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Werner Hug*

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1. A Historic Perspective which is also an Outlook

Two decades have elapsed since the modest experimental inception of vibrational Raman optical activity (VROA) [1][2]. What was looked at, by many chem-

ists and physicists alike, with the notable exception of some stereochemists, as a curious little effect, almost impossible to measure and prone to misinterpretation and artefacts, is gradually revealing its potential of becoming the most powerful chiroptical method of the future. This

achievement is owed to the perseverance of a small group of chemical physicists who never wavered in their belief in the potential of vibrational Raman optical activity.

We will elaborate in some detail on the potential of VROA in the following paragraphs and give a general argument here. Without wanting to take away from the merit of people working in the field of classical optical activity, our view can certainly be justified that the spectroscopic situation for determining chiral structures, apart from special X-ray techniques, has actually been for decades a dismal one, the only generally available techniques being the circular dichroism of electronic transitions and optical rotatory dispersion. This is equivalent to a situation where for ordinary structure determination the only spectroscopic method available would be visible and UV absorption spectroscopy and the measurement of the

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We will elaborate in some detail on the potential of VROA in the following paragraphs and give a general argument here. Without wanting to take away from the merit of people working in the field of classical optical activity, our view can certainly be justified that the spectroscopic situation for determining chiral structures, apart from special X-ray techniques, has actually been for decades a dismal one, the only generally available techniques being the circular dichroism of electronic transitions and optical rotatory dispersion. This is equivalent to a situation where for ordinary structure determination the only spectroscopic method available would be visible and UV absorption spectroscopy and the measurement of the

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index of refraction. Clearly, methods accessing molecular vibrations for the determination of chiral structures simply cannot fail with time to eclipse, though not supplant, conventional optical activity. But what about even much more powerful methods, such as nuclear magnetic resonance optical activity? This is a naive question which chemists have been asking me from time to time. The answer derives from the well-known fact that optical activity measures the variation of the vector potential, characterising the probing electromagnetic radiation, over the molecular dimension. This implies what one might call the $1/\lambda$ problem [3]. While it is always unwise to try to predict the future, a statement which safely can be made is that any NMR optical activity for ordinary sized molecules is expected to be exceedingly small due to the long wavelength of the probing radiation of this spectroscopic method.

Chiroptical methods probing molecular vibrations can thus be expected to dominate in the future. Apart from VROA, there is vibrational circular dichroism (VCD) the first measurements of which [4][5] were achieved at almost the same time as VROA. While the $1/\lambda$ problem also exists, to a more limited extent, in VCD, instrumental development has been somewhat faster than in VROA, and VCD is consequently ahead in stereochemical applications [6] in spite of a steady trickle of interesting and generally reliable experimental VROA data recorded with scanning instruments [7][8]. This is not expected to last, however. As a matter of fact, were it not for the destruction of the first high-performance multi-channel VROA instrument [3][9] by unfortunate circumstances, the situation might well be different. This instrument had allowed the rapid measurement of polarized [10], depolarized [11] and, in its latest version, backscattered [3] VROA spectra. Fortunately, some of the know-how gained in its design and operation found its way into the construction of an instrument now operating at Glasgow University [12], apparently in a safe environment and with sustained local support.

VROA can potentially provide a lot more information than VCD even though the two methods are complementary to a certain extent, as is the case for Raman spectroscopy and IR spectroscopy. Yet, a wealth of information also means a wealth of work to properly understand and interpret it. From a stereochemists' and biochemists' point of view, the scientists who are assumed to be using the method for structural elucidation in the future, the

basic theory of VROA [13] with all its esoteric optical tensors and their derivatives with respect to vibrational atomic displacements might look almost impenetrable. Though the elaborate calculations chemical physicists have been doing in the past will remain essential in the future, we will nevertheless try to make the point that some of the most fruitful applications of VROA might be based on its empirical application, with only a qualitative understanding of a few molecular vibrations and of the electronic structure of molecular fragments required.

