Catalysis in complex media
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Chapter 2

Off-line reaction monitoring of the oxidation of alkenes in water using drop coating deposition Raman (DCDR) spectroscopy

The application of drop coating deposition Raman (DCDR) spectroscopy to the field of reaction progress monitoring is addressed in this contribution. Although, DCDR spectroscopy has seen recent application in the study of biological fluids, its application in other areas has not yet been explored. Here we apply the technique to the catalysed oxidation of alkenes to epoxides in aqueous solutions at concentrations < 10 mM. The effect of surface characteristics, background interferences, homogeneity of distribution of analytes, drying time, as well as instrumental limits of detection and calibration are discussed. It was demonstrated that reproducible spectra can be obtained routinely, with relatively little variance, with short acquisition times and samples volumes of 2-10 µl and as little as 1 µg of analyte. The utility of the technique compared with online reaction monitoring by ¹H NMR and Raman spectroscopy is demonstrated in the excellent correlation between data obtained off and on-line.

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Chapter 2

Introduction

Over the last decades Raman spectroscopy\(^1\) has proven to be a versatile and cost effective analytical method in all fields of chemical sciences for qualitative and quantitative chemical analysis, in particular for multi-component samples.\(^2\) The technique has developed rapidly over the last decade due to the decrease in the cost of lasers and detectors and the increase in detector sensitivity. More importantly, the relatively weak Raman scattering cross-section of water and the spectrally rich ‘chemical fingerprint’ presented by compounds and materials allows for analysis of mixtures as complex as whole cells. Further advantages presented by Raman spectroscopy is the absence of or only minimal sample preparation required, non-destructiveness and rapidity of both on- and off-line analyses.\(^3\) These characteristics have led to the extensive application of Raman spectroscopy for reaction monitoring and, in particular in industrial settings, for process control.\(^4\)

The inherent weakness of the Raman scattering, however, means that species present at low concentration are difficult to detect both qualitatively and quantitatively (e.g., < 0.1-50 mM; the actual concentration limit being dependent both on the analyte in question and the system S/N characteristics). Several approaches can be taken to overcome this, including enhancement either by excitation at a wavelength resonant with an analytes electronic absorption band, i.e. resonance Raman (RR) spectroscopy,\(^5\) surface enhanced Raman spectroscopy (SERS)\(^6\) or a combination of both, i.e. surface enhanced resonance Raman spectroscopy (SERRS).\(^7\) Although RR, SERS and SERRS are powerful methods to obtain valuable structural and electronic information, they are not universally applicable methods and often require the use of non-standard excitation wavelengths and roughened metal surfaces or nanoparticles exhibiting surface plasmon bands at the appropriate wavelength.

More recently, an alternative approach has been taken by several groups in the drop coating deposition Raman (DCDR) method.\(^8\) The DCDR method takes a relatively simple approach to overcoming the detection limit of Raman spectroscopy, by pre-concentration of dilute solutions (i.e. solvent removal) prior to analysis. The technique is based on the well-known coffee stain effect,\(^9,10\) when a microdroplet is applied to a surface under certain conditions, the majority of nonvolatile materials concentrate at the edge of an evaporating droplet,\(^8,9,11\) thereby allowing for an increase by several orders of magnitude in the mass of the analyte present in the volume sampled by a Raman microspectrometer. The DCDR method has been applied recently in the analysis of biomaterials including human, bovine, and porcine insulin,\(^12\) lysozyme,\(^13\) glucose, glycan, taxane,\(^14\) domoic acid,\(^15\) human tear fluid\(^16\) and the synovial fluid of osteoarthritis patients, among other biomaterials that are present in serum at low concentrations.\(^17\) An important feature of the DCDR method was demonstrated by Zhang et al. in the segregation of proteins and salts during the drying process, which further enhances the signal strength achievable in complex biological matrices.\(^12,14\)

Although increasingly applied in the qualitative and sometimes quantitative analysis of biological serums and fluids, the DCDR method presents potential opportunities in the analysis of synthetic and catalytic reaction mixtures also. This is especially the case for reactions carried out in water where the solubility of substrates is generally poor and in high-throughput screening of, e.g., enzymatically, catalysed
reactions where the volume and mass of analyte available are often limited. Furthermore, when substrates are soluble in water, and especially when they do not bear chromophoric moieties, their polarity often presents problems in analysis with other techniques such as HPLC or GC.

In this chapter, the potential, to the best of my knowledge for the first time, of the DCDR technique to monitor reaction progress is explored. As a test case the catalytic epoxidation of alkenes by manganese catalysts in water was examined both quantitatively as well as qualitatively. This class of functional group transformation is especially suited for analysis by Raman spectroscopy since scattering from the alkene stretching mode is relatively intense as are the ring breathing modes of the epoxide product. Here we show that the DCDR method is suitable for such analysis and we address issues including the effect of surface characteristics, background interferents, homogeneity of distribution of analytes as well as instrumental limits of detection and calibration. The utility of the technique in reaction monitoring is demonstrated by direct comparison with on-line monitoring by $^1$H NMR and Raman spectroscopy.

