ELSEVIER

Carbohydrate Research 315 (1999) 35–47

CARBOHYDRATE RESEARCH

Amylose–dye complexes in cationic micelles: an optical spectroscopy study

Krzysztof Polewski *, Danuta Napierała

Institute of Physics, Agricultural University, ul. Wojska Polskiego 38/42, PL-60 637 Poznań, Poland

Received 27 April 1998; accepted 12 November 1998

Abstract

The formation of amylose complexes with rose bengal (RB), erythrosine B (ER), and phenolphthalein (PP) in the presence of the cationic detergent tetradecyltrimethylammonium bromide (TTABr) was studied using optical spectroscopy methods. Absorption spectroscopy, steady-state fluorescence spectroscopy and picosecond time-resolved fluorescence spectroscopy were used to derive association constants k_s of the dyes, critical micelle concentration (CMC) values and structural information on the complexes formed. It seems that PP fits very well into amylose sites, where it forms an efficient inclusion complex with $k_s = 44{,}500\ M^{-1}$. The molecular diameter of RB is too big to fit the amylose cavity. Only part of the xanthene unit may be adopted in the helical cavity of amylose, whereas most of the interaction occurs through electrostatic and/or dipole–dipole interactions with the amylose chain. The ER molecule is an intermediate case, because it may fit the amylose cavity or adsorb on the amylose surface to form a complex. The presence of a surfactant in the amylose–ligand system increases the association constant for all dyes. In the presence of amylose, a decrease of the detergent CMC value of about one order of magnitude is observed. It is probable that the increased number of micelles incorporate more dyes into the amylose vicinity, which finally changes the structure of the amylose chain. On a macro scale, it was noted that the samples with dyes and detergent have a lower tendency to precipitate and the gelation process is delayed compared to that in water.

Keywords: Amylose; Rose bengal; Erythrosin B; Phenolphthalein; Absorption spectroscopy; Fluorescence spectroscopy

1. Introduction

Micelles are formed when the concentration of an amphiphilic compound exceeds the critical micelle concentration (CMC). When ligands present in the micellar solution exhibit some hydrophobic property, they are partitioned between these two phases. Part of the molecule will be incorporated into the micellar interior whereas the rest will remain in aqueous solvent. In water, many types of macromolecules bind to the surface of ionic surfactant micelles [1]. The elementary step of binding wraps a section of the macromolecule around the micelle, or an interaction between a glycosidic unit and detergent molecules builds the micelle around that site. Usually, the energy of interaction is at least one order of magnitude less than the cohesive free energy of the detergent molecule in the micelle, and thus the binding does not interrupt micellar structure. Such processes often lead to a decrease in the CMC value of the surfactant, which increases the concentration of micelles in solution.

Substances such as dyes or preservatives [2], introduced into food during technological

* Corresponding author.

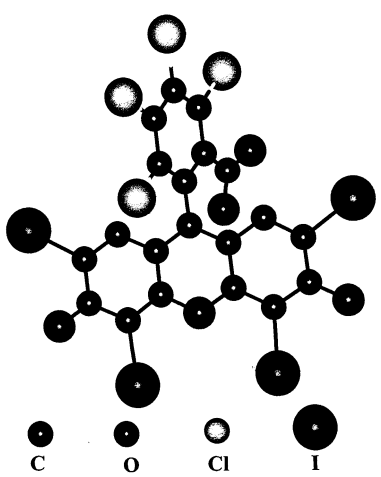
0008-6215/99/$ - see front matter
PII: S0008-6215(98)00303-6

processes, or compounds from a polluted environment, may affect the properties of food components such as carbohydrates [3,4]. It is known that the presence of compounds such as proteins, drugs, dyes or detergents induce conformational changes of amylose in solution [4–9]. It has been found that the major factors involved in the amylose complexation are the hydrophobicity of the substrate and the polarity of the solvent [10–13]. Several studies indicate that hydrogen bonding between host and guest also influences the stabilization of the helical form of the amylose and determines its reactivity towards substrates [11,14]. This interaction is believed to induce the formation of a helical inclusion complex at the secondary structure level [15–19]. The presence of ligands, which alters the secondary structure and changes the gelation properties of the starch, may have some influence on the foods containing such active ligands.

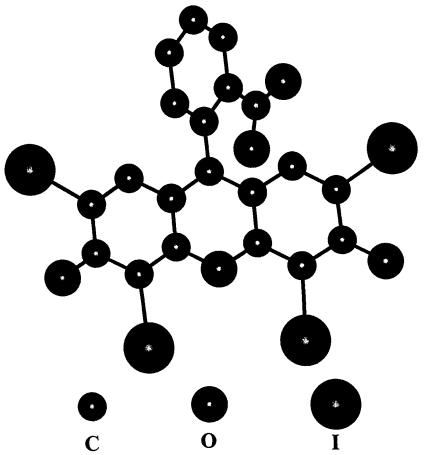
Many of the compounds which interact with starch have their absorption and fluorescence spectra in the visible range of the spectrum. This gives us the opportunity to investigate the conformational and structural changes of amylose in the presence of detergent and dye using optical spectroscopy methods. The binding of dyes to biopolymers induce changes in the physical properties of the dyes, mostly due to the different dielectric constants in aqueous solution and in the interior of the biomolecule or detergent. This may include shifts of the maxima of the absorption and fluorescence spectra, changes of fluorescence intensity, increases in the fluorescence quantum yield and fluorescence lifetimes, and changes in the emission polarization. For many dyes, including rose bengal (RB) and erythrosine (ER), such changes have been observed [20–22]. It is known that RB and ER form inclusion complexes with α-, β- and γ-cyclodextrins [23,24]. Our previous studies [12,25] have also shown that RB can induce the formation of helical structures in amylose in aqueous solution. RB and ER exhibit luminescence properties that are strongly dependent on the hydrogen-donating ability of the medium. This makes these dyes good probes for testing microenvironmental properties [26,27]. Both steady-state and time-resolved spectroscopy of RB have been used to derive information on stoichiometry, association constants, and structures of the complexes [28–34]. Data on the fluorescence lifetimes of the inclusion complexes formed by RB and ER can give detailed information on the microenvironment experienced by the probe molecule.

To the best of our knowledge no reports have been published addressing the problem of interaction between ligands and amylose in the presence of detergent. Because of the potential impact on food quality arising from such modified starch, we think that the problem is worth a preliminary investigation. The subject of this study is an attempt to characterize amylose complexes with RB, ER and PP, in the presence of a cationic detergent, using optical spectroscopy methods.