2. These Esoteric Tensors and Their Invariants...

The appropriate formulation of VROA intensities for isotropic samples is in terms of invariants of the relevant transition tensors [14]. For the anisotropic invariants, there has been a numerical redefinition [13] so that one has [15]

$$\gamma^2 = \beta(G')^2 = \gamma_G^2 \quad (1)$$

$$\delta^2 = \beta(A)^2 = \frac{1}{3}\gamma_A^2 \quad (2)$$

for the anisotropic invariants of the electric dipole-magnetic dipole and electric dipole-electric-quadrupole optical activity tensor products, respectively. To avoid confusion, the anisotropic invariant of the polarizability tensor α given as γ^2 in [14] is now written as β^2 or $\beta(\alpha)^2$. Formulas for these invariants as well as $\alpha G'$, the isotropic part of the electric dipole-magnetic dipole optical activity tensor, may be found in [13][14].

There are five distinguished scattering arrangements, namely

- a) three 90° scattering geometries with an analyzer oriented, with respect to the scattering plane
 - 1) perpendicular (polarized VROA spectra)
 - 2) parallel (depolarized)
 - 3) at $\pm 54.74^\circ$ ('magic angle', '*')
- b) two collinear geometries with no analyzer
 - 4) backscattering (180°)
 - 5) forward scattering (0°)

The differences for the differential scattering cross sections for left (L) and right (R) circularly polarized exciting light for these arrangements are readily obtained from published formula [3][13][16]

$$\Delta d\sigma = d\sigma^L - d\sigma^R \quad (3)$$

$$\Delta d\sigma_\perp = -\frac{4K}{3c} [135\alpha G' + 21\gamma^2 + 3\delta^2] d\Omega \quad (4)$$

$$\Delta d\sigma_\parallel = -\frac{4K}{3c} [18\gamma^2 - 6\delta^2] d\Omega \quad (5)$$

$$\Delta d\sigma_* = -\frac{4K}{3c} [90\alpha G' + 20\gamma^2] d\Omega \quad (6)$$

$$\Delta d\sigma(180) = -\frac{4K}{3c} [72\gamma^2 + 24\delta^2] d\Omega \quad (7)$$

$$\Delta d\sigma(0) = -\frac{4K}{3c} [540\alpha G' + 12\gamma^2 - 12\delta^2] d\Omega \quad (8)$$

while, for the average differential-scattering cross sections, one has

$$d\sigma_\perp = K [45\alpha^2 + 7\beta^2] d\Omega \quad (9)$$

$$d\sigma_\parallel = K 6\beta^2 d\Omega \quad (10)$$

$$d\sigma_* = K \left[30\alpha^2 + \frac{20}{3}\beta^2 \right] d\Omega \quad (11)$$

$$d\sigma(180) = d\sigma(0) = 2d\sigma_\perp \quad (12)$$

with

$$K = \frac{1}{90} \pi^2 \bar{v}_m^4 c^4 \mu_o^2 \quad (13)$$

$$\bar{v}_m = \frac{\omega_o}{2\pi c} \pm \Delta \bar{v}_m \quad (14)$$

ω_o being the angular frequency of the exciting light and $\Delta \bar{v}_m$ frequency shifts in wave numbers.

The formulas are valid for exciting light with ω_o well outside electronic absorption bands, *i.e.*, in the nonresonant case, and the tensors are considered transition tensors. Model interpretations and direct calculations need, therefore, in general be based on writing the tensor components as derivatives of the components of electronic molecular tensors with respect to the molecular vibrations.

Chemists are not generally at ease with methods which require the handling of intricate tensors. Yet, the above invariants, being scalar quantities, are actually as easy to handle as, say, the dipole strength extracted from an electronic absorption band of an UV spectrum. If only, and this is a complication with the above expressions, the three VROA invariants $\alpha G'$, γ^2 , and δ^2 , would not occur in combination but individually. From Eqns. 4–8, which

have been written with a common prefactor, it is easily seen, however, that appropriate combinations of $\Delta d\sigma$ values can be formed which depend on a single invariant only, as exemplified in the following section.

It is easy also, even for the nonspecialist, to qualitatively appreciate the occurrence of invariants of, say, the magnetic dipole – electric dipole tensor. For isotropic samples, liquids in general, rotationally invariant combinations of tensor elements only can survive. And as chiral information is carried by the radiation emitted by oscillating magnetic dipole moments induced in the sample by the electric component of the probing light, and by the electric dipole moments induced by the magnetic component, the corresponding dynamic distortion tensor occurs in the theoretical expression for VROA scattering. The same holds for the quadrupole contribution.

