Absorption Flattening as One Cause of Distortion of Circular Dichroism Spectra of Δ-RuPhen₃ · H₂TPPS Complex

ETTORE CASTIGLIONI,[1,2]* SERGIO ABBATE,[2] GIOVANNA LONGHI,[2] ROBERTO GANGEMI,[2]
ROSARIA LAUCERI,[3] AND ROBERTO PURRELLO[3]

1JASCO Corporation, Hachioji-shi, Tokyo, Japan
2Dipartimento di Scienze Biomediche e Biotecnologie, Università di Brescia, viale Europa 11, 25123 Brescia, Italy
3Dipartimento di Scienze Chimiche, Università di Catania, viale A. Doria 6, 95125 Catania, Italy

ABSTRACT   To extend the model that explains why and how much absorption flattening (AF) influences circular dichroism (CD) signals, we have investigated the interesting case of exciton CD in the Soret region of a noncovalent complex formed by $(\Delta\text{-RuPhen}_3)^{2+}$ and the tetraanionic porphyrin H_2TPPS. Different concentrations have been studied by using an AF emulator and spectra simulation. The CD spectra of this compound occasionally show distortions in the solution sampling mode with the increase of concentration; the inhomogeneous distribution in the cell volume is due to aggregation and is the source of the AF effect. On the basis of these results, we conclude that AF is an important cause of distortions in CD spectra for $\Delta\text{-RuPhen}_3 \cdot H_2TPPS$ complexes and might affect the CD bands of other aggregated systems as well. *Chirality 19:642–646, 2007.*   © 2007 Wiley-Liss, Inc.

KEY WORDS: circular dichroism; chiral porphyrins; absorption flattening; stray-light; bandshape analysis

INTRODUCTION

We recently proposed[1] a very simple sampling approach to generate absorption flattening[2,3] (AF) and to monitor its effects on the UV–vis absorption and CD spectra of chiral compounds in solution. In our search to verify the effect on bisignate exciton-coupling CD signals, we have selected a complex formed by $(\Delta\text{-RuPhen}_3)^{2+}$ (Fig. 1a) and the tetraanionic porphyrin H_2TPPS (Fig. 1b) since it has both strong absorption and strong CD signals in the Soret region, where influence from solvent is negligible. This specific sample was suggested also because similar preparations are occasionally giving spectra (Fig. 1c) in which the negative CD band of the bisignated signal tends to split into two components.

MATERIALS AND METHODS

We emulate an "absorption flattening" (AF) sample in the following way: we prepare a 2.5 mM citrate/phosphate buffer solution of $\Delta\text{-RuPhen}_3$ 5 μM · H_2TPPS 5 μM. Reference spectra have been first taken in standard 10 mm path-length quartz cells, which are 10 mm wide; the samples for monitoring AF effects have been prepared by filling the same solution in a 10-mm path-length rectangular quartz semimicro- and microcells (inside width 4 and 2 mm).

In this mode, the sample beam ($\cong$ 9 mm diameter) is only partially passing through the solution, while a sizeable part of it is free to pass through the transparent walls of the cell (Fig. 2c); in this way we emulate a sample which is not uniformly distributed in the cell total volume.

All CD and absorption spectra have been recorded on a JASCO J-710/715 spectrometer, using 1 nm SBW, 100 nm/min scanning speed, 0.5 sec of integration time and 0.2 nm of data pitch. Absorption spectra have been collected as HT voltage and converted into absorbance by JASCO Spectra Manager standard software. All data are blank subtracted following conventional procedures.