RB belongs to the group of halogeneted xanthenes, which are widely used as singlet oxygen photosensitizers, photoinitiators of polymerization, and as the active media in solar energy conversion and sensitizers of photodynamic action in biological material [20,28–30,35–38]. RB (Scheme 1) contains two functional groups, phenoxide and carboxylate, which are both capable of interacting with amylose and/or detergent to form complexes while simultaneously acting as a probe for the dye environment. In aqueous solution it exists as a dianion. RB exists as a monomer in solution only at low concentra-



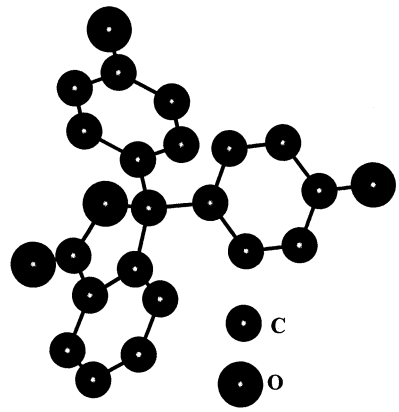
Scheme 1. Molecular structure of the RB dianion $C_{20}H_2Cl_4I_4O_5$(2 Na) with hydrogen atoms omitted for clarity. Symbols for iodine, chlorine, oxygen, nitrogen and carbon atoms are shown.



Scheme 2. Molecular structure of the ER dianion $C_{20}H_6I_4O_5$(2 Na) with hydrogen atoms omitted for clarity. Symbols for iodine, chlorine, oxygen and carbon atoms are shown.

tion i.e., below 20×10^{-6} M. At higher concentrations it undergoes aggregation. This process shifts and broadens the absorption spectra and results in a changing ratio for the two absorption bands. Aggregation may also result in a negative deviation from the Beer–Lambert law. Usually, the ratio of the observed absorbances of the two peaks, $\lambda_{548}/\lambda_{512}$, is used as an indicator regarding the state of the dye in solution. In water, this ratio is greater than 3 for the monomer form and for the dimer/aggregate form it may vary from 3 to 1. Solvent effects also influence the position of the absorption spectra. The less polar the solvent, the greater the red shift for the position of λ_{max} in the spectrum. Generally, similar solvent-effect changes apply to fluorescence spectra regarding their λ_{max} position, but the intensity of fluorescence emission increases as the solvent becomes less polar. The fluorescence lifetime of RB is very sensitive to protonation, which substantially decreases its lifetime in water to 90 ps. In less polar solvents the lifetime is about ten times longer. A longer lifetime leads to higher fluorescence intensity. Because both the micellar interface and interior are protic, the relative fluorescence should be higher as compared to the area where it has access to water molecules.

ER also belongs to the group of halogenated xanthenes and has an unsubstituted benzoic ring, whereas in RB the benzoic ring is substituted with chlorine atoms, which increases its diameter from 5 to 8 Å (Scheme 2). ER was used because its spectral properties are very similar to those of RB and it is used in the food industry as a colorant, or dye, known as Food Red No. 3.

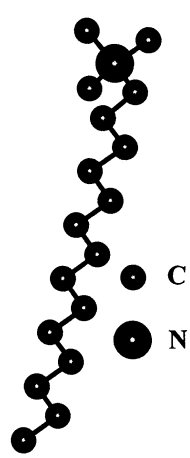


Scheme 3. Molecular structure of PP $C_{20}H_{14}O_4$ with hydrogen atoms omitted for clarity. Symbols for oxygen and carbon atoms are shown.

PP is known to form complexes with cyclodextrins and amylose [4] and was used as a reference in our measurements regarding the molecular dimension and association constant with amylose (Scheme 3).

2. Experimental

Materials.—Amylose Type III from potato (degree of polymerization, 970; molecular weight, 1.57×10^5 M), rose bengal (RB), erythrosin B (ER), phenolphthalein (PP) and te-



Scheme 4. Molecular structure of the TTABr cation $C_{17}H_{38}N(Br)$ with hydrogen atoms omitted for clarity. Symbols for nitrogen and carbon atoms are shown.

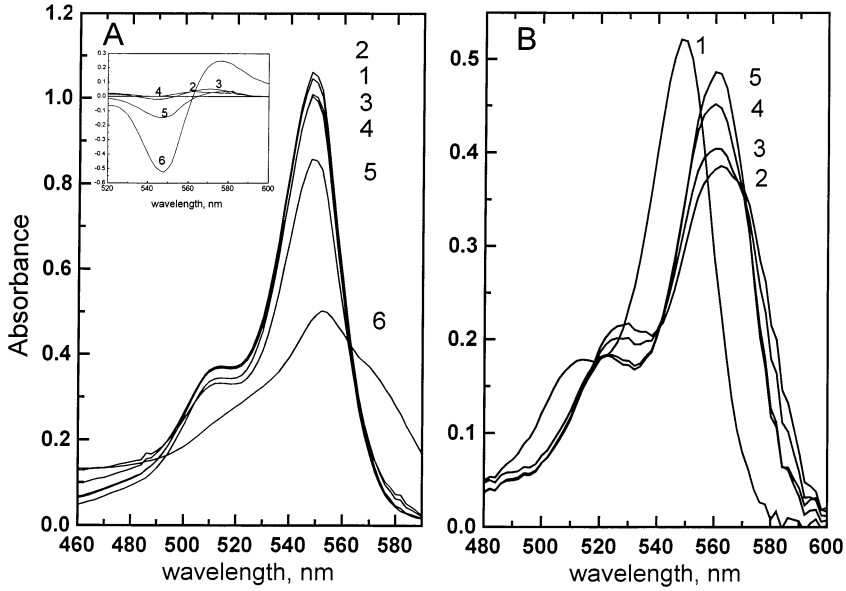


Fig. 1. (A) Absorption spectra of 11 μM RB in water in the presence of increasing amounts of amylose: 1, no amylose; 2, 0.01%; 3, 0.02%; 4, 0.05%; 5, 0.1%; 6, 0.2%; inset in (A) shows differential spectra of RB, i.e., in the presence of amylose minus RB in water (1). (B) Absorption spectra of 5 μM RB in the presence of 0.1% amylose with increasing concentration of TTABr: 1, 0.1% amylose (no TTABr); 2, 0.5 mM; 3, 1.2 mM; 4, 2.5 mM; 5, 6 mM.

tradecyltrimethylammonium bromide (TTABr, Scheme 4) were purchased from Sigma Chemical Co. RB and ER were dissolved in water to a concentration of 1×10^{-4} M as a stock solution. The dyes were recrystallized from methanol before use. The cationic detergent TTABr was used as purchased (CMC in water, 4.5×10^{-3} M). A stock solution of 100 mM in water was used and then diluted as required.

