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The absorption spectrum of *cis*-azobenzene†

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Azobenzene is a prototypical photochromic molecule existing in two isomeric forms, which has numerous photochemical applications that rely on a precise knowledge of the molar absorption coefficients (ϵ). Careful analysis revealed that the previously reported absorption spectra of the “pure” isomers were in fact mutually contaminated by small amounts of the other isomer. Therefore, the absorption spectra of both *trans*- and *cis*-azobenzene in methanol were re-determined at temperatures of 5–45 °C. The thermodynamically more stable *trans*-azobenzene was prepared by warming the solution in the dark. To obtain the spectrum of *cis*-azobenzene three methods were used, which gave consistent results within the limits of error. The method based on the subtraction of derivative spectra coupled with a global analysis of the spectra recorded during thermal *cis*–*trans* isomerization is shown to give slightly more reliable results than the method using isomeric ratios determined by ¹H-NMR. The described methods are readily generalizable to other azobenzene derivatives and to other photochromic systems. The practical implication of the re-determined ϵ values is demonstrated by a very high precision of spectrophotometric species analysis in azobenzene isomeric mixtures. The new ϵ values imply that the previously reported quantum yields must be revised.

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1 Introduction

The existence of the *cis*-isomer of azobenzene was first reported by Hartley in 1937 in a *Nature* paper entitled: “The *Cis*-form of Azobenzene”.¹ The discovery was based on the increased solubility of *cis*-azobenzene in polar solvents compared to that of the *trans*-isomer and its high dipole moment. Although the photochemical and thermal isomerization of azobenzene and its derivatives became textbook examples of the process, the details of the reaction mechanism are still under dispute.^{2–8} The reversible photoisomerization reaction (Fig. 1) of azobenzene derivatives has been exploited in numerous applications of photochemistry such as photoswitching to

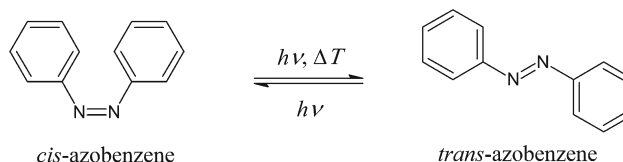


Fig. 1 The photoisomerization of azobenzene.

provide effective photocontrol of a large variety of biomolecules,^{9,10} molecular receptors,¹¹ logic units,¹² smart materials¹³ and devices,¹⁴ holographic materials,¹⁵ for molecular motion,¹⁶ and last but not least, azobenzene is frequently used as a convenient actinometer to count incident photons. Azobenzene was proposed as an actinometer for monochromatic light sources at wavelengths up to 450 nm by Gauglitz.^{17–20} Besides ferrioxalate, azobenzene is arguably the most common and frequently used actinometer,²⁰ however, sometimes with inconsistent results from different laboratories.^{21–24} Accurate knowledge of both the quantum yields and the molar absorption coefficients is a prerequisite for spectrophotometric actinometry.

In an effort to determine photon fluxes by using both the ferrioxalate and azobenzene actinometers we obtained significant discrepancies in the results and we noted a few pitfalls in the recommended procedures of quantum yield determination about which we wish to warn potential users. In this paper, we address the first problem that needs to be resolved: the molar

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† Electronic supplementary information (ESI) available: The values of the ϵ_{trans} , ϵ_{cis} , Gaussian peaks analysis. Minimization procedure for obtaining the concentrations of *trans*- and *cis*-azobenzenes from the absorption spectrum. Fig. S1–S16. See DOI: 10.1039/C7PP00314E

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absorption coefficients of the two isomers of azobenzene. We re-determined the molar absorption coefficients of both isomers in methanol, that of the *cis*-azobenzene by using three methods. Quantum yield measurements will be reported in the accompanying paper.²⁵

2 Experimental

2.1 Chemicals

For measurements of the molar absorption coefficients freshly recrystallized azobenzene (Reachim, pure) was used as described in the accompanying paper.²⁵ Methanol (Merck, Uvasol for spectroscopy) was used without further purification. For the NMR measurements the methanol-*d*₄ was stored over dried 3 Å molecular sieves (812 mesh) under dry N₂.

2.2 Instrumentation

The optical bench used for the preparation of *ca.* 97% *cis*-azobenzene in solution for the determination of the molar absorption coefficients consisted of a monochromator (MSH 150, LOT), a cuvette holder with a stirrer, and a Xe lamp, XBO 450 W, Osram. The light beam passing through the cuvette was collimated by quartz lenses. UV-Vis spectra were recorded using a double beam spectrophotometer Cary 5000-Agilent with a thermostated sample holder (the reference was at laboratory temperature), or a Shimadzu 1602 spectrophotometer in the case of the NMR/UV-Vis ratios. The ¹H-NMR spectra were recorded on a Bruker 500 MHz spectrometer without rotating the cell.

