INFLUENCE OF TEMPERATURE ON THE ABSORPTION PROPERTIES OF DYE—DETERGENT SYSTEMS

by

E. BÁLINT, E. LEHOCZKI, J. HEVESI

(Institute of Biophysics, Attila József University of Szeged, Hungary)

In this paper the dependence of the absorption properties of detergent solutions on temperature was investigated. Experiments were made for studying the changes in the properties of the different kinds of dyes due to the increase in detergent concentration and in temperature, too. It was found that the increase in temperature shifts the „critical micelle concentration" toward higher detergent concentrations and promotes the dissociation of the water-insoluble complex salts and dimers. From the experimental results it can be concluded that the solubilization of the complex salts and dimers contributes to the increase in the effectivity of the energy migration in mixed dye solutions.

Introduction

Photosynthesis begins with the photochemical process following the absorption of the light energy. The absorbed energy is transferred from pigment to pigment, from the site of absorption to the molecules actually taking part in the photosynthesis, to the so-called reaction centrum [1]—[2]. The determination of the mechanism of light absorption and energy migration in *in vivo* systems is rather complicated and difficult, because even molecules identical from the chemical point of view may be present in different physical states, and aggregates as well as bondings to other constituents of the chloroplast may lead to further absorption bands in comparison with *in vitro* systems. The investigations are further complicated by the fact that *in vivo* systems are very sensitive to light, to heat and to experimental conditions, and in most cases, the composition of the pigment systems is not known. These difficulties can be partly eliminated by performing the investigations in model-systems sufficiently approximating the structure of the photosynthetizing system [3]—[6].

Models often used to this purpose are solutions containing micelles. The micelles are formed by dissociation of paraffin compounds containing a sufficient number of carbonium atoms in water [7]—[8]. In the course of dissociation these compounds in water break up into negative sulphate-ions and positive carrier-ions. The negative sulphate-ions will form micelles, arrays of lamellar or of spherical symmetry in order to decrease their surface energy. According to our knowledge, about 80 to 100 ions of the detergent used associate to form a micelle [7]. The forming of the micelles usually occurs at a well defined detergent concentration, characteristic for the detergent, the so-called "critical micelle concentration" (c.m.c.).

If light absorbing dyes are added to solutions containing micelles, they will be adsorbed within the micelles, forming systems very similar to the structure of chloroplasts. By choosing adequate dye concentrations, the distance of the dye-ions within the micelles can be made commensurable with the main distance of the photo-synthetizing pigments in the *in vivo* systems [9].

In the case of model systems, the properties of the model (the similarity of the model to the modellized system), the dependence of the properties of the model on the c.m.c., and, on the other hand, the dependence of the latter on the dyes used, on their concentration, on the temperature of the system examined have to be known for studying the mechanisms of the processes taking place in the system [10]—[13].

The aim of this paper is to examine the dependence of the absorption properties of the solutions containing micelles on the properties of dyes used, as well as to study the dependence on temperature of the absorptivity of the dye-detergent systems.

Composition of the systems examined. Experimental methods

Sodium lauryl sulphate (NaLS; technical purity from Fluka) was used as detergent without further purification. Water of very low conductivity, obtained by re-distillation of distillated water on the three degrees quartz distillation apparatus, was used as solvent. All parts of the apparatus and also the flasks containing the solutions were purified from the water-soluble impurities by the method of ABEGG [14]. The conductivity of the water obtained with the described method was about 1 to 2×10^{-6} ohm^{-1} cm^{-1}; this value did not change even after storing for a very long time.

Rhodamin 6G (Rhod 6G), Thionin (Th) and Methylene Blue (MB) were used as luminescing dyes. The mixed solutions contained equimolar quantities of Rhod 6G (donor) and Th (acceptor), and Th (donor) and MB (acceptor), respectively. The dyes used were purified by recrystallization from ethanol until unchanging absorption spectra were obtained. In preparing the solutions, the required volumes of stock solution of the dyes and the detergents were pipetted into the flask (first Rhod 6G than Th, and Th and MB, respectively). Finally the detergent was added to the solutions and the mixtures made up to the mark. Purification and preparation of the solutions were made in the Chemical Laboratory of the Institute of Experimental Physics.

Each set of the measurements was completed within 48 hours. The experiments were repeated several times and the results obtained were within the range of the experimental error. For drawing the conclusions, the mean values of the results were used. The absorption spectra were recorded on a spectrophotometer Optica Milano CF—4DR in the spectral range of 450 to 750 nm, at temperatures of 25 °C, 50 °C and 70 °C. The recording unit was a compensograph Type Specdomax C. The experimental temperatures were kept constant with an Höppler ultrathermostat. The changes in the temperature of the solutions did not exceed ± 1 °C.

