INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES
[ISSN: 0975-4725; CODEN(USA): IJPS00]
Journal Homepage: https://www.ijpsjournal.com

Review Article

Circular Dichroism Spectroscopy of Voclosporin: Principles, Structural Characterization, and Pharmaceutical Significance

Arunlal V. B.*, Sana Dileep

National College of Pharmacy, Manassery, Mukkam, Kozhikode, Kerala 673602

ARTICLE INFO
Published: 31 May 2026
Keywords:
Circular dichroism spectroscopy, voclosporin, cyclosporin A, calcineurin inhibitor, chiral analysis, cyclic peptide conformation, lupus nephritis, chiroptical spectroscopy, vibrational circular dichroism, pharmaceutical analysis
DOI:
10.5281/zenodo.20472577

ABSTRACT

Voclosporin (ISA247, E-ISA247, brand name Lupkynis) is a semisynthetic cyclic undecapeptide calcineurin inhibitor approved by the United States Food and Drug Administration (USFDA) in January 2021 for the treatment of active lupus nephritis (LN) in adult patients.[4,10] As a structurally modified analogue of cyclosporin A (CsA), voclosporin harbours multiple chiral centres and exhibits a complex three-dimensional conformation that is intimately linked to its pharmacological efficacy, metabolic stability, and binding selectivity.[9] Circular dichroism (CD) spectroscopy, a chiroptical analytical technique that measures the differential absorption of left- and right-circularly polarized light, represents one of the most powerful tools available for the stereochemical and conformational characterization of such cyclic peptide drugs.[12,8] This review provides a thorough examination of the principles underlying CD spectroscopy and its application to the structural analysis of voclosporin. Topics addressed include the physicochemical and stereochemical properties of voclosporin, the theoretical foundations of electronic CD (ECD) and vibrational CD (VCD), instrumental considerations, spectral interpretation of cyclic undecapeptides, the relevance of CD data to voclosporin's binding with cyclophilin A, comparative analysis with parent cyclosporin A, and the pharmaceutical quality control implications of CD measurements for chiral drug substances.[5,8,19] Clinical context, regulatory considerations, and future perspectives in the application of advanced chiroptical techniques to voclosporin and related immunosuppressants are also discussed.

INTRODUCTION

The advent of calcineurin inhibitors (CNIs) in clinical medicine has fundamentally transformed immunosuppressive therapy, enabling both the successful management of solid organ transplantation and the treatment of a range of autoimmune disorders.[22] Cyclosporin A (CsA), introduced in the early 1980s, was the prototypical CNI that reshaped transplant medicine; however,

---

***Corresponding Author:** Arunlal V. B.
Address: *National College of Pharmacy, Manassery, Mukkam, Kozhikode, Kerala 673602*
Email ✉: arunlalvb@gmail.com

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

its clinical utility has been constrained by significant nephrotoxicity, narrow therapeutic windows, variable pharmacokinetics, and erratic metabolite profiles.[10,26] The discovery and development of voclosporin represents a major advancement over its predecessor: through a single, strategically placed chemical modification at the first amino acid residue of the cyclosporin scaffold, this novel molecule achieves enhanced calcineurin binding affinity, improved metabolic stability, and a more predictable pharmacokinetic profile that eliminates the need for routine therapeutic drug monitoring.[5,9]

From an analytical chemistry perspective, voclosporin presents several unique challenges and opportunities. As a cyclic undecapeptide with multiple stereocentres and a complex macrocyclic backbone, its conformational behaviour in solution, its interaction with biological targets such as cyclophilin A, and its stereochemical purity are all critical determinants of pharmacological activity.[9,19] Circular dichroism (CD) spectroscopy is ideally positioned to address these analytical needs. Unlike X-ray crystallography, which provides static, solid-state structural information, or NMR spectroscopy, which demands higher sample concentrations and extensive data processing, CD spectroscopy delivers rapid, solution-state conformational insights that are highly sensitive to changes in secondary structure, chirality, and molecular interactions.[12,14,21]

This review article is structured to provide a comprehensive account of the principles, instrumentation, and applications of CD spectroscopy as they pertain specifically to voclosporin. The review begins with an overview of the pharmacology and chemical architecture of voclosporin, followed by a detailed exposition of CD spectroscopic principles, the known conformational properties of cyclosporin-class molecules, and the specific utility of CD in characterizing the voclosporin–cyclophilin complex.[9,19,5] Pharmaceutical quality control considerations and future perspectives are addressed in the concluding sections.

2. Voclosporin: Chemical Identity, Structure, and Pharmacological Background

2.1 Chemical Identity and Classification

Voclosporin is classified as a cyclic undecapeptide of the cyclosporin class with molecular formula $C_{63}H_{111}N_{11}O_{12}$, and a molecular weight of approximately 1214.61 g/mol.[4,10] The USFDA has assigned it the designation of a first-in-class calcineurin inhibitor for the treatment of lupus nephritis.[4] Structurally, voclosporin is a semisynthetic analogue of cyclosporin A differing by virtue of a single modification at the N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine (MeBmt) residue at amino acid position 1, which is replaced by N-methyl-(4R)-4-[(2E,4E/Z)-2,4-pentadienyl]-4-methyl-L-threonine (E-MePmt1). The addition of one carbon to the butenyl side chain creates a conjugated diene moiety that extends into the binding site of cyclophilin A, resulting in enhanced van der Waals contacts and, consequently, a higher binding affinity than that of the parent cyclosporin molecule.[9,5]

The drug exists as a single trans isomer (E-isomer, or E-ISA247), in contrast to the racemic ISA247, which was the original synthesis product developed by Isotechnika Inc.[10] The transition from racemic synthesis to enantioselective production of the pure E-isomer was a critical pharmaceutical development step, as the E-isomer demonstrates superior immunosuppressive activity over the Z-isomer (Z-ISA247). The binding affinity measurements determined by fluorescence spectroscopy revealed that the E-isomer (voclosporin) binds cyclophilin A with a dissociation constant (Kd) of 15 nM, compared to 61 nM for the Z-isomer, a roughly four-fold difference that underscores the critical importance of geometric isomerism in determining biological potency.[9]

Voclosporin is administered orally as 7.9 mg hard gelatin capsules under the brand name Lupkynis, with the approved dose for lupus nephritis being 23.7 mg (three capsules) twice daily. The drug must be taken on an empty stomach to achieve optimal bioavailability, achieving a median time to peak plasma concentration (Tmax) of 1.5 hours. Its apparent volume of distribution is approximately 2154 L, indicative of extensive tissue distribution. The major metabolic pathway involves cytochrome P450 3A4/5-mediated hydroxylation in the liver, producing the IM9 metabolite, which is approximately eight times less potent than the parent molecule and accounts for approximately 16.7% of total drug-related exposure.[10,5]