**Results**

**Model reactions**

In the present study, the catalysts $[\text{Mn}_2\text{O}_3(tmtacn)_2](\text{PF}_6)_2\cdot\text{H}_2\text{O}(1)$ and $[\text{Mn}_2\text{O}(\text{OAc})_2(tmtacn)_2](\text{PF}_6)_2\cdot\text{H}_2\text{O}$ (2), where tmtacn is $N,N',N''$-trimethyl-1,4,7-triazacyclononane (Figure 1), were employed in the epoxidation of water soluble alkenes with $\text{H}_2\text{O}_2$.

![Figure 1](image-url)

**Figure 1** $[\text{Mn}_2\text{O}_3(tmtacn)_2](\text{PF}_6)_2\cdot\text{H}_2\text{O}$ (1), $[\text{Mn}_2\text{O}(\text{OAc})_2(tmtacn)_2](\text{PF}_6)_2$ (2) and the ligand, tmtacn.

Catalyst 1 was identified as a highly effective catalyst for low temperature bleaching of clothing and for the epoxidation of styrene in water in the 1990s and was later applied in a wide range of oxidative functional group transformation in organic solvents. Currently our group is investigating the application of these catalysts in aqueous media for alkene oxidation. For many substrates the relatively low solubility of the substrate and/or products in aqueous media has posed a considerable challenge in terms of analysis, both on- and off-line, especially in large scale reaction conditions and in high-throughput screening programs. This prompted us to investigate the possibility of applying the DCDR method for both the analysis of overall conversion and also in obtaining kinetic data for reactions where the reaction times are > 1 h.
Scheme 1 Model reactions examined; the oxidation of 4-vinylbenzoic acid (VBA) and styrene-p-sulfonate (SS).

We examined the oxidation of two model substrates (Scheme 1); i.e. 4-vinylbenzoic acid (VBA) and styrene-p-sulfonate (SS) in water with H$_2$O$_2$ catalyzed by 1. Reactions were carried out in a total volume of 3 ml of aqueous NaHCO$_3$ (10$^{-1}$ M). Typical concentrations employed were 10 mM substrate, 10 µM catalyst and 50 mM H$_2$O$_2$. In the case of VBA, the substrate dissolved in the carbonate buffer only upon addition of H$_2$O$_2$ and hence time zero is taken to be the point at which the catalyst is added. Unless stated otherwise all reactions were performed at ambient temperature (20-23 °C).

Solid state Raman spectroscopy

Raman spectra of VBA and its epoxide product (OBA) are shown in Figure 2 and those of SS and OS in Figure 3. Of particular interest with regard to analysis are the differences between the spectra in the ranges 1200-1300 cm$^{-1}$ and 1600-1650 cm$^{-1}$ and to a lesser extent the range between 600 and 900 cm$^{-1}$, as these regions do not suffer interference from Raman scattering from carbonates (vide infra). The bands in the range 1600–1650 cm$^{-1}$ are characteristic of C=C stretching vibrations of vinyl and aryl groups and in particular the band at 1630 cm$^{-1}$ is useful in monitoring reaction progress.

Figure 2 Solid state Raman spectra (λ$_{exc}$ 785 nm) of (a) VBA and its epoxide product (b) OBA. Bands of most interest with respect to analysis are indicated.
Effect of surface pretreatment on drying pattern and Raman analysis

A range of surfaces for DCDR studies have been employed previously by several groups, including quartz,\textsuperscript{12,20,21} calcium fluoride,\textsuperscript{13} coated or uncoated glass, PTFE coated stainless steel, and gold foil.\textsuperscript{8} The primary consideration in the choice of surface is that it is chemically inert with regard to the analyte and solution, presents little or no background signal and has a low optical absorbance to limit sample heating during measurements. Quartz slides are readily available and were chosen to avoid the fluorescence at $\lambda_{\text{exc}}$ 785 nm observed with borosilicate glass. The pattern formed by non-volatile reaction components upon drying depends on the characteristics of the surface used. Hence plasma treated quartz slides were compared with slides that were subsequently silanised with alkyl or perfluoroalkyl silanes.

Figure 3 Solid state Raman spectra ($\lambda_{\text{exc}}$ 785 nm) of (a) SS and (b) its epoxide product OS. Bands of most interest with respect to analysis are indicated.

Figure 4 Dependence of the drying pattern (2 µL of a 10 mM solution of VBA in 0.1 M NaHCO$_3$ (aq), pH 8, mass of analyte ca. 3 µg) on surface pre-treatment (a) plasma cleaned surface (contact angle 41°) and (b) plasma cleaned surface followed by silanisation with alkyl silanes (contact angle 103°).

