

INFLUENCE OF TEMPERATURE ON THE STRUCTURE OF DYE-  
-DETERGENT SYSTEMS

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The influence of temperature on the structure of dye-detergent systems and on the migration of excitation energy was investigated. The "critical micelle concentration" (c. m. c.), the absorption and the fluorescence spectra of the systems at three different temperatures are determined. The system studied exhibits intermolecular transfer of excitation energy between dye molecules (thionine ( $\text{Th}^+$ ) and methylene blue ( $\text{MB}^+$ )) adsorbed on the surface of the micelles. From the results we can conclude that the efficiency of the energy transfer from  $\text{Th}^+$  to  $\text{MB}^+$  is the highest at c. m. c. of detergent and at a temperature of  $25^\circ\text{C}$ . It is suggested that in the case of  $\text{Th}^+$  and  $\text{MB}^+$  ions adsorbed on the micelles the quenching of the  $\text{Th}^+$  luminescence is mainly due to an intermolecular energy transfer.

*Introduction*

The amphiphilic compounds possess two groups in the same molecules [12]: a hydrophilic group tending to be water-soluble and hydrocarbon insoluble and a lipophilic group tending to be hydrocarbon soluble and water-insoluble. Due to these groups in solutions of amphiphiles there is a tendency for a specific grouping of like molecules, the grouping termed by McBain [7] as micelle. The micellization in aqueous solution of a detergent takes place because it is accompanied by a decrease in free energy of the system [3].

Hartley believed [4] that the micelles are spherical, while McBain [7] advanced the idea much earlier that they exhibit several different forms from spherical to flat plates. As it was shown by Harkins *et al.* [3] the most widely occurring type of micelles has a lamellar structure. A simple form of lamellar micelle was given in [3] and [5]. It is now generally accepted that a planar lamella has a fluid aqueous environment on one side and a fluid organic environment on the other one. The lamellar character of these solutions clearly indicates that the amphiphilic monolayers (lamellae) in the solution show no predominant tendency to become either concave or convex toward their environment. Within uniform domains the micelles themselves are parallel and dispersed in a regular two-dimensional hexagonal array with

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intervening water [12]. This structure predominates in isotropic aqueous detergent solutions at rather low concentration of detergent. It is, however, a very mobile structure and on dilution of the solution it dissociates very rapidly. Also the form of micelle fluctuates locally with the thermal motion and the average form probably changes with the temperature, too.

Due to this lamellar structure the micelle imitates, at least to some extent, the structure of chloroplast in the photosynthetic *in vivo* systems. Therefore, one can use this system as model system for studying the energy migration between pigments. In earlier works [5], [9] the energy transfer between cations of thionine ( $\text{Th}^+$ ) and methylene blue ( $\text{MB}^+$ ) adsorbed on micelles of sodium lauryl (or dodecyl) sulfate was investigated. The distance of the neighbouring dyestuff molecules was of the order of  $10 \text{ \AA}$ , approximate to the distance between neighbouring pigment molecules in the photosynthetic pigment assemblies.

Energy transfer between adsorbed dye molecules in colloidal micelles first was observed by Teale [11], who found energy transfer from fucoxanthol to chlorophyll *a* by comparing the excitation spectra of chlorophyll *a* fluorescence in such adsorbates with and without fucoxanthol.

In the present paper the influence of temperature on the structure of dye-detergent systems is studied. The "critical micelle concentration" (c. m. c.), the absorption and the fluorescence spectra of the systems at different temperatures are determined.

### *Experimental*

Thionine and methylene blue were re-crystallized from ethanol. Sodium lauryl sulfate (NaLS),  $\text{C}_{12}\text{H}_{25}\text{SO}_4\text{Na}$ , of 96% purity was used as detergent without further purification. The concentration of detergent was varied from 0 to  $8 \cdot 10^{-3} \text{ M}$ . The details of the determination of the conductivity, the absorption and luminescence spectra are given in [5]. The fluorescence was observed from the light-exposed side of the sample. The wavelength of the exciting light was 549 nm absorbed in the mixture, to about 80% by thionine ( $\text{Th}^+$ ) and 20% by methylene blue ( $\text{MB}^+$ ). The fluorescence spectra were corrected for reabsorption [2] and for spectral response of the photomultiplier. In this way, since the conditions given in [1] were fulfilled, practically the true fluorescence spectra were obtained. The temperature of the systems were kept constant in a proper sample holder within  $\pm 0.1^\circ\text{C}$  by an U-10 Höppler ultrathermostat.

