Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Revisiting Absorption and Electronic Circular Dichroism spectra of cholesterol in solution: a joint experimental and theoretical study

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SUPPORTING INFORMATION

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1. Experimental details

1.1. Solvents cut-off range

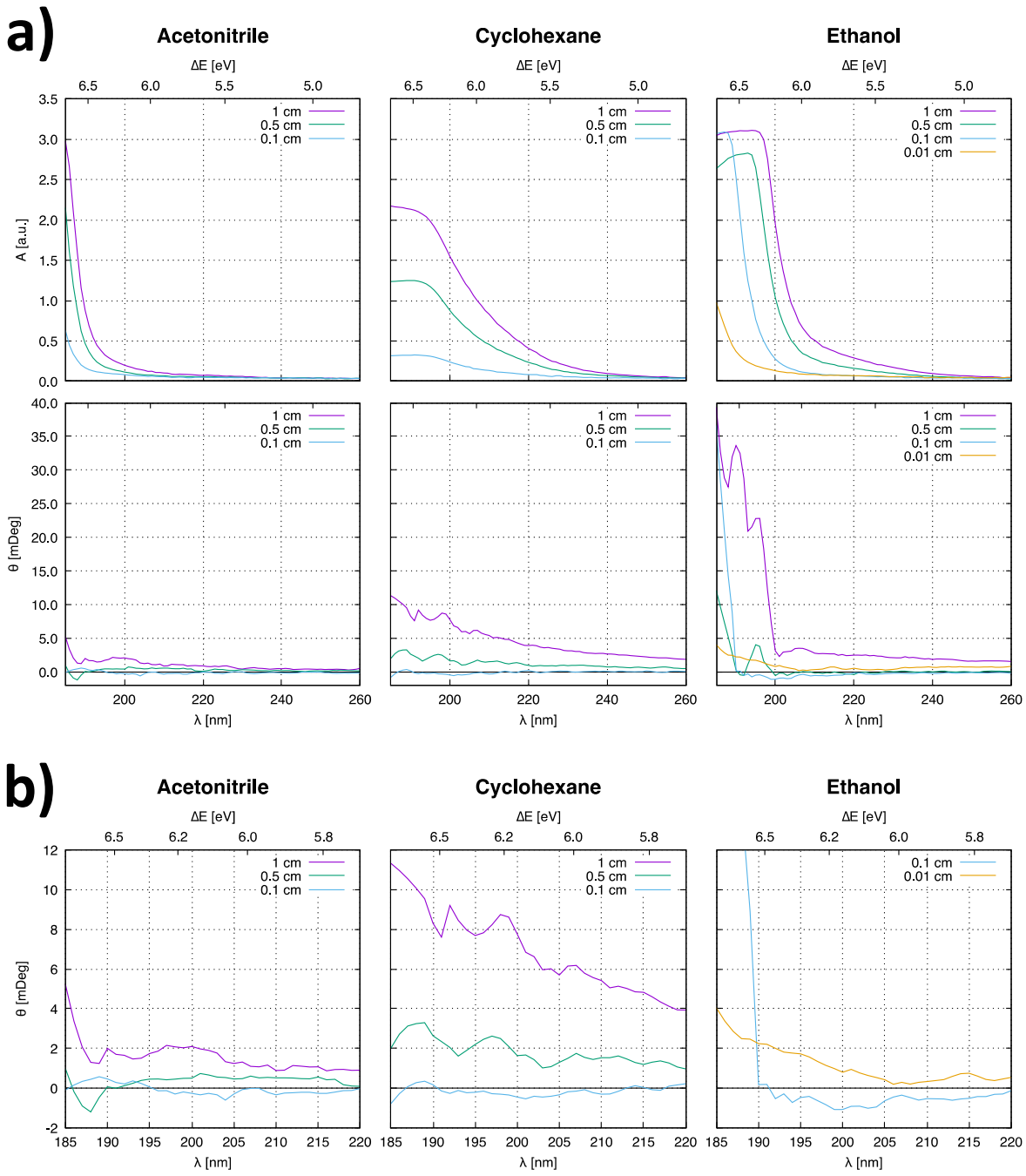

Figure 1: a) On top: absorption spectra vs. wavelength for acetonitrile, cyclohexane and ethanol, measured with different pathlengths. On bottom: ECD spectra (ellipticity (mDeg) vs. wavelength) for acetonitrile, cyclohexane and ethanol, measured with different pathlengths. b), zoom in the ECD spectra reported in a) on the [-2:12 mDeg] and the 185-220 nm ranges.