2.2 Mechanism of Action

Voclosporin exerts its pharmacological effects through the inhibition of calcineurin, a calcium- and calmodulin-dependent serine-threonine phosphatase. The mechanism begins with the intracellular binding of voclosporin to cyclophilin A (CypA), a cytoplasmic peptidyl-prolyl cis-trans isomerase. This binary complex then engages with and inhibits calcineurin, preventing the dephosphorylation of the transcription factor nuclear factor of activated T cells (NFAT).[9,25,27] Under normal immune activation conditions, calcineurin dephosphorylates NFAT, enabling its translocation from the cytoplasm to the nucleus where it drives the transcription of interleukin-2 (IL-2) and other pro-inflammatory cytokines. By blocking this pathway, voclosporin curtails T cell proliferation and the cytokine-mediated inflammatory cascade characteristic of lupus nephritis.[5,22]

Beyond its immunosuppressive effects, voclosporin demonstrates a direct antiproteinuric action in the kidney. In glomerular podocytes, calcineurin dephosphorylates synaptopodin, a protein critical for the structural integrity of the actin cytoskeleton at the slit diaphragm. Inhibition of calcineurin by voclosporin preserves synaptopodin phosphorylation, stabilizing the podocyte cytoskeleton and thereby reducing proteinuria.[25] This dual mechanism of action—immunosuppressive via T cell inhibition and antiproteinuric via podocyte stabilization—distinguishes voclosporin from conventional immunosuppressants that lack direct glomerular protective activity.[5,10]

An important mechanistic feature of voclosporin relates to its structural interaction with the calcineurin complex. X-ray crystallographic studies by Kuglstatter and colleagues demonstrated that the unique extended side chain of E-MePmt1 in voclosporin interacts with the so-called 'latch region' of calcineurin, formed by both the catalytic and regulatory subunits of the enzyme.[9] This interaction pattern differs subtly from that of cyclosporin A, and it is postulated that the modified binding induces conformational changes in calcineurin that contribute to the enhanced inhibitory potency of voclosporin relative to the parent molecule.[9,26]

2.3 Stereochemistry and Chirality of Voclosporin

Voclosporin contains eleven amino acid residues arranged in a macrocyclic ring, seven of which are N-methylated. These include sarcosine (N-methylglycine) at position 3, N-methyl-leucine at multiple positions, N-methyl-valine, and the critical E-MePmt1 residue at position 1. The molecule possesses multiple stereocentres, and the overall three-dimensional conformation of the macrocycle is dictated by the interplay of cis-trans peptide bond isomerism, intramolecular hydrogen bonding, N-methylation patterns, and the geometric configuration of the side chain double bond.[9,19,26]

In a polar solvents such as chloroform, cyclosporin-class molecules, including voclosporin, adopt a closed conformation characterized by a maximum number of intramolecular hydrogen bonds and near-C2 symmetry. This conformation, which closely resembles the crystal structure, is maintained by three intramolecular hydrogen bonds involving amide NH groups of residues 4, 6, and 8, and the carbonyl oxygens of residues 2, 4, and 6.[19] In polar solvents, the molecule can adopt more open conformations, with water molecules competing for the intramolecular hydrogen bond donors. This solvent-dependent conformational flexibility is of direct pharmaceutical relevance, as the biologically active conformation of voclosporin is the one that is recognized by and binds to cyclophilin A.[19,9]

The geometric isomerism at the C2=C3 bond of the pentadienyl side chain of E-MePmt1 (E-configuration in voclosporin versus Z-configuration in Z-ISA247) is the defining structural feature that distinguishes voclosporin from its less potent stereoisomer. In the crystal structure of the CypA–E-ISA247 complex, the extended E-configuration allows it to make optimal hydrophobic contacts with residues in the cyclophilin A binding cavity, an interaction sterically precluded in the Z-isomer due to the bent geometry of the Z-configured double bond.[9] This exquisite structure-activity relationship highlights the importance of geometric isomers in cyclic peptide pharmacology and underscores the critical role of chiroptical methods in confirming stereochemical identity.[12,8]

3. Circular Dichroism Spectroscopy: Theoretical Foundations

3.1 Historical Context and Physical Basis

The phenomenon of circular dichroism was first systematically described by Aimé Cotton in 1895, who observed that chiral chromophores exhibit differential absorption of left- and right-circularly polarized light in the vicinity of their electronic absorption bands.[24] The associated spectral feature—a dispersive change in sign and magnitude near an optical absorption band—became known as the Cotton effect, which remains a fundamental concept in the interpretation of CD spectra.[24,12] Despite this early discovery, the development of modern electronic CD spectroscopy as a practical analytical tool had to await the advent of appropriate light sources, photoelastic modulators, and sensitive detector systems.[8]

The physical basis of CD rests upon the interaction of circularly polarized light with chiral molecules. Linearly polarized light may be decomposed into two counter-rotating circularly polarized components of equal amplitude—left-circularly polarized light (L-CPL) and right-circularly polarized light (R-CPL). When such light traverses an optically active (chiral) medium, the differential absorption of L-CPL and R-CPL by the chiral chromophore gives rise to the phenomenon of circular dichroism, expressed as:[8,14]

$$\Delta A = AL - AR = (\varepsilon L - \varepsilon R) \times c \times l$$

where AL and AR are the absorbances of L-CPL and R-CPL, respectively; εL and εR are the corresponding molar extinction coefficients; c is the molar concentration; and l is the path length. In practice, CD spectra are presented as plots of molar ellipticity [θ] (degrees cm² dmol⁻¹) versus wavelength, related to ΔA by:[21,14]

$$[\theta] = 3298 \times \Delta\varepsilon$$

where $\Delta\varepsilon = \varepsilon L - \varepsilon R$ is the molar circular dichroism in units of M^{-1} cm^{-1}. For peptide and protein samples, molar ellipticity per residue is often reported to facilitate comparisons between molecules of different chain lengths.[21]

3.2 Electronic Chromophores in CD Spectroscopy

The CD signal arises from electronic transitions in chiral chromophoric groups. For peptides and proteins, the principal chromophores responsible for far-UV CD signals (approximately 180–250 nm) are the amide (peptide) bonds, which absorb through π→π* (around 190 nm) and n→π* (around 220 nm) transitions.[21,14] The coupling of amide chromophores in regular secondary structures gives rise to excitonic CD bands, first theorized by Moffitt in 1956 and elaborated by the exciton coupling model.[30] This coupling is the foundation for interpreting secondary structure from far-UV CD spectra.[21]

The characteristic CD spectral signatures for common secondary structure elements are well established: α-helices exhibit two negative bands at approximately 222 nm (n→π*) and 208 nm (π→π*, perpendicular) and a positive band near 193 nm (π→π*, parallel); β-sheets display a broad negative band near 218 nm and a positive band near 195 nm; random coils show a weak positive band near 217 nm and a large negative band near 195 nm.[21,17] Beta-turns have CD signals in the 190–210 nm region, though the precise pattern is turn-type dependent.[17] For cyclic peptides such as voclosporin, which do not adopt the regular secondary structures of globular proteins, the direct application of standard protein deconvolution algorithms is problematic. The development of specialized CD deconvolution models using reference sets based on cyclic peptide structures of known solution conformation (determined by NMR) is an active area of analytical method development.[1,17] Near-UV CD (approximately 250–350 nm) provides information about the tertiary environment of aromatic amino acid side chains and, for voclosporin specifically, CD signals from the conjugated pentadienyl diene system of the E-MePmt1 side chain, whose sign and magnitude reflect the absolute configuration and conformation of this diene in its macrocyclic context.[23,12]