Droplets of 10 mM solutions of the analytes (2 µl) were allowed to dry on quartz slides to achieve solute deposition in patterns that were found to be dependent on the type of surface treatment employed. Evaporation was accelerated by applying a low vacuum (10-20 mmHg). The deposited analytes were observed by optical microscopy and in all cases the deposition was in the shape of a ring (coffee stain pattern) with some material
deposited in the centre of the ring (Figure 4). Although the shape of the deposition on the more hydrophobic surface is less uniform, the majority of the analyte was deposited on the outermost edge of the ring, whereas on the hydrophilic surface the analyte is more thinly deposited in a series of thin rings. An additional factor affecting deposition is ionic strength. In the case of the more water soluble SS, deposition from aqueous solutions did not show the desired coffee stain pattern except when the ionic strength was increased by addition of NaHCO$_3$ or NaCl.

In general it can be concluded that uniform ‘coffee stain patterns’ cannot be assumed for a particular system and care should be taken to study not only the drying patterns under ideal conditions but also the effect of side reactions and additional reaction components.$^{22}$ For example, the irregular shape of the residue deposited upon drying of the droplet indicates that the droplet is not pinned during the drying process.

**Raman spectra of compounds deposited on hydrophobic surfaces**

Solutions of VBA and OBA in NaHCO$_3$ buffer were deposited on hydrophobic quartz surfaces and Raman spectra were recorded at the edges of the depositions (Figure 5). The Raman confocal volume (with a 50x objective) has a waist of approximately 5-10 microns$^{23}$ while the diameter of the deposition is approximately 250-300 microns and the width of the ring is 40 microns (see Figure 4).

![Raman spectra](image)

**Figure 5** Raman spectra ($\lambda_{exc}$ 785 nm) of (a) VBA and (b) OBA obtained on a treated hydrophobic surface. Regions of most interest are noted.

The DCDR analysis method relies on uniform co-deposition of the reaction components. Hence it is essential to verify the uniformity or otherwise of the distribution of various components across the deposition and the reproducibility of spectra both at the same point, at different points on a particular deposition and over several depositions. The distribution was investigated using solutions of a range of mole fractions of VBA and OBA. Three depositions were made at each of the three different mole fractions, followed by recording Raman spectra at eight points across each of the nine depositions (Figure 6). Each sample point was recorded using a 100x objective and the laser spot size and therefore waist of the confocal volume was < 5 microns. Visual inspection of the dried spots of VBA, OBA and mixtures of both indicates that most of the material deposits in a 30-40 micron wide ring with a diameter of 250 microns, which is confirmed
by comparison of Raman spectra recorded at the edge of the spot (i.e. points a-f) and at the centre (i.e. points g and h); the latter spectra being essentially featureless.

Figure 6 Raman spectra ($\lambda_{exc}$ 785 nm) recorded at eight points across a deposit. The spectra are overlaid in the lower panel and demonstrate the spectral consistency at multiple points on the outer ring except for contributions (both shape and intensity) from the carbonate buffer. The star indicates a spectral artefact.

As expected, the absolute intensity of the individual spectra is highly variable due to the lack of uniformity in terms of thickness of the deposited material in the deposition. Nevertheless, after normalisation (to the maximum in the 1600-1650 cm$^{-1}$ region where carbonates do not contribute), the standard deviation of the Raman spectra recorded at various points around the ring of each sample and between several spots for three mole fraction mixtures (0.2, 0.6 and 0.8) was found to be ca. 2-3 %, which indicates that the distribution can be treated as uniform for the two components VBA and OBA. By contrast, the distribution with respect to carbonates was not uniform as can be seen from the comparison of the average spectrum and the standard deviation at each wavenumber (Figures 19 to 24). Furthermore, the contributions of the various carbonate species to the spectra were found to be highly variable over the entire deposition.

The ratio of the two components (VBA and OBA) was calculated by fitting the average spectra with a model, based on spectra obtained using DCDR from (carbonate free) aqueous solutions of VBA and OBA. The spectra were processed by offset correction and normalization. Each spectrum was then fitted with a model to obtain the best fit in the region between (1600-1650 cm$^{-1}$), which is dominated by the alkene and vinyl C=C stretching modes and the 1100-1200 cm$^{-1}$ region. These regions were selected since the variability in other regions (e.g., between 1000-1100 cm$^{-1}$) precluded their use in fitting, due to the presence of varying contributions from carbonates. Fitting of the average spectra at 20 mol%, 60 mol% and 80 mol% of OBA w.r.t VBA shows a good correlation with the expected values, which indicated that a calibration curve can be prepared in this manner (vide infra).

Quantitative analysis

A calibration curve for VBA/OBA was prepared by the method of continuous variation holding the total concentration to that employed in the catalysed oxidation reactions (10 mM, vide infra). Samples were deposited on a hydrophobic surface (see experimental section) and Raman spectra were obtained at $\lambda_{exc}$ 785 nm. Fitting provided good agreement with the expected values, albeit close to the extremes (<0.2 and >0.8) the accuracy decreases as the limits to detection of one of the components are approached.
Figure 7 Raman spectra obtained from various mixtures of VBA and OBA (2 µL droplets, the total concentration, i.e. [VBA] + [OBA] was held at 10 mM) in NaHCO$_3$ (0.1 M) buffer and the calibration curve obtained. The uncertainties due to fitting are estimated to be ± <5 %.