1.2. Time evolution of samples

A preliminary assay on different cholesterol samples in cyclohexane was made taking into account only the possible degradation of cholesterol after light absorption. The samples were thus kept in closed glass vials wrapped in aluminium foil between measurements.

The following spectra show the absorption spectra recorded for the same sample (*[CHOL]*= 6.05 10^{-4} M) at two different times (the second was recorded 5 hours after the first one) and in different cells. The first measurement was made in a 0.05 cm cell, while the second spectrum was recorded in the 0.10 cm cell. An apparent shift of λ_{max} and a modification of its relative intensity were observed (Figure 2). The increase of intensity is probably due to solvent evaporation (increasing cholesterol concentration) during the time elapsed between the two measurements. The λ_{max} shift might be due to solution degradation after light exposure and/or sample evolution.

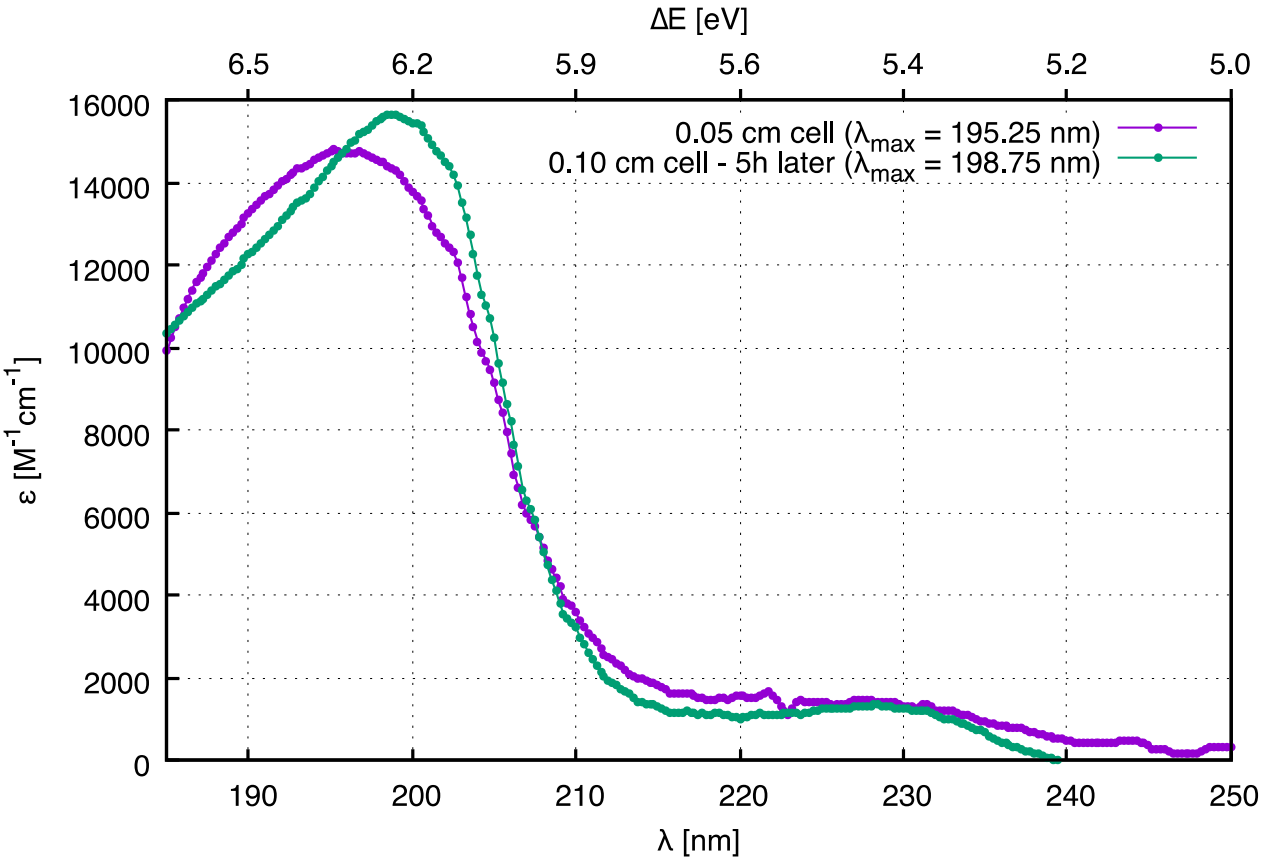

Figure 2: Two sequential absorption spectra recorded for the same sample at different times (5 hours later in the 0.10 cm path length cell). Cholesterol concentration for this sample is 3.45 10^{-4} M in cyclohexane.

1.3. Cholesterol Absorption and ECD spectroscopy in cyclohexane

- *Cholesterol concentrations with cyclohexane as solvent.*