RESULTS
Analysis of CD Data

Figure 2a shows the CD spectra of $\Delta\text{-RuPhen}_3$ 5 μM · H_2TPPS 5 μM in the standard 10 × 10 mm cell (solid line, A) compared with the one obtained with a 4-mm semimicro-cell (dotted line, B) and a 2-mm microcell (point line, C). AF-induced distortions here are very evident: the negative CD band of the bisignated signal is collapsing in intensity and splitting into two bands. As proposed in Ref. 1 we explain this fact by observing that the absorption spectra of Figure 2b have their maxima at a wavelength close to the minimum of the CD band. Indeed, applying the same computational algorithm presented in Ref. 1, we come up
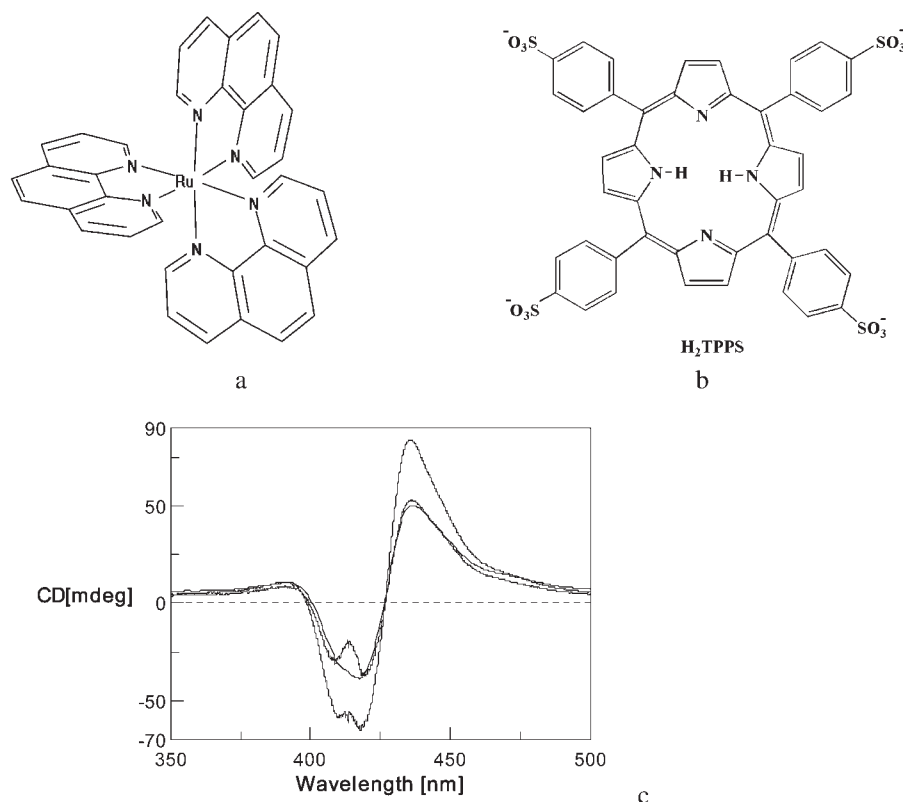
Fig. 1. Chemical structures of Δ-RuPhen₃ (**a**) and H₂TPPS (**b**), together with CD spectra of different batches of Δ-RuPhen₃ ≈ 10 µM · H₂TPPS ≈ 10 µM in 5 mM buffer (**c**).

with the simulations of Figure 3, where a few results are presented for some values of the parameter f; "f" was introduced in Ref. 1 and is the fraction of the incoming photons that interact with the absorbing particles (see