The detection limits in Raman spectroscopy are highly dependent on the power of the excitation laser, the efficiency of the light collection optics and the characteristics of the detector employed. Nevertheless it is informative to consider the effect of initial sample volume (2 -10 µl) and concentration of the analyte, VBA (0.1 mM to 10 mM), on the limits of detection in the presence of 0.1 M NaHCO$_3$ buffer (Figure 18). Depositing 10 µl of a 0.5 mM solution of VBA (i.e. 740 ng of substrate) still allowed for detection above the noise. The dependence of the final spot size after drying on the sample volume is shown in Figure 8. In general, a larger spot diameter is obtained with increased sample volume; however, the width of the outer ring, in which the components of interest are concentrated, is relatively unaffected. Hence, by increasing the volume of analyte sampled the absolute intensity of the Raman spectrum obtained and hence the signal to noise ratio can be increased, albeit at the cost of requiring larger sample volumes.

Figure 8 Images of deposits formed using different volumes of solution containing 0.5 mM VBA and 0.1 M NaHCO$_3$. The density of material in the areas where VBA is deposited in the ring indicated in mg/m$^2$ was estimated geometrically. The dimensions of the area imaged in each case are 200 µm by 155 µm.

Distribution of compounds of differing solubility

The DCDR analysis method relies on the uniform co-deposition of reaction components of interest with respect to the sampled volume. Precipitation of compounds occurs upon evaporation of water from the droplet and although the polarity VBA and OBA are similar, this is not necessarily the case for other reaction products such as the analogous diol product or benzaldehyde. The differences in solubility that can be tolerated by the technique was explored by DCDR analysis of mixtures of VBA and SS, the latter showing
orders of magnitude higher solubility in the applied media than the former (Figure 9). As above, the spectra were analysed using spectra obtained from DCDR of solutions containing only VBA or SS to determine the mole fraction of each component, which was plotted against the calculated mole fraction. A reasonably good distribution about the best fit was obtained and importantly, the spectra obtained from different points in individual deposits and for different deposits obtained from the same mixture were uniform in the ratio of the spectral contribution of each of the two components (see Figure 19 to 24).

![Figure 9 Raman spectra obtained from various mixtures of VBA and SS obtained by drying 2 µL of 10 mM (total concentration) solutions in 0.1 M carbonate buffer (sample mass ca. 3 µg)](image)

**Reaction progress monitoring by DCDR spectroscopy**

The time taken for the solvent (i.e. H₂O) to evaporate and for the deposit to form is, potentially, a limiting factor in the time resolution that can be achieved using the DCDR method. This limitation is reduced to a certain extent by the use of vacuum to accelerate evaporation (from ca. 20 min under ambient conditions to ca. 5 min). For reactions that proceed over relatively long periods (i.e. > 1 h) direct online analysis of large numbers of reactions is relatively expensive in terms of the resources required. With the DCDR method, however, analysis can be carried out in batch fashion assuming, of course, that the reaction stops upon drying.

The effect of drying rate on the analysis was examined by comparison of two samples deposited simultaneously but with one dried under ambient conditions (evaporation time ca. 20 min) and the second dried in vacuo (evaporation time < 5 min). From the spectra shown in Figure 10, it is apparent that for the sample dried under ambient conditions the reaction progressed further (38 %) than for the sample dried in vacuo (20 %); note the differences in the band structure at ca. 1610 cm⁻¹. These differences can be understood by considering that the concentration of reactants increases upon drying and hence reaction rate should increase; however, the increase is counterbalanced to some extent by the simultaneous deposition of substrate and product that occurs.
Figure 10 Comparison of spectra obtained from (a) a reaction mixture form which the catalyst was omitted, a reaction mixture with catalyst present with the droplet sample dried (b) under ambient conditions and (c) dried in vacuo.

Comparison of on-line vs. off-line (DCDR) methods

The utility of the DCDR method for off-line reaction progress monitoring was assessed by direct comparison with on-line reaction monitoring by both Raman and $^1$H NMR spectroscopy. In Figure 11, conversion calculated by the DCDR method and on-line reaction monitoring by Raman spectroscopy, show good agreement and indeed both techniques allowed for the observation of changes in reaction rates overtime (the induction period is due to minor changes in pH as the reaction proceeds). A discrepancy (ca. < 3%) in the conversion determined by both methods is ascribed to additional conversion which takes place during droplet drying (vide supra).

