MIGRATION OF EXCITATION ENERGY IN DYE—DETERGENT SYSTEMS*

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In dye-detergent solutions of methylene blue and thionine the migration of electron excitation energy from thionine (donor) to methylene blue (acceptor) can be observed. This process was studied by the examination of the fluorescence spectra of $2 \cdot 10^{-6}$ to $1 \cdot 10^{-4}$ equimolar solutions with sodium lauryl sulphate detergent. For each dye concentration there is an "optimal" detergent concentration at which the energy transfer is most efficient. This transfer is observed at the critical micelle concentration ($3.5 \cdot 10^{-3}$ M detergent concentration) in $2 \cdot 10^{-6}$ M solution and shifts to higher concentration with increasing dye concentration. This result proves that the effectivity of the energy transfer in dye-detergent systems depends on the dye concentration.

Introduction

It is well known that micelle systems are suitable models for studying the processes—first of all the migration of electron excitation energy—taking place in photosynthesizing *in vivo* systems [1]. Micelles were applied as model for studying the energy transfer by TEALE [2]. SINGHAL *et al.* [3] studied the migration of electron excitation energy from thionine (Th) to methylene blue (MB) in micelles. The most efficient energy transfer was observed at the critical micelle concentration (c.m.c.) of the detergent and at equivalent concentrations of the dyes applied.

The influence of temperature on the structure of dye-detergent systems and on the migration of electron excitation energy has been investigated by HEVESI *et al.* [4]. The results showed that the efficiency of the energy transfer from Th to MB was the highest at c.m.c. and at 25 °C temperature. This conclusion was drawn from the experimental results that, in the case of Th and MB ions adsorbed into the micelles, the quenching of Th luminescence was mainly due to intermolecular energy transfer from Th to MB. The influence of temperature on the energy migration was also studied in [5] and the results reported in [4] were corroborated. It was shown further in [5] that the effectivity of the energy migration is very closely connected with the number of the micelles present in the system and with the structure of the dye-detergent solution.

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The aim of the present work was to investigate the influence of changes in dye concentration on the effectivity of the excitation energy transfer. Since this process can be tracked by the changes in luminescence intensities of the donor and those of the acceptor, the luminescence properties of the dye-detergent solutions had to be studied.

Experimental results and discussion

The systems examined were aqueous solutions of sodium lauryl sulphate (SLS) containing thionine (Th) and methylene blue (MB) in equal concentrations. The concentrations of the dyes were varied from $2 \cdot 10^{-6}$ to $1 \cdot 10^{-4}$ M, that of the detergent between 2 and $8 \cdot 10^{-3}$ M. The experimental methods and conditions used are described in [1] and [5].

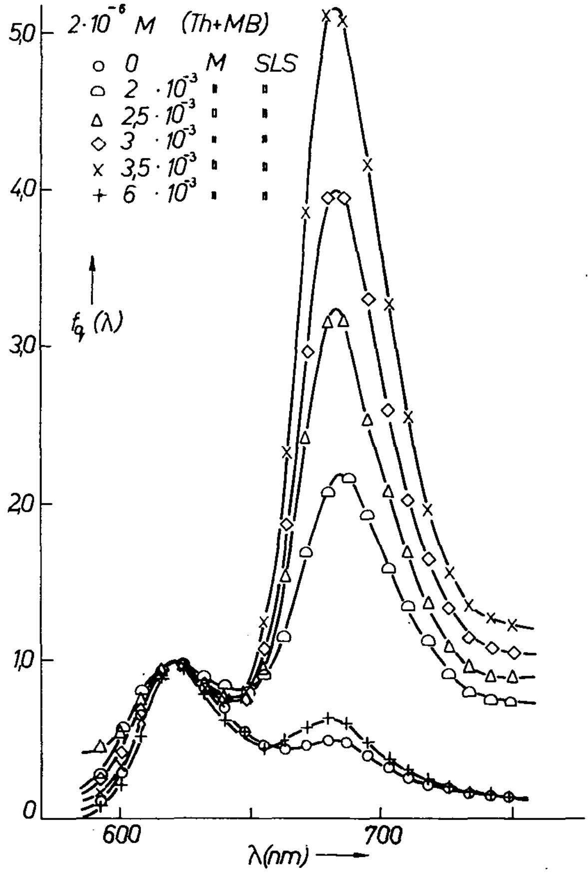

Fig. 1. Fluorescence spectra of mixed solutions of Th+MB of $2 \cdot 10^{-6}$ mol/1 dye concentration at different SLS concentrations