3 Results

3.1 Molar absorption coefficients of *trans*-azobenzene

Three samples of thrice recrystallized azobenzene (*ca.* 22.8 mg, weighed independently) were transferred to 50 mL volumetric flasks that were filled with methanol (HPLC grade). From these solutions aliquots of 2.00 mL were delivered to 50 mL volumetric flasks and filled with methanol. Samples of 4.7 mL were placed in a quartz cuvette (Hellma 117.100-QS), which was tightly closed using a cap with a Teflon septum and then wrapped with Parafilm. The cell was filled almost completely to minimize the evaporation of the solvent. The cuvette was kept in the dark at 45 °C in a thermostated cell holder for two weeks to completely convert any remaining contamination by the *cis*-isomer to the thermodynamically more stable *trans*-isomer (see section 4), and UV-Vis spectra were subsequently recorded at 5, 15, 25, 35, and 45 °C using methanol as the reference. The samples were held at each new temperature until changes in the absorption spectra were within the instrumental noise. The concentrations of azobenzene at the specified temperatures were calculated considering the thermal expansion of methanol based on the temperature-dependent thermal expansion coefficient $\alpha/K^{-1} = (0.10787 + 0.00367T/K) \times 10^{-3}$, which was determined as a linear approximation from three specific volumes determined in the range of 298.15 to 333.15 K by Machado *et al.*²⁶ Because of the large spread of

molar absorption coefficients at different wavelengths, two series of concentrations were used ($c \sim 2.5 \times 10^{-3}$ M and $c \sim 1 \times 10^{-4}$ M, 2 and 3 independent weighings, respectively) and the spectra were joined at 360 nm. The spectrum of *trans*-azobenzene at 25 °C is shown in Fig. 2, the values at particular wavelengths are given in Table 1, and full spectra are given in the ESI† together with a Gaussian peak fit (Fig. S1 and Table S1†). Variations of the spectra with temperature are shown in Fig. S3.†

3.2 Molar absorption coefficients of *cis*-azobenzene

While the method described above easily provides accurate spectra of *trans*-azobenzene, additional work is required to obtain those of the *cis*-isomer. Pre-irradiation of azobenzene in a methanol solution at 348 nm produces a mixture containing mostly the *cis*-isomer, but its precise composition is unknown. The irradiation time required to reach the photostationary state (PSS) on our setup was *ca.* 18 hours. Three approaches were used to determine ϵ_{cis} , which gave consistent results.

3.2.1 TEM. Firstly, we used the simple method known by the acronym TEM (for Thulstrup, Eggers, and Michl).²⁸ In our case, there is a noticeable vibronic structure in the absorption spectrum of *trans*-azobenzene, whereas the *cis*-azobenzene is known to have none.²⁹ Hence, difference spectra of the 2nd derivatives

$$\frac{d^2 \frac{A_{PSS}}{C_{tot} \times l}}{d\lambda^2} - c \times \frac{d^2 \epsilon_{trans}}{d\lambda^2}$$

were plotted, where A_{PSS} is the absorbance at the PSS, C_{tot} is the total concentration of azobenzene, and l is the optical path

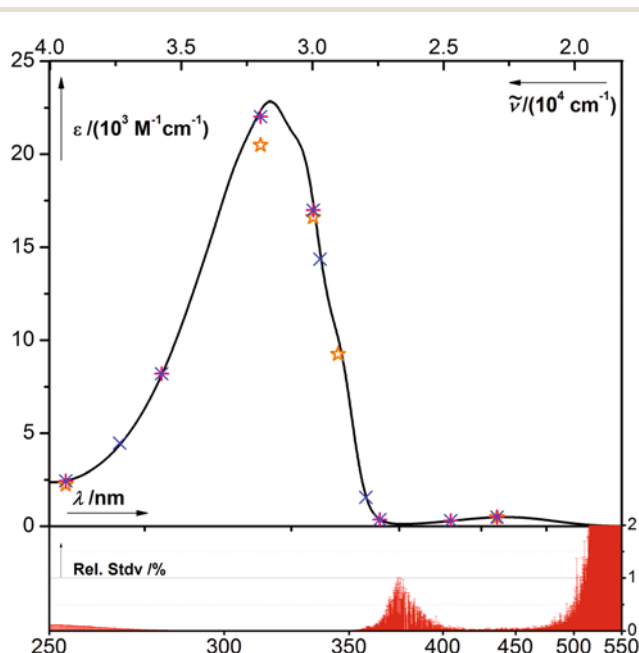


Fig. 2 The molar absorption coefficients (ϵ_{trans}) of *trans*-azobenzene in methanol at 25 °C and their comparison with published data: orange stars,²⁷ pink plus sign,¹⁹ and blue x.¹⁸ Bottom: Relative standard deviations among independent samples in %.

Table 1 The molar absorption coefficients ($\epsilon_{trans}/(\text{M}^{-1} \text{cm}^{-1}) \pm$ sample standard deviation) of *trans*-azobenzene in MeOH at 25 °C at representative wavelengths compared to reported values

λ/nm	Ronayette ²⁷	Gauglitz ¹⁹	Hubig ¹⁸	This work
254	2240	2450	2450	2446.0 \pm 2.6
268	—	—	4460	4365.4 \pm 2.0
280	—	8200	8200	8146.9 \pm 1.5
313	20 500	22 020	22 020	22337.4 \pm 1.0
334	16 600	16 980	16 980	17383.6 \pm 0.7
337	—	—	14 360	14733.4 \pm 0.6
345	9260	—	—	10077.4 \pm 0.7
355 ^a	—	—	—	3098.1 \pm 0.7
358	—	—	1550	1658.1 \pm 0.7
365	—	360	360	374.0 \pm 0.6
405	—	305	305	287.3 \pm 0.1
436	475	490	490	486.2 \pm 0.3