Preparation of sample solution for absorption and emission of dyes.—Amylose solutions ranging from 0.005 to 0.2% were prepared by incubating amylose in water at 80 °C for 10–30 min with stirring. On cooling down to room temperature (r.t.), the solution was filtered, the residue on the filter was dried and weighed, and the final concentration of amylose in the solution was calculated. The required amounts of detergent and dye were then added to the amylose solution and the total volume of the sample was 1 mL. The experiments involving PP were conducted in 20 mM carbonate buffer (pH 11).

Measurements.—Prepared samples were measured directly after mixing, then stored in the dark at r.t. for further measurements, which were taken 30, 60 and 120 min after mixing. No differences between these samples have been found in absorption and fluorescence spectra at these time intervals for RB and ER. For the titration experiments PP was added to the amylose solution and the absorption was measured at 553 nm after 2 h, whereby the PP concentration was held constant at 1.1×10^{-6} M. All absorption spectra were measured in a 1 cm cuvette. Fluorescence spectra for both dyes were measured in a 3 mm cuvette and the excitation wavelength was 510 nm. Absorption spectra were measured using an HP4 photodiode array spectrophotometer (Hewlett–Packard) with a resolution of 2 nm; however, the spectral repeatability and reproducibility is better than 0.05 nm.

Fluorescence spectra and fluorescence lifetimes of RB and ER with amylose were recorded simultaneously using the Omnilyzer instrument at the National Synchrotron Light Source at Brookhaven National Laboratory at port U9B; details of this instrument are described elsewhere [39].

3. Results

Absorption spectra.—As has been pointed out in the Introduction, the absorption spectra of RB and ER are sensitive to the environ-

ment where the dye is located. We used this property to study the distribution of dye in aqueous solutions in the presence of amylose and detergent.

Rose bengal. Fig. 1 shows the spectra of RB in water (1.1×10^{-6} M) with increasing concentration of amylose, from 0.01 to 0.2% (0.28×10^{-6} to 5.7×10^{-6} M) in water. For the low amylose concentration (0.01%) a small increase in the absorption intensity at its maximum is observed and the peak is shifted to 549 nm compared to 548 nm in water. This means that a few percent of the dye was located inside the amylose helix, a low polar environment which shifts the absorption maximum to red (inset in Fig. 1(A)). A further increase of the amylose concentration decreases the absorption maximum and changes the ratio of the absorption intensities at $\lambda_{550}/\lambda_{514}$ from 3 for the dye in water to 2.55 for 0.1% amylose and 2 for 0.2% amylose. However, a 0.2% amylose solution is slightly opaque and not as optically clear and transparent as the other samples with lower amylose concentrations. Simultaneously, the increasing amylose concentration is accompanied by the formation of a new band at 582 nm. These spectra are shown in the inset in Fig. 1(A), as a difference between the spectra of amylose/RB and RB in water only. These changes include the decreasing absorption at λ_{max}, the changing ratio of λ_{550}:λ_{514}, and the appearance of a new band indicating the formation of an RB–amylose complex. The isosbestic point is the place where optical densities (OD) of the substrate and product are equal and indicates a direct 1:1 complex formation. The lack of an isosbestic point indicates that the complex formation is accompanied by another process such as dimerization of the dye or the formation of an inclusion complex with the amylose helix. This last process may be responsible for the observed increase in absorption and the shift of the maximum to 549 nm at the low amylose concentrations.

The absorption spectrum of RB in the presence of amylose and a cationic detergent depends on the surfactant concentration. The spectra of 5 μM RB and 0.1% amylose with varying concentrations of detergent are shown in Fig. 1(B). The titration with detergent started with premicellar concentration and stopped after no changes in intensity and position of the spectra were noticed. The observed phenomenon of premicellar aggregation originates from the formation of hydrophobic ion pairs aggregates that include two surfactant molecules and RB [34]. This broadens the absorption spectrum, shifts the maximum peak to 564 nm, and decreases the fluorescence significantly. When more detergent is added, these premicellar aggregates deaggregate easily and transform into micelles where there is apparently a better stabilization for the anionic dye. The increasing detergent concentration shifts the position of the maximum from 564 nm [premicellar aggregate Fig. 1(B) (curve 2)] to 558 nm [RB in micelle, Fig. 1(B) (curve 5)] and increases the intensity until all the RB molecules are embedded into the micelle. Changes in the spectrum are also accompanied by changes in the λ_{550}:λ_{514} ratio from 1.74 to 2.49 for 0.1% amylose, compared to 1.75 to 3.26 without amylose. At a higher detergent concentration (>15 mM) all spectra are similar, indicating that all the dye molecules, regardless of their position, are located inside the micelle.

Phenolphthalein. PP is known to form complexes with β- and γ-cyclodextrins [4] and stable complexes with amylose with 1:2 stoi-

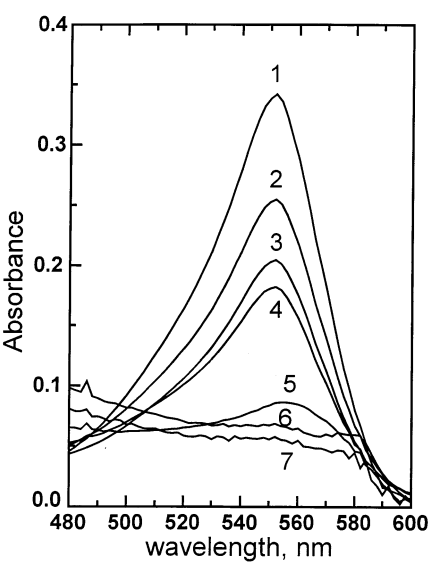


Fig. 2. Absorption spectra of 1.4 μM PP in carbonate buffer at pH 11 with increasing amylose concentration: 1, no amylose; 2, 0.02%; 3, 0.05%; 4, 0.1% and in the presence of 14 mM TTABr and amylose: 5, 0.005%; 6, 0.01%; 7, 0.02%.