Experimental results

In order to study the dependence of the absorption properties of the systems examined on the detergent concentration and on temperature, three different sets of systems were examined:

1. 2×10^{-5} M/l concentration of Th, of MB and of Th + MB,
2. 5×10^{-6} M/l concentration of Th, of MB and of Th + MB,
3. 5×10^{-6} M/l concentration of Rhod 6G, of Th and of Rhod 6G + Th.

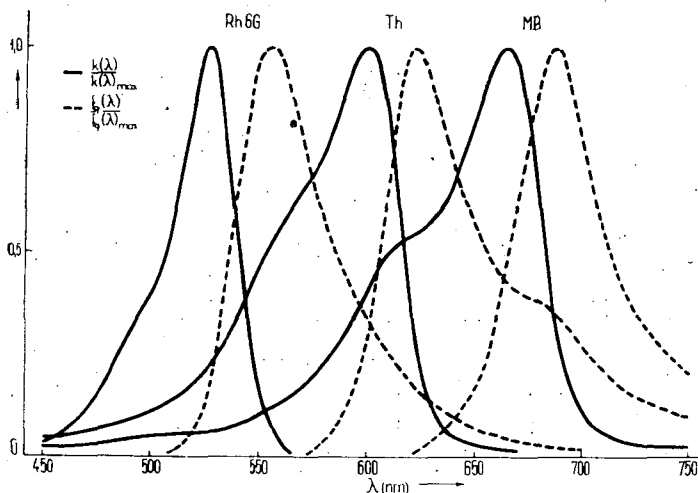The detergent concentration in all solutions was varied between 0 and 8×10^{-3} M/l.

Fig. 1. Absorption (———) and luminescence (— — — —) spectra of Rhod 6G, Th and MB

Fig. 1 shows the absorption and the luminescence spectra of water solutions of the dyes at 25 °C. It can be seen from the position of the spectra that in the case of mixed solutions the energy absorbed by the Rhod 6G is transferred to the Th, that absorbed by Th to the MB, respectively.

It is well known from earlier experiments that the absorption spectra of the solutions are changed by adding detergent to the solution. In Fig. 2 the values of $k(\lambda)/k(\lambda)_{max}$ of the mixed solutions of Rhod 6G + Th are plotted as a function of the detergent concentration at different temperatures. The figure shows, that the addition of the detergent shifts the maximum of the spectra towards longer wavelengths by 2—10 nm, but further increases in detergent concentration do not essentially affect the position of the maximum. Increase in temperature exerts no substantial influence on the position of the maxima of the absorption spectra. It can be also seen from the figure that the absorption of Th at $3,5 \times 10^{-3}$ M/l concentration of the detergent is essentially less than it was in water-solutions, and it also substantially increases with increasing detergent concentration. With increasing tempera-

tures, the absorption maxima increase in the case of low detergent concentration, whereas at higher detergent concentrations decreases in the maxima are found.

In the case of solutions containing low concentrations ($\sim 2 \times 10^{-3}$ M/l) of the detergent, beside the bands of monomers (α) and dimers (β), a new band (γ) occurs in the shorter wavelength region of the absorption spectrum. This band, according to MUKARJEE et al. [13], is due to the presence of water-insoluble dyedetergent complex salts in the solution.

Fig. 3 shows the dependence of the peak absorptions [α at 600 nm, β at 565 nm and γ at 465 nm] of 5×10^{-6} M/l Th solutions on detergent concentraction. It can be seen that the maxima of the α-bands increase up to the c.m.c.; above the c.m.c. they practically do not change. The β-band due to the dimers systematically decreases above the c.m.c. The γ-band has a maximum at the smallest detergent concentration (2×10^{-3} M/l), above which it decreases up to the c.m.c., to remain constant at higher concentrations. The disappearing of the γ-band at the c.m.c is due to the solubilization of the dye-detergent complex salts, i.e. to their incorporation into micelles [15].