Figure 11 On-line reaction monitoring at 532 nm$^{24}$ (solid squares) overlaid with results from off-line analysis with DCDR (open circles) of the same reaction mixture. Reaction conditions: VBA (10 mM), H$_2$O$_2$ (50 mM), NaHCO$_3$ (0.1 M). t = 0 is the point at which the catalyst (2, 2 μM) was added. Raman spectra were acquired on-line with 4 min intervals. Samples for off-line analysis were taken at indicated time points, drying times were < 4 min.
Comparison of reaction progress monitored by the DCDR method and by in situ $^1$H NMR spectroscopy, highlighted an important point in regard to obtaining kinetic information by direct measurement. Immediately after addition of H$_2$O$_2$ to the reaction mixture a sample was withdrawn for on-line $^1$H NMR analysis, with spectral acquisition at intervals equal to the rate of sampling of the reaction mixture by DCDR. The reaction progress determined from the sample held in the NMR spectrometer showed a significant discrepancy to that determined by DCDR. The origin of the discrepancy was identified by taking a second sample from the reaction mixture for $^1$H NMR analysis at the end of the monitoring period, which showed that conversion in the NMR tube used for online analysis was different to that obtained in the bulk reaction mixture from which DCDR analysis was performed. Sampling of the reaction mixture at set intervals for analysis by both techniques was therefore performed to avoid this problem. Comparison of the conversion determined by both methods shows good agreement over the entire course of the reaction (Figure 12).

Use of DCDR in high-throughput screening of reaction conditions

The application of DCDR for reaction progress monitoring is demonstrated in the oxidation of VBA to OBA and in the oxidation of SS to OS with H$_2$O$_2$ catalysed by 1 or 2. The reaction rate shows a dependence on catalyst concentration. The intensity of the Raman band at 1631 cm$^{-1}$ can be seen to decrease as the reaction progresses where 8 μM of 1 was used. Fitting the spectra obtained by DCDR as described above allows for determination of the mole fraction of VBA at each time point. At lower catalyst concentrations the reaction rate decreases. The data obtained by the DCDR method for five reactions in parallel (55 data points in total) is shown in Figure 13. The data indicate that the reaction is non-linearly dependent on catalyst concentration.

![Figure 12 Off-line monitoring of reaction progress in the oxidation of VBA (0.01 M) with H$_2$O$_2$ (0.05 M) catalysed by 2 (10 μM) in NaHCO$_3$ (aq) (0.1 M) in D$_2$O (the VBA was solubilised by addition of H$_2$O$_2$ prior to addition of 2). 700 μL and 2 μL aliquots were removed at the times indicated for analysis by $^1$H NMR spectroscopy (with tert-butanol as internal standard) and DCDR spectroscopy, respectively. Analysis of the Raman spectra employed fitting with the spectra of VBA and OBA as described above.](image)
Figure 13 (a) Raman spectra of a reaction mixture obtained at 30 min time intervals at 8 µM of 2. (b) Substrate conversion with time for catalyst 2 concentrations between 0 and 8 µM. The reactions were performed in parallel and spectral acquisition for the entire DCDR sample set was carried out in approximately 1 h.

Conclusions

Previous studies have shown that inhomogeneous deposition can be a problem in DCDR for large molecules such as proteins.\textsuperscript{16,21} For smaller molecules, it was confirmed that drop coating deposition of compounds of substantially differing solubility show essentially homogeneous deposition.\textsuperscript{23} The spectra obtained in this study using the DCDR technique are reproducible with relatively little variance (<2%) and can be obtained with short acquisition times and small samples volumes. The time taken for the volatiles (i.e. H\textsubscript{2}O) to evaporate and the deposit to form is a potentially limiting factor in the time resolution that can be achieved using the DCDR method. However, for reactions that proceed over relatively long periods (i.e. > 1 h) off-line analysis of large numbers of reactions by the DCDR method offers a relatively inexpensive approach both in terms of facilities required and time, in comparison with commonly employed techniques such as GC and HPLC or online monitoring. A key advantage in the catalytic reaction studied in the present report in comparison with on-line Raman or off line \textsuperscript{1}H NMR spectroscopy is that rapid drying results in quenching of the reaction, which allows for analysis after the reaction at a later time and that much lower sample volumes are required.

The catalysed reactions studied here are highly suited to the DCDR technique. For other reactions control experiments must be made to ensure that the observations made here hold for those reactions also, especially with regard to homogenous deposition of reaction components over the sampled area. Nevertheless, in many cases we expect that this approach to reaction monitoring can be applied generally to reactions carried out under aqueous conditions. Extension of this method to non-aqueous conditions will be explored in future studies.
Experimental

Synthesis

4-vinyl benzoic acid (VBA), 4-(oxiran-2-yl)benzoic acid (OBA), and styrene sulfonate sodium salt [SS], $^1$H NMR (D$_2$O, 400 MHz): 7.79 (2H, d, 9 Hz), 7.62 (1H, d, 9 Hz), 6.85 (2H, dd, 11 Hz and 17 Hz), 5.95 (1H, dd 1 Hz and 17 Hz), 5.43 (1H, dd 1 Hz and 11 Hz) ppm] were obtained from Sigma-Aldrich (Steinheim, Germany). Commercially available chemicals were used without further purification unless stated otherwise. H$_2$O$_2$ was 50% w/w in water. [Mn$_2$O$_3$(tmtacn)$_2$](PF$_6$)$_2$.H$_2$O (1) and [Mn$_2$(O)(OAc)$_2$(tmtacn)$_2$](PF$_6$)$_2$(2) where tmtacn is N,N',N''-trimethyl-1,4,7-triazacyclononane (Figure 1), were available from earlier studies.\textsuperscript{25}

Caution. The drying or concentration of solutions that potentially contain H$_2$O$_2$ should be avoided. Prior to drying or concentrating, the presence of H$_2$O$_2$ should be tested for using peroxide test strips followed by neutralization on solid NaHSO$_3$ or another suitable reducing agent. When working with H$_2$O$_2$, suitable protective safeguards should be in place at all times.

