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Short communication

Influence of sampling mode on ATR-FTIR spectra of peptide APIs: comparison of solution and dry film measurements

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Introduction

Fourier-transform infrared (FTIR) spectroscopy is a valuable analytical tool for characterization of active pharmaceutical ingredients (APIs) with peptide structure, as it provides structural insight through analysis of several characteristic bands sensitive to molecular conformation and secondary structure. Among them, the Amide I region (1600-1700 cm^{-1}) is particularly important due to its strong dependence on secondary structure, while the Amide II region (1500-1600 cm^{-1}) provides complementary structural information. The Amide I band is especially useful for distinguishing structural elements such as α-helices, β-sheets, turns and random coils (Tintor et al., 2024). However, interpretation of this region is often complicated by interference from water absorption in aqueous media (Rutherford et al., 2023).

Among the different FTIR sampling approaches, attenuated total reflectance (ATR)-FTIR spectroscopy is widely used in pharmaceutical analysis due to its minimal sample preparation and suitability for aqueous and solid samples. However, the sampling mode may significantly affect the acquired spectra. ATR-FTIR spectra of peptide solutions can be acquired directly from solutions or from dry films obtained after solvent evaporation on the ATR crystal. These approaches differ not only because of the overlap of the water bending vibration with the Amide I region, but also due to changes in hydrogen-bonding, possible alterations in peptide secondary structure upon drying, and ATR-specific effects associated with the penetration depth of the evanescent wave. Consequently, the choice of sampling mode can significantly influence spectral appearance and data interpretation (Van de Weert et al., 2001, Tintor et al., 2024).

The aim of this work is to highlight the influence of sampling mode (solution versus dry film) on ATR-FTIR spectra of peptide APIs and discuss the implications for structural characterization and spectral interpretation.

Solution versus dry film measurements

ATR-FTIR measurements performed directly in aqueous solution offer the advantage of reflecting the solution-state secondary structure of peptide APIs that more closely resembles the native environment. However, water introduces a major analytical challenge due to its intense absorption overlaps with the Amide I region, which contains the most valuable structural information for secondary structure analysis (Barth, 2007). Although solvent-

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background subtraction can partially reduce this interference, the resulting spectra often exhibit a significantly reduced signal due to the relatively low concentration of peptides compared with the solvent contribution (Zhao et al., 2023).

To overcome these limitations, ATR-FTIR spectra can be acquired from dry films obtained after evaporation of the solvent directly on the ATR crystal. This approach effectively minimizes water interference, concentrates the analyte and generally results in stronger, sharper amide bands with better resolution (Kafle et al., 2023). The improved spectral quality supports band assignment, spectral comparison and subsequent chemometric analysis. In addition, differences between solution and dry-film measurements may also arise from the varying penetration depth of the evanescent wave, which can influence band intensities and overall spectral appearance (Tintor et al., 2024).

Despite the analytical advantages, drying may influence peptide structure. Water plays an important role in stabilizing peptide conformations through hydrogen-bonding interactions, and removal of hydration layers can disrupt secondary structural elements. This can influence band position, shape and intensity in the amide regions (Tintor et al., 2024). Spectra acquired from dried samples may differ substantially from those obtained in solution, suggesting that dehydration can affect the apparent secondary structure composition. Similar observations have been reported in more recent studies, where improved spectral quality was accompanied by evidence of structural changes following drying (Van de Weert et al., 2001, Kafle et al., 2023, Tintor et al., 2024).

Therefore, differences observed between solution and dry-film ATR-FTIR spectra cannot be attributed only to the elimination of water absorption. They may also reflect changes in hydrogen-bonding interactions and peptide conformation induced by dehydration. Therefore, the choice of sampling mode should be based on the objective of the analysis. Measurements in solution should be considered when investigating peptides in physiologically relevant conditions, and in conformations that more closely resemble their native state. On the other hand, dry-film measurements can be advantageous in quality control analysis where spectral quality is critical for sample discrimination and chemometric performance.

Conclusion

Sampling mode represents a critical experimental parameter in ATR-FTIR analysis of peptide APIs. Measurements performed in solution provide information that is more representative of the native peptide conformation but are affected by significant water interference. Dry-film measurements offer improved spectral quality, enhanced band resolution and reduced interference from water, but dehydration can induce secondary structure changes. Therefore, the selection of the sampling approach should balance the need for physiologically relevant structural information against the benefits of improved spectral quality and data interpretation. Careful consideration of sampling mode is essential for obtaining reliable ATR-FTIR data and ensuring accurate structural characterization of peptide APIs.

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