**Diffusion kinetics of vitamin B6 released from bicontinuous and phase-inverted gelatin-agarose gels using blending law for diffusion modelling.**

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In order to keep up with the increasing competition, the food industry is continuously being challenged to improve products in terms of cost efficiency and contribution to maintaining a healthy lifestyle (Kasapis, 2008). The use of proteins and polysaccharides in the food industry is widespread but when used in combination, these polymers exhibit thermodynamic incompatibility leading to phase separation. The interplay of gel formation and phase separation has been successfully studied using techniques like light scattering, rheology, calorimetry, and microscopy. Rheology-based blending laws are being used extensively to ascertain the mechanical properties of the composites from the shear storage modulus and the phase volume of the individual phases in the mixture (Mhaske et al., 2020).

The diffusion kinetics of single-phase systems have been studied by various researchers using diffusion theory (Rubilar et al., 2017; Whitehead et al., 2019). To date, work has not been carried out to rationalize the diffusion kinetics of a two-phase system. This study builds on previous work modelling diffusion of vitamin B6 from a phase-separated system of dispersed agarose phase within a continuous gelatin network. Increasing concentrations of agarose relative to gelatin yields composite gels of protein (gelatin) and polysaccharide (agarose) exhibiting bicontinuous and phase inversion systems from which kinetics of vitamin B6 diffusion are studied via blending law for diffusion modelling. In this study, gelatin 1% (w/w) in combination with various concentrations of agarose (2%, 2.5%, 3%, and 3.5%, w/w) was used to study two-phase systems showing bicontinuous and phase inversion conditions. Fourier-transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), polarized light microscopy and small-deformation oscillation in shear were used to analyse the structural properties of composite gels. UV-vis spectroscopy was employed to study the diffusion kinetics of vitamin B6 from the gelatin-agarose gels.

FTIR confirmed that no chemical interactions occurred between gelatin and agarose in the mixture. XRD was used to study the morphology of the gelatin-agarose-vitamin B6 system, showing that it remained largely amorphous. Polarized light microscopy provided tangible evidence of the phase-separated nature of the two polymeric constituents in the mixture. Blending law predictions were used to calculate the phase volume and effective concentrations of the individual components in the gel. Using UV-vis spectroscopy, the diffusion of vitamin B6 was measured experimentally from the composite gels prepared from the estimated phase volumes and effective concentrations of the individual components according to blending law modelling. These blending laws were modified to provide corresponding relationships for the diffusion coefficients of each phase and the composite. Thus, theoretical diffusion coefficients were calculated, which were compared favourably with those from the experimental studies. Results argue for the presence of a blending law-based diffusion theory that can predict the diffusion kinetics of bioactive compounds in aqueous composite gels of biopolymers.

References:

Kasapis, S. (2008). Phase separation in biopolymer gels: A low- to high-solid exploration of structural morphology and functionality. *Critical reviews in food science and nutrition*, *48*(4), 341-359. https://doi.org/10.1080/10408390701347769

Mhaske, P., Condict, L., Dokouhaki, M., Farahnaky, A., & Kasapis, S. (2020). Phase volume quantification of agarose-ghee gels using 3D confocal laser scanning microscopy and blending law analysis: A comparison. *LWT*, *129*, 109567. https://doi.org/https://doi.org/10.1016/j.lwt.2020.109567

Rubilar, J. F., Cruz, R. M. S., Zuñiga, R. N., Khmelinskii, I., & Vieira, M. C. (2017). Mathematical modeling of gallic acid release from chitosan films with grape seed extract and carvacrol. *International Journal of Biological Macromolecules*, *104*(Pt A), 197-203. https://doi.org/10.1016/j.ijbiomac.2017.05.187

Whitehead, F. A., Paramita, V. D., Teimouri, S., Young, S., & Kasapis, S. (2019). Controlled release of ascorbic acid from genipin-crosslinked gelatin matrices under moving boundary conditions. *Food hydrocolloids*, *89*, 171-179. https://doi.org/10.1016/j.foodhyd.2018.10.026