The fact that the maximum of the α-band in detergent solutions above the c.m.c. is higher than it was in water-solutions makes probable that under these circumstances the dye molecules become free from the mixed micelles and/or the dye-aggregates present in the water-solutions are solubilized [12]. These changes in the system promote the equal distribution of the dye molecules within the micelles.
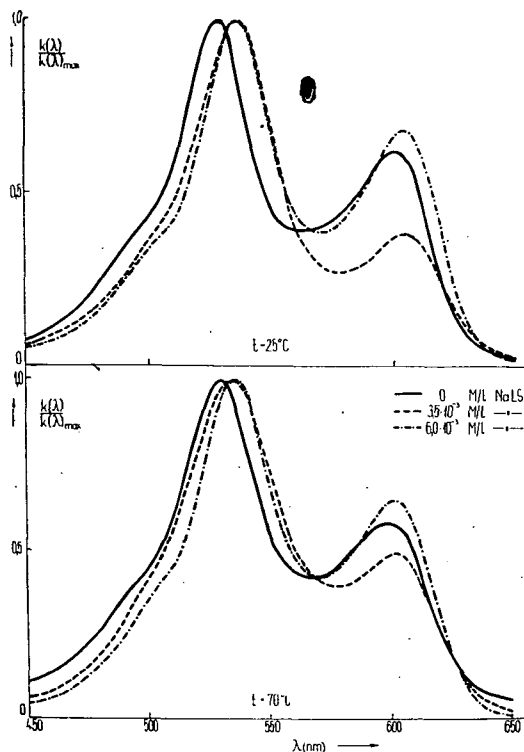

Fig. 2. Absorption spectra of Rhod 6G and Th with identical concentration of $5 \cdot 10^{-6}$ M/l at different temperatures and at different detergent concentrations

In the case of MB solutions with concentration of 2×10^{-6} M/l one can observe a small γ-band of much less intensity than in Th solutions; this band is totally absent in MB solutions of 5×10^{-5} M/l concentration. The dependence of the bands α and β on the dye concentration fully agrees with that found in Th solutions. These changes can be explained in the same way as given in the case of Th solutions. The dependence on detergent concentration of the maxima volues of the absorption bands α and β in MB solutions of 5×10^{-6} M/l concentration can be seen from the data listed in Table I.

In solutions of Rhod 6G, beside the γ-band also the β-band is totally absent. In the case of solutions containing very small amounts of detergent (2×10^{-3} and
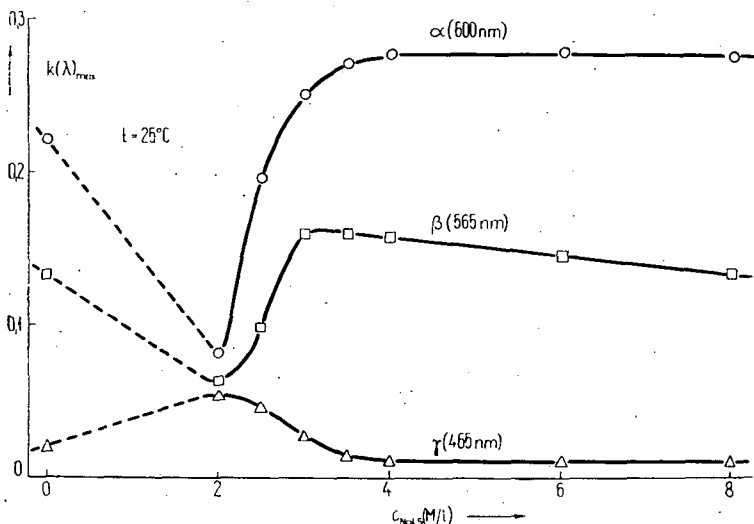
Fig. 3. Absorption bands of Th with $5 \cdot 10^{-6}$ M/1 concentration
at different detergent concentrations

$2,5 \times 10^{-3}$M/1) a little "shoulder" appears in the absorption spectrum around
500 nm, which gradually disappears with increasing detergent concentration.

Table I

*Absorption maximum values, $k(\lambda)_{max}$, in the α- and β-bands of MB solutions with $5 \cdot 10^{-6}$ M/1.
concentration at different temperatures and different detergent concentrations*

$C_{NaLS} \cdot 10^3$ (M/1)	α			β		
	25 °C	50 °C	70 °C	25 °C	50 °C	70 °C
0	0,760	0,752	0,743	0,392	0,403	0,423
2,0	0,483	0,631	0,678	0,495	0,474	0,469
2,5	0,772	0,716	0,695	0,459	0,467	0,469
3,0	0,730	0,771	0,759	0,440	0,457	0,468
3,5	0,857	0,832	0,794	0,437	0,457	0,469
4,0	0,940	0,867	0,831	0,434	0,452	0,467
6,0	0,950	0,918	0,891	0,432	0,445	0,469
8,0	0,960	0,940	0,920	0,435	0,447	0,463

In Fig. 4 the maximum values of absorption bands measured in the case of
system 2 are plotted as a function of detergent concentration. It can be seen from
the figure that in these mixed systems (Th and MB with identical concentrations
of 5×10^{-6} M/1) a γ-band due to the significant overlap of the absorption spectra
can also be observed.

