Hyaluronic Acid Dipeptide Gels Studied by Raman Spectroscopy, Atomic Force Microscopy and DFT Calculations

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We studied hyaluronic acid dipeptide gels as a model system for some antibacterial gel. Since one of the requirements for the active pharmaceutical ingredient is that it does not change when applied in formulation, it was important to monitor gels for any changes in vibrational spectra. N-Acetyl-Alanine-Methyl Amide (NAcAlaNHMA) and N-Acetyl-Tyrosine-Methyl Amide (NAcTyrNHMA) display different crystal packing (Fig. 1a and 1b), due to different hydrogen bond networks. Both dipeptides show good solubility in water [1], and form well miscible gels with hyaluronic acid (Fig. 1c and 1d).

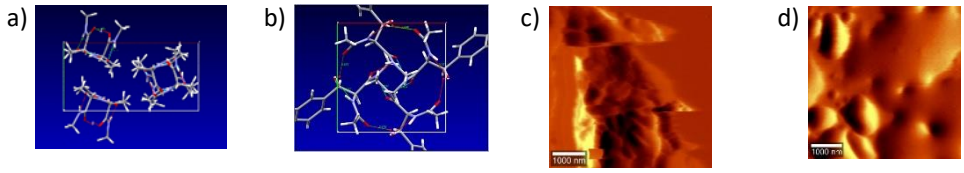

Figure 1. a) crystal structure of NAcAlaNHMA and b) NAcTyrNHMA powder, AFM images of c) NAcAlaNHMA/hyaluronate and d) NAcTyrNHMA/hyaluronate gels

Phonons of the NAcAlaNHMA crystal were calculated using CRYSTAL09 program [2], while normal modes of NAcTyrNHMA and two basic disaccharide units of hyaluronic acid (HA), N-acetyl-β-D-glucosamine-β-(1→4)-D-glucuronic acid sodium salt, and β-D-glucuronic acid-β-(1→3)-N-acetyl-β-D-glucosamine sodium salt [3] were obtained using Gaussian16. Most of the observed gel bands correspond either to HA or to dipeptides in the crystalline form, while in one particular NAcTyrNHMA gel different spectrum was observed (Fig.2).

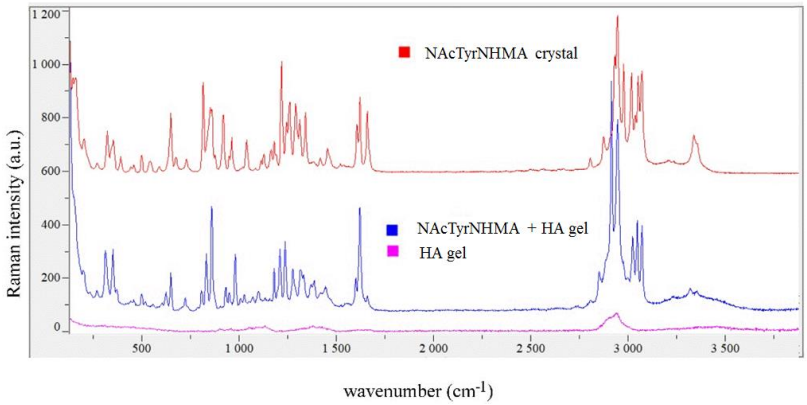

Figure 2. Raman spectra of NAcTyrNHMA powder and mixed with HA gel compared to the spectrum of HA gel

Gels with a dipeptide-to-disaccharide molar ratio of 2:1 exhibited distinct shift in key vibrational modes most notably in the N–H stretching and C–O–H bending regions of the spectrum suggesting the formation of hydrogen bonds between the dipeptide and the HA matrix. These spectral shifts were consistent with the hypothesis that NAcTyrNHMA engages in specific binding interactions with the carboxyl and hydroxyl groups present on the hyaluronic acid backbone. The data support a model where one dipeptide molecule binds to the glucosamine carboxyl group and another to the glucuronic acid carboxylate group, forming a stabilized complex within the gel network. This behavior was not observed for NAcAlaNHMA, likely due to its simpler, less polar side chain, which lacks the phenolic hydroxyl group of tyrosine that facilitates hydrogen bonding.

References:

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