3.3 The Cotton Effect and Exciton Chirality Method

A Cotton effect is observed as a CD signal in the vicinity of an absorption band, characterized by a positive or negative extremum at the wavelength of maximum absorption.[24] For cyclosporin-class molecules with multiple peptide chromophores, Cotton effects arise from the chiral superposition of these transitions, with sign, amplitude, and position determined by the absolute configuration of the stereocentres, the geometry of the molecular backbone, and coupling interactions between neighbouring chromophores.[24,12]

The exciton chirality method, developed by Harada and Nakanishi, is particularly useful for determining the absolute configuration of molecules bearing two or more interacting chromophores.[24] When two chromophores are in close spatial proximity and oriented in a chiral (non-planar) arrangement, their electronic transitions couple to produce a characteristic bisignate CD spectrum—two bands of opposite sign at slightly different wavelengths. The sign of the first Cotton effect (at longer wavelength) in this bisignate pattern determines the sense of the twist between the two chromophores.[24,12] Application of this method to voclosporin would in principle be informative for the diene chromophore of E-MePmt1 and any interacting amide chromophores, potentially providing a direct chiroptical readout of the geometric configuration of the pentadienyl side chain.[12]

3.4 Instrumentation and Experimental Considerations

A CD spectropolarimeter comprises: (i) a high-intensity xenon arc lamp light source generating continuous UV radiation; (ii) a monochromator for wavelength selection; (iii) a photoelastic modulator (PEM) alternating between L-CPL and R-CPL at approximately 50 kHz; (iv) a sample compartment accommodating cuvettes of varying path lengths; and (v) a phase-locked photomultiplier tube (PMT) detector isolating the CD signal.[14,8] Critical experimental variables for cyclic peptides such as voclosporin include sample concentration (typically micromolar range for far-UV measurements), solvent selection, path length, scan speed, spectral bandwidth, and data accumulation (multiple scans averaged for improved signal-to-noise ratio).[21,14]

For synchrotron radiation circular dichroism (SRCD), the much higher photon flux from synchrotron sources enables measurements at shorter wavelengths (down to approximately 130 nm), providing enhanced information about secondary structure composition.[3] SRCD has been applied to membrane proteins, intrinsically disordered peptides, and structurally unusual macrocycles; its application to voclosporin and related cyclosporin analogues represents a productive avenue for future research.[3]

4. Vibrational Circular Dichroism (VCD): Principles and Application to Cyclosporins

4.1 Principles of VCD

Vibrational circular dichroism (VCD) is the infrared analogue of electronic CD, measuring the differential absorption of left- and right-circularly polarized infrared radiation by chiral vibrational transitions.[15] While electronic CD probes transitions between electronic energy levels and is sensitive to overall secondary structure, VCD probes transitions between vibrational energy levels and is exquisitely sensitive to the local geometry and absolute configuration of individual functional groups, making it uniquely powerful for de novo determination of absolute configuration of chiral molecules, particularly when complemented by density functional theory (DFT) calculations.[15,19]

The theoretical basis of VCD is grounded in the interaction of infrared radiation with oscillating molecular dipoles and magnetic moments associated with molecular vibrations. For a VCD signal to arise, there must be a nonzero rotational strength R, which depends on the scalar product of the electric dipole transition moment (μ) and the magnetic dipole transition moment (m): $R = \mathrm{Im}(\mu \cdot m^*)$. Only chiral molecules can have a nonzero rotational strength for an individual transition, because only in a chiral molecular environment can a vibrational motion simultaneously generate both translating charges (electric dipole) and circulating charges (magnetic dipole) along the same axis.[15]

For cyclosporin-class molecules, the most informative VCD bands are found in the amide I region (approximately 1620–1700 cm^{-1}, arising from C=O stretching of amide bonds), the amide II region (approximately 1510–1560 cm^{-1}, arising from N-H bending and C-N stretching), and the N-

H stretching region (approximately 3300–3450 cm^{-1}). The N-H stretching region is highly informative for distinguishing intramolecularly hydrogen-bonded NH groups (shifted to lower wavenumbers) from free NH groups. Collectively, these vibrational signatures report on the backbone geometry, hydrogen bonding pattern, and the specific arrangement of amide bonds in the macrocyclic ring.[19,15]

4.2 VCD Studies of Cyclosporin Analogues

The application of VCD spectroscopy to cyclosporin A and its analogues has yielded detailed insights into solution conformation. VCD studies using $CDCl_3$ as solvent have demonstrated that cyclosporin A in this non-polar medium adopts the same conformation as seen in its crystal structure, a finding supported by good agreement between experimental VCD spectra and DFT-calculated VCD spectra using the BPW91 functional with the 6-31G* basis set.[19] The VCD spectra in the amide I region exhibit a characteristic bisignate pattern that is sensitive to the specific hydrogen bonding topology of the crystal conformation, distinguishing it from more open, solution-state conformers.[19]

VCD studies have also examined the conformational heterogeneity of cyclosporin analogues in polar solvents, where disruption of intramolecular hydrogen bonds promotes more open, flexible states. The formation of metal ion complexes with cyclosporin A (e.g., with Mg^{2+} or Ca^{2+}) has been shown to alter the VCD spectrum in characteristic ways, reflecting changes in peptide backbone geometry induced by metal coordination to carbonyl oxygens of the macrocycle.[19] These metal-induced conformational transitions are of biological relevance, as the interaction of CsA with calcium and magnesium is implicated in altered mineral homeostasis associated with cyclosporin toxicity.[19,10]

For voclosporin specifically, the introduction of the E-MePmt1 residue with its extended pentadienyl diene introduces an additional chromophore with characteristic vibrational signatures (C=C stretching modes near 1620 cm^{-1} may overlap with amide I modes). DFT-based prediction and experimental measurement of VCD for the E-MePmt1 residue within the full macrocycle would provide critical information about the geometry of this stereochemically critical side chain. The distinction between the E and Z isomers of ISA247 by VCD, alongside electronic CD, represents a powerful analytical strategy for the stereochemical quality control of voclosporin.[15,9]

5. Circular Dichroism in the Structural Analysis of Voclosporin

5.1 CD Spectral Characteristics of Cyclic Undecapeptides

Cyclic undecapeptides of the cyclosporin class present a unique challenge for CD spectroscopic analysis. Unlike linear peptides or globular proteins, which adopt relatively well-defined secondary structures, cyclic undecapeptides are constrained by the macrocyclic ring topology and display a mixture of structural elements that do not map cleanly onto standard protein-based CD reference libraries.[16,1] The backbone of cyclosporin A, and by close analogy voclosporin, includes segments with β-turn-like geometry, portions of extended strand, and regions of bend. The N-methylated amide bonds, which lack the NH group, cannot serve as hydrogen bond donors and introduce additional conformational constraints; in addition, the absence of the NH signal removes their contribution from the amide I CD band, simplifying spectral interpretation.[19,9]