^a $\epsilon_{trans}^{355 \text{ nm}}/(\text{M}^{-1} \text{cm}^{-1}) = 2992 \pm 89$.²²

length of the cuvette. The coefficient c was chosen such that the vibronic features in the range of 300–380 nm disappeared from the difference spectrum (Fig. S4†). This procedure allowed us to estimate the relative amount of the *trans*-isomer in the spectra obtained for different samples close to the PSS. For individual runs it ranged between 2 and 4%. Then ϵ_{cis} was calculated as

$$\epsilon_{cis} = \left(\frac{A_{\text{PSS}}}{C_{\text{tot}} \times l} - c \times \epsilon_{trans} \right) \times \frac{1}{1 - c}.$$

The factor $1/(1 - c)$ is the correction for the subtracted amount of *trans*-azobenzene. The molar absorption coefficients obtained by this method are denoted as $\epsilon_{cis}^{\text{TEM}}$ showing very good reproducibility as exemplified in Fig. S5.† The corrected spectra of *cis*-azobenzene, which were obtained by triplicate independent weighing and measured at the temperatures of 5, 15, 25, 35, and 45 °C, are shown in Fig. S6.†

3.2.2 NMR. Secondly, we used ¹H-NMR to determine the ratio

$$p_t = c_{cis,t}/c_{trans,t}$$

in a sample of azobenzene ($6\text{--}8 \times 10^{-4} \text{ M}$) in deuterated methanol as a function of the total time t of irradiation at 348 nm to approach the PSS containing predominantly the *cis*-isomer. The irradiation wavelength was chosen based on the anticipated ratio of molar absorption coefficients of *cis*- and *trans*-azobenzene (see Fig. 5), which should give the highest concentration of the *cis*-isomer in the PSS. The temperature of the sample was around 25 °C (NMR measurements were done at 30 °C and UV-Vis measurements at RT, 22–25 °C). Experiments allowing the reaction progress to be monitored both by ¹H-NMR and UV-Vis spectroscopy were done using an optical quartz cuvette (0.1 cm path length) that was collinearly fused to an NMR tube of 5 mm diameter. This allowed UV-Vis absorption and NMR measurements of the same solution as well as intermittent irradiation at 348 nm with minimal exposure to ambient light or losses upon sample manipulation. Typical ¹H-NMR spectra at varying *cis/trans* ratios are depicted in Fig. S7.†

The absorbances A_t at time t can be described by eqn (1)

$$\frac{A_t(\lambda)}{l} = c_{trans,t}\epsilon_{trans}(\lambda) + c_{cis,t}\epsilon_{cis}(\lambda) \quad (1)$$

where l is the path length of the cell, $c_{trans,t}$ and $c_{cis,t}$ are the concentrations of the *trans*- and *cis*-azobenzene at time t , and ϵ_{trans} and ϵ_{cis} are the molar absorption coefficients of *trans*- and *cis*-azobenzene, respectively. Because the total concentration of azobenzene remains constant during the series of measurements,

$$C_{\text{tot}} = C_{trans,t} + C_{cis,t} \quad (2)$$

and the ratio of the concentrations of *cis* and *trans* forms p_t is known from the NMR measurements, eqn (1) can be transformed to

$$\frac{A_t(\lambda)}{l} = C_{\text{tot}}\epsilon_{trans}(\lambda) \frac{1}{1 + p_t} + C_{\text{tot}}\epsilon_{cis}(\lambda) \frac{p_t}{1 + p_t}. \quad (3)$$

For the purposes of numerical analysis, eqn (3) will be rewritten as

$$y_t(\lambda) = a(\lambda)x_{trans,t} + b(\lambda)x_{cis,t} \quad (4)$$

where

$$y_t = \frac{A_t}{l}$$

$$a = C_{\text{tot}}\epsilon_{trans}$$

$$b = C_{\text{tot}}\epsilon_{cis}$$

$$x_{trans,t} = \frac{1}{1 + p_t}$$

$$x_{cis,t} = \frac{p_t}{1 + p_t}.$$

Using the least squares method the parameters a and b were calculated separately for each wavelength:

$$a = \frac{\left(\sum_t x_{trans,t} y_t \right) \left(\sum_t x_{cis,t}^2 \right) - \left(\sum_t x_{cis,t} y_t \right) \left(\sum_t x_{trans,t} x_{cis,t} \right)}{\left(\sum_t x_{trans,t}^2 \right) \left(\sum_t x_{cis,t}^2 \right) - \left(\sum_t x_{trans,t} x_{cis,t} \right)^2}$$

$$b = \frac{\left(\sum_t x_{cis,t} y_t \right) \left(\sum_t x_{trans,t}^2 \right) - \left(\sum_t x_{trans,t} y_t \right) \left(\sum_t x_{trans,t} x_{cis,t} \right)}{\left(\sum_t x_{trans,t}^2 \right) \left(\sum_t x_{cis,t}^2 \right) - \left(\sum_t x_{trans,t} x_{cis,t} \right)^2}.$$

From the known ϵ_{trans} the total azobenzene concentration is calculated as the slope of the equation $a(\lambda) = C_{\text{tot}} \times \epsilon_{trans}(\lambda)$. The molar absorption coefficient of *cis*-azobenzene at each wavelength is calculated as $\epsilon_{cis}(\lambda) = b(\lambda)/C_{\text{tot}}$.