### *Results and discussion*

Determination of the "critical micelle concentration" (c. m. c.) was carried out conductometrically in the absence and in the presence of dyes. Fig. 1 shows a plot of conductivity *versus* detergent concentration for NaLS solutions. In these solutions, at temperatures of 30 and  $50^\circ\text{C}$ , a dip appears in the curves at a concentration of  $3.5 \cdot 10^{-3} \text{ M}$  of detergent. According to Mukarjee and Mysels [8] the presence of this dip is due to the disappearance of the charge carriers in consequence of the formation of micelles. This concentration of detergent is called c. m. c. At a temperature of  $70^\circ\text{C}$  there is practically no dip, which means that at this temperature the micellization begins at a higher concentration of detergent.

Fig. 2 shows that in the presence of  $MB^+$  the c. m. c. was lowered to about  $3 \cdot 10^{-3} M$ . This may be ascribed to the formation of neutral dye-detergent molecules, in the other words, the presence of dye induces an earlier micellization of the detergent [9]. It is also to be

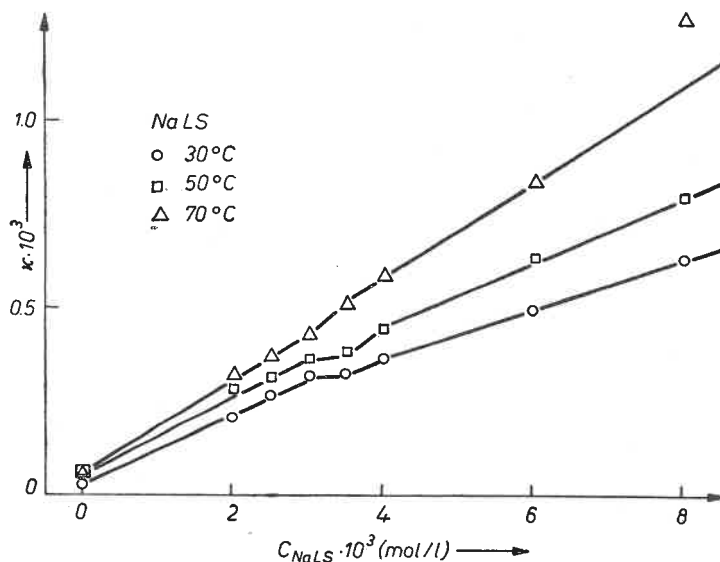


Fig. 1. Conductivity of detergent solutions at different temperatures

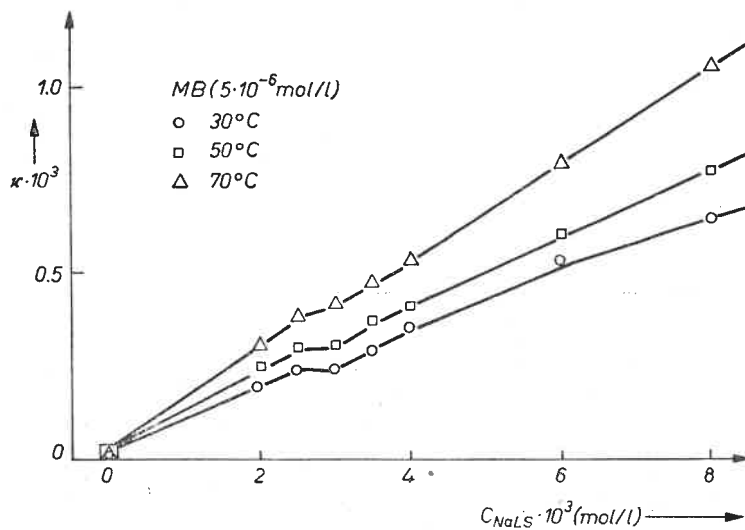


Fig. 2. Conductivity of detergent solutions in the presence of  $MB^+$  at different temperatures