Figure 2c. Here, in case A, $f = 1$; in case B, $f = 0.57$; in case C, $f = 0.28$). We selected small f values to match the three experimental situations, but we are also showing data for f values close to 1 to illustrate that, also in case of moderately
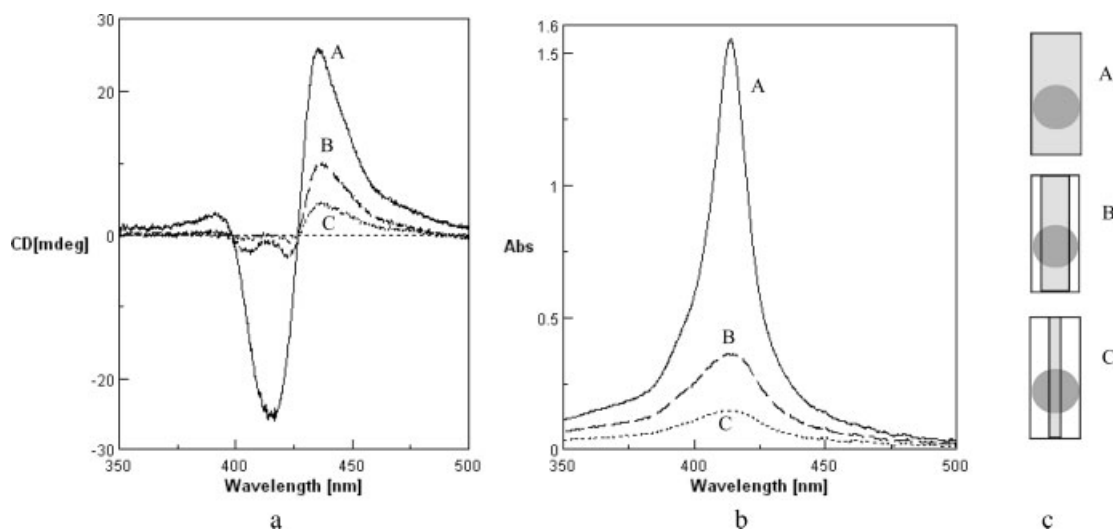
Fig. 2. a: CD spectra of Δ-RuPhen₃ 5 µM · H₂TPPS 5 µM in standard cell (A; ——), in 4 mm semimicro cell (B; - - - -) and in 2 mm microcell (C; .....).
b: Corresponding absorption spectra in standard cell (A; ——), in 4 mm semimicro cell (B; - - - -) and in 2 mm microcell (C; .....). **c**: Panels A, B, and C show the drawings for the employed cells with the principle of the AF emulator; (A) light beam passes through the solution in a normal 10 mm wide cell; (B) and (C) the sample is restricted in the 4- and 2-mm apertures of the microcells.
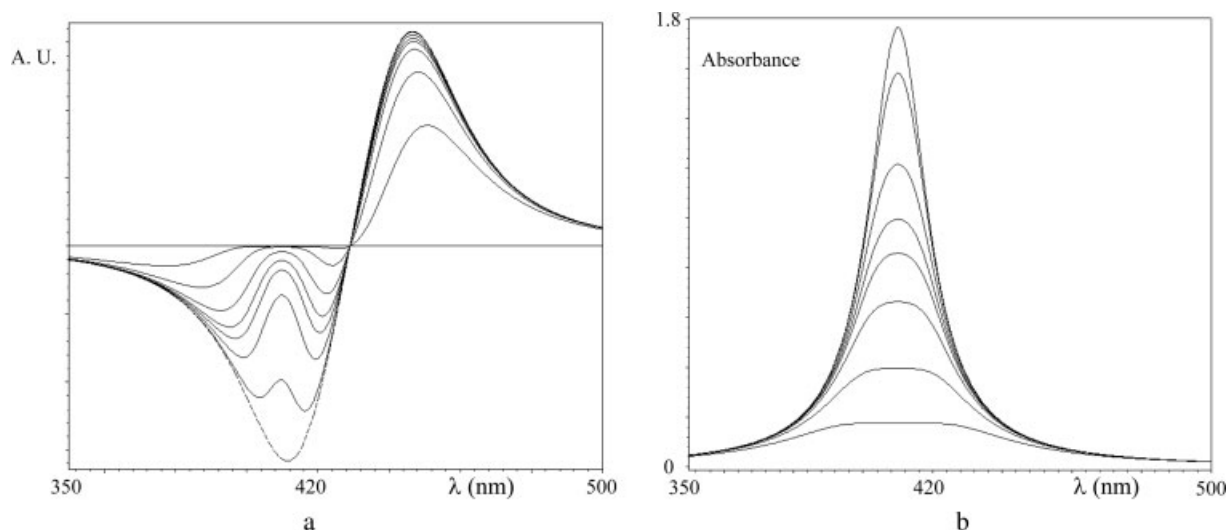
Fig. 3. CD spectra (**a**) and theoretical absorption (**b**) for an exciton couplet. In both graphs, spectral bands represented by the superposition of two Lorentzian functions (lines with highest maxima and lowest minima) are progressively modified by AF. Indeed, several traces in each graph, which correspond to f = 1, 0.95, 0.91, 0.87, 0.79, 0.60, and 0.33 (see Ref. 1), clearly show how a decrease in f is accompanied by a decrease in absorption and CD maxima. The Lorentzian functions composing the absorption band are centered at 411 and 409 nm and both have 10-nm half-bandwidths (i.e., the parameter of the Lorentian function γ is 10 nm). CD components are centered at 414 (−) and 444 (+) nm with 20-nm half-bandwidth. Absorbance units are the same as for experiments of Figure 2; CD units are arbitrary.

inhomogeneous distribution, AF will distort spectrum shapes. The absorption and CD bands are simulated by two superimposed Lorentzian bands, the center band frequencies of which, along with bandwidths, are given in the figure captions. (The bands are both positive in absorption and negative and positive in CD). Different bandwidths have been used for absorption and CD, according to what is gathered from experiments. As may be observed, when the absorption peak and the negative CD peak are close, in the presence of AF, one obtains the splitting of the negative band.

As a further step, we have considered a more concentrated solution (Δ-RuPhen$_3$ 10 μM · H$_2$TPPS 10 μM) in which the splitting of the negative band is already present in a normal width (10 mm) cell. Diluting the sample, the artifact disappears (Fig. 4). This figure shows clearly how distortions are induced at high concentration, due to AF, even though we cannot exclude other concomitant factors.