The experimental results prove, that with increasing temperatures the α- and
the β-bands increase, while the γ-band decreases in all examined systems. This change
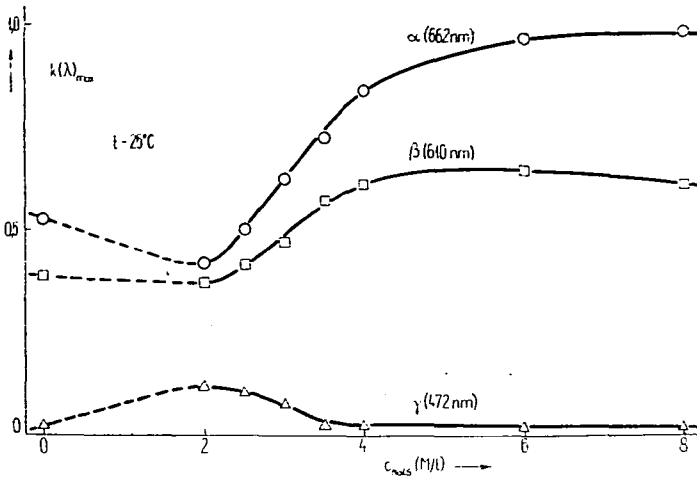in the bands can be well observed in solutions containing low dye concentration.

2*

Fig. 4. Absorption bands of Th and MB with equivalent concentrations
of $5 \cdot 10^{-6}$ M/1

Fig. 5 shows the changes of the values of $k(\lambda)_{max}$ due to the influence of temperature for the α- and β-bands of Th solutions of 5×10^{-6} M/l concentration. The decrease in the maximum of the γ-band with increasing temperature can be explained by more complete solubilization of the water-insoluble complex salts. This change observed at low detergent concentrations causes the increase in monomer dye concentration, which can be seen from the changes in the α-band.

In the range of detergent concentrations above the c.m.c. the maximum values of the α-band (and similarly of the β-band) decrease with increasing temperature.
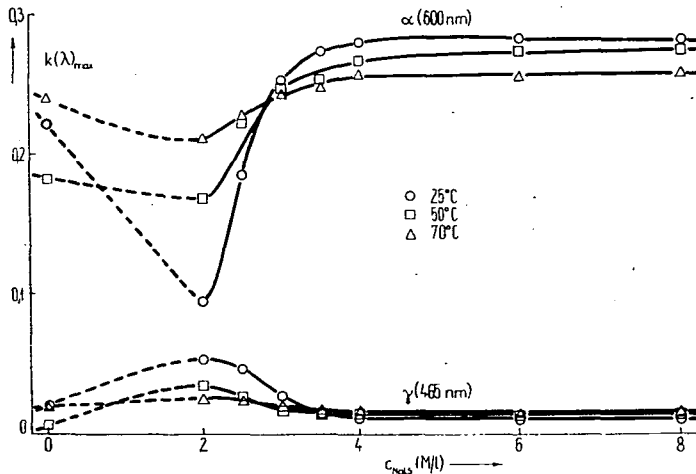

Fig. 5. Absorption bands of Th with $5 \cdot 10^{-6}$ M/l concentration
at different temperatures and different detergent concentrations

In this concentration range the solubilization of the dye-detergent complex salts and that of the dimers is probably completed and therefore an increase in temperature causes the decrease of the absorption coefficients [16]. The very little increase in the γ-band which can be observed is probably due to the broadening of the spectrum under the influence of temperature. Such a broadening of the spectrum due to increasing temperature can be observed in Fig. 2, too.