The far-UV CD spectrum of cyclosporin A in aqueous solution typically displays negative Cotton effects in the 220–230 nm region and positive signals near 195–200 nm, but the precise spectral shape is highly solvent- and concentration-dependent. The ratio of hydrogen-bonded to free amide NH groups in the macrocycle determines the relative contribution of closed (compact) versus open (expanded) conformers to the observed spectrum.[19] In a polar organic solvents (e.g., $CDCl_3$), the cyclosporin molecules exist predominantly in the compact crystal conformation; in polar solvents (e.g., water), an equilibrium mixture of conformers is observed, with open forms progressively more populated as solvent polarity increases.[19,16]

For voclosporin, the E-MePmt1 side chain introduces a UV-absorbing diene chromophore that may contribute to the near-UV and far-UV CD signals. The conjugated (E,E)-pentadienyl system absorbs in the 220–260 nm region, overlapping with the amide transitions of the peptide backbone. The sign and magnitude of the CD bands contributed by this diene will reflect its absolute configuration and the dihedral angle between it and the adjacent carbonyl. Careful spectroscopic deconvolution, potentially aided by comparison with TD-DFT computations of the ECD spectrum, is required to disentangle the diene and amide contributions to the overall observed CD signal.[12,9,15]

5.2 Conformational Equilibria and Solvent Dependence

One of the most important features of cyclosporin-class molecules is their pronounced conformational variability as a function of solvent polarity. In the crystalline state and in non-polar solvents, CsA and voclosporin adopt the compact crystal conformation, stabilized by three intramolecular hydrogen bonds and characterized by a trans configuration at all peptide bonds except the Ser-Pro bond.[19,9] In water and other polar solvents, this compact structure partially unfolds, and populations of alternative conformers with different hydrogen bonding patterns appear.[19]

CD spectroscopy is uniquely well suited to monitor these conformational equilibria in real time, as a function of temperature, pH, ionic strength, or solvent composition.[14,21] Thermal denaturation experiments—in which the CD signal at a given wavelength is recorded as a function of temperature—can provide thermodynamic parameters (melting temperature Tm, enthalpy of unfolding ΔH) for conformational transitions of voclosporin. These data are directly relevant to formulation development and stability assessment.[21,14]

A particularly informative application of CD is the comparison of the conformation of voclosporin in free solution with that in the cyclophilin A-bound state. When voclosporin binds to CypA, it adopts the all-trans peptide bond conformation required for calcineurin engagement, which is distinct from the ground-state solution conformation.[9,27] CD measurements of the free drug versus the drug-CypA complex reveal characteristic changes in the far-UV spectral pattern that reflect this conformational rearrangement. Induced circular dichroism (ICD) signals may also arise when voclosporin binds to cyclophilin A, as the chiral protein environment may distort and amplify the CD signals of the bound peptide ligand.[11]

5.3 The Voclosporin–Cyclophilin A Interaction: Structural Insights from X-ray Crystallography and Implications for CD

The crystal structure of the E-ISA247 (voclosporin)–cyclophilin A complex, determined at 2.2 Å resolution by Kuglstatter and colleagues in 2011 (PDB code 3ODI), provides the most detailed atomic-level picture of the bound conformation of voclosporin. In this structure, voclosporin adopts a specific macrocyclic conformation with all peptide bonds in the trans configuration, forming several intermolecular hydrogen bonds with key residues of the CypA binding site.[9] The extended E-MePmt1 side chain makes hydrophobic van der Waals contacts with the side chains of Phe60, Met61, Arg55, Lys82, and Ala101 of CypA—interactions sterically inaccessible to the Z-isomer due to the kinked geometry of Z-MePmt1.[9]

The higher binding affinity of voclosporin for CypA (Kd = 15 nM) relative to the Z-isomer (Kd = 61 nM) can be rationalized on the basis of superior van der Waals complementarity between the E-configured pentadienyl side chain and the hydrophobic binding pocket of CypA. Comparison with the known CsA–CypA crystal structure reveals that the butenyl side chain of CsA occupies a smaller portion of this hydrophobic cavity than does the extended pentadienyl chain of voclosporin, consistent with the higher potency of the latter.[9,26,27]

These structural findings carry important implications for the interpretation of CD spectra of the free and CypA-bound forms of voclosporin. The conformational transition from the solution-state equilibrium mixture of conformers to the all-trans, CypA-bound conformation should produce characteristic changes in the amide CD bands, reflecting the reorganization of the backbone hydrogen bonding pattern.[19,9] Stopped-flow CD experiments, in which the binding reaction between voclosporin and CypA is rapidly initiated, could in principle provide kinetic information about the rates of conformational rearrangement associated with this binding event, complementing ITC and SPR binding studies.[14,11]

5.4 Differentiation of E- and Z-Isomers of ISA247 by CD

The E- and Z-isomers of ISA247 differ in the geometric configuration of the C2=C3 double bond in the pentadienyl side chain of residue 1. This geometric difference is expected to produce distinguishable CD spectra for several reasons: (i) the dihedral angles between the diene chromophore and the adjacent chiral carbon differ in the two isomers, leading to different exciton coupling patterns; (ii) the extended versus bent shape of the E and Z side chains may impose different constraints on the overall macrocyclic conformation, altering amide CD bands; and (iii) the two isomers may adopt different populations of backbone conformers in solution.[9,12]

Electronic CD spectra of E-ISA247 and Z-ISA247 would be expected to show differences in both the near-UV region (from the diene chromophore) and the far-UV region (from the perturbed backbone amide geometry). The sign of the Cotton effect associated with the diene transition may differ between the two isomers, providing a direct chiroptical signature of geometric isomeric identity.[12,8] This is analytically important because the USFDA and ICH guidelines require rigorous demonstration of the stereochemical purity of chiral drug substances; the ability to discriminate E- and Z-isomers of ISA247 by CD provides a rapid, non-destructive, and orthogonal analytical tool to complement chiral HPLC and NMR methods.[8]

The combination of electronic CD and VCD spectroscopy, in conjunction with TD-DFT calculations of predicted ECD spectra for both isomers, provides a comprehensive chiroptical fingerprinting approach for voclosporin stereochemical quality control. Such an approach does not require the resolution of isomers by chiral chromatography and can be performed on small quantities of material in a rapid, non-destructive manner.[15,12,8]

6. Circular Dichroism in Pharmaceutical Quality Control of Voclosporin

6.1 ICH Guidelines and Regulatory Framework for Chiral Drugs

The regulatory landscape for the development and quality control of chiral drug substances is governed by the ICH guideline Q6A, which requires that the enantiomeric purity and stereochemical integrity of chiral active pharmaceutical ingredients (APIs) be documented and controlled. For voclosporin, as a molecule containing multiple stereocentres and existing as a single enantiomeric and geometric isomeric form (E-isomer), the most critical stereochemical quality attribute is the absence of the corresponding Z-isomer and the maintenance of all L-amino acid configurations throughout synthesis and purification.[8,4]

The use of CD spectroscopy in the quality control of chiral drugs has been growing steadily, driven by its speed, non-destructive nature, high sensitivity to stereochemical information, and compatibility with both API batches and finished dosage forms.[8] Regulatory acceptance of CD-based methods for chiral drug characterization is increasing, and several compendial pharmacopoeias have begun to include CD as an accepted technique for structural and conformational characterization of biopharmaceuticals and complex molecules.[8] From a validation standpoint, a CD-based method for the determination of enantiomeric purity of voclosporin would need to meet standard pharmaceutical analytical method validation criteria as outlined in ICH Q2(R1): specificity, linearity, accuracy, precision, range, and limits of detection/quantification.[8]

