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CHIRALITY ANALYSIS OF „FLYING ELEPHANTS“

Physico-chemists at the Philipps-Universität Marburg have developed a new method for analyzing the molecular chirality of amino acids based on a combination of electrospray ionization (ESI) with detection of the photoelectron circular dichroism (PECD) in the source of a chirality spectrometer allowing for the detection of electrons and ions (c.f. Fig. 1). The approach may open new access to studying proteins or pharmaceuticals as relevant in biology or medicine.

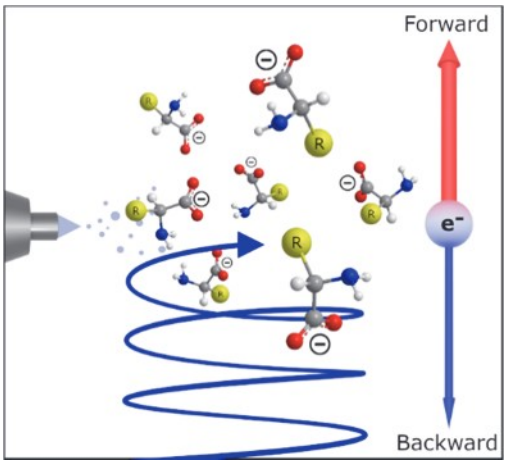

Figure 1: Sketch of the ESI-PECD approach implemented. Taken from ref. [13]

Ever since the discovery of optical activity at the beginning of the 19th century and the mechanical separation of mirror-image crystals of a tartrate salt by Pasteur in the mid of the 19th century, the phenomenon of chirality (a term coined by Lord Kelvin) has fascinated scientists and in fact also non-scientists. For an introduction into the history of chirality in chemical language the reader is pointed to a very informative review by Cintas [1]. That review even builds an intriguing bridge to the novel “Through the Looking-Glass (and what Alice found there) by Lewis Carroll. The novel is a sequel to “Alice’s adventures in wonderland” and describes Alice moving through a mirror into an unreal world, eventually saying to her cat “Perhaps looking-glass milk isn‘t good to drink”. That novel was written in 1871. The original citations can be found in ref. [1].

Today we define chirality as a property of rigid objects defined by the absence of symmetry elements of the second kind (a mirror plane, σ=S_1, a center of inversion, i=S_2, or a rotation-reflection axis, S_{2n}) [2]. As a consequence these objects are non-superposable on their mirror image. This concept applies to macroscopic objects but it is most important for molecules and matter dominated by molecular properties.

Scientific interest in chirality today is (at least) twofold. We first mention the topic of homochirality, meaning equal or similar handedness (from the greek words ὁμός and χειρ). All natural amino acids (except glycine) and all natural sugars are chiral. In nature the L-enantiomer of amino acids and the D-enantiomer of sugars dominate by far, although the respectively other enantiomers may also exist [3]. Biological homochirality refers to the fact that life as we know it is dominated by biopolymers constructed from L-amino acids and D-sugars. The origin of homochirality is fiercely disputed with some scientists favoring energy differences due to parity violation and others favoring a sudden imbalance by chance perhaps from an extra-terrestrial source both requiring what is in general called *chiral amplification* [4,5,6].

Secondly, interest in molecular chirality is connected to the fact that most pharmaceutical compounds are chiral. Often only one of the enantiomers is bioactive or has the desired pharmacological effect. Therefore, the analysis of molecular chirality is still one of the pivotal challenges in analytical chemistry accompanying the route to enantiopure chemical synthesis. There are plenty of good reasons to strive for enantiopure synthesis. However, caution is recommended when selecting arguments for motivation. From what

is known today, enantiopure preparation would not have prevented the Contergan tragedy due to the fact that (+)-R-thalidomide and (-)-S-thalidomide racemize in human blood within a few hours [7], and the deep truth is probably even more complex [8].

While experts in chemical synthesis usually employ traditional analysis techniques as e.g. chiral chromatography or the formation of diastereomers, physico-chemists in general prefer to apply optical variants of chirality analysis. Classical chiroptical concepts include the measurement of circular dichroism, CD, i.e. the difference between extinction coefficients for left and right circular polarized light, optical rotatory dispersion and Raman optical activity [9,10]. Here, in recent years we have witnessed tremendous progress in measuring the circular dichroism in total ion yields in a mass spectrometer (PICD) and the angular distribution of photoelectrons (PECD) in an electron spectrometer, in particular with a coincident detection of PICD and PECD [11].

So far, all these approaches have mainly been applied to rather small molecules exhibiting a sufficiently high vapor pressure enabling the analysis in the gas phase. What was missing was the advancement allowing to measure PICD or PECD of molecules with biological relevance, i.e. of "molecular elephants" to use a phrase coined by John Fenn [12]. Having cited this phrase it is basically clear where the solution to the challenge is to be found. We are searching for a combination of electrospray ionization with a chiroptical detection of chirality. This is exactly what Krüger and Weitzel demonstrate in reference [13]. Here, the combination of ESI with PECD analysis provides unambiguous distinction of the enantiomers of glutamic acid and DOPA, two molecules that can hardly be transferred to the gas phase without the means of ESI. Glutamic acid (GLU) was investigated as a prototype for proteinogenic amino acids and 3,4-dihydroxyphenylalanine (DOPA) was selected due to its pharmaceutical relevance.

Anions of GLU and DOPA have been sprayed into the source of a chirality spectrometer, where eventually all electrons and ions can be detected, the latter with mass analysis (c.f. Fig. 2). Electrons are set free from the anions by photodetachment and detected by a spectrometer projecting forward and backward scattered parts of the signal (relative to the laser beam propagation) onto different detectors.