The resulting 7 spectra of molar absorption coefficients of *cis*-azobenzene were determined from seven independent experiments of six independently weighed samples of azobenzene in deuterated methanol; each experimental ϵ_{cis} was calculated from 5 to 23 input data sets of UV-Vis absorption

spectra and the corresponding isomer ratios obtained by NMR. The minimization was performed in the spectral range of 235 to 600 nm. Trials using wavelengths below 230 nm resulted in errors due to the absorbance by methanol. The resulting molar absorption coefficients of *cis*-azobenzene are presented in Fig. S8† and the averaged ϵ_{cis}^{NMR} is depicted in Fig. 4.

3.2.3 Thermal isomerization. A third estimate of ϵ_{cis} was then obtained from the series of spectra recorded during the thermal isomerization from the PSS induced by 348 nm irradiation towards the *trans*-isomer at 35 °C. Care was taken to minimize the concentration changes caused by solvent evaporation and condensation on the lid by filling the cuvette as much as possible. The UV-Vis spectra were recorded at regular intervals, e.g., every 10 min up to 1000 min, then every 30 min up to 4000 min, and every 60 min up to 7960 min (ca. 5.4 days overall). The matrix of the absorption spectra (more than 250 spectra) was subjected to singular value decomposition.²⁰ Two overwhelming eigenvectors were obtained. To account also for

Table 2 The average rate constants of thermal *cis* to *trans* isomerization of azobenzene at various temperatures

$T/^\circ\text{C}$	k/s^{-1}
20	$(7.240 \pm 0.075^a) \times 10^{-7}$
25	$(1.092 \pm 0.004^a) \times 10^{-6}$
35	$(3.462 \pm 0.058^b) \times 10^{-6}$
45	$(1.168 \pm 0.004^a) \times 10^{-5}$

^a Standard deviation of a single kinetic trace. ^b Standard error of the sample mean from five kinetic traces.

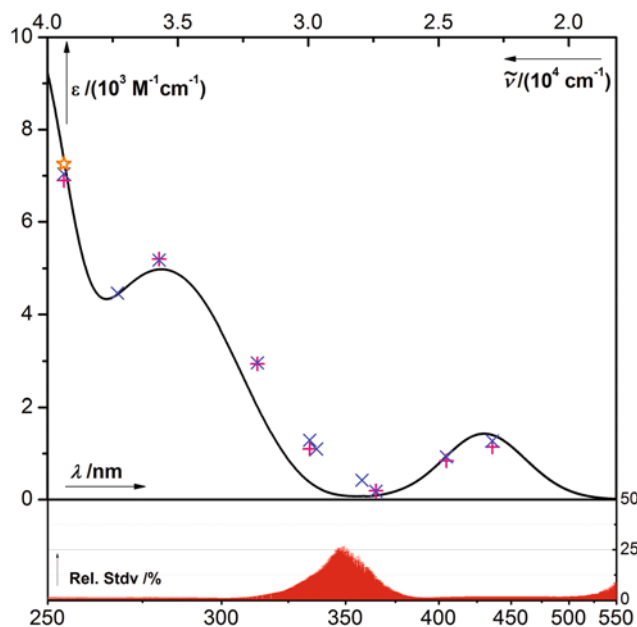


Fig. 3 The molar absorption coefficients (ϵ_{cis}^t) of *cis*-azobenzene in methanol at 35 °C and comparison with published data: orange stars,²⁷ pink plus sign,¹⁹ and blue x.¹⁸ Bottom: Relative standard deviations among independent samples in %.

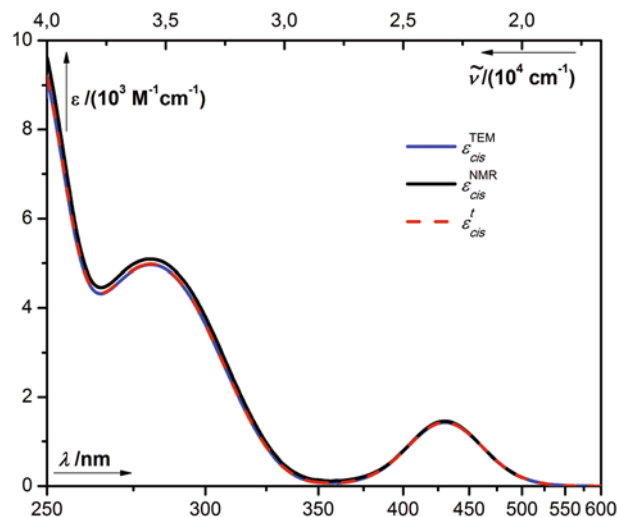


Fig. 4 Comparison of the molar absorption coefficients (ϵ_{cis}) of *cis*-azobenzene in methanol obtained by three methods (TEM – blue, NMR – black, and thermal isomerization – red). Spectra are determined at 35, 25 and 35 °C, respectively.

the noise, three eigenvalues were allowed and the matrix of absorbances was reconstructed using three eigenvectors. The target factor analysis was performed using the known initial concentrations of the *trans*- and *cis*-isomers that were obtained by the TEM method. The global analyses of the series of spectra obtained during the thermal isomerization (*cis* to *trans*) at various temperatures in methanol accurately followed the first-order rate law (representative examples are shown in Fig. S9 to S12†) and thus provided for the spectra of the starting *cis*-azobenzene and the rate constants (Table 2). The spectra obtained from three independent samples are shown in Fig. S13;† their average ϵ_{cis}^t is depicted in Fig. 3 and 4, and is also used for fitting with Gaussian peaks (Fig. S2, Table S2†).