noted that in the presence of dye there is a small dip in the curve even at the temperature of 70°C. This corroborates the statement that the lowering of the c. m. c. is due to induced micellization. On micellization the dye-cations begin to distribute among the micelles present, *i. e.* the dye-cations will incorporate into micelles.

It was shown in [5] that in the case of systems examined at equivalent concentration of  $\text{Th}^+$  and  $\text{MB}^+$ , at c. m. c. of the detergent, each micelle carries about 16–20 ions in an average distance of about 10–14 Å. This distance approximates that of the neighbouring pigment molecules in the photosynthetic pigment systems. This result is in a good accordance with Stigler's data [10].

The absorption spectra of dyes for a water-solution at the equivalent concentrations ( $2 \cdot 10^{-5}$  M) of  $\text{Th}^+$  and  $\text{MB}^+$  is given in [5]. The positions,  $\lambda_{\text{max}}(\text{nm})$ , and the intensities,  $k(\lambda)_{\text{max}}$ , of the main absorption bands of dyes used in detergent solutions, at three temperatures, are tabulated in Table I (for  $\text{Th}^+$ ) and in Table II (for  $\text{MB}^+$ ). It can be seen from the

TABLE I  
Positions and intensities of main absorption bands of thionine in detergent solutions at different temperatures

NaLS (mole/l) $\times 10^3$	25°C		50°C		70°C	
	$\lambda_{\text{max}}(\text{nm})$	$k(\lambda)_{\text{max}}$	$\lambda_{\text{max}}(\text{nm})$	$k(\lambda)_{\text{max}}$	$\lambda_{\text{max}}(\text{nm})$	$k(\lambda)_{\text{max}}$
0	600	0.246	600	0.220	600	0.230
2.0	603	0.130	603	0.152	602	0.196
2.5	603	0.240	603	0.212	602	0.220
3.0	603	0.260	603	0.249	602	0.253
3.5	603	0.272	603	0.254	602	0.268
4.0	603	0.273	603	0.265	602	0.249
6.0	603	0.288	603	0.273	602	0.272
8.0	603	0.275	603	0.264	602	0.265

TABLE II  
Positions and intensities of main absorption bands of methylene blue in detergent solutions at different temperatures

NaLS (mole/l) $\times 10^3$	25°C		50°C		70°C	
	$\lambda_{\text{max}}(\text{nm})$	$k(\lambda)_{\text{max}}$	$\lambda_{\text{max}}(\text{nm})$	$k(\lambda)_{\text{max}}$	$\lambda_{\text{max}}(\text{nm})$	$k(\lambda)_{\text{max}}$
0	665	0.790	665	0.750	665	0.743
2.0	660	0.610	660	0.631	660	0.678
2.5	660	0.770	660	0.710	660	0.690
3.0	660	0.810	660	0.770	660	0.759
3.5	660	0.870	660	0.830	660	0.790
4.0	660	0.900	660	0.850	660	0.820
6.0	660	0.980	660	0.918	660	0.900
8.0	660	0.980	660	1.015	660	0.920

Tables that a very small amount of the detergent strongly bleaches the solution, *i. e.* the formation of neutral dye-detergent salt molecules affects the optical properties of the dye. This is in good accordance with results by Mukarjee and Mysels [8]. There is an evidence that these dye-detergent salts are highly water-insoluble. These effects are reversed with the formation of micelles around and above the c. m. c.