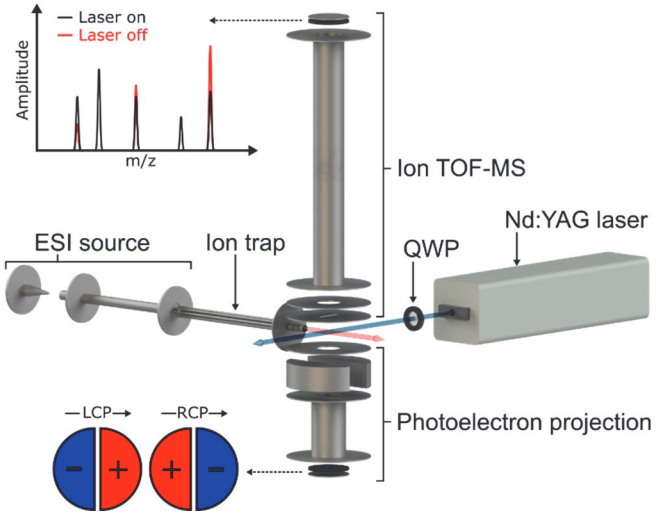

Figure 2: Schematic of the experimental setup, including the ESI beam and the laser beam for inducing photodetachment as well as the electron and the ion spectrometer with an illustration of typical observables. Taken from ref. [13].

As an example the mass spectrum of the ESI beams operated in the negative mode is shown in Fig. 3 for L-DOPA. The MS is dominated by the deprotonated molecular anion $[M\text{-}H]^-$ ion (m/z=196) (c.f. Fig. 3). Photodetachment is induced by intersecting a 355 nm pulse from a tripled Nd-YAG laser with the ESI beam. Formation of photoelectrons is accompanied by a depletion of the dominant anion signals in the mass spectrum (blue line in Fig. 3). Using photodetachment from anions bears the advantage of circumventing classical resonance requirements, since it exploits a bound-free transition. The magnitude of the PECD observed is here on the order of 5%, with the value being positive for D-DOPA (c.f. Fig. 3) and for L-GLU. For details, see ref. [13].

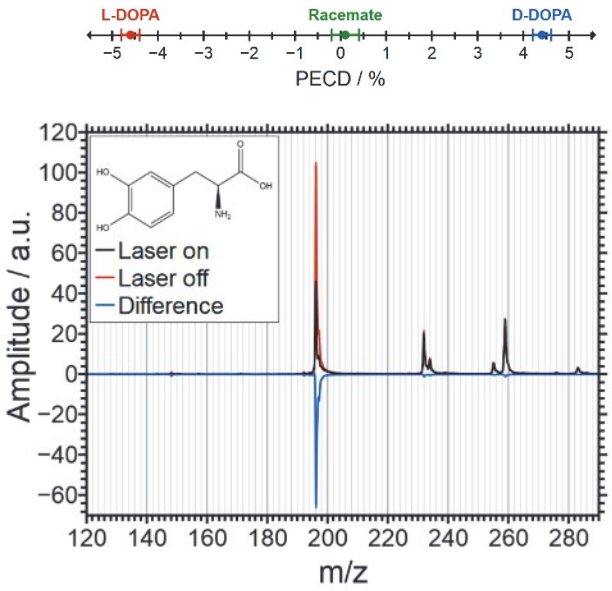

Figure 3: Bottom: Typical electrospray mass spectrum of L-DOPA in the negative ion mode. In black the spectrum with the *laser on* (mean of left and right circular polarized light and in red with the *laser off* is displayed, respectively. The corresponding *difference* in blue illustrates the yield changes caused by photodetachment at 355 nm. For L-DOPA a depletion of the $[M-H]^-$ ion (m/z = 196) is dominant. Top: Illustration of PECD for L-DOPA, D-DOPA and the racemate. For further details see ref. [13].

Since ESI hardly has any relevant mass limitation - there have been reports on spraying viruses - the ESI-PECD approach implemented in reference [13] can be expected to be applicable to a wide range of matter, with properties determined by the molecular character. The authors group currently applies this approach for analyzing the chirality of gramicidin. Gramicidin is an antibiotic peptide consisting of 15 amino acids with a mass of approximately 2000 u.

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Prof. Dr. Karl-Michael Weitzel

Karl-Michael Weitzel is professor for physical chemistry at the Philipps-Universität Marburg.
Research in the Weitzel group is focussed on experimental and theoretical studies of the formation, transport and reactivity of ions. This research touch all aspects of physical chemistry including thermodynamics, spectroscopy, kinetics and electrochemistry. The research projects cover fields ranging from chirality analysis to ion-molecule-reaction dynamics and ion (and electron) transport in solid state materials.
KMW studied chemistry in Marburg. He obtained his doctoral degree in physical chemistry with Jürgen Troe at the Universität Göttingen. Subsequently he spent close to two years as a postdoc with Tomas Baer in Chapel Hill (NC, USA). Right after the unification he returned to Germany joining the group of Helmut Baumgärtel in Berlin, where he worked for his Habilitation and obtained the venia legend in Physical chemistry. Since 2002 KMW is full professor in Marburg. KMW has served as director of the institute of physical chemistry and as a dean of the chemistry department. KMW has been member of the editorial board of Zeitschrift für Physikalische Chemie for about 10 years, of that several years as editor-in-chief. KMW also served as a member of the topical committee (Themenkommission) of the Deutsche Bunsen-Gesellschaft (DBG) for about 10 years, 7 of those years as chairman. Currently KMW is member of the advisory board (Ständiger Ausschuß) of the DBG. He is also spokes person of the DFG research unit FOR 5065, Energy Landscapes and Structure in Ion Conducting Solids (ELSICS).

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