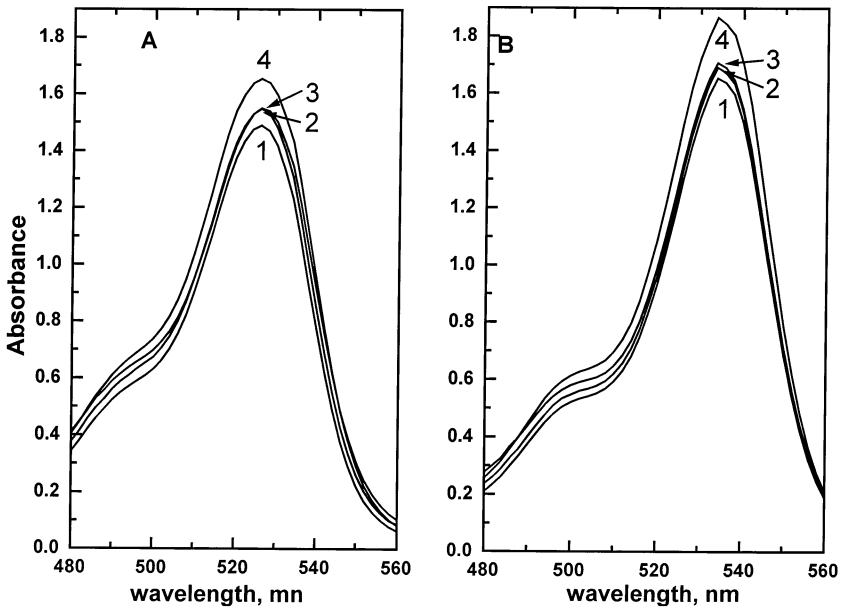


Fig. 3. Absorption spectra of 10 μM ER with increasing concentration of amylose (A) in water: 1, no amylose; 2, 0.02%; 3, 0.1%; 4, 0.2%; or (B) in 28 mM TTABr: 1, no amylose; 2, 0.02%; 3, 0.1%; 4, 0.2%.

chiometry. The absorption spectra of the PP–amylose complex in buffer at pH 11 in aqueous solution and in the presence of the cationic detergent TTABr are given in Fig. 2. PP in water has an absorption maximum at 553 nm and during complex formation this maximum slowly decreases. In the presence of a cationic detergent the peak maximum is shifted to 558 nm. When amylose is added to a detergent–PP system, we observe a significant decrease of the absorption spectrum. At a 0.02% amylose concentration and 14 mM TTABr the features of the absorption spectrum of PP disappear. This indicates that all of the dye molecules have been included into the formed amylose–dye–detergent complex. The interaction is strong enough that the dye undergoes a chemical change that leads to the disappearance of the absorption spectrum of the ionic form of PP. This behavior is very different as compared to the aqueous solution, where at 0.02% amylose only 20% of the dye molecules are involved in the complex. Thus, the added surfactant facilitates complex formation.

Erythrosine B. Absorption spectra of ER in water with an increasing amylose concentration are given in Fig. 3(A). The enhancement of the intensity for the absorption maximum at 526 nm is observed up to 0.12% amylose without changing the shape or the position of the maximum. This indicates that ER in this complex is located inside the amylose structure, which offers a less polar environment than water. In the presence of 28 mM TTABr and 0.12% amylose, the peak/shoulder ratio increases to 3 compared to 2.58 in water, and the maximum shifts to 534 nm (Fig. 3(B)). Figs. 4(A) and (B) present the changes in the absorption spectra of ER when an increasing amount of detergent is added to both the ER–water and ER–amylose systems. The main difference between these two systems as comparing the curves on Figs. 4(A) and (B) is that the detergent concentration at which the premicellar aggregate starts to form micelles, when no shift of the peak maximum occurs, is lower (0.5 mM) in the presence of 0.1% amylose, compared to that in water (1.2 mM). This shows that the presence of amylose decreases the CMC of the detergent in such a system. The values of CMC for all dyes calculated from the titration experiment are given in Table 3.

Fluorescence spectra.—The fluorescence spectra of 10 μM RB in the presence of amylose are given in Fig. 5(A). It displays a peak with the maximum at 568 nm, which changes with an increasing amount of amylose. At a low concentration of amylose the intensity increases about 8% and when the amylose

concentration is increased the intensity diminishes. For 0.2% amylose the intensity is 85% compared to that without amylose. In this case the fluorescence intensity variations are similar to those of the absorption spectra, where for the low amylose concentration an increase in absorbance intensity was observed. However, the decrease of fluorescence intensity is lower than that for the absorption observed at 0.2% amylose. This is an indication, except for the formation of a complex which is non-fluorescent or weakly fluorescent, that another process involving RB may occur, as mentioned during the presentation of the absorption spectra. In a cationic detergent the fluorescence emission intensity of RB increased almost ten times as compared to water, and the peak is shifted from 568 to 583 nm in water. This indicates that the dye was transferred to a more hydrophobic micellar environment. The fluorescence emission spectra of such a system with increasing amounts of amylose are presented in Fig. 5(B). Again, we can observe that the presence of amylose quenches the fluorescence of RB much more strongly than in water. At higher amylose concentrations the peak is shifted to 579 nm. This indicates that fluorescent molecules are still in the micelles, however, with the environment modified by the amylose chain.

Fluorescence lifetimes.—Fluorescence lifetime measurements allow us to determine the number of emitting components in the fluorescent solution and also provide information about the location and the distribution of the dye in the investigated system. The calculated lifetimes of RB and ER in different systems are given in Table 1. For RB and ER in water, in glucose and in detergent (TTABr), these were fitted as a single exponential with 90, 95 and 690 ps for RB and 95, 98 and 610 ps for ER, respectively. In the presence of amylose, the emission decay for the above systems could be fitted as a linear combination of two exponentials. When the system exhibits biexponential decay the lifetime of the shorter component is usually close to one of the compounds in water and is assigned to the uncomplexed form. The longer lifetime is assigned to the more hydrophobic environment, like the interior of the micelle or ethanol. It is interesting to notice that in the presence of amylose the lifetimes of both dyes, which are not participating in the amylose inclusion complex, increased by 20% compared to water, from 90 to 115 ps. The presence of amylose changes the homogeneous distribution of these hydrophobic dyes in solution and tends to move them closer to the amylose chain. The fluorescence lifetime of RB in the amylose cavity is