6.2 CD for Stereochemical Purity Assessment

The theoretical basis for using CD in enantiomeric purity assessment rests on the linear relationship between CD signal amplitude and the enantiomeric excess (ee) of a sample: for a pure single enantiomer, the CD signal is maximal; for a racemic mixture, the CD signals from the two enantiomers cancel and the net CD signal is zero.[8,12] Plotting the differential molar extinction coefficient ($\Delta\varepsilon$) at the wavelength of maximum CD signal versus enantiomeric purity yields a linear standard curve that can be used for quantitative purity assessment. For drugs with multiple stereocentres, such as voclosporin, the relationship between stereochemical composition and CD signal depends on the specific nature of the diastereomeric impurities present.[8,12]

The electronic CD spectra of voclosporin and its geometric isomer Z-ISA247 are not mirror images of each other (as would be expected for a pair of true enantiomers), because E- and Z-ISA247 are geometric isomers (diastereomers with respect to double bond geometry), not enantiomers.[9,12] They will therefore produce non-mirror-image CD spectra, potentially with different signs and intensities for corresponding Cotton effects. This spectral non-equivalence is advantageous for analytical purposes: even small amounts of one isomer in the presence of the other can be detected as deviations from the expected spectrum of the pure isomer, without requiring complete spectral resolution of the two components.[8]

6.3 CD for Conformational Stability and Formulation Studies

The conformational stability of voclosporin in different formulation environments—aqueous buffers, organic co-solvents, cyclodextrin complexes, lipid-based formulations—is directly relevant to its bioavailability and therapeutic consistency. CD spectroscopy provides a rapid means of assessing whether a given formulation condition perturbs the preferred macrocyclic conformation, which could affect the rate and extent of absorption as well as bioequivalence of different formulations.[14,8]

Thermal stability studies using CD can identify the temperature at which voclosporin undergoes significant conformational changes. Such experiments involve recording CD spectra as the temperature is incrementally raised, typically from 5°C to 80–90°C. A sigmoidal decrease in signal intensity as a function of temperature indicates a cooperative conformational unfolding transition.[21,14] Aggregation is another concern in the long-term storage and stability of peptide therapeutics. The formation of aggregates is often accompanied by conformational changes detectable by CD, particularly a reduction in far-UV CD signal intensity and a shift in spectral shape consistent with increased beta-sheet or random coil content. Routine CD monitoring of voclosporin solutions during accelerated stability studies could serve as an early warning indicator of aggregation-related stability failure, complementing dynamic light scattering and size-exclusion HPLC methods.[8,14]

6.4 Comparison with Other Analytical Methods

While CD spectroscopy is a powerful technique for the stereochemical and conformational characterization of voclosporin, it is most informative when used in conjunction with complementary analytical methods. Nuclear magnetic resonance (NMR) spectroscopy, particularly two-dimensional methods (COSY, NOESY, ROESY, HSQC), provides atomic-resolution information about the solution conformation of voclosporin and can unambiguously assign all proton and carbon environments.[19,9] The coupling constants (J values) of peptide NH-αCH pairs are directly related to the φ backbone dihedral angles, providing quantitative conformational information. CD spectroscopy is faster, requires less material, and is better suited to rapid screening and quality monitoring.[19]

Mass spectrometry (MS), particularly ion mobility MS and hydrogen-deuterium exchange MS (HDX-MS), provides information about the shape and conformational dynamics of voclosporin molecules. The integration of CD spectroscopy with HDX-MS has been described by Adpressa and colleagues as a powerful combined workflow for global peptide conformation analysis in solution.[1] The integration of CD spectroscopy with these complementary techniques in a multi-orthogonal characterization strategy represents the state of the art in the structural analysis of complex cyclic peptide APIs.[1,12]

7. Clinical Development and Therapeutic Context

7.1 Phase 2 Trial: AURA-LV

The clinical development of voclosporin proceeded through a well-designed series of trials. The AURA-LV (Aurinia Urinary Protein Reduction Active Lupus with Voclosporin) trial was a Phase 2, multicentre, randomised, double-blind, placebo-controlled trial that evaluated two doses of voclosporin (23.7 mg and 39.5 mg, each twice daily) versus placebo, in combination with mycophenolate mofetil (2 g/day) and rapidly tapered low-dose oral corticosteroids. The primary endpoint was complete renal response (CRR) at 24 weeks. Results showed that the low-dose voclosporin group (23.7 mg BID) achieved statistically superior CRR rates compared to placebo at 24 weeks, and this superiority persisted at 48 weeks.[13] A higher rate of serious adverse events and more deaths were observed in the low-dose voclosporin group compared to placebo, which necessitated careful surveillance in subsequent trials.[13]

7.2 Phase 3 Trial: AURORA 1

The pivotal Phase 3 AURORA 1 (Aurinia Renal Response in Active Lupus with Voclosporin) trial was a multicentre, double-blind, randomised, placebo-controlled study conducted across 142 hospitals and clinics in 27 countries.[4] Eligible patients had biopsy-proven lupus nephritis of class III, IV, or V and active renal disease. Participants were randomised 1:1 to receive oral voclosporin (23.7 mg twice daily) or placebo, on a background of mycophenolate mofetil (1 g twice daily) and rapidly tapered low-dose steroids, for 52 weeks. The primary endpoint was complete renal response at 52 weeks, defined as a urine protein-to-creatinine ratio (UPCR) of ≤0.5 mg/mg, stable renal function, no use of rescue medication, and no more than 10 mg/day prednisolone equivalent.[4]

The results of AURORA 1 demonstrated that voclosporin combined with mycophenolate mofetil and low-dose steroids achieved a significantly superior complete renal response rate compared to mycophenolate mofetil and low-dose steroids alone (40.8% versus 22.5%, $p<0.001$).[4] Importantly, the improved efficacy was achieved with a substantially lower cumulative steroid dose than used in any previous lupus nephritis trial. Secondary endpoints, including UPCR ≤0.5 mg/mg at 24 weeks, were also met. The safety profile of voclosporin was comparable to that of the placebo group, with no increase in serious adverse events or deaths.[4,5]

The successful results of AURORA 1 formed the basis for the January 2021 USFDA approval of voclosporin (Lupkynis) at a dose of 23.7 mg twice daily for the treatment of adults with active lupus nephritis, in combination with background immunosuppressive therapy.[4,6,7] The European Medicines Agency (EMA) subsequently approved voclosporin in September 2022 under the brand name Lupkynis.[5]

7.3 Long-term Data: AURORA 2

The AURORA 2 extension study provided follow-up data for patients who completed AURORA 1 and continued voclosporin treatment for up to three years. Long-term data demonstrated progressive improvement in proteinuria over the extended follow-up period, with CRR rates of 50.9% in the voclosporin group versus 39% in the placebo group at three years.[5] The safety profile remained consistent with that observed in AURORA 1, supporting the long-term tolerability of voclosporin. Continued monitoring of renal function (eGFR) and management of dose-adjustments in patients with declining renal function remained key clinical management considerations throughout the extension study.[5,22]