3. ... and Their Measurement

Ideally, the data obtained by VROA experiments should be the relative, if not the absolute, size and sign of the five invariants entering expressions 4–12 for all vibrations of a molecule. A maximum of information on each vibration, and thus on the structure of a molecule, would then be available. The comparison of measured and calculated data would become really meaningful, what is now by no means always the case as calculated and measured VROA spectra are often compared for one particular measuring arrangement only. In principle, given an instrument with sufficient sensitivity and lack of artefacts, this should be readily accomplished. In practice, quite apart from instrumental drifts and sample degradation during measurements, there are some real difficulties, namely:

- instrument sensitivity for different scattering arrangements is different;
- stray light varies;
- fluorescent and solvent background, if present, are different;
- resolution can vary even for identical instrument settings because optics are not filled homogeneously;
- the finite size of the light collection cone has not the same effect on the measurement of different invariants;
- isotropic and anisotropic contributions may have different spectral widths;
- bands often overlap.

Some of these problems, like instrument response, are amenable to comparatively simple solutions, if tackled by a

skilled instrumentalist, others might be disregarded in view of the limited precision of VROA data. Still, it is understandable that only recently the first quantitative measurement of the relative size of the anisotropic magnetic dipole contribution γ^2 and the anisotropic electric quadrupole contribution δ^2 has been published [17]. An elaborate double modulation technique was employed to obtain the data [18]. This is a beautiful achievement, but for reasons given in [12] we have doubts that the applied technique lends itself to the rapid routine acquisition of VROA data. Indeed, the results of [17] already represent a compromise between data acquisition speed and precision on account of the finite light collection cones which were chosen for the two realized scattering geometries [19].

A method which allows the elimination of the quadrupole contribution is the so-called magic angle scattering [20]. From Eqns. 4, 5, and 6, it is seen that one has

$$\Delta d\sigma_{\perp} = \frac{2}{3}\Delta d\sigma_{\perp} + \frac{1}{3}\Delta d\sigma_{\parallel} \quad (15)$$

Eqn. 15 shows that, its originally somewhat elaborate presentation in terms of the Stokes-Mueller formalism notwithstanding [20], magic angle scattering is an ordinary 90° scattering experiment with the polarization analyzer setting chosen to yield the particular linear combination of $\Delta d\sigma_{\perp}$ and $\Delta d\sigma_{\parallel}$ in which δ^2 cancels.

Apart from double-modulation techniques [17], a combination of $\Delta d\sigma$ (180) and $\Delta d\sigma_{\parallel}$ can also yield γ^2 and δ^2 separately:

$$\Delta d\sigma(180) + 4\Delta d\sigma_{\parallel} = -\frac{4K}{3c} 144\gamma^2 d\Omega \quad (16)$$

$$\Delta d\sigma(180) - 4\Delta d\sigma_{\parallel} = -\frac{4K}{3c} 48\delta^2 d\Omega \quad (17)$$

It is gratifying that the two measurements needed are the easier ones with respect to artefact suppression. As a matter of fact, artefact suppression for $\Delta d\sigma_{\parallel}$ is trivial with a double lens light collection system, and the use of unlaminated Polaroid foil as a polarization analyzer [21], and the already favorable situation for $\Delta d\sigma$ (180) can be further improved by the use of a fibre optics cross-section transducer with statistically mixed fibres [3].

It should perhaps be mentioned here that there is a skew-angle scattering geometry which yields γ^2 directly. The scattering angle is 109.47° and the analyzer setting $\pm 30^\circ$ to the scattering plane:

$$\Delta d\sigma_{30}(109.5) = -\frac{4K}{3c} 24\gamma^2 d\Omega \quad (18)$$

The implementation might be delicate, and similar arrangements for δ^2 and $\alpha G'$ do not exist – the appropriate equations, which are readily derived from the formulae given in [16], have no solutions. Maybe one should call this arrangement ‘dual magic angle’ or, in view of the possible experimental difficulties, ‘bewitched angle’ scattering.