The concentrations details for the samples are presented in the following Table 1.

Sample #	[CHOL] [M]	Δc/c [%]	λ_{max} [nm]	A(λ_{max})	Experiment
1	5.18E-04	4.6%	197.0	0.749	Abs.
2	4.31E-04	4.2%	196.0	0.621	Abs.
3	3.02E-04	4.0%	196.5	0.463	Abs.
4	2.16E-04	5.1%	196.5	0.296	Abs.
5	1.73E-04	4.8%	196.5	0.248	Abs.
6	6.47E-04	3.5%	/	/	3 ECD
7	4.31E-04	5.3%	/	/	3 ECD
8	5.39E-04	5.3%	/	/	3 ECD

Table 1: Concentration (and relative percentage error) for each sample. The λ_{max} value relative to each spectra is specified. The "Abs." label indicates that an absorption spectrum was recorded. The "3 ECD" label indicates that for each sample, 3 consecutives scans were recorded to obtain an ECD spectrum. Each sample was used for the recording of an unique spectrum.

- *Circular dichroism*

Due to solubility problems and instrumental noise, cholesterol in cyclohexane does not show linearity in ellipticity values with respect to concentration. In particular, a iso-dichroic point is found near 192 nm but has no vanishing (negative) ellipticity value (Figure 3). This is probably due to two factors: i) the very weak positive CD band becomes too weak when lowering the concentration and reaches the instrumental noise; (ii) we can not increase cholesterol concentration due to solubility problems.