Tables I and II show that the temperature does not affect the position of the main absorption band of dyes used and the location of the absorption maxima does not depend significantly on the concentrations of detergent. It is, however, an interesting fact that with the detergent the location of the absorption maximum is shifted towards longer waves for  $\text{Th}^+$  and towards shorter waves for  $\text{MB}^+$  as compared to the location of maxima for solution without detergent. As a consequence, in detergent solutions the overlap of the emission spectra of thionine and the absorption spectra of methylene blue is larger than that in water-solutions. This larger overlap is — at least partly — responsible for the higher efficiency of the energy migration observed in mixed detergent solutions.

The values of  $k(\lambda)_{\text{max}}$  in the Tables show a gradual increase with the concentration of detergent. For thionine  $k(\lambda)_{\text{max}}$  increases from 0.246 to 0.288, for methylene blue from 0.790 to 0.980 at temperature of 25°C. A similar increase in  $k(\lambda)_{\text{max}}$  can be observed at temperatures of 50 and 70°C, too. These changes are shown for methylene blue in Fig. 3. At temperature of 25°C there is a significant increase of  $k(\lambda)_{\text{max}}$  with the detergent concen-

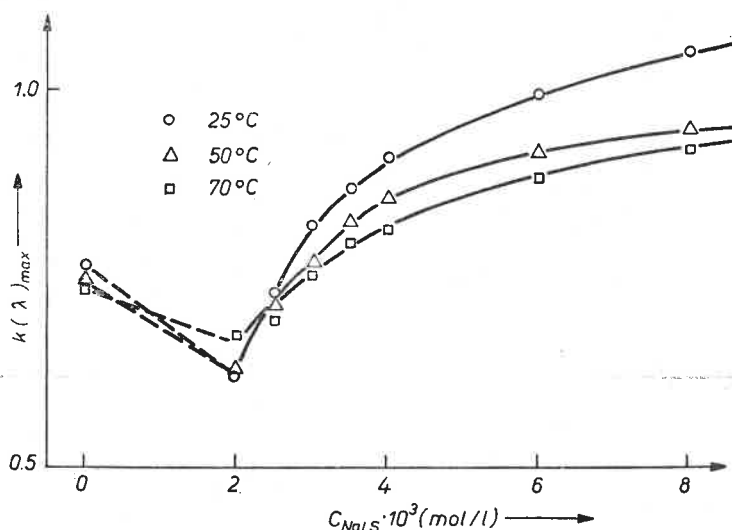


Fig. 3. Absorption coefficient of methylene blue solutions versus detergent concentration at different temperatures

tration, which weakens with increasing temperatures. The value of  $k(\lambda)_{\text{max}}$  in water solution is reached around the c. m. c. in NaLS solutions and at higher concentrations  $k(\lambda)_{\text{max}}$  reaches an almost steady state. This is partly due to the solubilization of the dye-detergent water-insoluble salts, *i. e.* to their incorporation into micelles.

Another possible explanation of the higher  $k(\lambda)_{\text{max}}$  values is that even in water solution some aggregates are present and these dissociate at higher detergent concentrations.

Comparing the conductivity of systems (see Fig. 2) and the value of  $k(\lambda)_{\text{max}}$  (see Fig. 3) at the temperature of 70°C one can explain the slow increase in  $k(\lambda)_{\text{max}}$  by attributing this increase to the absence of micellization occurring at this temperature at higher detergent concentrations.

It was shown in [5] and [9] that the energy transfer from the adsorbed  $\text{Th}^+$  ions to the  $\text{MB}^+$  ions can be studied by observing the quenching of fluorescence of donor ( $\text{Th}^+$ ) and the sensitization of fluorescence of acceptor ( $\text{MB}^+$ ). From the quenching of  $\text{Th}^+$  fluorescence we came to the conclusion that the efficiency of excitation energy transfer is 85% at the equal concentration of  $2 \cdot 10^{-5}$  M of dyes and at c. m. c. of  $3 \cdot 10^{-3}$  M.