4 Conclusions

In previous work, the *cis*-isomer of azobenzene was isolated after exhaustive irradiation of saturated solutions of *trans*-azobenzene in glacial acetic acid³⁰ or acetone¹ at ca. (440 ± 30) nm and was recrystallized from petroleum ether at room temperature or ice-salt freezing temperature (−10 to −20 °C) as small, bright red crystals. The isomers were also separated by liquid chromatography¹⁹ or preparative TLC, for which a contamination of 1% was estimated.^{31,32} The *cis*-isomer is reported to be stable in its crystalline form in the dark.³⁰ It absorbs more strongly than the *trans*-form throughout the visible region,³³ in accord with the fact that the $n \rightarrow \pi^*$ transition of azobenzene is symmetry-allowed for the *cis*-isomer, but forbidden for the *trans*-isomer.²⁹

As the *cis*-form is estimated to be (42–55) kJ mol^{−1} thermodynamically less stable than the *trans*-isomer,^{34–36} the thermal isomerization to the *trans*-form is essentially irreversible.

Therefore the method applied here for obtaining the *trans*-isomer by heating the solution prepared from recrystallized *trans*-azobenzene to an elevated temperature under careful exclusion of light leads to very pure samples of *trans*-azobenzene in solution. The molar absorption coefficients of *trans*-azobenzene (Fig. 2, Table 1) largely agree with previously reported data, but they are somewhat higher for the $S_0 \rightarrow S_2$ (300–365 nm) and lower for the $S_0 \rightarrow S_1$ (405–436 nm) transition than those reported. This indicates a minor contamination by the *cis*-isomer in the samples of *trans*-azobenzene used in previous investigations. The precision, indicated by the standard deviation among independent samples, of our repeated analyses is comparable to that of repeated solution preparation by independent weighing. The relative standard deviations of the values are largest for wavelengths with very low ϵ_{trans} , as could be expected, but they never exceed 3% in the measured spectral range. The spectra of *trans*-azobenzene were found to be temperature-dependent (Fig. S3†) as mentioned in the literature.³⁷

The spectra of the *cis*-isomer obtained here by three methods give results that are very similar to each other (Fig. 4); the values of individual ϵ_{cis} lie within the confidence intervals of the others, and ϵ_{cis}^t and ϵ_{cis}^{TEM} are almost identical. Our best estimate of the true *cis*-azobenzene absorption spectrum is the one obtained from the global analysis of the thermal reaction, based on the following discussion.

The TEM method assumes that the spectrum of *cis*-azobenzene does not show any vibronic structure similar to that of *trans*-azobenzene. This assumption is justified by published research, where no vibronic structure was found even at 77 K for *cis*-azobenzene,²⁹ whereas the vibronic structure is apparent even at room temperature for *trans*-azobenzene (Fig. 2 and S4†). This is also supported by the number of fitted Gaussian curves (see ESI section 3†). The TEM method gave good values of molar absorption coefficients, which were almost identical to those determined by thermal isomerization, ϵ_{cis}^t (Fig. 4 and S5†), but the noise level was a little higher for the former. The TEM method is not time demanding and permits the determination of ϵ_{cis} at lower temperatures, which is impractical using thermal isomerisation due to a very slow rate of isomerisation. The ϵ_{cis} at various temperatures showed little thermal dependence (Fig. S6†).

In the NMR method, the spectral changes induced by irradiation at 348 nm with the simultaneous monitoring of the isomeric ratio by ¹H-NMR were analyzed. Replicate analyses averaged well to the spectrum obtained by the other methods but some spectra from individual experiments showed a vibrational structure and a corresponding spread in values especially at wavelengths where *trans*-azobenzene exhibits a vibrational structure (Fig. S8†). This method may be sensitive to imprecise control of the sample temperature and possibly to some exposure to laboratory illumination even though we operated with the special fused UV-Vis-NMR cuvette and took all precautions to avoid any unwanted illumination by working at night and covering the windows and the instrument's LEDs.

The *cis*-azobenzene absorption spectra obtained by the thermal method show relatively high values at the spectral maxima (282 and 430 nm), low absorptions at the spectral troughs (around 360 nm), and very low scatter between independent samples (Fig. S13†). This analysis is influenced by the TEM estimate of the PSS composition of the first spectrum but it ought to be more reliable because it is averaged from hundreds of spectra creating the kinetic trace. The measured samples were kept all the time at the desired temperature in the darkness of the UV-Vis spectrometer; therefore no unwanted photochemical isomerization occurred. The intensity of the monitoring light in the double beam spectrometer did not cause any measurable photochemical transformation. Therefore, we consider this spectrum the best estimate of the absorption spectrum of *cis*-azobenzene, use it in the following discussion, show it in Fig. 3, and list the $\epsilon_{cis}^t(\lambda)$ in Table 3. Our attempts of using the thermal isomerization method at lower temperatures were hampered by long measurement times and problems with the baseline stability. As we did not find any temperature dependence of ϵ_{cis} by the TEM method, we use this spectrum of *cis*-azobenzene obtained at 35 °C also for lower temperatures.

