Tracking Calcium-Induced Assembly and Phase Transitions in Sodium Alginate via Circular Dichroism

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Sodium alginate (SA) forms ionically crosslinked hydrogels in the presence of divalent cations through coordination with guluronic acid (G) blocks [1-2]. Despite extensive studies on alginate gelation, the early - molecular events governing nucleation and intermediate assembly remain insufficiently understood due to the limited sensitivity of conventional techniques to early-stage conformational changes [3-5].

In this work, conventional circular dichroism (CD) and synchrotron radiation circular dichroism (SRCD) were used to monitor Ca^{2+}-induced conformational changes and phase transitions of SA in solution and hydrogel states.

The mannuronic/guluronic (M/G) ratio of the investigated SA was determined from CD spectral features, revealing a slightly M-rich composition (M/G ≈ 1.32). Systematic titration of Ca^{2+} (0–4.0 mM) across SA concentrations (1.0–6.0 mg/mL) showed sigmoidal variations in CD ellipticity, enabling identification of transition points associated with nucleation and the onset of the intermediate phase. At SA concentrations ≤ 2.0 mg/mL, the CD spectra reached a plateau after the stoichiometric Ca^{2+}–GG coordination limit, indicating completion of early assembly stage. For instance, at SA = 2.0 mg/mL, sigmoidal curve reached a plateau with a transition point at approximately 2.75 mM Ca^{2+} (**Figure 1a,b**). Transition points obtained at different SA concentrations were then plotted in the phase diagram together with the minimum gelation concentration (MGC) values (**Figure 1c**).

To validate these findings, complementary FTIR-ATR, SAXS, and AFM analyses were performed. FTIR spectra of dried films confirmed Ca^{2+} coordination through splitting and red-shift of the carboxylate band, while SAXS and AFM revealed fibrillar growth and network formation (*data not shown*).

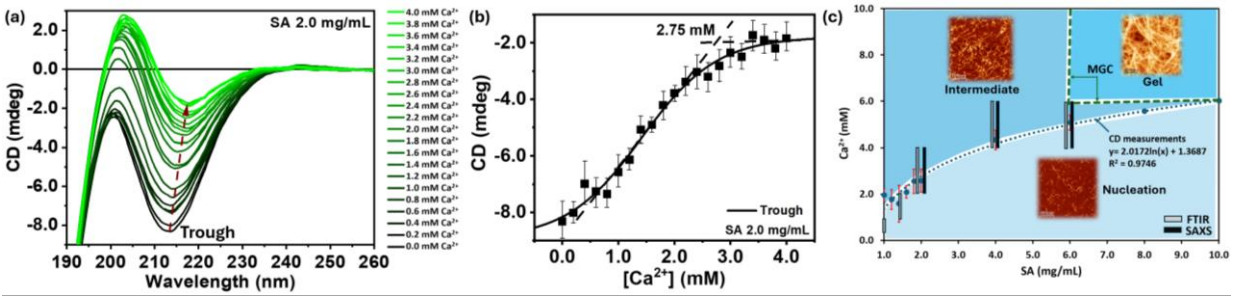

FIGURE 1. (a) CD spectra showing Ca^{2+}-induced assembly of SA (2.0 mg/mL) in the non-gel state; (b) the corresponding ellipticity changes at the trough (λ ≈ 210-215nm) derived from panel (a), highlighting the transition point for 2.0 mg/mL at the plateau region (~2.75 mM Ca^{2+}); and (c) the SA–Ca^{2+} phase diagram derived from CD transition points across various SA and Ca^{2+} concentrations, with SAXS, FTIR, and AFM results overlaid to demonstrate cross-technique consistency.

Transition boundaries obtained from all techniques showed consistent concentration-dependent trends, confirming that CD is highly sensitive to early nucleation events in solution. This multi-technique approach provides a unified phase diagram for the SA–Ca^{2+} system and highlights the capability of SRCD to detect subtle conformational rearrangements during early hydrogel assembly (**Figure 1c**). The results contribute to a deeper understanding of alginate gel formation relevant to biomaterials, drug delivery, and tissue engineering applications.

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