In our opinion, the separate measurement of $\alpha G'$ might have particular significance, because sizeable values might be characteristic for particular vibrations [22]. Three measurements are required to neatly separate it from γ^2 and δ^2 , namely, in addition to $\Delta d\sigma$ (180) and $\Delta d\sigma_{\parallel}$, either $\Delta d\sigma$ (0) or $\Delta d\sigma_{\perp}$:

$$\Delta d\sigma(180) + 6\Delta d\sigma(0) - 8\Delta d\sigma_{\perp} = -\frac{4K}{3c} 3240\alpha G' d\Omega \quad (19)$$

$$5\Delta d\sigma(180) + 24\Delta d\sigma_{\perp} - 8\Delta d\sigma_{\parallel} = -\frac{4K}{3c} 3240\alpha G' d\Omega \quad (20)$$

In practice, one might do with $\Delta d\sigma$ (0) [22] or even $\Delta d\sigma_{\perp}$ [10]. A better compromise, δ^2 being small, might be either

$$6\Delta d\sigma(0) - \Delta d\sigma(180) = -\frac{4K}{3c} [3240\alpha G' - 96\delta^2] d\Omega \quad (21)$$

or

$$6d\sigma_{\perp} - 7\Delta d\sigma_{\parallel} = -\frac{4K}{3c} [810\alpha G' + 60\delta^2] d\Omega \quad (22)$$

with other combinations also possible. Indeed, the simultaneous detection of VROA in two or even three scattering arrangements might be entirely feasible in the future in view of the now easy availability of compact spectrographs and light detection systems. There is, after all, as we have already demonstrated with the dual light collection system [21], no reason to throw away well over 95% of the precious scattered light! It should well be seen that the presently fastest instrument [12] with which at last acquisition times, compared on an equal wavenumber, laser power, and resolution basis, are achieved equal to or better than with our earlier design [3], does this with considerably less collected light, and this feat opens up real possibilities for simultaneous multiple scattering arrangement instruments.

As a last remark in this section, we point out that a test on the internal consist-

ency of the data is possible if $\Delta d\sigma(180)$, $\Delta d\sigma(0)$, and $\Delta d\sigma_{\perp}$ are measured as one has

$$\Delta d\sigma(180) + \Delta d\sigma(0) - 4\Delta d\sigma_{\perp} = 0 \quad (23)$$

Obviously then, in spite of the present euphoria over the ease of the measurement of $\Delta d\sigma(180)$, the independent measurement of $\Delta d\sigma_{\parallel}$ will always be required for a complete analysis of VROA data, with an additional two measurements for either $\Delta d\sigma(180)$ and $\Delta d\sigma(0)$, or $\Delta d\sigma(180)$ and $\Delta d\sigma_{\perp}$, or $\Delta d\sigma(0)$ and $\Delta d\sigma_{\perp}$ inevitable. But a reasonable compromise, aiming not at the separation of quadrupole and magnetic dipole contributions but rather the isotropic and anisotropic contributions, appears to be the measurement of either $\Delta d\sigma(180)$ and $\Delta d\sigma(0)$, or $\Delta d\sigma_{\parallel}$ and $\Delta d\sigma_{\perp}$.

4. Interpretation and Applications

Stereochemists and biochemists on the one side, and spectroscopists and theoreticians on the other, will to a certain extent be interested in different aspects of the information contained in VROA data. Either group will have its rich pickings, a fact which is underscored with each new type of molecule measured: small model compounds, amino acids [23], peptides and proteins [24][25], carbohydrates [26][27].

A theoretically inclined spectroscopist would want to have a VROA instrument capable of routinely measuring $\Delta d\sigma_{\parallel}$, $\Delta d\sigma_{\perp}$, and either $\Delta d\sigma(180)$ or $\Delta d\sigma(0)$, or both, and it should reliably yield all the tensor invariants discussed above. In conjunction with model calculations, e.g. with the bond polarizability theory [13], or direct *ab initio* calculations, a more thorough understanding can be obtained of the mechanisms which underly the generation of Raman optical activity. This certainly provides guidance for establishing rules relating the sign and the size of VROA to the geometric molecular structure, but it also refines the knowledge of molecular vibrations and of the electronic structure of molecular fragments. For this latter aspect, we suspect that the comparison of the sign and size of γ^2 and δ^2 , even if the latter is generally small, might be useful. A lot of work has already been done along these lines and the reader is here referred to the specialized literature.