Fig. 4 shows the luminescence spectra of the equimolar mixtures of  $\text{Th}^+$  and  $\text{MB}^+$  ( $5 \cdot 10^{-6}$  M) at different detergent concentrations and at different temperatures. It can

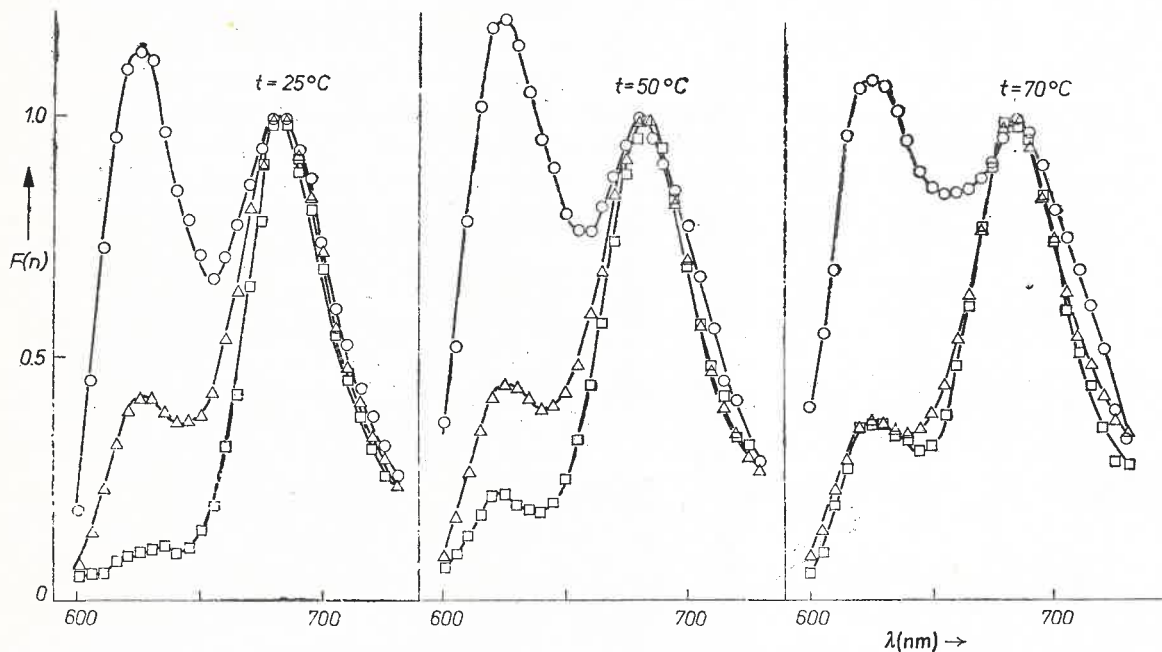


Fig. 4. Luminescence spectra of the mixture of  $\text{Th}^+$  and  $\text{MB}^+$  for different detergent concentrations (0.0 mole/l —  $\circ\circ\circ$ ;  $3.5 \cdot 10^{-3}$  mole/l —  $\square\square\square$  and  $6 \cdot 10^{-3}$  mole/l —  $\triangle\triangle\triangle$ ) at different temperatures

be seen from the figure that in the case of water solutions the luminescence intensity of  $\text{Th}^+$  is always higher than that of  $\text{MB}^+$ . At a detergent concentration of  $3.5 \cdot 10^{-3}$  M the intensity of thionine luminescence is almost completely quenched and the intensity of luminescence becomes higher with increasing detergent concentration, *i.e.* the efficiency of the energy transfer decreases with increasing detergent concentration. This may be interpreted by assuming a transition from dye-rich micelles to the normal type, containing less and less dye-ions per micelle. This should be expected since normal micelles will form above the c. m. c. in large number and the dye-ions should be distributed uniformly among the micelles present.