Figs. 1, 2 and 3 show the fluorescence spectra at different SLS concentrations for $2 \cdot 10^{-6}$, $1 \cdot 10^{-5}$ and $5 \cdot 10^{-5}$ M dye concentrations excited at 546 nm, in the region of Th absorption. They show that in the aqueous solutions the fluorescence intensity of Th is about double compared to that of MB for each dye concentration. Adding, however, detergent to the solutions, the relative fluorescence intensity of MB increases with increasing detergent concentration up to a maximum which depends on the dye concentration. For $2 \cdot 10^{-6}$ M concentrations of the dyes the

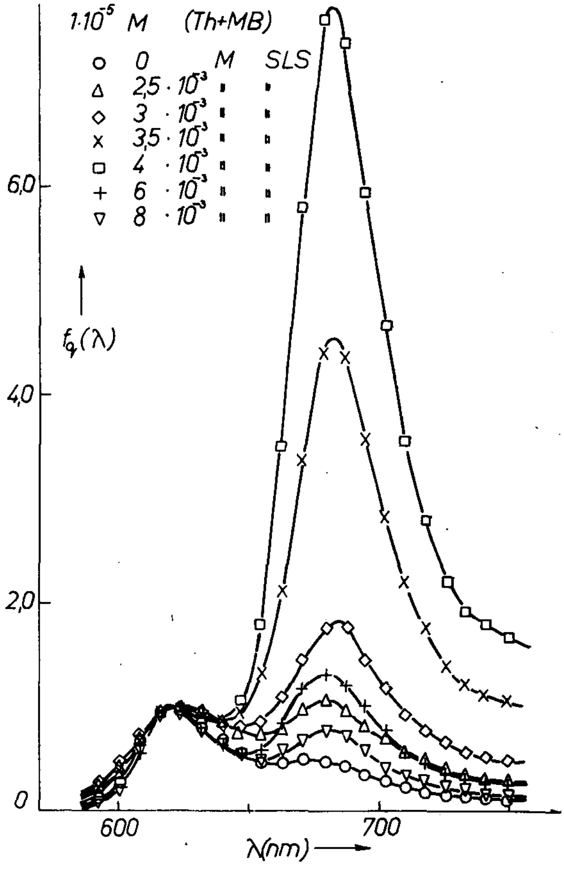

Fig. 2. Fluorescence spectra of mixed solutions of Th+MB of $1 \cdot 10^{-5}$ mol/l dye concentration at different SLS concentrations

maximum is at $3.5 \cdot 10^{-6}$ M SLS concentration, and the fluorescence intensity of MB is about five times higher than that of Th (Fig. 1). The increase in detergent concentration causes a decrease in the relative intensity of MB fluorescence. The relative fluorescence intensity of MB in solutions of $1 \cdot 10^{-5}$ M dye concentration has a maximum at $4 \cdot 10^{-3}$ M SLS concentration and its value is almost eight times higher (Fig. 2). At higher SLS concentration, a decrease in the fluorescence intensity of MB can be observed again. In solutions of $5 \cdot 10^{-5}$ M dye concentration the fluorescence intensity of MB has a maximum at $6 \cdot 10^{-3}$ M SLS concentration and it is more than eight times higher than that of Th (Fig. 3). The observation of the high increase in the fluorescence intensity of MB at a given SLS concentration is explained by the transfer of electron excitation energy of Th to MB. The effectivity of this transfer depends on the concentration of SLS. For each dye concentration there is an "optimal" SLS concentration at which the transferred energy is most