Table II

Absorption maximum values, $k(\lambda)_{max}$, in the α- and β-bands of Th solutions with $2 \cdot 10^{-5}$ M/l concentration at different temperatures and different detergent concentrations

$C_{NaSL} \cdot 10^3$ (M/l)	α			β		
	25 °C	50 °C	70 °C	25 °C	50 °C	70 °C
0	0,995	0,887	1,266	0,599	0,576	0,836
2,0	0,232	0,355	0,834	0,142	0,269	0,637
2,5	0,322	0,584	0,486	0,208	0,391	0,659
3,0	0,520	0,729	0,949	0,249	0,532	0,682
3,5	0,759	0,928	1,071	0,476	0,645	0,744
4,0	1,106	1,129	1,172	0,649	0,726	0,764
6,0	1,280	1,320	1,338	0,686	0,756	0,770
8,0	1,382	1,414	1,446	0,660	0,730	0,781

It can be seen from the data of Table II that the values of the coefficients in the α-band increase with increasing temperature. This clearly shows that the solubilization of the dye-aggregates is not complete at room-temperature even at the detergent concentration of 8×10^{-3} M/l and the solubilization is going on under the influence of the temperature.

In order to clear up the changes in the absorption properties of mixed systems, the difference spectra of the systems were also studied. These spectra were calculated from the relation

$$[k(\lambda)_D - k(\lambda)_A] - k(\lambda)_K = D(\lambda) \tag{1}$$

using the measured values of the wavelength dependent absorption coefficients. Here $k(\lambda)_D$ means the absorption coefficient of the donor, $k(\lambda)_A$ that of the acceptor and $k(\lambda)_K$ that of the mixture measured at the same detergent concentration, $D(\lambda)$ is the value of the calculated difference spectrum depending on the wavelength. As well known, these spectra show very sensitively the changes in the system, the formation of the dimers and of the water-insoluble complex salts. It is also known that the value of $D(\lambda)$ is only positive if there is some kind of interaction between the dye molecules present in the solution [5].

In Table III the values of the difference spectra for mixed solutions of Rhod 6G and Th, both of 5×10^{-6} M/l concentration, and 2×10^{-3} M/l detergent concentration are tabulated for two different temperatures. It can be seen from the Table that with increasing temperatures, i.e. with increasing solubilization of the dimers and of the complex salts, the positive values of the difference spectra show a significant decrease.

Table III

Calculated difference spectra of mixed solutions of Rhod 6G and Th with identical concentrations of $5 \cdot 10^{-6}$ M/l at different temperatures

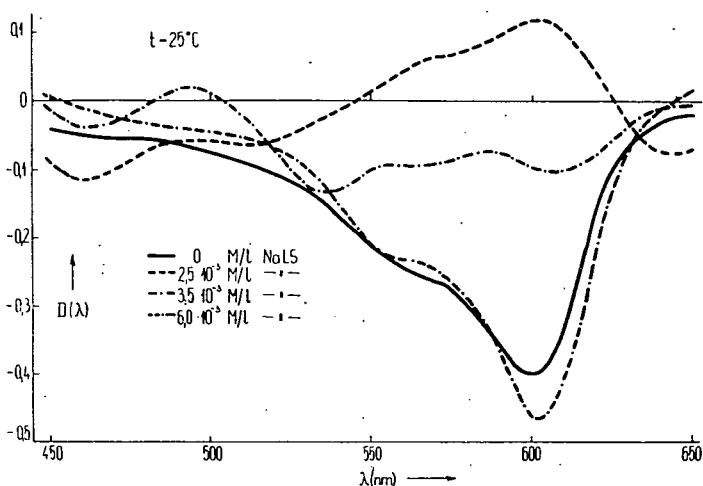| λ (nm)	$D(\lambda) \cdot 10^2$		λ (nm)	$D(\lambda) \cdot 10^2$	
	25 °C	70 °C		25 °C	70 °C
450	1,37	2,0	580	13,6	5,5
460	—1,54	2,2	590	17,9	9,1
470	—3,06	1,2	600	23,3	14,7
480	—2,01	1,0	610	23,3	16,5
490	—0,86	1,4	620	19,2	14,7
500	0,61	1,7	630	15,0	11,0
510	0,19	1,9	640	13,1	10,8
520	2,2	2,0	650	14,2	11,0
530	2,9	2,4	660	14,5	11,4
540	4,2	2,0	670	13,4	10,3
550	6,9	3,4	680	9,1	8,3
560	9,1	4,0	690	5,6	4,8
570	11,0	3,8	700	3,8	2,4

Fig. 6. Calculated difference spectra of Rhod 6G and Th with identical concentrations of $5 \cdot 10^{-6}$ M/l at different detergent concentrations *versus* wavelength

Fig. 6 shows the difference spectra for mixtures of Rhod 6G and Th, both of 5×10^{-6} M/l concentration measured at 25 °C for four different detergent concentrations. It can be seen that in solutions containing very low detergent concentration the difference spectra have a broad positive region, consequently, at this detergent concentration, there exists a strong interaction between the detergent and the dye particles. With increasing detergent concentration the positive region decreases and

the values of $D(\lambda)$ at detergent concentration of 6×10^{-3} M/l are very close to those obtained with water-solution. This means that at this temperature the solubilization of the complex salts is completed.