From the use of our AF emulator and from spectra simulation, we can reasonably believe that the band splitting observed in the spectra of several preparations at relatively high concentration/high absorbance is coming from absorption flattening.

It must be stressed clearly that the interaction between H$_2$TPPS porphyrin with the Δ-RuPhen$_3$ chiral compound is
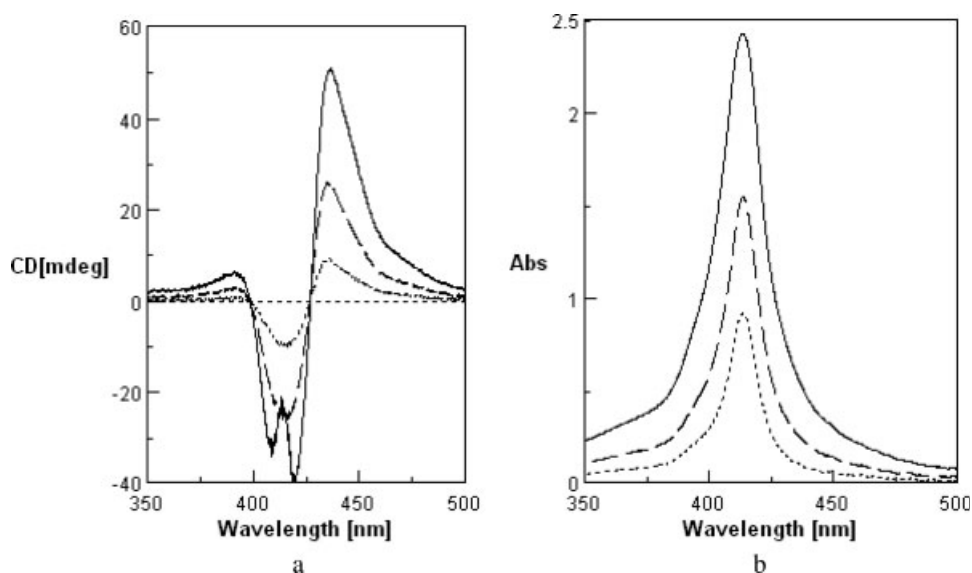
Fig. 4. **a**: CD spectrum of Δ-RuPhen$_3$ 10 μM · H$_2$TPPS 10 μM in standard 10 mm cell overlayed to CD spectra for diluted samples. **b**: Corresponding absorption spectra.
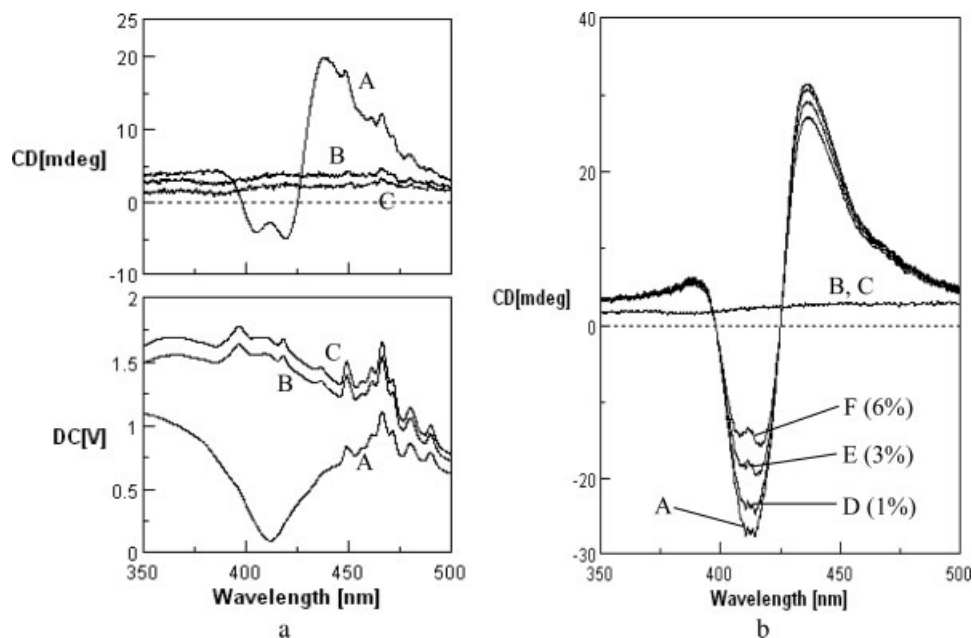
Fig. 5. a: AC (uncorrected CD) and DC spectra of Δ-RuPhen₃ 7 μM · H₂TPPS 7 μM (A), buffer (B), and air (C) obtained with constant HT on the photomultiplier tube. **b**: Calculated (AC/DC) CD spectra of the above solution and buffer baseline overlayed to (AC/DC) CD spectra for ≈1, 3, and 6% stray light obtained as explained in the text.

