

CHAPTER 2
MATERIALS AND METHODS

Materials

Glycine ethyl ester - $\text{NH}_2\text{CH}_2\text{COOC}_2\text{H}_5$ (EtGly) and Glycine methyl ester $\text{NH}_2\text{CH}_2\text{COOCH}_3$ (MeGly) were obtained from Sigma and DiMethyl Glycine ethyl ester $(\text{CH}_3)_2\text{NCH}_2\text{CO}_2\text{C}_2\text{H}_5$ (diMeGly) and Phenyl Glycine methyl ester $\text{C}_6\text{H}_5\text{CH}(\text{NH}_2)\text{CO}_2\text{CH}_3$ (PhGly) were obtained from Fluka, Germany and was used without any further purification.

Ferric chloride (FeCl_3) and Copper chloride (CuCl_2) were obtained from Loba Chemie, India and Zinc Chloride (ZnCl_2) and Manganese chloride (MnCl_2) from Qualigen's, India. 2,2'Bipyridyl (bipyl) and O-Phenanthroline (Ophen) were obtained from Merk, India and diethylene triamine (DET) from Loba Chemie, India.

Cetyl trimethyl ammonium bromide (CTAB) and Sodium dodecyl sulphate (SDS) were procured from Qualigen's India and Brij35 from Merk, India and used as received.

Pyrene was obtained from Fluka, Germany and was used as received. Cetyl pyridinium chloride (Cpycl) obtained from Loba Chemie, India and was recrystallised from Benzene.

Experimental Techniques

a) *Critical Micelle Concentration*

Critical Micelle Concentration was determined using surface tension and conductivity method.

i) *Surface Tension:*

The critical micelle concentrations of surfactants were determined at 40°C by surface tension measurements using a du Nauty ring tensiometer (S.C.Dey and Co., India). The temperature was maintained constant, by circulating thermostated water through jacketed vessel containing the solution. Surface tension decreases with increase in surfactant concentration and reached a constant value. The concentration of solution was varied from adding aliquots of concentrated stock solution to a known volume of solution in the

jacketed vessel by means of a 5 ml Hamilton micro syringe. The ring was thoroughly cleaned before each measurement.

The CMC was determined from the cross point of the curve obtained from the plot of measured surface tension against the logarithm of surfactant concentration. The reproducibility of the surface tension concentration curves were checked by duplicate runs.

ii) Conductivity

The critical micelle concentration (CMC) of ionic surfactants was determined by conductivity method using a Mullard conductivity bridge. A dip type cell of cell constant 0.6645 cm^{-1} was used. The conductance was measured for a solution (10 ml) containing 0.001M glycine ester and 0.1M surfactant, placed in thermostated water bath maintained at constant temperature. The experiment was repeated by removing 5 ml surfactant -glycine ester solution and adding 5 ml aqueous solution containing 0.001M glycine ester. CMC was determined from the break point of the plot of specific conductance vs surfactant concentration

b) Aggregation number of micelle

Flourescence Quenching

Flourescence quenching method was used to determine the micellar aggregation number (N_{agg}). The spectra was recorded on a Shimadzu RF5000 spectrofluorophotometer. Cetyl pyridinium chloride was used as a quencher and pyrene was used as the lumincence probe. The spectra was collected at 390 nm.

Preparation of Solution: An aliquot of a stock solution of pyrene in acetone was transferred in to a flask and the solvent was evaporated by passing a stream of nitrogen. The surfactant solution was added to it. After thorough shaking of the solution in the flask, the probe solubilised in the micelle. The concentrations of pyrene (10^{-6} M) and surfactant (5 mM) were kept constant, while the quencher concentration was varied from 0 to $1 \times 10^{-4} \text{ M}$. The ratios of the concentration of pyrene and micelle as well as quencher and micelle were 0.01 and < 0.8 respectively, which assumes a Poisson distribution.

For the equilibrium of solubilize between aqueous and micellar phases, the equation used is,

$$\begin{aligned} \ln I_0 &= \ln I_0 - [Q]/[M] \\ &= \ln I_0 - N_{agg} \frac{[Q]}{[S]-[CMC]} \end{aligned}$$

Therefore, $\ln I_0/I = N_{agg} [Q] / [[S]-[CMC]]$

[Q] Quencher concentration

[M] Micellar concentration

[S] Total surfactant concentration

I_0 and I are the intensities of the spectra in the absence and presence of quencher. From the slope of the plot of $\ln I_0/I$ vs $[Q]$, the aggregation number (N_{agg}) was computed. The experiment was carried out at room temperature.

c) Kinetic measurements:

Kinetic measurements for the hydrolysis of glycine esters in presence and absence of surfactant and metal were carried out in pseudo first order conditions. The rate was monitored by pH stat method using a digital pH meter (Systronics India).

Glycine ester - micelle system:

Stock solutions of surfactants and NaOH were prepared. 50 ml reaction mixture (Glycine ester and surfactant) was prepared in a 100 ml beaker, and placed in a thermostated water bath to maintain constant temperature. The pH of the system was brought to a desired value, using the addition of small aliquot of base or acid without making much change in the volume of reaction medium. The progress of hydrolysis reaction was monitored by pH meter. The pH was brought to the original constant value, by adding 0.001M NaOH to the reaction mixture from a micro burette at every short interval of time for about 1 hr. then the reaction mixture was kept in a close bottle over night and again the volume of NaOH required to bring the pH to original value was determined.

In order to see the effect of concentration of surfactants, the same experimental method was repeated by varying concentration of surfactant in the range $0-2 \times 10^{-1}$ M. Rate

constants in presence of metal/metal complex were determined in presence of 0.01 M metal salt solution or 0.01M metal complex (1:1) using the same procedure.

A known quantity of water in beaker was placed in thermostat to attain the constant temperature ($\pm 0.1^\circ\text{C}$) Glycine ester (0.001M) was weighed and added to the water. pH was adjusted (4, 6.8 or 11) by the addition of acid or base. As the reaction proceeds the pH decreases. The pH was brought to original pH by the addition of NaOH (0.001M) at definite constant interval of time for 1 hr. The volume of NaOH added and time were noted. The reaction mixture was kept in a closed container and next day again the volume of NaOH to bring the pH to its original value was noted down.

The rate constant for the hydrolysis were determined by plotting ($V_\infty - V_t$) vs time where V_∞ is the total volume of NaOH required to maintain the pH till the hydrolysis of ester is completed and V_t is the volume of NaOH at time t for bringing the pH to a definite pH.

This procedure was repeated in presence of surfactants (CTAB, SDS and Brij 35) using glycinate in surfactant solution. The concentration of surfactant was varied from $0 - 2 \times 10^{-1}$ M.

In order to see the effect of Metal/Metal complex, to a metal salt / (1:1) metal complex solution 0.01 M kept at temperature bath, glycine ester (0.001M) was added and the above procedure was repeated to determine the rate constant in absence and presence of CTAB.

Rate constants in presence of mixed surfactant were determined using different concentrations of mixture of CTAB and Brij 35 at 40°C and at pH 6.8 and 11.