pH 3.0. The content of CPCl or CTABr was estimated via potentiometric titration with NaTPB using SPCPEs as

sensing electrode. One sachet of Femigin B was dissolved in appropriate amount of water and the content of SDS was estimated via potentiometric titration with standardized CPCl solution. The obtained results were compared with that of the commercial surfactant electrodes and with that of British pharmacopeia [265].

II.11.2. Determination of anionic surfactants in detergent products

An accurate appropriate amount of the detergents was dissolved in 50 ml water and different aliquots of the sample solution were transferred to a 10 ml beaker containing a 2 ml citrate buffer of pH 3.0. The content of anionic surfactant was estimated via potentiometric titration with CPCl using SPCPEs and commercial surfactant electrode as sensing electrodes in addition to the two-phase titration method [266-267].

II.11.3. Determination of anionic surfactant in water sample

A 3 ml aliquot of water sample was transferred to a 10 ml beaker containing 2.0 ml citrate buffer of pH 3.0. The content of anionic surfactant was estimated via potentiometric titration with CPCl using SPCPE and commercial surfactant electrode as sensing electrodes in addition to the two-phase titration method [266-267].

II.11.4. Two phase-titration method

This has been described in detail elsewhere [266-267]. It consists in the titration of an aqueous sample mixed with chloroform phase containing a mixed indicator of dimidium bromide and Acid Blue 1 (Patent Blue VF CI 42045). The titrant is standard aqueous solution of the appropriate counter-ion surfactant, either CTAB or SDS. This displaces the appropriate indicator ion, shifting the organic phase color between pink and blue with a grey end-point.