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Review

Circular dichroism in functional quality evaluation of medicines

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ABSTRACT

Circular dichroism (CD) is a non-destructive and powerful technique for providing structure and ligand interaction information of small molecules as well as biotechnological medicines. While CD is a well-established technique in biomedical research, and different types and variants of CD do exist, the focus of this review is on the pharmaceutical quality control (QC) aspects of the classic electronic CD (ECD). The basic principles of the CD technique are initially described, followed by a systematic literature research on pharmaceutical aspects encompassing chiral small molecules, bio-polymers (*i.e.* proteins, peptides and nucleic acids), medicine-biotarget interaction (*i.e.* small molecule-albumin interaction, protein-receptor interaction and peptide-biotarget interaction) and medicine changes (*i.e.* chemical modification, biosimilar/bio-better with stability and aggregation). In addition, unstructured literature was also included covering the use of CD mainly in discovery and fundamental research, but which might shift towards the pharmaceutical QC field as well in the future.

Contents

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1. Principles of CD for pharmaceutical QC

1.1. Basic principles of CD

Plane polarized light can be viewed as being made up of two circularly polarized components of equal magnitude, with one rotating counter-clockwise (left-handed circularly polarized, LCP) and the other one rotating clockwise (right-handed circularly polarized, RCP). Circular dichroism (CD) refers to the differential absorption of LCP and RCP lights; this differential absorption occurs on chiral compounds. In a CD instrument, plane polarized light is split into LCP and RCP lights by passage through a modulator, subjected to an alternating electrical field. The modulator generally is composed of a piezoelectric quartz crystal and a thin plate of isotropic material. The alternating electrical field induces structural changes in the quartz crystal, which cause the plate to transmit circularly polarized light at the extremes of the field. As the transmitted light is switched between LCP and RCP lights, these are detected in turn by the photomultiplier. If one of the lights is absorbed by the sample to a greater extent than the other, the resultant lights would be elliptically polarized, i.e. the resultant would trace out an ellipse (Fig. 1) [1]. In practice, the CD instrument does not recombine the lights but detects the two lights separately; it will then display the dichroism at a given wavelength of light expressed as the difference in absorbance (ΔA) of the two light components. A CD spectrum is obtained when the dichroism is measured as a function of wavelength (Fig. 1).

1.2. Units

CD can be expressed in terms of absorbance: the unit is the differential absorbance ($\Delta A = A_L - A_R$) of LCP and RCP lights, while ΔA is dimensionless; however, it is sometimes reported in milliabsorbance units (mAU). The molar absorbance ($\Delta\varepsilon$, $cm^{-1}\,M^{-1}$) compensates for both the concentration and pathlength, and is defined as equation $\Delta\varepsilon = \varepsilon_L - \varepsilon_R = \frac{\Delta A}{C \times l}$ ε_L and ε_R are the molar extinction coefficients for LCP and RCP light respectively, C is molar concentration (M), and l is pathlength in centimetres (cm). However, CD is generally expressed in terms of the ellipticity (θ; = 32.982 × ΔA), and more often in millidegrees (m° or mdeg). θ is sometimes converted to be molar ellipticity ([θ]). Another principal unit is the mean residue ellipticity ($[\theta]_{MRW}$), which is specific for proteins and polypeptides, and reports the molar ellipticity for individual protein residues instead of whole protein molecules.

1.3. Data treatment

General signal processing is performed by comparing the signals from different CD spectra in order to figure out signal difference. However, until now, signal processing is hardly applied in CD studies; only some studies about the lots comparability of protein medicines or protein modification by evaluation of the complexation with the receptor protein are reported [2–4]. Moreover, the first derivative of the CD curve was used to study the melting temperature (T_m) [5]. T_m has been used as one parameter for the thermal stability tests for protein medicine, and for biological function studies of peptides [6–9].

Various algorithms and databases, established by well characterized standard proteins, have been developed for the secondary structure estimation of unknown proteins. These algorithms include least squares analysis (*e.g.* LINCOMB), ridge regression (*e.g.* CONTIN), singular value decomposition (*e.g.* SVD), single value decomposition with variable selection (*e.g.* VARSLC and CDSSTR), the self-consistent method (*e.g.* SELCON) and neural network analysis (*e.g.* CDNN, K2D and SOMCD) [10–18]. Detailed descriptions of the algorithms and datasets have already been given in different review articles [19–22].

1.4. Practical considerations

CD is essentially a spectrophotometric measurement, so the usual precautions for spectrophotometry should be considered for CD as well: the total sample absorbance should be below 1.0. Alternatively, the absorbance is conveniently monitored by the trace of the photomultiplier tube (PMT) voltage. In general, the voltage should be less than 700 V (this value depends on the used instrument), since S/N ratio diminishes once the voltage goes above 500 V [1,20]. Using proteins as one example, a typical concentration is around 0.1 mg/ml, applying a 1 mm cuvette at the far-UV region. In the near UV region, longer path length cuvettes (*e.g.*10 mm) are required with a high concentration such as 1–2 mg/ml. 10 mM phosphate buffer is a commonly used buffer for the proteins in the far UV studies; other suitable ions include sulphate, fluoride and borate [20]. The suitability of other buffer systems and organic solvents has been discussed in other articles [1,23–26]. Before a measurement, different instrument parameters shall be set such as the half-band width, wavelength interval and the data collection time to ensure the data acquisition. For the details of the settings, other articles can be consulted [1,20].

1.5. The peculiarity of CD

Like other optical spectroscopies, CD is a fast and non-destructive technique. A single protein far-UV CD spectrum over the 190–240 nm range can take as less as 3 min, while titration experiments (*e.g.*, ligand or DNA-protein bindings) and temperature-dependent measurements need more time, usually 1–2 h [27–29]. Many techniques aim at structural determination, most notably NMR and X-ray crystallography, which are based on the quantitative evaluation of peak amplitudes and positions, by analyzing numeral data in geometry optimization algorithms [29]. One advantage of CD is that the characterization can be performed in aqueous condition, which is a more actual condition to understand some molecules and their biological functions such as proteins, since the proteins actually operate in solution. CD studies can be performed under simulative physiological conditions and other widely aqueous conditions, i.e. different concentration, pH, temperature, ionic strength and detergents [27]. In contrast, NMR and X-ray crystallography characterization requires crystal forms of molecules. Another advantage of CD is that rigorous pre-treatment of the samples, such as glycoproteins and membrane proteins, is not required, since neither carbohydrate moieties nor residual lipids disturb CD signals of the protein component. However, one limit of CD is that, compared to NMR and X-ray crystallography, only low-resolution structural information can be provided both for small molecules and biopolymers [1]. More detailed information cannot be provided by CD, such as the structure in specific regions of the protein, the stereoscopic shape and amino acid composition of ligand binding clefts and pockets.