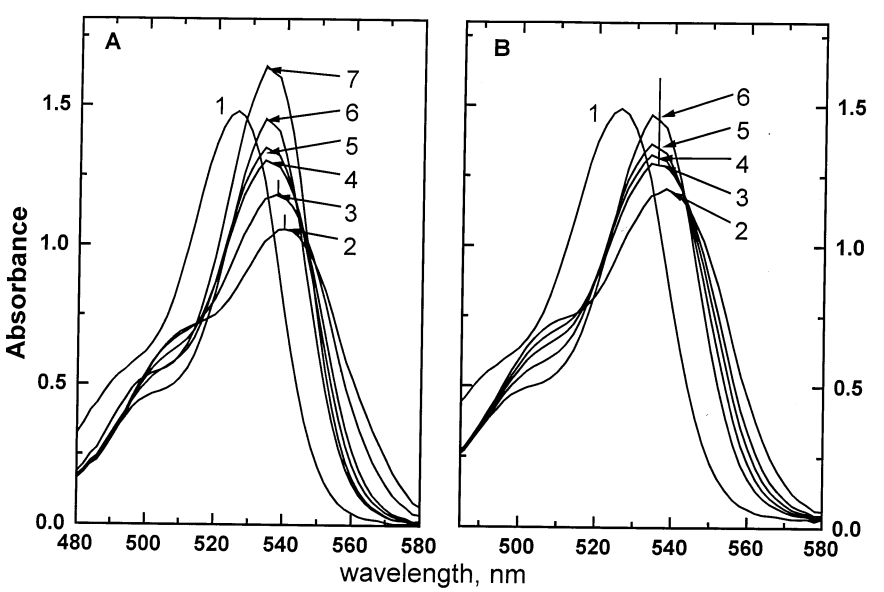


Fig. 4. Absorption spectra of 10 μM ER with increasing concentration of TTABr (A) in water: 1, no TTABr; 2, 0.25 mM; 3, 0.5 mM; 4, 1.2 mM; 5, 2.5 mM; 6, 6 mM; 7, 12 mM; and (B) in the presence of 0.1% amylose: 1, in water; 2, 0.25 mM; 3, 0.5 mM; 4, 1.2 mM; 5, 2.5 mM; 6, 6 mM.

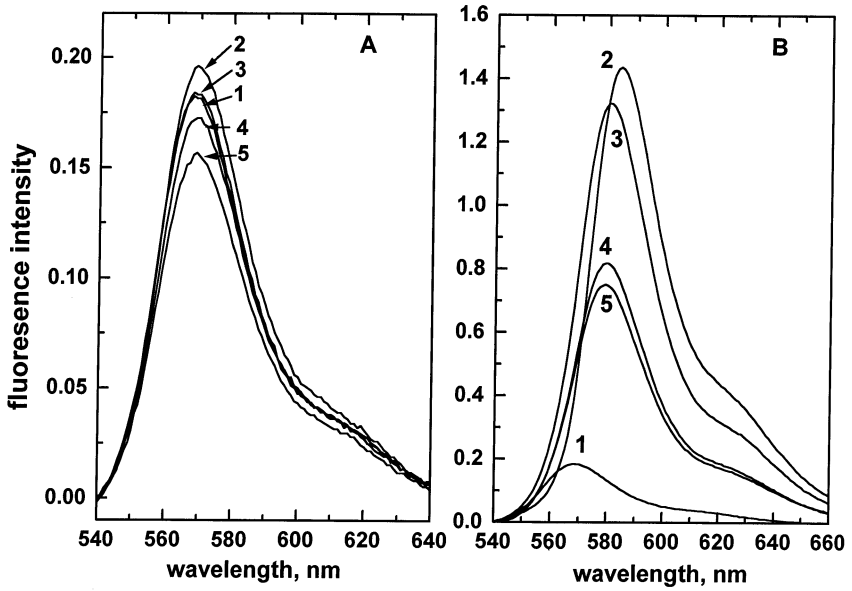


Fig. 5. Fluorescence spectra of 5 μM RB (A) in water with increasing concentration of amylose: 1, no amylose; 2, 0.02%; 3, 0.05%; 4, 0.1%; 5, 0.2%; and (B) in 12 mM TTABR with increasing concentration of amylose: 1, no amylose (in water); 2, no amylose (in TTABr); 3, 0.02%; 4, 0.1%; 5, 0.2%. Excitation at 510 nm. Fluorescence intensity in arbitrary units and this is the same for both graphs.

longer and is closer to the lifetime in a micellar environment than that of ER in similar conditions. This is an indication that part of the RB structure, i.e., the xanthene ring, as an inclusion complex with amylose, is buried deeper into amylose than that of ER.

ER exhibits increasing absorption, fluorescence and fluorescence lifetimes upon increasing amylose concentration and RB shows this behavior only at low amylose concentrations; thus, we may conclude that the similarity of behavior reflects the common features of both dyes. The complex formation involves inclusion of the common part of both dyes, which is the xanthenic moiety containing iodine substituents. From the comparison of the molecular diameters with the amylose helix, it can be deduced that only part of the xanthene system can be accommodated into amylose, leaving the rest of the molecule protruding into the surrounding water. There is also a possibility that the benzoic moiety enters the cavity. This kind of inclusion leaves the xanthene ring even more exposed to water than in the previous configuration. Due to the fact that the excitation is mainly localized on the xanthene ring [40], in order to observe longer lifetimes this portion must be localized in the amylose cavity, where it experiences a microenvironment similar to that in micelles. Additionally, in RB, a substitution of four chlorine atoms increases the diameter by about 50%, which may prevent the dye from entering the sites available to ER. Therefore, the mechanism of the complex formation for RB, where a decrease of both absorption and fluorescence is observed, should be different from that for ER.

The fluorescence lifetime measurements may be used to calculate the association constant of the dyes. The ratio of the pre-exponentials, in our conditions, is related to the concentration of the two fluorescent components and may also serve to estimate the equilibrium constant between amylose and dye, and is given by the following expression:

$$\alpha_1/\alpha_2 = k_s c_{am}$$

where α_1 and α_2 are the pre-exponential factors for components 1 and 2, respectively, k_s is the association constant and c_{am} is the amylose concentration. The pre-exponential values are given in Table 1. The values of the calculated association constant from fluorescence lifetime measurements and from absorption titration are given in Table 2.