8. Induced Circular Dichroism and Drug-Protein Interactions

8.1 Induced CD in Drug Binding Studies

Induced circular dichroism (ICD) refers to the CD signals that arise when an achiral or weakly CD-active molecule binds to a chiral macromolecule (or vice versa). The binding interaction places the molecule in a chiral environment, breaking the local symmetry and generating CD signals not present in either component in isolation. ICD spectroscopy has been widely used to characterize drug-protein interactions, particularly drug binding to serum albumin, where binding-induced CD signals provide information about the orientation, conformation, and binding mode of the ligand within the protein binding site.[11]

For voclosporin, ICD principles are relevant when considering the binding to cyclophilin A. The CD spectrum of the voclosporin–CypA complex will differ from the sum of the individual CD spectra of free voclosporin and free CypA, due to: (i) conformational changes in voclosporin upon binding; (ii) conformational changes in the CypA protein induced by voclosporin binding; and (iii) through-space coupling between the chromophores of voclosporin and the aromatic residues in the CypA binding pocket (Phe60, Trp121, His126). These effects collectively produce an ICD signal that is a sensitive reporter of the bound state of the complex.[11,9]

The use of ICD to monitor the binding kinetics of voclosporin to CypA in real time, particularly using stopped-flow CD techniques, represents a powerful strategy for characterizing the conformational pathway of drug-protein association. Such experiments can reveal whether voclosporin binds to CypA through an induced-fit mechanism or through a conformational selection mechanism, with important implications for understanding the thermodynamics and kinetics of calcineurin inhibition.[11,14]

8.2 Binding Studies with Human Serum Albumin

Human serum albumin (HSA) is the most abundant plasma protein and a major determinant of the pharmacokinetic behaviour of many drugs, including cyclic peptides. Voclosporin, with its predominantly hydrophobic character, would be expected to bind to HSA at the Sudlow site I or site II, the primary drug binding sites of this protein.[11] Characterization of the voclosporin–HSA interaction by ICD would provide information about the binding affinity (Ka), stoichiometry, and the mode of binding (orientation and conformation of voclosporin in the binding site). Competitive displacement experiments using established site-specific probes (e.g., warfarin for site I, ibuprofen for site II) in combination with ICD can localize the binding site.[11]

9. Advanced Chiroptical Spectroscopic Methods and Future Directions

9.1 Electronic CD with TD-DFT Calculations

A powerful approach for the interpretation of CD spectra of structurally complex molecules such as voclosporin involves the combination of experimental ECD spectroscopy with theoretical TD-DFT calculations of the expected ECD spectrum. In this approach, the three-dimensional structure of voclosporin (derived from X-ray crystallography, solution NMR, or computational conformational search) is used as input for quantum chemical calculations of the ECD spectrum using time-dependent density functional theory.[12,15] Hybrid functionals such as B3LYP and CAM-B3LYP with polarized triple-zeta basis sets have been widely used for peptide ECD calculations with good results. More recently, the use of solvent continuum models (PCM, SMD) to account for solvent effects on calculated ECD spectra has improved agreement with experimental data.[12]

The availability of crystal structure coordinates for the CypA–voclosporin complex (PDB codes 3ODI for E-ISA247 and 3ODL for Z-ISA247) provides a definitive starting structure for TD-DFT calculations.[9] Calculating and comparing the ECD spectra of the free (solution-state conformation) and bound (crystal structure conformation) forms of voclosporin provides a theoretical prediction of the CD spectral changes expected upon binding to cyclophilin A, which can be validated experimentally. This approach also allows the a priori prediction of the ECD spectra of voclosporin analogues in the absence of experimental crystal structure data, supporting structure-based drug design.[12,9]

9.2 Synchrotron Radiation Circular Dichroism (SRCD)

Synchrotron radiation circular dichroism (SRCD) extends the accessible wavelength range of CD measurements to below 190 nm, into the vacuum UV region (down to approximately 130–170 nm on state-of-the-art beamlines). This extended range provides additional spectral information particularly valuable for secondary structure deconvolution and for studying molecules with electronic transitions at short wavelengths.[3] SRCD has been applied to membrane proteins, intrinsically disordered proteins, and structurally unusual peptides. The coupling of SRCD with high-throughput automated sample handling systems has enabled large-scale conformational screening of peptide libraries, a capability that could be applied to systematic studies of voclosporin analogues and structure-conformation-activity relationships in the cyclosporin series.[3]

9.3 Raman Optical Activity (ROA)

Raman optical activity (ROA) measures the small differences in the intensity of Raman-scattered left- and right-circularly polarized light by chiral molecules. Like VCD, ROA provides vibrational signatures that are exquisitely sensitive to molecular chirality and three-dimensional structure, but through the Raman rather than infrared absorption mechanism. ROA is particularly powerful for studying aqueous solutions of biomolecules, as water (being non-chiral) contributes no ROA background signal, in contrast to its strong IR absorption bands that can interfere with VCD measurements.[15]

The application of ROA to cyclic peptide drugs such as voclosporin would provide complementary vibrational conformational information to VCD. ROA spectra of cyclosporin A and its analogues would reveal characteristic backbone fingerprint bands (peptide NH stretching modes, Cα-H stretching modes, amide I, II, and III vibrations) that are sensitive to the dihedral angles of the macrocyclic backbone. Combined ROA/VCD studies, correlated with NMR NOE constraints and X-ray structural data, represent the most comprehensive experimental approach to the solution conformational characterization of macrocyclic peptide drugs.[15,19]

9.4 CD in Formulation Screening and Drug-Excipient Interactions

The development of suitable oral formulations for cyclic peptide drugs—which commonly suffer from poor aqueous solubility and variable bioavailability—is a major challenge in pharmaceutical development. Voclosporin is formulated as hard gelatin capsules and its bioavailability is sensitive to fed/fasted state, as it must be administered on an empty stomach for optimal absorption.[10,5] CD spectroscopy can rapidly assess whether the addition of cyclodextrins, surfactants, co-solvents, or polymeric excipients to voclosporin solutions perturbs the drug's preferred conformation, as well as whether these excipients interact directly with the drug molecule (detectable as ICD signals or as spectral shifts). Such formulation screening studies identify excipients that stabilize the preferred pharmacological conformation of voclosporin versus those that promote undesired conformational transitions or aggregation.[8,11]

10. Comparative CD Analysis: Voclosporin versus Cyclosporin A and Tacrolimus

A comparative CD analysis of voclosporin, cyclosporin A, and tacrolimus—the three major calcineurin inhibitors in clinical use—illuminates the structure-activity relationships within this therapeutic class and demonstrates the utility of CD as a tool for differentiating structurally related immunosuppressants. Cyclosporin A and voclosporin share a cyclic undecapeptide backbone and, by extension, similar overall CD spectral shapes in the far-UV region dominated by amide transitions.[5,19] The primary spectroscopic difference between CsA and voclosporin is expected in the region of the pentadienyl diene absorption (approximately 220–260 nm), where voclosporin exhibits additional CD signals attributable to the extended conjugated chromophore of E-MePmt1.[9,12]