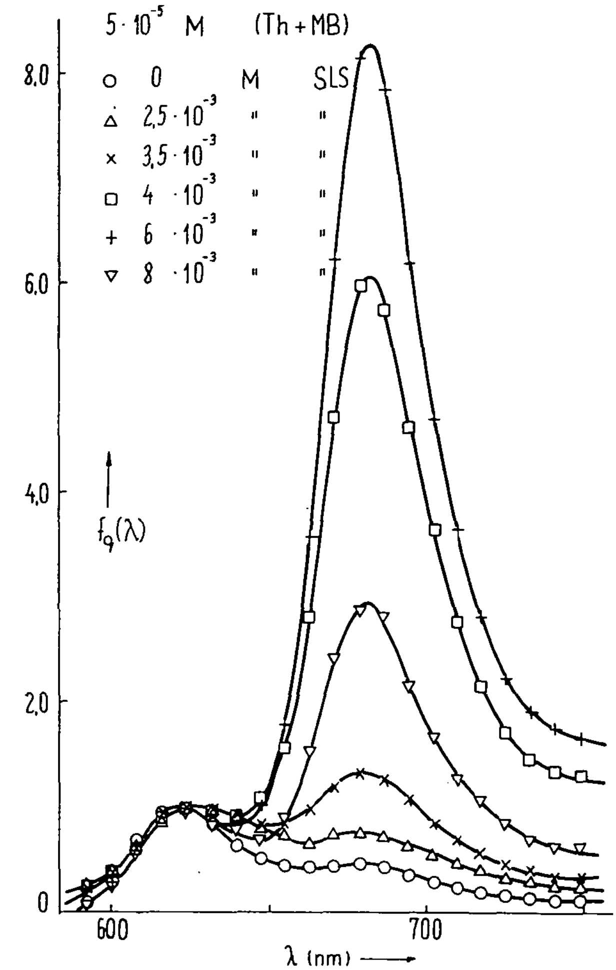

Fig. 3. Fluorescence spectra of mixed solutions of Th+MB of $5 \cdot 10^{-5}$ mol/l dye concentration at different SLS concentrations

efficiently used for the sensitization of the fluorescence of MB. Below c.m.c. there are no micelles and the dye molecules are randomly distributed with an avarage distance leading to a given transfer frequency. Above c.m.c. micelles are present in the solution, their number is increasing with the increase of the concentration of SLS. The dye molecules are concentrated within the micelles, therefore the mean distance of the molecules is less and the transfer frequency is higher. With the increase of concentration of SLS the number of micelles is increasing and the number of dye molecules within a micelle is smaller, their distribution is more random, therefore their mean distance is greater again. This leads to a smaller transfer frequency.

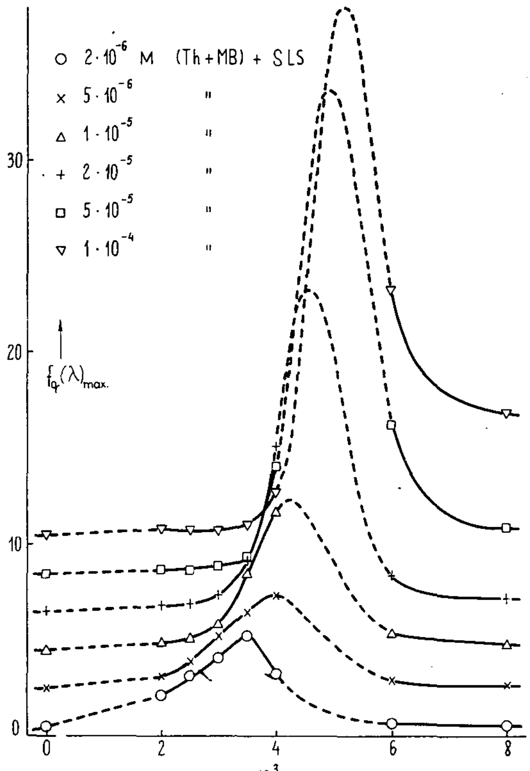

Fig. 4. Ratios of the fluorescence intensities of MB and Th as a function of SLS concentration at different dye concentrations

The picture is more complex due to the presence of aggregates, but the concentration-dependent aggregation leading to a quenching of fluorescence runs parallel with the change of the mean distances of molecules, therefore both transfer and quenching contribute to the changes of fluorescence intensity in the same sense.