Preparation of 4-(oxiran-2-yl)benzoic acid (OBA) from VBA

[Mn$_2$O$_3$(tmtacn)$_2$](PF$_6$)$_2$.H$_2$O\textsuperscript{26} (1, 0.8 mg, 1.0 µmol) in 1 ml of water was added to 4-vinylbenzoic acid (148 mg, 1 mmol) in 100 mL of NaHCO$_3$ (aq) (0.1 M) followed by addition of H$_2$O$_2$ (50 % in water, 283 µL, 5 mmol, 5 equiv. w.r.t. VBA) with stirring. The reaction mixture was stirred overnight. After the H$_2$O$_2$ had been consumed, the solution was acidified to pH 3 with dilute HCl and extracted into CH$_2$Cl$_2$. The organic layer was dried over Na$_2$SO$_4$ (anhydr.) and the solvent removed in vacuo. Elem. Anal. Found (calculated) C 65.7 (65.85) %, H 4.94 (4.91) %

Preparation of 4-oxirane-phenyl-sulfonate (OS) from SS

The epoxide formed from the sodium salt of styrene sulfonate (SS) was prepared using a procedure adapted from that described earlier by de Boer et al.\textsuperscript{25b} H$_2$O$_2$ (50 %, 7 µL) was added to a solution of 1 (2.0 mg, 2.5 µmol) and salicylic acid (3.5 mg, 25 µmol) in acetonitrile (1 mL) at room temperature. The mixture was stirred for 20 min after which 0.4 mL was added to VS (206 mg, 1 mmol) in water/acetonitrile (6 mL, 7:3, v/v). The mixture was cooled to 0 °C and H$_2$O$_2$ (50 % in water, 63 µL, 1.1 mmol, 1.1 equiv. w.r.t. substrate) was added via syringe pump at a rate of 7.8 µL/h. The mixture was stirred and allowed to the reach room temperature over 16 h. $^1$H NMR analysis of a 1 mL aliquot indicated approximately 50 % conversion of SS. A second portion of freshly prepared catalyst containing solution and a second portion of peroxide was added (by syringe pump) and stirring continued for a further 14 h. The solvent was removed by lyophilisation and the product was purified by flash precipitation into acetonitrile, yielding the product 4-oxirane-phenyl-sulfonate, 130 mg, 0.59 mmol, 59 %. $^1$H NMR (D$_2$O, 400 MHz): 7.82 (2H, d, J = 16 Hz), 7.49 (2H, d, J = 16 Hz), 4.14 (1H, t, 4 Hz), 3.32 (1H, t 4 Hz), 3.08 (1H, dd 3Hz and 4 Hz) ppm.


Preparation of 4-(1,2-dihydroxyethyl)phenylsulfonate sodium salt (DS) from SS

The diol (DS) formed from styrene sulfonate (SS) was synthesised according to a method described by Lam et al.\textsuperscript{27} A mixture of styrene sulfonate (340 mg, 1.65 mmol) and mCPBA (415 mg, 2.40 mmol) were dissolved in water/ethanol (40 ml, 1:1, v/v). The mixture was stirred for 2 h at 65 °C at which point all oxidant had been consumed (determined by testing with bromine water). The solution was allowed to cool and the solvent was removed \textit{in vacuo}. Residual m-chlorobenzoic acid was dissolved in acetone (300 mL) and the remaining white product was isolated by filtration, dissolved in water and purified by flash precipitation into acetonitrile, yielding the product; 378 mg, 1.57 mmol, 95 %. \textsuperscript{1}H NMR (D\textsubscript{2}O, 400 MHz): 7.83 (2H, d, \textit{J} = 9 Hz), 7.56 (2H, d, \textit{J} = 9 Hz), 4.89 (1H, t, \textit{J} = 5.5Hz), 3.79 (1H, m) ppm.

Instrumentation

\textsuperscript{1}H NMR (400.0MHz) and \textsuperscript{13}C NMR (100.6 MHz) spectra were recorded on a Varian Avance400. Chemical shifts are relative to DOH (4.79 ppm). Contact angles were measured on a Device Dataphysics instrument with SCA20, software version 3.60.2. A 2 \textmu L drop of doubly distilled deionized water was used as the measuring liquid (Sessile drop method). A minimum of five spots on each sample were probed and the contact angles averaged. Analysis consisted of applying a baseline and elliptical curve fitting of the water-air contact profile. The uncertainty in the measurements is (± 3°). Drying of samples under vacuum was carried out by placing slides in a desiccator followed by attaching to a membrane vacuum pump.