Fig. 4 shows also a significant influence of the temperature on the migration of energy. It can be seen that at the temperature of  $50^\circ\text{C}$  and especially at  $70^\circ\text{C}$  the decrease in the luminescence intensity of  $\text{Th}^+$  is not so high at a detergent concentration of  $3.5 \cdot 10^{-3}$  M than it was at a temperature of  $25^\circ\text{C}$ . Further, it can be seen from Fig. 4 that at the temperature of  $70^\circ\text{C}$  there is no difference between the intensity of luminescence of  $\text{Th}^+$  at detergent con-

TABLE III

Fluorescence maxima,  $\lambda_{\max}(\text{nm})$ , and intensities,  $f_q(\lambda)_{\max}$ , of thionine at different temperatures

NaLS (mole/l) $\times 10^3$	25°C		50°C		70°C	
	$\lambda_{\max}(\text{nm})$	$f_q(\lambda)_{\max}$	$\lambda_{\max}(\text{nm})$	$f_q(\lambda)_{\max}$	$\lambda_{\max}(\text{nm})$	$f_q(\lambda)_{\max}$
0	620	7.93	620	7.88	620	6.04
2.0	625	2.61	625	5.28	625	7.13
2.5	625	4.51	625	6.26	625	6.99
3.0	625	7.44	625	8.00	625	7.28
3.5	625	9.73	625	9.40	625	8.07
4.0	625	11.04	625	11.01	625	7.61
6.0	625	13.86	625	12.50	625	9.09
8.0	625	14.80	625	13.10	625	9.58

TABLE IV

Fluorescence maxima,  $\lambda_{\max}(\text{nm})$ , and intensities,  $f_q(\lambda)_{\max}$ , of methylene blue at different temperatures

NaLS (mole/l) $\times 10^3$	25°C		50°C		70°C	
	$\lambda_{\max}(\text{nm})$	$f_q(\lambda)_{\max}$	$\lambda_{\max}(\text{nm})$	$f_q(\lambda)_{\max}$	$\lambda_{\max}(\text{nm})$	$f_q(\lambda)_{\max}$
0	687	6.30	687	5.73	687	5.19
2.0	685	0.45	685	2.07	685	1.42
2.5	685	1.85	685	3.21	685	2.57
3.0	685	5.08	685	5.40	685	3.80
3.5	685	10.57	685	8.56	685	6.00
4.0	685	16.23	685	11.05	685	8.15
6.0	685	21.96	685	15.54	685	14.60
8.0	685	23.40	685	15.91	685	17.20

centrations of  $3 \cdot 5 \cdot 10^{-3}$  M and  $6 \cdot 10^{-3}$  M. This is probably due to the absence of the micellization as was mentioned above. From these results one can conclude that the efficiency of the energy transfer from  $\text{Th}^+$  to  $\text{MB}^+$  is the highest at c. m. c. of detergent and at a temperature of 25°C.

As it was mentioned above the micellization (shown by the dip in the conductivity curves) occurs at markedly lower detergent concentrations in the presence of dye. In other words, micelles are induced by the presence of dye. Due to this induced micellization also the intensity of luminescence of dyes is weak at detergent concentrations below c. m. c. (Table III and Table IV), because the NaLS anions form neutral molecules with dye cations.

It can be seen from the Tables that at temperature of 25°C the luminescence of  $\text{Th}^+$  and  $\text{MB}^+$  is almost quenched. At higher temperatures due to the absence of the normal micellization this quenching becomes less. Over the c. m. c., after the solubilization of the neutral non-fluorescent molecules by the detergent anions, the fluorescence reappears.

The formation of non-fluorescent asymmetric dimers ( $\text{Th}^+\text{MB}^+$ ) can hardly be expected [1]. This is supported by the fact that in mixed solution of dyes the intensity of luminescence

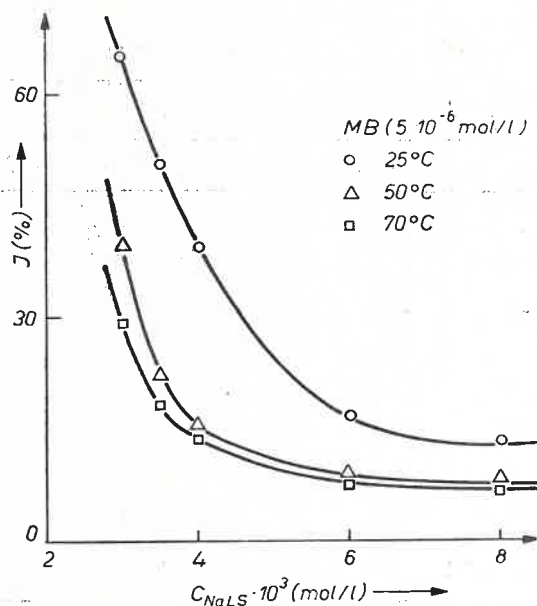


Fig. 5. Decrease of luminescence intensity of  $MB^+$  as a function of detergent concentration at different temperatures