Tacrolimus (FK506), by contrast, is a macrocyclic lactone structurally unrelated to the cyclosporin scaffold. It acts through binding to FK-binding protein 12 (FKBP12) rather than cyclophilin A, and the resulting FKBP12-tacrolimus complex inhibits calcineurin by an analogous but distinct mechanism.[5,22] The far-UV CD spectrum of tacrolimus would be expected to differ substantially from that of voclosporin, as it lacks the amide backbone generating characteristic peptide CD signals. Instead, tacrolimus displays UV absorbance from its conjugated triene and carbonyl chromophores, which in a chiral macrolide environment give rise to ECD signals in the near-UV and visible regions. These fundamental spectroscopic differences make CD a robust method for distinguishing voclosporin from tacrolimus even in complex mixtures.[12,8]

The improved metabolic stability of voclosporin relative to cyclosporin A is reflected in the reduced formation of nephrotoxic metabolites. Cyclosporin A undergoes extensive metabolism to yield multiple metabolites, including the nephrotoxic AM1 and AM19 metabolites, whose concentrations in transplant patients can approach or exceed those of the parent drug. Voclosporin's primary metabolite (IM9), produced by hydroxylation at amino acid position 9, is approximately eight times less potent than voclosporin and accounts for a smaller fraction of total drug-related exposure.[10,5] The CD spectra of voclosporin and its IM9 metabolite would differ due to the introduction of a hydroxyl group at position 9, which alters the local electronic environment and potentially the overall macrocyclic conformation. CD monitoring of metabolism in microsomal incubations could thus provide a rapid assessment of voclosporin metabolic stability.[12,8]

11. Challenges, Limitations, and Considerations in CD Analysis of Voclosporin

11.1 Spectral Complexity of Cyclic Undecapeptides

The interpretation of CD spectra of cyclic undecapeptides is inherently more complex than that of conventional proteins or simple linear peptides. The macrocyclic constraint imposes unique backbone geometries not captured by standard protein secondary structure reference databases, making direct application of classical CD deconvolution algorithms unreliable.[1,17] Second, the presence of N-methylated amide bonds, which are achiral chromophores in isolation but become chiral in the context of the macrocyclic ring, complicates the assignment of individual spectral contributions. Third, the conformational heterogeneity of cyclic peptides in solution, with multiple interconverting conformers of similar energy, means that the observed CD spectrum is a population-weighted average of all conformers.[16,19]

Addressing these challenges requires the development of cyclic peptide-specific CD reference databases and deconvolution algorithms, as well as the combined use of CD with solution NMR, X-ray crystallography, and computational molecular dynamics simulations to constrain the conformational space.[1,16] Progress in this area has been reported: Adpressa and colleagues have developed a combined workflow integrating size exclusion chromatography with hydrogen-deuterium exchange mass spectrometry and CD spectroscopy, which provides complementary information about the overall conformation and hydrogen bonding dynamics of cyclic peptides.[1]

11.2 Sensitivity and Detection Limits

While CD spectroscopy is a sensitive technique, its detection limits for cyclic peptide impurities (such as the Z-isomer of ISA247) are generally inferior to those of HPLC combined with UV or mass spectrometric detection. The minimum detectable level of the Z-isomer in a voclosporin sample by CD will depend on the magnitude of the spectroscopic difference between the two isomers and on the achievable signal-to-noise ratio.[8,12] In favourable cases, where the E- and Z-isomers exhibit substantially different ECD spectra, sub-1% levels of the minor isomer may be detectable. However, in cases where the spectra are very similar, HPLC-CD (the coupling of HPLC separation with online CD detection) may be necessary to achieve adequate analytical sensitivity and selectivity.[8]

11.3 Solvent and Environmental Interference

CD measurements are susceptible to artefacts arising from several sources: optical activity of the solvent itself (a concern if impure or chiral solvents are used), birefringence from strain in optical components, linear dichroism (LD) artefacts arising from oriented or aggregate samples, and fluorescence emission from aromatic impurities.[14,8] For voclosporin, which is only sparingly soluble in water, adequate aqueous concentrations for CD measurement may require the use of organic co-solvents (methanol, DMSO) that may shift the conformational equilibrium. The careful selection of solvent conditions that maintain voclosporin in solution while preserving its biologically relevant conformation is therefore an important experimental design consideration.[19,14]

CONCLUSION

Voclosporin is a chemically sophisticated cyclic undecapeptide immunosuppressant whose remarkable therapeutic properties—enhanced calcineurin inhibitory potency, improved metabolic stability, predictable pharmacokinetics, and dual immunosuppressive/antiproteinuric mechanism in lupus nephritis—are intimately tied to its three-dimensional conformational architecture and precise stereochemical identity.[4,5,9,10] Circular dichroism spectroscopy, encompassing electronic CD, vibrational CD, and related chiroptical methods, occupies a central position in the analytical characterization of this molecule throughout its lifecycle: from early-stage structural elucidation and conformational analysis, through pharmaceutical quality control and formulation development, to the investigation of drug-protein interaction mechanisms.[8,12,19]

The combination of electronic CD with TD-DFT calculations provides a powerful approach for the absolute configuration assignment and conformational characterization of voclosporin and for distinguishing it from its geometric isomer Z-ISA247.[12,9] Vibrational CD, with its sensitivity to local geometry and hydrogen bonding, complements electronic CD by providing detailed information about the amide backbone conformation and the geometry of the critical E-

MePmt1 side chain.[15,19] Induced CD in the voclosporin–cyclophilin A and voclosporin–HSA complexes offers mechanistic insights into the molecular basis of calcineurin inhibition and plasma protein binding.[11]

Future developments in the application of chiroptical spectroscopy to voclosporin should focus on: the development and validation of CD-based pharmaceutical quality control methods; the application of SRCD to achieve extended wavelength range measurements; the integration of ROA spectroscopy for complementary vibrational conformational information; and the use of time-resolved and stopped-flow CD to investigate the kinetics of conformational change associated with cyclophilin A binding.[3,15,11] As the clinical use of voclosporin expands—potentially to other autoimmune indications beyond lupus nephritis—the demand for comprehensive analytical characterization tools will grow, and CD spectroscopy is ideally positioned to meet this need.[5,22]

The broader relevance of voclosporin as a pharmaceutical subject for CD spectroscopy extends to the general problem of characterizing macrocyclic peptide drugs, a rapidly growing class of therapeutic agents that includes natural product-derived cyclopeptides, stapled peptides, and designed macrocycles. The lessons learned from the conformational analysis of voclosporin—regarding the challenges of spectral interpretation, the importance of solvent-dependent conformational equilibria, and the value of complementary analytical methods—are applicable across the wider field of macrocyclic drug development and analysis.[16,1,12]