Kinetic studies.—The process of complex formation between amylose and dye occurs instantly after mixing. No changes in the absorption or fluorescence spectra were observed up to 2 h after mixing. Because the processes of gelation and precipitation in the samples are on the scale of days, we assume that after mixing the system is in a quasi-equilibrium state. This allows us to calculate the association constant from the absorption and fluorescence measurements. The disappearance of the color of the solution, in the case of PP and RB, as well as the observed quenching of the RB fluorescence and the fluorescence enhancement for ER during the complex formation, allow the stability constant of such a complex, $k_{s,}$ to be determined spectroscopically. Titration of amylose in the presence of dye, or dye and detergent, covered the range from 3×10^{-7} to 6×10^{-6} M. Fig. 6 shows the measured curves for PP, RB and ER. According to Wulff and Kubik [4,18] the complex formed between amylose and PP has a stoichiometry of 1:2 and the shape of the curve is described by a cubic equation. Since we observe similar shapes for the other dyes, we assume this same stoichiometry for the RB and ER complexes with amylose. The minimum observed for curve 4 in Fig. 6, RB with amylose in water, reflects the formation of the inclusion complex at low amylose concentration. At the higher amylose concentration the mechanism of complex formation is changing to Type II. Only those points were selected to calculate the association constant for the amylose–RB complex. The final equation is given below:

$$k_s = k_{s_1} k_{s_2} = [A{-}D_2]/([A][D]^2) \quad (1)$$

where $[A] = [A]_0 - [A{-}D_2]$, $[D] = [D]_0 - 2[A{-}D_2]$, [A–D] is the amylose–dye complex, $[A]_0$ and $[D]_0$ are the initial concentrations of amylose and dye, [A] and [D] are the actual concentrations in solution, $[A{-}D_2]$ is the amylose complex with two dye molecules, and k_{s_1} and k_{s_2} are the stability constants for the formation of amylose complex with one and two dye molecules, respectively. By replacing the above symbols with known absorbance values and solving a simple cubic equation, we can calculate the stability constant of the system. For a detailed discussion, see Wulff and Kubik [4]. Using a fitting program we were able to obtain the binding constant for each dye. The calculated values of the association constant for amylose and dyes, and for amylose and dyes with a detergent, are presented in Table 2.

The results from both methods only give similar values for RB. The fluorescence lifetimes method overestimates the k_s value for ER, probably because most of the interaction with amylose is formed by both types of inclusion complexes. Very probably each complex possesses a different fluorescence quantum yield and in this case fluorescence, which is a much more sensitive method than absorption, gives a higher value for k_s. This means that the value calculated from absorption measurements should be used for the further discussion. However, both methods show an increase of the k_s value in a micellar environment.

Detergent concentration study.—In order to determine the influence of detergent on the amylose–dye complex, a titration experiment with the detergent and a fixed concentration

Table 1
Fluorescence lifetimes, τ_1 and τ_2[a], and pre-exponential factors α_1 and α_2 of RB and ER in neat solvents, detergent and amylose solutions

	10 μM RB				10 μM ER			
	τ_1	τ_2	α_1	α_2	τ_1	τ_2	α_1	α_2
Water	90				95			
D-Glucose 0.5 M	95				98			
TTABr 56 mM	690				610			
Amylose 0.1%	115	535	0.97	0.03	115	331	0.87	0.13
Amylose and TTABr	522	693	0.04	0.96	310	612	0.15	0.85

[a] τ_1 and τ_2 in ps ± 5 ps.

Table 2
Association constant k_s[a] for amylose with dyes in water and detergent calculated from absorption titration and fluorescence lifetime methods

Method	Absorption		Fluorescence lifetime	
	Amylose	Am+TTABr	Amylose	Am+TTABr
Rose bengal	11,100	22,300	14,000	15,700
Erythrosine	5200	6500	58,800	67,000
Phenolphthalein	44,500	71,000	Non-fluorescent	Non-fluorescent

[a] k_s measured in M^{-1}.

of amylose and dye has been carried out using the absorption and fluorescence method. In a typical experiment, as a surfactant was slowly added to the solution of dye and amylose, the absorption or fluorescence began to saturate at the point where each dye molecule started to occupy one micelle. A further increase of the detergent did not change the absorption or fluorescence intensity level. After reaching the saturation point, the surfactant can form empty micelles not containing dye. At the initial saturation point, the micelle concentration was equal to the dye concentration and was plotted as a function of the total surfactant concentration to yield the values of the CMC (Fig. 7(A,B)). The initial saturation point was calculated as a crossing point between the two tangents where the saturation point was reached and that before that point. The method is shown in Fig. 7(A). Changes in the CMC, when 0.1% amylose was added to the system, were observed for all the dyes used and these value are listed in Table 3. The absorption method was used to calculate the CMC values only for PP (Fig. 7(A)) which does not fluoresce. The fluorescence method was used to calculate the CMC values for RB and ER. In the case of RB (Fig. 7(B)), the change in the CMC value depends on the amylose concentration. For a low amylose concentration the changes were negligible, whereas for 0.1% amylose the CMC changes from 5.5 to 1.1 mM. For ER, the character of the changes is similar to that of RB and the plot is omitted. At 0.2% amylose, the sample is slightly opaque, which indicates the presence of a gelation process, and in the presence of TTABr the CMC is 0.66 mM for both the xanthenic dyes used. The absorption titration gives essentially similar results for both fluorescent dyes; however, the CMC values are less exactly defined and data obtained from the fluorescence measurements are used for further discussion. These experiments show that in the presence of amylose the CMC of the detergent decreases, which suggests that the amylose may serve as a condensation point and facilitates the micelization process.

4. Discussion

In the present study, the interaction of dyes with amylose in the presence of a cationic

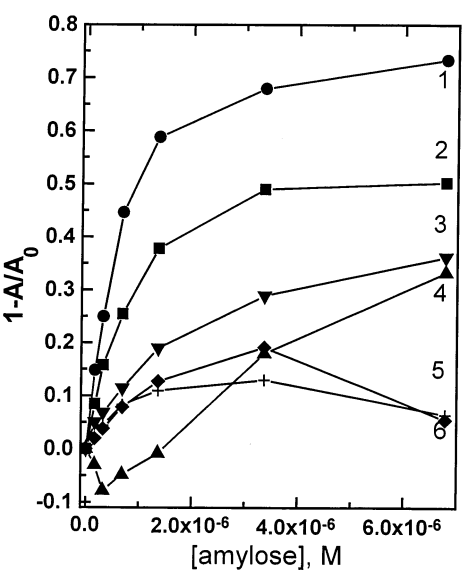


Fig. 6. Amylose concentration studies for dyes in the presence of the detergent and in amylose solution: 1, PP in 12 mM TTABr; 2, PP at pH 11; 3, RB in 12 mM TTABr; 4, RB in water; 5, ER in 12 mM TTABr; 6, ER in water; A_0, absorption measured without amylose; A, actual absorption measured in the solution.