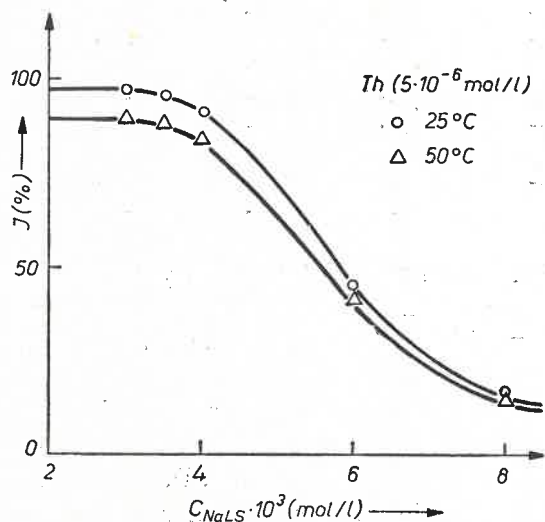


Fig. 6. Decrease of luminescence intensity of  $Th^+$  versus detergent concentration at different temperatures

is lower than that in pure dye solutions. The percentage decrease of the intensity of luminescence as a function of the concentration of detergent, above the c. m. c., at different temperatures is plotted in Fig. 5 for  $MB^+$  and in Fig. 6 for  $Th^+$ .

Fig. 5 shows that at a temperature of 25°C the intensity of luminescence decreases to a value of 60% of the initial solution at detergent concentration of  $3 \cdot 10^{-3}$  M, finally with increasing concentration of NaLS the intensity falls to 13%. This shows clearly that at



equal concentrations of  $\text{Th}^+$  and  $\text{MB}^+$  ( $5 \cdot 10^{-6} \text{ M}$ ) the formation of assymmetric dimers plays a very important role in the system. The excitation energy absorbed and collected by  $\text{MB}^+$  dissipates when  $\text{MB}^+$  accidentally collides with a non-fluorescent assymmetric dimer. At higher temperatures the maximal decrease of the intensity of luminescence is around 35% becoming less and less with increasing concentration of detergent. This proves the significant influence of the temperature on the formation of dimers.

It can be seen from Fig. 6 that the decrease of the intensity of luminescence of  $\text{Th}^+$  is less than that of  $\text{MB}^+$  and it depends also on the concentration of detergent. However, the plots give typical quenching curves [6] clearly showing that in the case of  $\text{Th}^+$  the migration of the excitation energy from  $\text{Th}^+$  to  $\text{MB}^+$  plays a more important role than the presence of assymmetric non-fluorescent dye-dimers. Fig. 6 also shows that in the case of  $\text{Th}^+$  the temperature does not influence significantly the migration of the excitation energy.

### Concluding remarks

Our results support the conclusion that in the case of  $\text{Th}^+$  and  $\text{MB}^+$  ions adsorbed on the micelles the quenching of the  $\text{Th}^+$  luminescence is mainly due to an intermolecular energy transfer. The system which we have studied thus exhibits intermolecular transfer of excitation energy between dye molecules adsorbed on the surface of the micelles. Therefore, this system could serve as model for studying the energy transfer in chloroplast lamellae. The influence of temperature on the migration of the excitation energy seems to be very important in the system studied. Further experiments are needed, however, to clarify the mechanism of this intermolecular transfer of excitation energy and to determine the influence of temperature on the transfer mechanism. Experiments to study these problems are in progress.

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