Fig. 4 shows the relative fluorescence intensities of MB *vs.* SLS concentration for different dye concentrations. (The zero points of the curves are shifted by two units for better discernment.) It can be seen from the figure that at increasing dye concentrations, the optimal detergent concentration becomes higher and higher. For a given dye concentration the optimal distance depends on the number of micelles present in the solution, *i.e.* on the detergent concentration. At higher dye concentration more micelles are necessary for ensuring this optimal distance between the dye molecules. Consequently, the effectivity of the excitation energy transfer is influenced by the change of the dye concentration, too.

The maxima of fluorescence intensities for Th measured in solutions of Th+SLS (above the dotted line) and that of Th++MB+SLS (under the dotted line) are listed in Table I. The intensities measured in aqueous solutions of Th and those of Th+MB are arbitrarily considered to be 100%. For Th+SLS systems the fluorescence intensity at the optimal detergent concentration is between 25 and 81% (depending on the dye concentration.) For mixed solutions of Th and MB the intensity of Th fluorescence is between 2 and 15%. This means that in mixed solutions the Th fluorenscence is almost totally quenched at the optimal detergent concentration, due to the migration of the excitation energy from Th to MB.

Table I.

$c_{SLS}\cdot 10^{-3}$ (M)	$c_{Th}(M)$ / $c_{Th+MB}(M)$					
	$2\cdot 10^{-6}$	$5\cdot 10^{-6}$	$1\cdot 10^{-5}$	$2\cdot 10^{-5}$	$5\cdot 10^{-5}$	$1\cdot 10^{-4}$
2	9.7	6.7	3.2	2.0	1.2	0.9
	2.7	1.3	0.6	0.4	0.4	0.5
2.5	22.6	11.1	4.8	3.3	1,2	0.9
	4.0	1.4	0.7	0.5	0.4	0.5
3	41.9	15.6	7.9	5.2	1.4	1.0
	7.3	2.3	0.8	0.7	0.5	0.5
3.5	77.4	28.9	12.7	7.2	2.1	1.1
	15.0	4.1	1.3	0.8	0.7	0.5
4	96.8	80.0	49.2	24.8	7.8	3.3
	39.8	13.8	3.7	1.7	0.9	0.6
6	132.3	148.9	134.9	143.1	79.2	55.0
	152.9	105.7	80.9	48.9	9.0	4.1
8	151,6	148.9	141.3	166.7	118.4	122.2
	162.0	121.4	120.0	102.5	43.8	13.3

References

[1] *Hevesi, J., E. Lehoczki, E. Bálint:* Zh. Prikl. Spektr. **13**, 458 (1970).
[2] *Teale, F. W. I.:* Nature **181**, 416 (1958).
[3] *Singhal, G. S., E. Rabinowitch, J. Hevesi, V. Srinivasan:* Photochem. Photobiol. **11**, 531 (1970).
[4] *Hevesi, J., E. Bálint, E. Lehoczki:* Acta Phys. Polon. **A38**, 829 (1970).
[5] *Lehoczki, E., E. Bálint, J. Hevesi:* Zh. Prikl. Spektr. **16**, 97 (1972).

МИГРАЦИЯ ЭНЕРГИИ ВОЗБУЖДЕНИЯ В СИСТЕМЕ КРАСИТЕЛЬ—ДЕТЕРГЕНТ

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В растворах метиленового голубого и тионина типа краситель-детергент наблюдается миграция энергии возбуждения от тионина (донор) к метиленовому голубому. Этот процесс был изучен путём исследования спектра флуоресценции ($2 \cdot 10^{-6} - 1 \cdot 10^{-4}$) эквимолярных растворов с детергентом натрий-лаурилсульфатом. Для каждой концентрации красителя существует оптимальная концентрация детергента, при которой перенос энергии происходит наиболее эффективно. Этот перенос наблюдается при критической концентрации мицеллообразования (концентрация детергента $3{,}5 \cdot 10^{-3}$) в растворе $2 \cdot 10^{-6}$ моль$\cdot$л$^{-1}$ и смещается к более высоким концентрациям красителя. Результаты свидетельствуют о том, что эффективность переноса энергии в системе краситель—детергентзависит от концентрации красителя.