Raman spectra were recorded using a Raman microscope (Perkin Elmer Raman station with an Olympus BX-51 microscope and long working distance objectives) at 785 nm (typically 20 mW at sample with a 50x long working distance objective) at room temperature. Raman spectra were recorded typically with 10 exposures of 8 s duration. Raman spectra at 532 nm were recorded using a homebuilt system consisting of an Andor Technology iDus-420-OE CCD camera, a Shamrock163 spectrograph and a 532 nm (300 mW, Cobolt) laser, both fibre coupled to an Inphotonics 532 nm Raman probe. Raman spectra were recorded typically with 20 exposures of 2 s duration. Reactions were carried out in a quartz 3 cm\textsuperscript{3} volume 1 cm pathlength cuvette during on-line monitoring.

Catalysed oxidation of VBA and SS

Oxidation of VBA and SS with H\textsubscript{2}O\textsubscript{2} (50% w/w in water) catalysed by 1 or 2, were carried out in 50 mL round bottomed flasks at 20 °C. pH was adjusted prior to addition of oxidant using with H\textsubscript{2}SO\textsubscript{4}(aq) or NaOH(aq) to pH 8.5.

Preparation of surfaces

Prior to modification, quartz slides were rinsed in turn with 10 % hydrochloric acid, water, acetone and then ethanol. The slides were subjected to air plasma cleaning (Diener electronic, Femto) at 100 W, for 1 min (at 1.7×10\textsuperscript{-3} mbar air). Quartz slides were functionalized with a 4 mM solution of 1H,1H,2H,2H-perfluoroctytriethoxysilane or octyltriethoxysilane in toluene with heating at reflux overnight. After functionalization,
the surface was rinsed with ethanol followed by dichloromethane and dried under a nitrogen gas stream at r.t.

**Analysis of Raman spectra obtained using DCDR**

Spectra obtained following DCDR were analyzed in the spectral range 1800 to 600 cm\(^{-1}\). The inconsistencies in the contribution of the carbonate buffer both spectrally and in terms of intensity precluded the reliable use of chemometric analysis and instead a manual data reduction and fitting approach using Microsoft Excel\(^{TM}\) was taken. Spectra of the main reaction components, *i.e.* the alkene, epoxide and diol, were recorded by DCDR from carbonate buffered solutions at the pH used under reaction conditions. Fitting of a weighted sum of the substrate and product spectra provided the mole fraction of each component in a mixture.

The data analysis began with an offset correction followed by normalization of the spectra to the area of the bands between 1600 and 1650 cm\(^{-1}\), which includes contributions from the reactant and products only. The raw spectra of mixtures of alkene and epoxide were then fitted with a weighted sum of the spectra of the pure components and an offset correction to provide the mole fraction of substrate and product in the mixture. Spectral fitting involved minimization of the bands of the substrate and product in the residual spectra (*i.e.* real spectrum – calculated spectrum) in spectral regions where the carbonate or other components do not show signals. A calibration curve was constructed with mole fraction increments of 0.05 for both VBA and SS. The advantage of this approach to the analysis lies in the absence of a need to apply a baseline correction and the effect of spectrum to spectrum variations in absolute intensity and background signals.

**Comments on the synthesis and characterisation of 4-oxirane-phenyl-sulfonate**

The detergent properties of sodium alkylbenzene sulfonates frequently do not allow the use of conventional solvents and methods of organic chemistry for isolation and purification. The preparation of 4-oxirane-phenyl-sulfonate from styrene-p-sulfonate was achieved readily by epoxidation however, the generation of the unwanted diol product rendered separation and purification of the epoxide a challenge. The synthesis of the epoxide of styrene sulfonate was attempted in three ways.