Our values of ϵ_{cis} compare well with those reported below 270 nm, but are lower at 300–365 nm, and higher at 405–436 nm (Table 3). A comparison of the spectrum of *cis*-azobenzene with those reported previously^{18,19} indicates that all of the latter were contaminated by some amount of *trans*-azobenzene.

The ϵ_{cis} uncertainties of the thermodynamically unstable *cis*-isomer are much larger than those of the *trans*-isomer because the spectrum of the *trans*-isomer is used to correct that of impure samples of the *cis*-isomer. Still, the relative standard deviation does not exceed 25% even for very low ϵ_{cis} values below 100 M⁻¹ cm⁻¹.

The obtained absorption spectra can be characterized by their absorption maxima at 316 and 443 nm for the *trans* isomer and at 280 and at 430 nm for the *cis* isomer, and also by the ratio of the molar absorption coefficients presented in Fig. 5. This ratio is helpful in the choice of light sources for azobenzene switching in particular applications. The maximal

Table 3 The molar absorption coefficients ($\epsilon_{cis}/M^{-1} \text{ cm}^{-1} \pm$ sample standard deviation) of *cis*-azobenzene in MeOH at 35 °C at representative wavelengths compared to reported values

λ/nm	Gauglitz ¹⁹	Hubig ¹⁸	This work
254	6900	6890	7342 ± 93
268	—	4460	4431 ± 53
280	5200	5070	4976 ± 59
313	2940	2950	2016 ± 37
334	1100	1275	378 ± 43
337	—	1100	276 ± 37
358	—	280	73 ± 13
365	190	190	89 ± 9
405	840	920	942 ± 12
436	1140	1290	1402 ± 23

$$\epsilon_{cis}^{254 \text{ nm}}/M^{-1} \text{ cm}^{-1} = 7260.^{27}$$

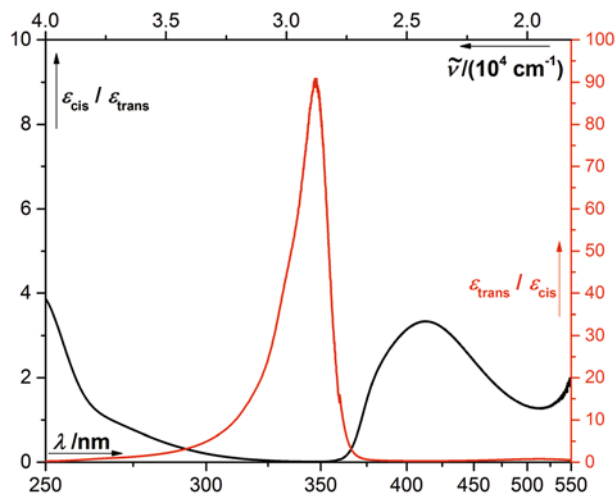


Fig. 5 The ratio of molar absorption coefficients $\epsilon_{cis}/\epsilon_{trans}$ (black) and $\epsilon_{trans}/\epsilon_{cis}$ (red, dashed) of azobenzene in methanol.

values for *trans*-azobenzene are reached at 347 nm, where it absorbs 91 times more than the *cis*-azobenzene, and for *cis*-azobenzene at 413 nm, where it absorbs 3.3 times more than the *trans* isomer. The isosbestic points are located at 268 and 372 nm.

The spectra of the molar absorption coefficients of the two isomers were used in minimization to determine their individual concentrations from the measured absorption spectra of their mixtures (for details see ESI section 1†). The method works perfectly without any sign of systematic error judging from the inspection of the residuals of the fit (Fig. S14–S16†). In the analysis of spectral series starting from 98% *cis*-azobenzene and reaching less than 15% of *cis*-azobenzene, the calculated change of the total concentration was always less than 1.5%. Such an analysis validates the obtained molar absorption coefficients. For the described procedure of finding the concentrations of individual isomers we used the discussed best estimates of ϵ_{trans} and ϵ_{cis}^t or ϵ_{cis}^{TEM} . Nevertheless, using the ϵ_{cis}^{NMR} values also provides a good fit to the trial data (the calculated change of the total concentration during the conversion was smaller than 4%). Therefore, all of the described methods lead to a spectrum of *cis*-azobenzene with satisfactory reliability.

In conclusion, we present molar absorption coefficients of the *trans* and *cis* isomers of azobenzene whose true values are within experimental uncertainty as verified by applying them in the global analysis of the absorption spectra of various isomeric mixtures. Because both these spectra have lower absorbances than those reported previously in the wavelength regions where the impurity isomer absorbs more strongly, we are convinced that the spectra reported previously for both isomers were mutually contaminated by small amounts of the other isomer. Multiple approaches to obtain the spectra of the *cis*-isomer with consistent results further validate their correctness. The use of the simple TEM method in the analysis of UV-Vis spectra is particularly interesting and

novel. It resulted in data only a bit more noisy than those obtained with the analysis by lengthy kinetic measurement. The presented method for the separation of spectra in mixtures, which does not rely on the physical separation of isomers, may well be applicable for other photochromic systems or other solvents. The advantages and limitations of individual methods have been discussed.

