

Preparation, Topography, Nanomechanical Properties and Crosslink Density of Soft Swollen Hydrated Micron-Sized Polypeptide Microgels

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We report the innovative preparation of soft micron-sized polypeptide microgels by horseradish peroxidase (HRP)-mediated crosslinking in inverse suspension[1]. The prepared microgels were based on poly[*N*⁵-(2-hydroxypropyl)-L-glutamine)-*ran*-(*N*⁵-propargyl-L-glutamine)-*ran*-(*N*⁵-(6-aminohexyl)-L-glutamine)]-*ran*-(*N*⁵-[2-(4-hydroxyphenyl)ethyl]-L-glutamine)] polymer precursor (P2HPG-Tyr). We tested the effect of surfactants sorbitan monooleate (SPAN 80), polyoxyethylenesorbitan trioleate (TWEEN 85), and dioctyl sulfosuccinate sodium salt (AOT), on microgelation in inverse suspension without or with pre-emulsification step. The morphology, size, and particle size distribution of the P2HPG-Tyr microgels were evaluated by light microscopy technique. The crosslinking procedure employing surfactant SPAN 80 and 1-hour pre-emulsification yielded high-quality, spherical, and colloidally stable $\sim 80 \mu\text{m}$ P2HPG-Tyr microgels, which were subsequently studied by cryo-SEM and atomic force microscopy (AFM). To evaluate the topography and nanomechanical properties of the developed P2HPG-Tyr microgels in the hydrated swollen state, the large swollen hydrated P2HPG-Tyr microgels were immobilized on Mica and glass substrates for investigation by atomic force microscopy (AFM) in PeakForce QNM mode in Q-H₂O and PBS buffer (pH 7.4). The AFM investigation revealed surface irregularities of the P2HPG-Tyr microgels and proved their viscoelasticity and softness, documented by Young's moduli in the range of tens of kPa derived from force-separation curves. Finally, the crosslink density of the P2HPG-Tyr microgels was evaluated, revealing the concentrations of elastically active network chains (EANCs) in the range of 0.489×10^{-3} to $0.812 \times 10^{-3} \text{ mol cm}^{-3}$. Following this work, the new series of P2HPG-Tyr microgels, which were prepared in the presence of the surfactant SPAN 80 and 1-hour pre-emulsification step, were prepared with various H₂O₂/Tyr ratio, and they are being tested for encapsulation of Lactobacillus, Streptococcus, and Saccharomyces.

Keywords: AFM, microgel, nanomechanical properties, polypeptide, topography.

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References

[1] Kotko, O.; Šálek, P.; Dvořáková, J.; Dušková Smrčková, M.; Šomvarský, J.; Bonvent, J.J.; Brochsztain, S.; Proks, V. Soft Micron-sized Polypeptide Microgels: Preparation, Crosslink Density, Topography and Nanomechanics in Swollen State. *Mater. Adv.* **2024**, *5*, 5984-5997.