As a stereochemist or biochemist on the other hand, I would want to own an instrument measuring VROA simultaneously in backscattering ($\Delta d\sigma(180)$) and forwardscattering ($\Delta d\sigma(0)$). To maximize light collection and to minimize artefacts

without lengthy adjustments, and also to render the instrument as compact as possible, I would insist on coupling the entrance slits of my two spectrographs with carefully calculated fibre optics cross-section transformers to the forward and backward light-collection optics. I would be aware that sample preparation needed some effort to exclude dust, gas bubbles, and as much as possible fluorescence, and that in routine work stray light in a collinear experiment would in general prevent me from measuring frequencies below 350 cm^{-1} [27].

A chemist thus equipped would be in a position to distinguish the isotropic and anisotropic contributions (Eqns. 21 and 7) to VROA, even though, for the anisotropic contribution, he would be limited to the somewhat arbitrary combination of $3\gamma^2 + \delta^2$ for the magnetic dipole and electric quadrupole part. This limitation is less severe than it might appear. From the very first VROA spectra measured in the seventies for $\Delta d\sigma_{\parallel}$ and $\Delta d\sigma_{\perp}$, we concluded that the quadrupole contribution δ^2 did not determine the sign of VROA bands and seemed generally much smaller than the magnetic dipole contribution $3\gamma^2$ [10]. Likewise, we found that the isotropic part $\alpha G'$ contributed little to the polarized spectra (Eqn. 4), except for two partially polarized bands at 755 cm^{-1} and 1205 cm^{-1} , where it appeared important. As this general early conclusions on the relative importance of $\alpha G'$, γ^2 , and δ^2 have so far been confirmed without exception [17][22], a VROA instrument for the determination of the structure of molecules which yields $\Delta d\sigma(180)$ and $\Delta d\sigma(0)$ looks like a good compromise. Such an instrument should, however, also be equipped to measure conventional depolarization ratios, which, within the limited precision admitted in VROA spectroscopy, is nothing difficult at all. We do not understand why this valuable information has only occasionally been used in the interpretation of VROA spectra. Even if a fully polarized band will never occur for a chiral molecule, this limiting case, for which $\gamma^2 = \delta^2 = 0$ holds, provides a valuable piece of information. Similarly, the fact that the mean derived polarizability α , and thus $\alpha G'$, vanishes for a depolarized band is often helpful [10].

If contributions from $\alpha G'$ are small or even negligible except for a few partially polarized bands, why then should one try to measure them? It is precisely because sizeable values for $\alpha G'$ occur for particular vibrations only that their measurement is important [22]. Firstly, spectra and interpretation are dramatically simplified by

the display of those VROA bands only which are generated through $\alpha G'$, and secondly some of these bands appear characteristic for chirally perturbed vibrations on achiral molecular fragments. The olefinic methylene twist vibration has been suggested to be such a probe [20][22]. In a structure of C_{2v} symmetry, the methylene twist transforms as A_2 , and this irreducible representation is spanned by G'_{xz} , G'_{yy} , and G'_{zz} , but not by α_{xz} , α_{yy} , and α_{zz} . A chiral perturbation allowing α to contribute then will lead to large and characteristic values of $\alpha G'$. The situation is the vibrational analogue to the chirally perturbed $n\pi^*$ transition of the carbonyl group with which stereochemists are well acquainted.

Another case where the determination of anisotropic and isotropic contributions would doubtless yield a better understanding is the couplet often observed for the antisymmetric deformation mode of the methyl group in chiral arylethanes [2][28]. It was unambiguously shown that the couplet is present in polarized and depolarized VROA spectra [10], and that it originates in a coupling of the methyl vibrations with a vibration of the H-atoms of the aromatic part of the molecule, and not in a steric deformation of the methyl group [11]. Later discussions [29] centred on the influence quadrupole contributions might have on its relative size in polarized and depolarized spectra, while assuming $\alpha G'$ to be negligible. We feel that a more fruitful approach might consist in considering in detail the contributions made by the components of the various tensors spanning different irreducible representations of the group C_{3v} of the methyl group.