Initially, the epoxidation of styrene sulfonate was carried out using a modified method described by Hage *et al.*\(^{28}\) Styrene sulfonate (2.06 g, 10 mmol) and \([\text{Mn}_2\text{O}_3(\text{tmtn})_2]\text{[PF}_6\text{]}_2\cdot\text{H}_2\text{O} (80 \text{ mg, 0.1 mmol, 1 mol%})\) was dissolved in double distilled water (100 ml). The reaction mixture was stirred and cooled in ice for 30 min. Hydrogen peroxide (50 %, 2.83 mL, 50 mmol, 5 equiv.) was added dropwise over 1 h. The reaction mixture was stirred for a further 2 h and then the water removed by lyophilisation. \(^1\)H NMR and Raman spectroscopic analysis indicated the formation of both epoxide and diol products, however. This reaction was repeated but with only 0.1 mol% (8 mg) of 1, and the ice bath was allowed to warm to room temperature over 19 h followed by lyophilisation. This did not lead to an improvement in selectivity.
A second method, that reported by Lam et al.\textsuperscript{29} was attempted. This method involves the direct oxidation of styrene sulfonate with \textit{m}CPBA and is reported to form the epoxide after which the diol is formed in a subsequent reaction step by reaction in aqueous \textit{NaHCO}_3. In the present study a mixture of styrene sulfonate (340 mg, 1.65 mmol) and \textit{m}CPBA (415 mg, 2.40 mmol) were dissolved in water/ethanol (40 cm\textsuperscript{3}, 1:1, v/v). The reaction was stirred for 2 h at 65 °C and tested periodically with bromine water until the test was negative. The solution was allowed to cool and the solvent was evaporated in vacuo. Residual \textit{m}-chlorobenzoic acid was dissolved in acetone (300 mL) and filtered off leaving a white solid. \textsuperscript{1}H NMR and Raman spectroscopic analysis confirmed that the diol product was obtained exclusively, indicating that hydrolysis with base was not in fact necessary. Inspection of the \textsuperscript{1}H NMR data provided by the Lam et al.\textsuperscript{2} revealed that the product reported as the epoxide was in fact the diol product, 4-(1,2-dihydroxyethyl)phenylsulfonate. Ultimately the epoxide was obtained, despite the insolubility of styrene sulfonate in organic solvents, using the catalyst 1 in H\textsubscript{2}O/CH\textsubscript{3}CN under mildly acidic conditions (see main text for details).

\textbf{\textsuperscript{1}H NMR Spectroscopy}

The \textsuperscript{1}H NMR spectra of the sodium salts of 4-vinylbenzenesulfonate sodium salt (I), 4-oxirane-phenyl-sulfonate (II) and 4-(1,2-dihydroxyethyl)phenylsulfonate (III) are shown in Figure 14. The differences are apparent as the double bond is converted to the epoxide and then the diol. The aromatic protons give rise to two doublets around 7.6 ppm as expected. The vinyl protons give rise to three signals between 5 and 7 ppm. For the epoxide these protons are shifted upfield to 3 – 4.5 ppm. Ring opening of the epoxide to the diol results in a smaller shift in these proton signals.
Additional spectra and data

Figure 14 $^1$H NMR spectra of sodium salts of 4-vinylbenzenesulfonate sodium salt (I), 4-oxirane-phenyl-sulfonate (II) and 4-(1,2-dihydroxyethyl)phenylsulfonate (III) in D$_2$O.
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Effect of surface treatment on deposit pattern and Raman spectra obtained

Figure 15 Image of deposit from dried droplet containing 0.5 mM VBA in 0.1 M NaHCO$_3$(aq) and Raman spectra recorded from each spot.

Comparison of VBA, OBA and 4-formylbenzoic acid in the solid state and after deposition using the DCDR method

Figure 16 Solid state Raman spectra of VBA, OBA and 4-formylbenzoic acid

Figure 17 Raman spectra of VBA, OBA and 4-formylbenzoic acid obtained using DCDR method using 20 mM solutions in 0.1 M NaHCO$_3$(aq).
Effect on droplet size and concentration of analyte on spectra obtained by DCDR method

![Graph](image1)

**Figure 18** Raman spectra of VBA obtained using DCDR method from 10 to 0.1 mM solutions (in 0.1 M NaHCO₃) using the DCDR method.

Uniformity of co-deposition of mixtures of VBA and SS as 0.2, 0.6 and 0.8 mole fraction mixtures

![Graph](image2)

**Figure 19** Raman spectra (after normalisation for area under the peak at 1600 cm⁻¹ and baseline correction) obtained at 8 points on deposition ring (mole fraction 0.2 VBA/0.8 SS)
Figure 20 Average spectrum (blue) and standard deviation (red) from average spectrum obtained at 8 points on deposition ring (mole fraction 0.2 VBA/0.8 SS).

Figure 21 Raman spectra (after normalisation for area under the peak at 1600 cm$^{-1}$ and baseline correction) obtained at 8 points on deposition ring (mole fraction 0.6VBA/0.4SS)

Figure 22 Average spectrum (blue) and standard deviation (red) from average spectrum obtained at 8 points on deposition ring (mole fraction 0.6 VBA/0.4 SS)
Off-line reaction monitoring by DCDR spectroscopy

**Figure 23** Raman spectra (after normalisation to the area under the band at 1600 cm$^{-1}$ and baseline correction) obtained at 8 points on the deposition ring (mole fraction 0.8 VBA/0.2 SS)

**Figure 24** Average spectrum (blue) and standard deviation (red) from average spectrum obtained at 8 points on deposition ring (mole fraction 0.8 VBA/0.2 SS)

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23. The spot size was found to be sufficiently large to still allow for acquisition of spectra representative of the bulk sample even where components crystalize separately.
24. Raman spectra were recorded at 532 nm for online monitoring, as at 785 nm, as used for analysis of the DCDR system, was not sufficiently sensitive to provide a useful signal to noise ratio for in-line analysis (the laser power at sample was 200 mW at 532 nm in contrast to 80 mW at sample at 785 nm). In addition the detector used has a higher sensitivity in the visible region than in the NIR.