a process that takes place in a long-time scale and involves the formation of chiral aggregates. The phenomenon can be followed by the build-up of the CD exciton-coupled signal in the absorption wavelength region of the Ru compound; while interaction is always present, the generated aggregates may vary in dimensions. This may explain the variability of the final CD signal shape, when concentrations are high and AF may play a role.

Real Absorption Flattening or Instrumental Artifact?

We now wish to verify whether we are in presence of real AF or of instrumental artifacts, since it is known that at high absorbance levels, stray-light from the monochromator can be important and may influence the spectra shape. For this reason we have performed the following tests:

i. We have collected the CD spectra of Δ-RuPhen₃ 7 μM · H₂TPPS 7 μM not in the usual way (i.e., measuring CD as lock-in amplifier output, while the DC level is kept constant by dynode feedback), but measuring directly and separately the AC (corresponding to CD) and the DC components, while the high voltage on the photomultiplier tube is kept constant: these data are reported for air, for solvent, and for the sample in Figure 5a. The correct CD spectrum for the sample, obtained by ratioing AC over DC signals, is reported as trace A in Figure 5b, and is the strongest exciton couplet curve. Correspondingly data for air and solvent are traces B and C. Afterwards, we have emulated stray-light in the system, by offsetting the DC plot of 0.015, 0.05, and 0.1 V corresponding to about 1, 3, and 6% of

stray radiation. By proceeding in the same way as just described, we have obtained the CD spectra corresponding to the three stray-light modified DC situations (Figure 5b, traces D, E, and F). One may appreciate that the same spectral features/artifacts as we had noticed above appear also here. This confirms that instrumental stray-light will induce spectra distortions similar to AF. However, the needed amount of stray-light that may justify the distortions of Figure 1 is too high for double-monochromator instruments in this spectral range.