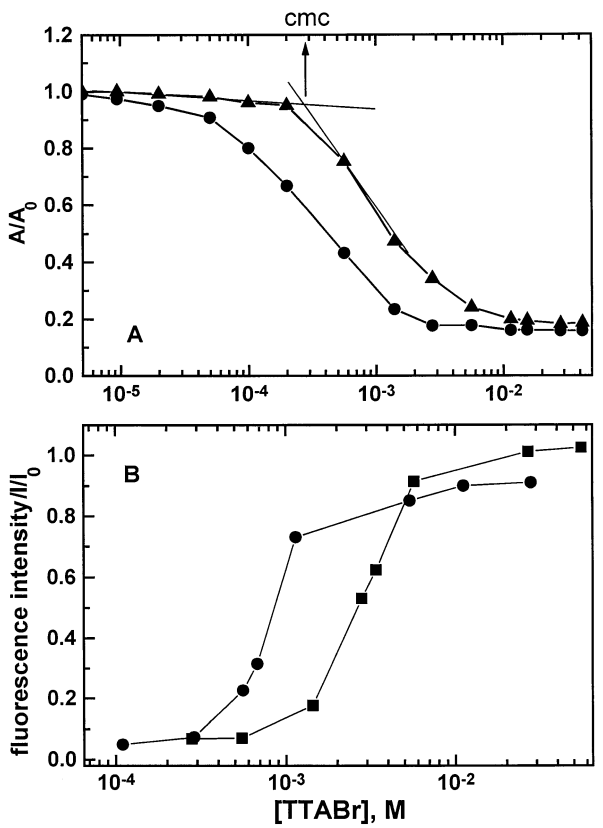


Fig. 7. Detergent concentration studies of dyes in water and in the presence of 0.1% amylose for (A) absorption of 5 μM PP (▲) in water (●) with amylose and (B) fluorescence of 10 μM RB (■) in water (●) with amylose. A_0 and I_0 are the absorbance and fluorescence intensities measured without amylose in the system, respectively. (A) shows how the CMC value was obtained from the plot.

detergent are compared to those in an aqueous solution. The differences found are ascribed to an enhanced interaction of the dyes with amylose due to the detergent micelles, which facilitate the inclusion of more dye into the amylose surface and/or helical cavities. An observed decrease of the CMC of detergent in the presence of amylose indicates that the amylose chain facilitates the formation of micelles, which wrap around the helical part of the amylose.

Table 3
Calculated CMC values[a] for TTABr in the presence of dyes and 0.1% amylose

	Buffer	Amylose
Phenolphthalein	0.28	0.068
Rose bengal	5.5	1.1
Erythrosine	5.2	1.2

[a] CMC values in mM.

The observed decreases in absorption for RB (Fig. 1) and PP (Fig. 2), and increase for ER (Fig. 3) appeared to be a good indicator for the formation of an amylose–dye complex. Each of the above dyes has a specific response to the increasing amylose concentration in solution. The location of the dye around the amylose chain is the main factor responsible for its behavior. The fluorescence emission spectra and fluorescence lifetimes for both halogenated xanthene dyes are sensitive to the microenvironment. This is reflected by differences in the fluorescence lifetimes (Table 1) and fluorescence intensities (Fig. 5). On the basis of absorption and fluorescence data, the existence of two types of complexes is evidenced. The first or Type I complex forms by the inclusion of part of the dye into the amylose helix. It is characterized by the increase in the absorption and fluorescence spectrum intensity. Also the fluorescence lifetime is closer to that found in the micelles than in water. Such changes are found for ER, which suggests that this dye forms a Type I complex with amylose. The second or Type II complex is formed by van der Waals and/or dipole–dipole inductive interactions between amylose and dye. This may involve direct interaction between oxygen atoms in the RB molecule with local high electron density spots of amylose provided by the glycosidic oxygen [13] or by the formation of the hydrogen bonds [11,14]. The formation of such a complex enhances the intersystem crossing constant, which decreases the number of fluorescent RB molecules. As a result, it shows a decrease of intensity for the absorption and fluorescence spectra upon bonding with amylose. It means that RB forms a Type II complex with amylose.

PP, which is the smallest molecule among those used in the experiment, exhibits the highest association constant with amylose as compared to RB and ER (Table 1) and the presence of cationic detergent enhances the process of dye intercalation (Fig. 2). Wulff and Kubik [4] have shown that the PP–amylose complex shows a circular dichroism spectrum similar to that of β-cyclodextrin, which corresponds to the diameter of β-cyclodextrin of about 6.5 Å. The PP molecule span is

about 8 Å; however, each of the 'wings' is about 5 Å in diameter (Scheme 3). This suggests that PP intercalates preferentially to the amylose chain where it can fit tightly, forming an efficient Type I complex with amylose.

The presence of a detergent in the amylose–dye solution at the micellar concentration changes the absorption (Figs. 1(B) and 3(B)) and fluorescence emission spectra (Fig. 5(B)), which indicates that all dye molecules are incorporated into the micellar environment. An enhanced binding constant (Table 1) found for all the dyes and a change in the CMC value (Table 3) with increasing amylose concentration indicate that detergent plays an active role in that process. In a homogeneous solution the relative positions of the micelles are determined only by their mutual repulsion and thermal agitation. In the presence of an amylose polymer the micelles become adsorbed to the amylose strand without disrupting their structures. At a low amylose concentration, each macromolecule is in the form of a random coil that limits the interaction of the dye to the ends of the amylose chain. As the concentration of amylose increases the molecules start to interact, which increases the number of sites where detergent and dyes may bind. Also the detergent molecules now have more possibilities to 'embrace' the helical segment and to form micelles around. Finally, at a high enough concentration the amylose molecules can form an irregular web in the solution, which leads to the gelation process [14,41]. This suggestion is supported by the observation that in the presence of dye and detergent the gelation process is delayed significantly compared to an aqueous amylose solution, and precipitation is decreased in favor of gelation.

Using our spectroscopic methods we cannot measure directly whether the presence of detergent induces the conformational changes in the amylose molecule, therefore we refer to the other publications which used other methods to investigate the interactions between polysaccharides and amphiphilic molecules [5,42,43]. It appears that the presence of detergent itself does not induce conformational changes in polysaccharides, but rather stabilizes the formation of the adsorbed micelles which are formed at a lower CMC. This increases the ability of such a system to incorporate more dye to the micelles and then to distribute them into or on the amylose chain. Such a process of increased intake of ligands into amylose changes its physico–chemical properties. The presented model of amylose behavior in heterogeneous environment explains our experimental results and is in general agreement with the picture of the polymers interacting with the detergents [41]. These results also indicate the potential influence on the food produced from amylose modified by the presence of ligands.

It seems obvious from the above results and discussion that there are several factors which determine the influence of detergent on the properties of amylose–dye complexes. We indicated in this paper the geometry and dimensional factors of the interacting dyes regarding the amylose sites that are available to form inclusion complexes. We also indicated that the formation of an adsorption complex requires an interaction between the amylose surface and the xanthene ring. Although the above factors are important, it seems that there are other factors, such as hydrophobic properties and charge distribution of the dyes or the amylose chain length, which may also influence these processes. Especially, to understand the 3D structure and the conformation of the formed complex, the investigation by other methods besides optical spectroscopy, such as NMR or Raman spectroscopy, may be necessary.