The conformational study of biomolecules requires the high sensitivity of collinear scattering. The reduction in measuring time is certainly less pronounced than one might conclude from Eqns. 4–8, because Raman scattering is a shot-noise-limited experiment, and the summed intensities are also higher in a collinear experiment, but the gain in time is nevertheless important. This is particularly true once $\alpha G'$ will be determined routinely by forward scattering. No such experiment has been published so far for biologically important molecules, but we are confident that, in view of the potential simplification of the VROA spectra, such data will eventually be measured.

Even in the absence of spectra for $\Delta d\sigma(0)$, VROA appears a far more incisive probe of biomolecular conformation than conventional vibrational spectroscopy. The reason for this rests with the fact that only those few vibrational coordinates within a

complicated normal mode which sample the skeletal chirality directly contribute to the corresponding VROA band intensity. In a first study intended to identify signals characteristic of an α -helical structure the VROA spectra of poly(L-lysine) in random coil and α -helix conformation were compared with spectra of bovine serum albumin and insulin, and several characteristic bands were tentatively identified [25]. In a parallel study, VROA bands characteristic of antiparallel β -sheets were found for concanavalin A, α -chymotrypsin and β -lactoglobulin when compared to the model β -turn peptide L-Pro-L-Leu-Gly-NH₂ [30].

Carbohydrates appear particularly well suited for VROA spectroscopy. First results on D-glucose and several partially deuterated D-glucoses [26] and disaccharides [27] indicate signals which reflect the α - and β -anomeric configuration of monosaccharides, distinguish α - and β -linked disaccharides and polysaccharides, signals characteristic for the relative orientation of substituents on the six-membered glucose ring, and signals which distinguish different rotamers of the exocyclic hydroxymethyl group. There is little doubt that VROA will prove invaluable for conformational studies of carbohydrates, even if some of the present vibrational assignments must be considered tentative and need confirmation by further studies of series of related molecules. One should obviously not lose sight of the fact that a monosaccharide like D-glucose, even discounting C-H and O-H stretching modes, represents a vibrational assignment problem with well over 50 normal mode!

5. Conclusions

The use of a collinear scattering geometry and recent advances in detector technology, in particular of backthinned CCD devices with very high quantum efficiencies and low read-out noise, have made possible VROA instruments with data acquisition speeds equalling and surpassing the best previous designs even while collecting substantially less scattered light. The reliable measurement of molecules as difficult to measure as biopolymers in solution has thus become a reality.

The relatively low amount of scattered light needed for these results, due to the quantum efficiency of the detector and the use of a comparatively simple spectrograph with a single high efficiency planar grating and a holographic notch filter, should make the construction straightforward of

instruments capable of measuring VROA simultaneously for more than one scattering arrangement. The simultaneous measurement of VROA in backward and forward scattering, straylight problems notwithstanding, appears particularly promising. Contrary to conventional Raman spectroscopy, where the scattering intensities are identical for these two configurations, VROA in backward scattering depends on anisotropic contributions only, while forward scattering is essentially determined by the isotropic part of the optical activity scattering tensor. A dramatic simplification of VROA data can result, and one expects their interpretation decisively facilitated. The situation is not unlike, but probably more favorable, to the use of depolarization ratios in ordinary Raman spectroscopy.

In conclusion, we point out that our emphasis in this brief account of the development of VROA has been on those aspects we consider most significant for the more imminent future applications of the technique. There might be other areas like the determination of absolute configurations by a comparison of measured and *ab initio* calculated VROA spectra which one might want to include in such a catalogue [31]. Likewise, *nonlinear techniques* have not even been mentioned here but might become very important, because they should allow the measurement of dilute solutions, impossible in spontaneous Raman scattering because of its limited dynamic range. Indeed, we see no reason why this should not be accomplished, once a skilled experimentalist gets interested in this area and chooses an appropriate thresholdless collinear phenomenon like Raman gain/loss spectroscopy, instead of the generally favored CARS.

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