ii. To further check that in the measurements of Figures 1 through 4, in correspondence of high absorbances, stray-light effects may be neglected, we have performed two different tests with suitable samples in the same wavelength region. Solutions of perfectly soluble chiral Δ-RuPhen₃ were tested for three different concentrations (Figs. 6a and 6b), which allowed us to draw the following conclusions: albeit the maxima for absorbance are larger than 2, there is an excellent linearity of both absorption and CD data with concentration. However, since in this case the absorption band is broad, we have run a further experiment. We placed in series to a standard 10 mm cell filled with Δ-RuPhen₃ 5 μM · H₂TPPS 5 μM porphyrin another 10 mm cell filled with the achiral porphyrin H₂TPPS. Figures 6c and 6d show the absorption and CD spectra of the separate chiral and achiral porphyrin solutions plus that of the two cells together. The resulting CD spectra become much noisier, but the proper shape of the CD spectrum of the chiral porphyrin solution is maintained.
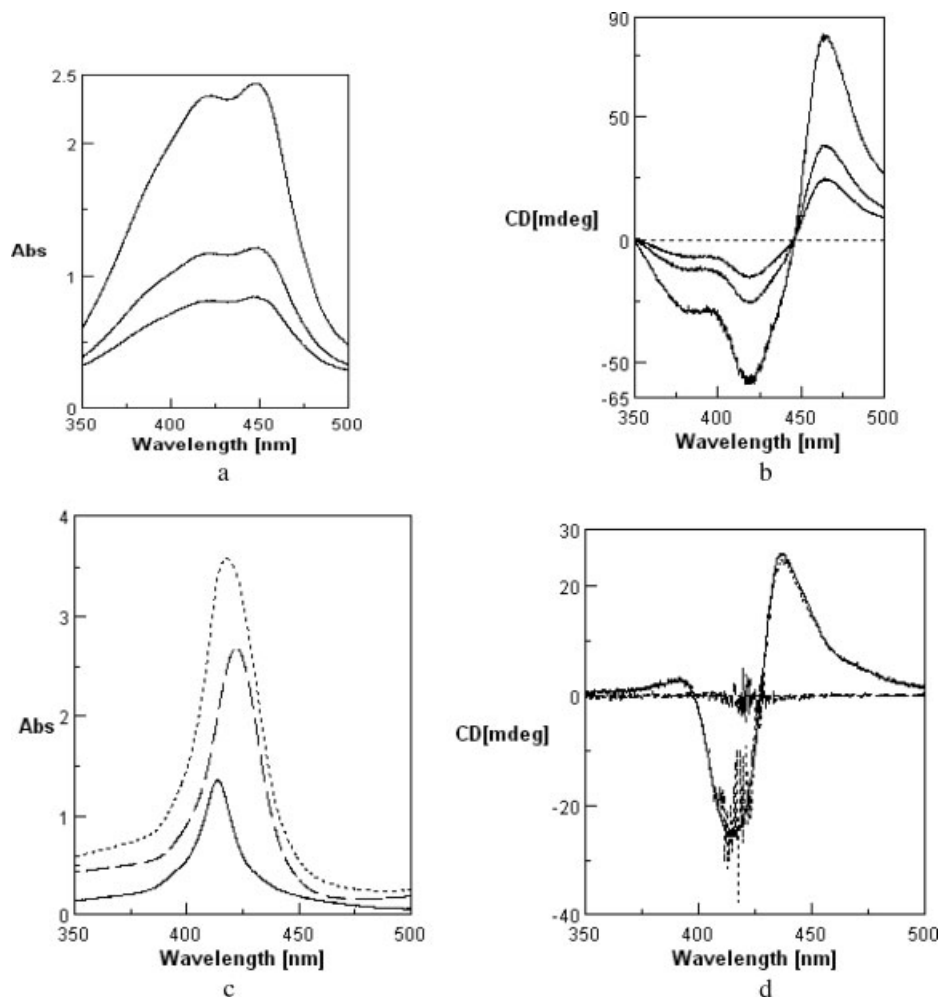
Fig. 6. Absorption (**a**) and CD (**b**) spectra of chiral Δ-RuPhen$_3$ solutions at different concentrations. Absorption (**c**) and CD (**d**) spectra of Δ-RuPhen$_3$ 5 μM · H$_2$TPPS 5 μM (———), achiral porphyrin (- - - -) and their sum measured experimentally (.......).

From these data we may convince ourselves that the observed distortions come from the sample itself, and not from the instrumentation used.

CONCLUSIONS

From the data reported above, we conclude that the occasional distortions noticed in the CD spectra of Δ-RuPhen$_3$ · H$_2$TPPS come from AF, due to sample inhomogeneous distribution, even in buffer solution, since the possibility of particle aggregation in some solutions has been proven.[4,5] Further evidence of this is based on our model calculations, which have allowed us to show for the first time (see Ref. 1) that a doublet is generated whenever AF takes place and the absorption bands' center is very close to the CD bands' center. In practical terms, this effect may be caused by aggregation or similar processes, which may induce other effects also on the spectral shape. Possible concomitant light scattering effects, as pointed out a long time ago by Ji and Urry (Ref. 6) have been evaluated here by placing the sample either close or very far

away from the detector (data not shown): no spectra distortions have been observed, except for a slight attenuation of both absorbance and CD spectra.

LITERATURE CITED

1. Castiglioni E, Abbate S, Longhi G, Gangemi R. Wavelength shifts in solid-state CD spectra: a possible explanation. Chirality 2007;19:491–496.
2. Duysens LMN. The flattening of the absorption spectrum of suspensions, as compared to that of solutions. Biochim Biophys Acta 1956;19:1–12.
3. Gordon DJ, Holzwarth G. Optical activity of membrane suspensions: Calculation of artifacts by Mie scattering theory. Proc Natl Acad Sci USA 1971;68:2365–2369.
4. Ribo JM, Crusats J, Farrera JA, Valero ML. Aggregation in water solutions of tetrasodium diprotonated meso-tetrakis(4-sulfonatophenyl)porphyrin. J Chem Soc Chem Commun 1994;6:681–682.
5. Micali N, Romeo A, Lauceri R, Purrello R, Mallamace F, Scolaro ML. Fractal structures in homo- and heteroaggregated water soluble porphyrins. J Phys Chem B 2000;104:9416–9420.
6. Ji TH, Urry DW. Correlation of light scattering and absorption flattening effects with distortions in the circular dichroism patterns of mitochondrial membrane fragments. Biochem Biophys Res Commun 1969;34:404–411.