Acknowledgements

This work is supported by the Polish Scientific Committee grant no. 5 PO6G 054 08. The authors greatly acknowledge the help of Dr J.C. Sutherland from the Biology Department at Brookhaven National Laboratory (BNL) and the assistance of his laboratory staff. BNL is supported by the US Department of Energy, Office of Materials Sciences and Office of Chemical Sciences, under Contract DE-AC02-76CH0016; the spectrophotometer at U9B is supported by the Office of Health and Environmental Research, US Department of Energy.

References

[1] I.D. Robb, in J. Lucassen, E.J. Reynders (Eds.), *Anionic Surfaces: Physical Chemistry of Surfactant Action*, Marcel Dekker, New York, 1981.
[2] M.L. Heijnen, J.M. van Amelsvoort, J.A. Weststrate, *Eur. J. Clin. Nutr.*, 49 (1995) 446–457.
[3] Y. Tezuka, *Biopolymers*, 34 (1994) 1477–1481.
[4] G. Wulff, S. Kubik, *Carbohydr. Res.*, 237 (1992) 1–10.
[5] P.V. Bulpin, N.A. Cutler, A. Lips, *Macromolecules*, 20 (1987) 44–49.
[6] F. Casset, A. Imberty, R. Haser, F. Payan, S. Pérez, *Eur. J. Biochem.*, 232 (1995) 284–293.
[7] M.C. Godet, V. Tran, M.M. Delage, A. Buleon, *Int. J. Biol. Macromol.*, 15 (1993) 11–16.
[8] W. Helbert, H. Chanzy, *Int. J. Biol. Macromol.*, 16 (1994) 207–213.
[9] W. Jacques, M.A. Mateescu, *J. Mol. Recognit.*, 8 (1995) 106–110.
[10] Y.-Z. Hui, J.C. Russell, D.G. Whitten, *J. Am. Chem. Soc.*, 105 (1983) 1374–1379.
[11] Y. Hui, Y. Gai, *Makromol. Chem.*, 189 (1988) 1281–1294.
[12] W. Maciejewska, K. Polewski, M. Wyspianska-Grunwald, *Carbohydr. Res.*, 246 (1993) 243–251.
[13] K. Polewski, W. Maciejewska, *Carbohydr. Res.*, 226 (1991) 179–183.
[14] M. Tako, S. Hizukuri, *J. Carbohydr. Chem.*, 14 (1995) 613–622.
[15] J. Jane, J.F. Robyt, *Carbohydr. Res.*, 132 (1984) 105–118.
[16] K. Sensse, F. Cramer, *Chem. Ber.*, 102 (1969) 509–521.
[17] X.-K. Jiang, X.-Y. Li, B.-Z. Huang, *Proc. Indian Acad. Sci.* (*Chem. Sci.*), 98 (1987) 423–429.
[18] G. Wulff, S. Kubik, *Makromol. Chem.*, 193 (1992) 1071–1080.
[19] G. Wulff, A. Steinert, O. Holler, *Carbohydr. Res.*, 307 (1998) 19–31.
[20] D.C. Neckers, *J. Photochem. Photobiol A*: *Chem.*, 47 (1989) 1–29.
[21] M.A.J. Rodgers, *Chem. Phys. Lett.*, 76 (1981) 509–514.
[22] M.A.J. Rodgers, *J. Phys. Chem.*, 85 (1981) 3372–3374.
[23] L. Flamigni, *J. Phys. Chem.*, 97 (1993) 9566–9572.
[24] D.C. Neckers, J. Paczkowski, *J. Am. Chem. Soc.*, 108 (1986) 291–292.
[25] K. Polewski, W. Maciejewska, *Carbohydr. Res.*, 246 (1993) 253–265.
[26] P. Bilski, R. Dabestani, C.F. Chignell, *J. Phys. Chem.*, 95 (1991) 5784–5791.
[27] K. Polewski, *Physiol. Chem. Phys. Med. NMR*, 28 (1996) 163–172.
[28] T.A. Dahl, W.R. Midden, M.C. Neckers, *Photochem. Photobiol.*, 46 (1988) 607–612.
[29] P.C.C. Lee, M.A.J. Rodgers, *Photochem. Photobiol.*, 45 (1987) 79–86.
[30] A. Seret, A. Van de Vorst, *J. Phys. Chem.*, 94 (1990) 5293–5297.
[31] T. Sarna, J. Zajac, M.K. Bowman, T.G. Truscott, *J. Photochem. Photobiol. A*: *Chem.*, 60 (1991) 295–310.
[32] A. Seret, A. Van de Vorst, *J. Photochem. Photobiol B*: *Biol.*, 17 (1993) 47–56.
[33] C. Lambert, T. Sarna, T.G. Truscott, *J. Chem. Soc., Faraday Trans.*, 86 (1990) 3879–3882.
[34] P. Bilski, R. Dabestani, C.F. Chignell, *J. Photochem. Photobiol. A*: *Chem.*, 79 (1994) 121–130.
[35] J.M. Allen, S. Engenolf, S.K. Allen, *Biochem. Biophys. Res. Commun.*, 212 (1995) 1145–1151.
[36] P. Bilski, C.F. Chignell, *J. Photochem. Photobiol. A*: *Chem.*, 77 (1994) 49–58.
[37] C. Giulivi, M. Sarcansky, E. Rosenfeld, A. Boveris, *Photochem. Photobiol.*, 52 (1990) 745–751.
[38] D.P. Valenzeno, J. Trudgen, A. Hutzenbuhler, M. Milne, *Photochem. Photobiol.*, 46 (1987) 985–990.
[39] L.A. Kelly, J.G. Trunk, K. Polewski, J.C. Sutherland, *Rev. Sci. Instrum.*, 66 (1995) 1496–1498.
[40] L.E. Cramer, K.G. Spears, *J. Am. Chem. Soc.*, 100 (1978) 221–227.
[41] A.M. Vallera, M.M. Cruz, S. Ring, F. Boué, *J. Phys. Condens. Matter*, 6 (1994) 311–320.
[42] N. Caram-Lelham, F. Hed, L.-O. Sundelöf, *Biopolymers*, 41 (1997) 765–772.
[43] P. Griffiths, R. Abbott, P. Stilbs, A. Howe, *J. Chem. Soc., Chem. Commun.*, (1998) 53–54.