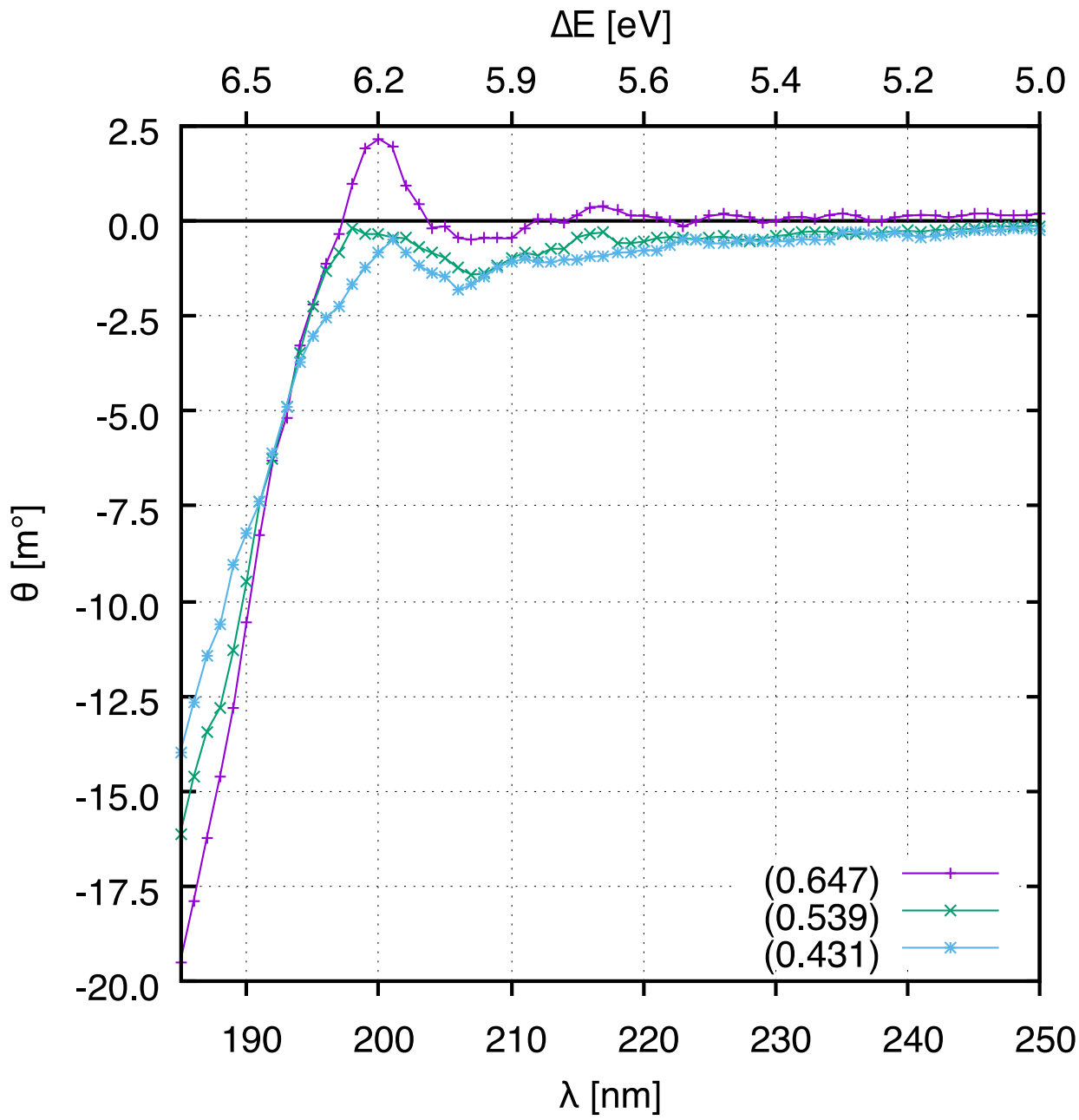

Figure 3: ECD spectra of Cholesterol at different concentrations. Ellipticity (mDeg) vs. wavelength (nm). The concentrations indicated on the caption are in mM.

1.4. Cholesterol Absorption and ECD spectroscopy in acetonitrile

- *Cholesterol concentrations with acetonitrile as solvent.*

Sample #	[CHOL] [M]	Δc/c [%]	λ_{max} [nm]	A(λ_{max})	Experiment
1	8.00E-04	2.8%	194.0	0.871	Abs.
2	7.00E-04	3.9%	194.0	0.746	Abs.
3	5.00E-04	3.3%	196.0	0.472	Abs.
4	4.00E-04	3.2%	194.5	0.257	Abs.
5	4.00E-04	3.7%	194.5	0.575	Abs.
6	2.00E-04	4.3%	196.0	0.439	Abs.
7	6.50E-04	4,5%	/	/	3 ECD
8	5.00E-04	4,3%	/	/	3 ECD
9	3.00E-04	2,9%	/	/	3 ECD

Table 2: Concentration (and relative percentage error) for each sample. The "Abs." label indicates that an absorption spectrum was recorded. The "3 ECD" label indicates that for each sample, 3 consecutives scans were recorded to obtain an ECD spectrum. Each sample was used for the recording of an unique spectrum.

2. Computational details

2.1. Basis-set effect

As already pointed out by Kongsted and coworkers[1], the use of diffuse basis sets in excited states calculations creates a mixing of valence- and Rydberg-type excited states. Consequently, many low-lying weak excited states are observed, and the first singlet excited state S_1 does not coincide anymore with the bright(test) excited state. We verify this using different basis sets for the same functional (CAM-B3LYP). The results are clearly visible in the following Figure 4.