Precise knowledge of ϵ values may be particularly interesting in many applications especially in photoswitching: the best choice of the irradiation wavelength allows the PSS to be reached with higher efficiency and therefore higher speed and would lead to a higher purity of the wanted isomer. The choice of a properly selected light source based on good knowledge of ϵ values thus may, for example, lead to more efficient molecular machines, or molecular logical units exhibiting less error; a better choice of writing and erasing light sources can increase the reliability of memory, and so on.

The quantum yields based on the presented re-determined molar absorption coefficients are reported in the accompanying article.²⁵

Conflicts of interest

There are no conflicts of interest to declare.

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References

- 1 G. S. Hartley, The Cis-form of Azobenzene, *Nature*, 1937, **140**, 281.
- 2 S. Tanaka, S. Itoh and N. Kurita, Possibility of nonequilibrium isomerization of azobenzene triggered by vibrational excitations, *Chem. Phys. Lett.*, 2002, **362**, 467–475.
- 3 T. Cusati, G. Granucci and M. Persico, Photodynamics and Time-Resolved Fluorescence of Azobenzene in Solution: A Mixed Quantum-Classical Simulation, *J. Am. Chem. Soc.*, 2011, **133**, 5109–5123.
- 4 L. Duarte, R. Fausto and I. Reva, Structural and spectroscopic characterization of E- and Z-isomers of azobenzene, *Phys. Chem. Chem. Phys.*, 2014, **16**, 16919.
- 5 L. Duarte, L. Khriachtchev, R. Fausto and I. Reva, Photoisomerization of azobenzenes isolated in cryogenic matrices, *Phys. Chem. Chem. Phys.*, 2016, **18**, 16802–16811.
- 6 H. M. D. Bandara and S. C. Burdette, Photoisomerization in different classes of azobenzene, *Chem. Soc. Rev.*, 2012, **41**, 1809–1825.