Conclusions

As our results prove, the absorption properties of the systems examined are very strongly changed by adding even small amounts of detergent to the solution. Dimerization and the formation of water-insoluble complex salts in the system, due to the influence of the detergent, change the absorption properties of the system. At detergent concentrations above the c.m.c. the inner structure of the system undergoes significant changes [18]; the lamellar structure developed and the energy migration between the dye molecules adsorbed in it make the system suitable for serving as a model for studying the mechanism of energy migration, which plays a very important role in photosynthesis.

The influence of the changes in the temperature is also very significant with respect to the absorption properties of the micelle-systems. Increases in temperature shift the value of c.m.c. toward higher detergent concentrations and promote the solubilization of dimers and of water-insoluble dye-detergent complex salts, increasing thereby the number of free dye molecules, which play an important role in the energy migration occurring in the system.

The absorption properties of the systems examined are also dependent on the quality of the dye used. The strongest changes in the absorption properties due to temperature were found in the case of Th and of MB, which both are susceptible to dimerization [17]. The influence of the detergent concentration on the absorption properties of the systems also appears to be the highest for these dyes.

Experiments for studying the physical properties of the model-systems, as well as for clearing up the dependence of the yield of the energy transfer on the concentration of the dyes in such systems are in progress.

* * *

References

[1] *Duysens, L. N. M.:* Progress in Biophysics **14**, 1 (1964).
[2] *Förster, Th.:* Ann. Physik **2**, 55 (1948).
[3] *Teale, F. W. I.:* Nature **181**, 416 (1958).
[4] *Cellarius, R. A., D. Manzerall:* Biochim. Biophys. Acta **112**, 235 (1966).
[5] *Singhal, G. S., E. Rabinowitch, J. Hevesi, V. Srinivasan:* Photochem. Photobiology **11**, 531 (1970).
[6] *Tweet, A. G., W. D. Bellamy, G. L. Gaines, Jr.:* J. Chem. Phys. **41**, 2068 (1964).
[7] *Harkins, W. D., R. W. Mattoon, M. L. Corrin:* J. Amer. Chem. Soc. **68**, 220 (1946).
[8] *McBain, J. W.:* Trans. Farad. Soc. **9**, 99 (1913).
[9] *Corrin, M. L., W. D. Harkins:* J. Chem. Phys. **14**, 641 (1946).
[10] *Lehoczki, E., E. Bálint, J. Hevesi:* Zh. Prikl. Spectr. (to be published).
[11] *Corrin, M. L., H. B. Klevens, W. D. Harkins:* J. Chem. Phys. **14**, 480 (1946).
[12] *Hevesi, J., E. Bálint, E. Lehoczki:* Acta Phys. Polon. Sci. **A38**, 185 (1970).

[13] *Mukarjee, P. M., K. J. Mysels:* J. Amer. Chem. Soc. **77**, 2937 (1955).

[14] *Erdey-Gruz, T., J. Proszt:* Fizikai-kémiai Praktikum (Tankönyvkiadó, Budapest, 1962).

[15] *Sheppard, S. E., et al.:* Rev. Mod. Phys. **14**, 303 (1942).

[16] *Hevesi J., L. Kozma:* Acta Phys. Hung. **20**, 351 (1966).

[17] *Förster, Th.:* Fluoreszenz organischer Verbindungen (Wandenhoeck und Ruprecht, Göttingen, 1951).

[18] *Hevesi, J., E. Lehoczki, E. Bálint:* Zh. Prikl. Spectr. **13**, 458 (1970).

ВЛИЯНИЕ ТЕМПЕРАТУРЫ НА АБСОРБЦИОННЫЕ СВОЙСТВА КРАСИТЕЛЬ-ДЕТЕРГЕНТ СИСТЕМ

Э. Балинт, Э. Лехоцки, Я. Хевеши

Изучалось изменение абсорбционных свойств растворов различных краситель-детергент систем при увеличении концентрации детергента и температуры. Установили, что при повышении температуры «критическая концентрация мицелообразования» смешается в сторону более высокой концентрации детергента и способствует распаду нерастворимых в воде комплексных солей и димеров. Результаты показывают, что растворение таких ассоциатов приведет к повышению эффективности переноса энергии происходящего в смешанных растворах.