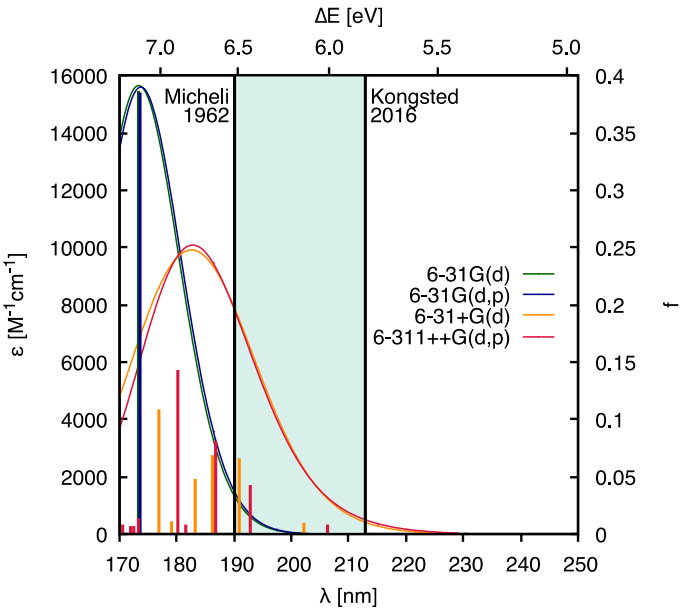

Figure 4: Calculated (convolution made with GaussView5.1 and HWHM parameter set to 0.333 eV) cholesterol absorption spectra with the CAM-B3LYP functional and different basis sets. The λmax values from the Micheli[2] and Kongsted[1] works are indicated for sake of comparison.

2.2. Geometries for vertical excitations

In the paper, the computation of the excited states with various exchange-correlation functionals were performed at the same molecular geometry obtained at the B3LYP-D3/6-31+G(d) level of theory. The following table show that the changes in the excited state energies (but also in their

oscillator and rotational strengths) when reoptimizing the ground state geometry at the same level of theory as that employed in the excited state calculation are negligible.

System:	PCM acetonitrile									
FC geometry found with:	PBE0/6-311++G(d,p)+D3					B3LYP/6-31+G(d,p)				
Excited states computed with:	PBE0/6-311++G(d,p)					PBE0/6-311++G(d,p)+D3				
	ΔE (eV)	*λ (nm)*	*f*	R_{vel}	R_{length}	*ΔE (eV)*	*λ (nm)*	*f*	R_{vel}	R_{length}
S_1	5.7955	213.93	0.0172	5.1653	5.311	5.7995	213.79	0.0184	5.6299	5.8101
S_2	6.0932	203.48	0.0085	6.9889	7.3097	6.1051	203.08	0.0125	7.2857	7.5352
S_3	6.1518	201.54	0.0149	-10.8598	-11.2796	6.1494	201.62	0.0135	-13.3313	-13.7109
S_4	6.1877	200.37	0.0024	2.5923	2.5807	6.1922	200.23	0.0034	4.127	4.1143
S_5	6.3584	194.99	0.1169	-16.981	-17.1571	6.3549	195.1	0.1257	-17.3919	-17.5229
S_6	6.4675	191.7	0.0542	8.7968	9.9591	6.4699	191.63	0.0637	8.5155	9.8479
S_7	6.5706	188.69	0.0045	-0.4593	-0.2185	6.5729	188.63	0.0046	-0.1875	0.0852
S_8	6.6099	187.57	0.0709	-6.4511	-7.0019	6.6104	187.56	0.0635	-5.6492	-6.1842
S_9	6.6651	186.02	0.0012	2.1786	2.2181	6.6617	186.12	0.0014	1.504	1.5486
S_{10}	6.7119	184.72	0.0239	7.1665	7.6499	6.7118	184.73	0.0209	5.4558	5.9219

Table 3: Transition energies (in nm), oscillator strengths f, and rotatory strengths (in its velocity representation R_{vel} and in its length representation R_{length} both in units of 10^{-40} esu^2 cm^2) for the first 10 singlet excited states computed in the PCM-acetonitrile system.

3. Bibliography

1 L. J. Nåbo, J. M. H. Olsen, N. Holmgaard List, L. M. Solanko, D. Wüstner and J. Kongsted, *J. Chem. Phys.*, 2016, **145**, 104102.

2 R. A. Micheli and T. H. Applewhite, *J. Org. Chem.*, 1962, **27**, 345–353.