- 7 J. Casellas, M. J. Bearpark and M. Reguero, Excited-State Decay in the Photoisomerisation of Azobenzene: A New Balance between Mechanisms, *ChemPhysChem*, 2016, **17**, 3068–3079.
- 8 M. Quick, A. L. Dobryakov, M. Gerecke, C. Richter, F. Berndt, I. N. Ioffe, A. A. Granovsky, R. Mahrwald, N. P. Ernsting and S. A. Kovalenko, Photoisomerization Dynamics and Pathways of trans- and cis-Azobenzene in Solution from Broadband Femtosecond Spectroscopies and Calculations, *J. Phys. Chem. B*, 2014, **118**, 8756–8771.
- 9 A. A. Beharry and G. A. Woolley, Azobenzene photoswitches for biomolecules, *Chem. Soc. Rev.*, 2011, **40**, 4422–4437.
- 10 M. Dong, A. Babalhavaeji, S. Samanta, A. A. Beharry and G. A. Woolley, Red-Shifting Azobenzene Photoswitches for in Vivo Use, *Acc. Chem. Res.*, 2015, **48**, 2662–2670.
- 11 M. Natali and S. Giordani, Molecular switches as photo-controllable smart receptors, *Chem. Soc. Rev.*, 2012, **41**, 4010.
- 12 T. Avellini, M. Baroncini, G. Ragazzon, S. Silvi, M. Venturi and A. Credi, Photochemically Controlled Molecular Machines with Sequential Logic Operation, *Isr. J. Chem.*, 2014, **54**, 553–567.
- 13 J. W. Brown, B. L. Henderson, M. D. Kiesz, A. C. Whalley, W. Morris, S. Grunder, H. Deng, H. Furukawa, J. I. Zink, J. F. Stoddart and O. M. Yaghi, Photophysical pore control in an azobenzene-containing metal organic framework, *Chem. Sci.*, 2013, **4**, 2858.
- 14 N. Yanai, T. Uemura, M. Inoue, R. Matsuda, T. Fukushima, M. Tsujimoto, S. Isoda and S. Kitagawa, Guest-to-Host Transmission of Structural Changes for Stimuli-Responsive Adsorption Property, *J. Am. Chem. Soc.*, 2012, **134**, 4501–4504.
- 15 S. Hvilsted, C. Sánchez and R. Alcalá, The volume holographic optical storage potential in azobenzene containing polymers, *J. Mater. Chem.*, 2009, **19**, 6641–6648.
- 16 E. Merino and M. Ribagorda, Control over molecular motion using the cis-trans photoisomerization of the azo group, *Beilstein J. Org. Chem.*, 2012, **8**, 1071–1090.
- 17 G. Gauglitz, Azobenzene as a convenient actinometer for the determination of quantum yields of photoreactions, *J. Photochem.*, 1976, **5**, 41–47.
- 18 S. Hubig, *Dissertation*, Eberhard-Karls, 1984.
- 19 G. Gauglitz and S. Hubig, Chemical Actinometry in The UV by Azobenzene in Concentrated-Solution - A Convenient Method, *J. Photochem.*, 1985, **30**, 121–125.
- 20 P. Klán and J. Wirz, *Photochemistry of Organic Compounds: From Concepts to Practice*, Wiley, Chichester, West Sussex, U.K., 2009, pp. 102–118.
- 21 M. Gaplovsky, Y. V. Il'ichev, Y. Kamdzhilov, S. V. Kombarova, M. Mac, M. A. Schwoerer and J. Wirz, Photochemical reaction mechanisms of 2-nitrobenzyl compounds: 2-Nitrobenzyl alcohols form 2-nitroso hydrates by dual proton transfer, *Photochem. Photobiol. Sci.*, 2005, **4**, 33–42.
- 22 P. Anstaett, A. Leonidova and G. Gasser, Caged Phosphate and the Slips and Misses in Determination of Quantum Yields for Ultraviolet-A-Induced Photouncaging, *ChemPhysChem*, 2015, **16**, 1857–1860.
- 23 P. Anstaett, A. Leonidova, E. Janett, C. G. Bochet and G. Gasser, Reply to Commentary by Trentham *et al.* on Caged Phosphate and the Slips and Misses in Determination of Quantum Yields for Ultraviolet-A-Induced Photouncaging by Gasser *et al.*, *ChemPhysChem*, 2015, **16**, 1863–1866.
- 24 J. E. T. Corrie, J. H. Kaplan, B. Forbush, D. C. Ogden and D. R. Trentham, Commentary on “Caged Phosphate and the Slips and Misses in Determination of Quantum Yields for Ultraviolet-A-Induced Photouncaging” by G. Gasser and Co-Workers, *ChemPhysChem*, 2015, **16**, 1861–1862.
- 25 V. Ladányi, P. Dvořák, J. Al Anshori, L. Vetráková, J. Wirz and D. Heger, Azobenzene photoisomerization quantum yields in methanol redetermined, *Photochem. Photobiol. Sci.*, 2017, DOI: 10.1039/C7PP00315C.
- 26 J. R. S. Machado and W. B. Streett, Equation of state and thermodynamic properties of liquid methanol from 298 to 489 K and pressures to 1040 bars, *J. Chem. Eng. Data*, 1983, **28**, 218–223.
- 27 J. Ronayette, R. Arnaud, P. Lebourgeois and J. Lemaire, Isomérisation photochimique de l'azobenzène en solution. I, *Can. J. Chem.*, 1974, **52**, 1848–1857.
- 28 (a) J. Michl and E. W. Thulstrup, Ultraviolet and Infrared Linear Dichroism - Polarized-light as a Probe of Molecular and Electronic Structure, *Acc. Chem. Res.*, 1987, **20**, 192–199; (b) J. Michl, E. W. Thulstrup and J. H. Eggers, Polarization Spectra in Stretched Polymer Sheets. III. Physical Significance of Orientation Factors and Determination of $\pi\text{-}\pi^*$ Transition Moment Directions in Molecules of Low Symmetry, *J. Phys. Chem.*, 1970, **74**, 3878–3884.
- 29 H. Rau, Spectroscopic Properties of Organic Azocompounds, *Angew. Chem., Int. Ed. Engl.*, 1973, **12**, 224–235.
- 30 G. S. Hartley, The cis-Form of Azobenzene and the Velocity of the Thermal cis - trans conversion of Azobenzene and Some Derivatives, *J. Chem. Soc.*, 1938, 633.
- 31 P. Bortolus and S. Monti, Cis-Trans Photoisomerization of Azobenzene - Solvent and Triplet Donor Effects, *J. Phys. Chem.*, 1979, **83**, 648–652.
- 32 C. L. Forber, E. C. Kelusky, N. J. Bunce and M. C. Zerner, Electronic spectra of cis- and trans-azobenzenes: consequences of ortho substitution, *J. Am. Chem. Soc.*, 1985, **107**, 5884–5890.
- 33 G. Zimmerman, L.-Y. Chow and U.-J. Paik, The Photochemical Isomerization of Azobenzene, *J. Am. Chem. Soc.*, 1958, **80**, 3528–3531.
- 34 A. R. Dias, M. E. M. Dapiedade, J. A. M. Simoes, J. A. Simoni, C. Teixeira, H. P. Diogo, M. Y. Yang and G. Pilcher, Enthalpies of Formation of Cis-azobenzene and Trans-azobenzene, *J. Chem. Thermodyn.*, 1992, **24**, 439–447.
- 35 K. Takeshita, N. Hirota and M. Terazima, Enthalpy changes and reaction volumes of photoisomerization reactions in solution: azobenzene and p-coumaric acid, *J. Photochem. Photobiol., A*, 2000, **134**, 103–109.

- 36 H. K. Cammenga, V. N. Emelyanenko and S. P. Verevkin, Re-investigation and Data Assessment of the Isomerization and 2,2-Cyclization of Stilbenes and Azobenzenes, *Ind. Eng. Chem. Res.*, 2009, **48**, 10120–10128.
- 37 L. De Boni, C. Toro, S. C. Zilio, C. R. Mendonca and F. E. Hernandez, Azo-group dihedral angle torsion dependence on temperature: A theoretical experimental study, *Chem. Phys. Lett.*, 2010, **487**, 226–231.