

Notes & Tips

# Redetermination of the extinction coefficient of camphor-10-sulfonic acid, a calibration standard for circular dichroism spectroscopy

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Camphor-10-sulfonic acid (CSA)<sup>1</sup> is routinely used for calibrating circular dichroism (CD) instruments. However, CSA is hygroscopic, so an accurate concentration can be obtained only by measuring its absorbance peak at 285 nm. Hence, calibrations are highly dependent on having a correct value for the extinction coefficient for this compound.

Following an extensive search of the literature, the origins of the commonly accepted value for the molar extinction coefficient for CSA of  $34.5 \text{ M}^{-1} \text{ cm}^{-1}$  at 285 nm can be ascribed to Chen and Yang [1], who cited Lowry and French [2], although Lowry and French reported the value at 284 nm to be  $35 \text{ M}^{-1} \text{ cm}^{-1}$ , following measurements at 286 and 292 nm [3]. (There was actually no data point at the peak maximum.) The value of  $34.52 \text{ M}^{-1} \text{ cm}^{-1}$  at 285 nm is reported in instrument manuals without reference, usually quoted in the form of a 1 mg/ml solution producing an absorbance of 0.743 in a 5-mm cell (also see [4]). Recently, more stringent methods of standardizing the calibration of conventional and synchrotron radiation CD instruments have been developed [5]. Considering that CSA is the most widely used standard for calibration and cross-correlation for both types of CD instruments, we decided to re-examine the measurement of the extinction coefficient of CSA, concentrating on reproducibility (error levels) and measurements at the actual peak maximum.

## Materials and methods

CSA of more than 99% purity was obtained from Sigma–Aldrich. Standardized 0.5-M KOH solution and

neutral red pH indicator were purchased from BDH Chemicals. In the experiment, 25 ml of a nominally (based on gravimetric measurements) 0.5-M aqueous solution of CSA were titrated against 0.5 M KOH using a 50-ml Goldline glassware burette (H.J. Elliot). Three different batches of CSA and three batches of KOH were used, with each titration being repeated five times. The extinction coefficient was determined at the absorption peak (285 nm) using a Cary 3 UV/Vis spectrophotometer calibrated for baseline stability, wavelength accuracy, and photometric accuracy within an absorbance range of 0.2–1.0. A number of dilutions and different cuvettes of 1-, 5-, and 10-mm path lengths were used to eliminate systematic errors. The cuvettes were calibrated using the chromate method (A.J. Miles et al., submitted). Dilutions were carried out using A-type volumetric flasks and calibrated pipettes. Each measurement was repeated five times. Fig. 1 shows the calibration curve obtained for the repeated measurements.

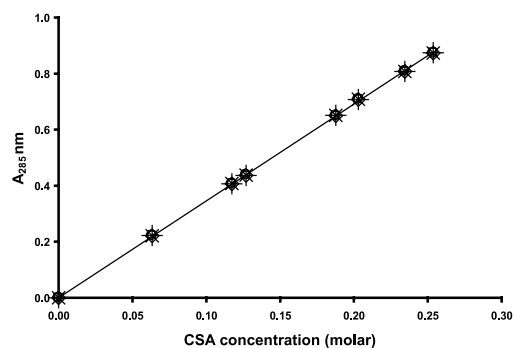


Fig. 1. Calibration plot for the CSA extinction coefficient. Five repeated measurements for each point are shown by the following symbols: open square, cross, open diamond, open circle and star.

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<sup>1</sup> Abbreviations used: CSA, camphor-10-sulfonic acid; CD, circular dichroism.

## Summary

The extinction coefficient of CSA at 285 nm was determined to be  $34.59 \pm 0.18$  with an error level of 0.5%. This corresponds to an  $A_{285}$  of 0.745 for a 10.0-mg/ml solution in a 5-mm pathlength cell.

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