REFERENCES

1. Adpressa D, Janecki E, Bhowmick S, et al. Combined workflow integrating size exclusion chromatography with hydrogen-deuterium exchange mass spectrometry and circular dichroism spectroscopy for global peptide conformation analysis. J Pharm Biomed Anal. 2024;251:116420. doi:10.1016/j.jpba.2024.116420.
2. Barman K, Islam M, Das K, et al. Recent advances in enantiorecognition and enantioseparation techniques of chiral molecules in the pharmaceutical field. Biomed Chromatogr. 2025;39:e6073. doi:10.1002/bmc.6073.
3. Miles AJ, Janes RW, Wallace BA. Tools and methods for circular dichroism spectroscopy of proteins: a tutorial review. Chem Soc Rev. 2021;50(15):8400–8413. doi:10.1039/D0CS01350A.
4. Rovin BH, Teng YKO, Ginzler EM, et al. Efficacy and safety of voclosporin versus placebo for lupus nephritis (AURORA 1): a double-blind, randomised, multicentre, placebo-controlled, phase 3 trial. Lancet. 2021;397(10289):2070–2080. doi:10.1016/S0140-6736(21)00578-X.
5. van Gelder T, Kers J, Hesselink DA. Voclosporin: a novel calcineurin inhibitor for the treatment of lupus nephritis. Expert Rev Clin Pharmacol. 2022;15(5):565–578. doi:10.1080/17512433.2022.2092470.
6. Clarke J. Voclosporin improves outcomes in lupus nephritis. Nat Rev Rheumatol. 2021;17(7):378. doi:10.1038/s41584-021-00638-7.
7. Moroni G, Ponticelli C. AURORA 1 reports efficacy of voclosporin in lupus nephritis. Nat Rev Nephrol. 2021;17:637–638. doi:10.1038/s41581-021-00460-0.
8. Yao H, Wynendaele E, Xu X, Kosgei A, De Spiegeleer B. Circular dichroism in functional quality evaluation of medicines. J Pharm Biomed Anal. 2018;147:50–64. doi:10.1016/j.jpba.2017.08.031.

9. Kuglstatter A, Mueller F, Kusznir E, et al. Structural basis for the cyclophilin A binding affinity and immunosuppressive potency of E-ISA247 (voclosporin). Acta Crystallogr D Biol Crystallogr. 2011;67(Pt 2):119–123. doi:10.1107/S0907444910051905.
10. Shaikh J, Mahadik T, Patel M, et al. Voclosporin: unique chemistry, pharmacology and toxicity profile, and possible options for implementation into the management of lupus nephritis. Cells. 2023;12(20):2440. doi:10.3390/cells12202440.
11. Ascoli GA, Domenici E, Bertucci C. Induced circular dichroism as a tool to investigate the binding of drugs to carrier proteins: classic approaches and new trends. J Pharm Biomed Anal. 2015;113:172–188. doi:10.1016/j.jpba.2015.03.010.
12. Bertucci C, Pistolozzi M, De Simone A. Circular dichroism in drug discovery and development: an abridged review. Anal Bioanal Chem. 2010;398(1):155–166. doi:10.1007/s00216-010-3959-2.
13. Rovin BH, Solomons N, Pendergraft WF 3rd, et al. A randomized, controlled double-blind study comparing the efficacy and safety of dose-ranging voclosporin with placebo in achieving remission in patients with active lupus nephritis. Kidney Int. 2019;95(1):219–231. doi:10.1016/j.kint.2018.08.025.
14. Kelly SM, Jess TJ, Price NC. How to study proteins by circular dichroism. Biochim Biophys Acta. 2005;1751(2):119–139. doi:10.1016/j.bbapap.2005.06.005.
15. Nafie LA, Freedman TB. Vibrational optical activity theory. In: Berova N, Nakanishi K, Woody RW, editors. Circular Dichroism: Principles and Applications. 2nd ed. Wiley-VCH; 2000. p. 97–131.
16. Ahlbach CL, Lexa KW, Bockus AT, et al. Beyond cyclosporine A: conformation-dependent passive membrane permeabilities of cyclic peptide natural products. Future Med Chem. 2015;7(16):2121–2130. doi:10.4155/fmc.15.78.
17. Sreerama N, Woody RW. Estimation of protein secondary structure from circular dichroism spectra: comparison of CONTIN, SELCON, and CDSSTR methods with an expanded reference set. Anal Biochem. 2000;287(2):252–260. doi:10.1006/abic.2000.4880.
18. Kolic J, Beet L, Overby P, et al. Differential effects of voclosporin and tacrolimus on insulin secretion from human islets. Endocrinology. 2020;161(11):bqaa162. doi:10.1210/endocr/bqaa162.
19. Urbanova M, Setnicka V, Devlin FJ, Stephens PJ. Solution conformations of cyclosporins and magnesium-cyclosporin complexes determined by vibrational circular dichroism. J Am Chem Soc. 2004;126(6):1882–1893. doi:10.1021/ja037466d.
20. Busque S, Cantarovich M, Mulgaonkar S, et al. The PROMISE study: a phase 2b multicenter study of voclosporin (ISA247) versus tacrolimus in de novo kidney transplantation. Am J Transplant. 2011;11(12):2675–2684. doi:10.1111/j.1600-6143.2011.03763.x.
21. Greenfield NJ. Using circular dichroism spectra to estimate protein secondary structure. Nat Protoc. 2006;1(6):2876–2890. doi:10.1038/nprot.2006.202.
22. Ponticelli C, Podesta MA. Calcineurin inhibitors in lupus nephritis. J Nephrol. 2021;34:399–402. doi:10.1007/s40620-020-00907-0.
23. Woody RW. Contributions of tryptophan side chains to the far-ultraviolet circular dichroism of proteins. Eur Biophys J. 1994;23(4):253–262. doi:10.1007/BF01185703.

24. Harada N, Nakanishi K. Circular Dichroic Spectroscopy: Exciton Coupling in Organic Stereochemistry. University Science Books; 1983.
25. Faul C, Donnelly M, Merscher-Gomez S, et al. The actin cytoskeleton of kidney podocytes is a direct target of the antiproteinuric effect of cyclosporine A. Nat Med. 2008;14(9):931–938. doi:10.1038/nm.1821.
26. Mikol V, Papageorgiou C, Borer X. The role of water molecules in the structure of cyclosporin A and derivatives bound to cyclophilin A. J Med Chem. 1994;37(22):3707–3714. doi:10.1021/jm00047a025.
27. Ke H, Mayrose D, Cao W. Crystal structure of cyclophilin A complexed with substrate Ala-Pro suggests a solvent-assisted mechanism of cis-trans isomerization. Proc Natl Acad Sci USA. 1993;90(8):3324–3328. doi:10.1073/pnas.90.8.3324.
28. Galat A. Structures of complexes between cyclophilins, macrolide immunosuppressants, and calcineurin. Curr Opin Biotechnol. 1993;4(6):666–673. doi:10.1016/0958-1669(93)90046-T.
29. Pflugl G, Kallen J, Schirmer T, et al. X-ray structure of a decameric cyclophilin–cyclosporin crystal complex. Nature. 1993;361(6407):91–94. doi:10.1038/361091a0.
30. Moffitt W. Optical rotatory dispersion of helical polymers. J Chem Phys. 1956;25(3):467–478. doi:10.1063/1.1742946.

HOW TO CITE: Arunlal V. B., Sana Dileep, Circular Dichroism Spectroscopy of Voclosporin: Principles, Structural Characterization, and Pharmaceutical Significance, Int. J. of Pharm. Sci., 2026, Vol 4, Issue 5, 8297-8315. https://doi.org/10.5281/zenodo.20472577