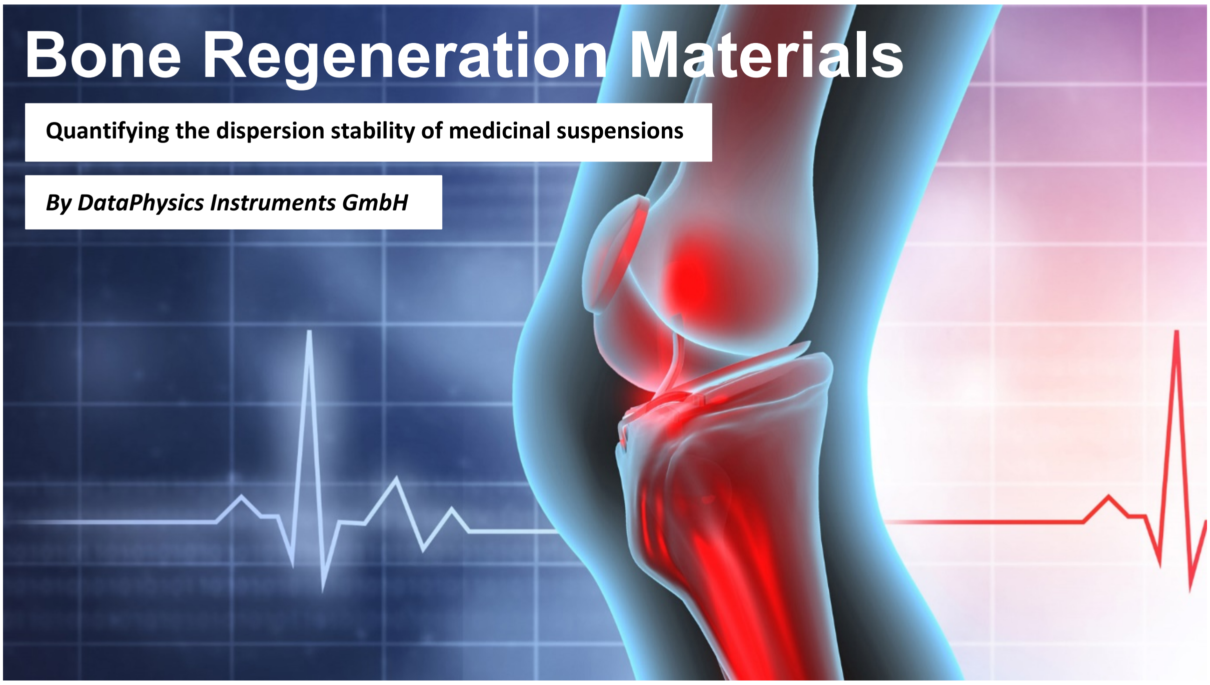
How optical dispersion stability analysis can help to develop bone regeneration materials.



Hydroxyapatite (HAp) is a widely used material with high biocompatibility, as well as great chemical and crystallographic similarity to bone. Thus, it has a high potential for being used in the regeneration of our skeletal system, to treat some common skeletal ailments like osteoporosis. However, the mechanical properties of HAp, like brittleness, low tensile strength and fracture toughness, severely limit its usability. To overcome these drawbacks HAp can be combined with a polymer phase to obtain hydrogels increasing its strength and flexibility without eliminating the bioactivity of HAp. The stability of these HAp-polymer suspensions is a critical parameter to characterize the homogeneity of the substance, which significantly influences the quality of the hydrogels. It is of great importance to study the stability in order to find a suitable particle size, solvent, concentration and reaction time. For the polymer phase, whey protein isolate (WPI) is a good candidate with impressive biocompatibility and good water solubility. In addition it can undergo a gelation process after heating to form a protein bond structured hydrogel. Therefore, a combination of HAp and WPI is an ideal material to create a modified hydrogel that can be applied in the regeneration of bone. Recently, Słota et al. have proposed a high performance biocomposite hydrogel for bone regeneration based on WPI reinforced with nano-HAp powder. The descent rate of the suspended HAp particles and their average diameter were determined as key parameters for the stability of these HAp suspensions.

In this work, the WPI based hydrogel was obtained from WPI solutions (40% WPI in distilled water) with nano-HAp powder via a gelation reaction (**Fig. 1**). Two HAp particles, i.e., HAp A and HAp B, used as the ceramic phase of WIP based hydrogels, were prepared by different precipitation methods.

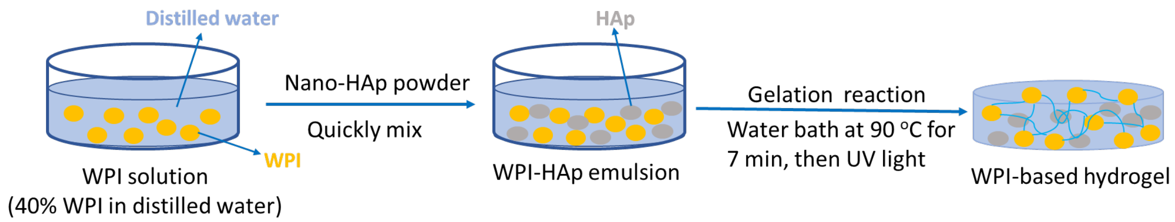
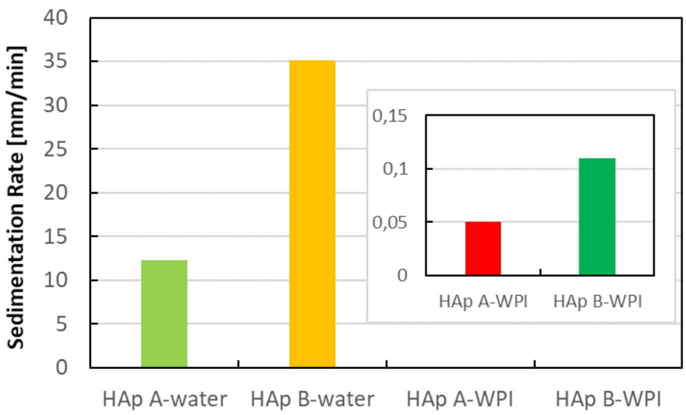


Fig. 1: *Schematic illustration of the preparation of the WPI based Hydrogels.*

To understand the stability of the utilized HAp suspensions, the sedimentation rates of HAp particles in a 40% WPI solution and in distilled water were determined with an optical dispersion stability analyzer (DataPhysics Instruments MS 20). **Fig. 2** shows that the sedimentation rates of HAp A in distilled water and 40% WPI are slower than that of HAp B, indicating that HAp A suspension are more stable (lower sedimentation rate).



Particle	Average diameter (µm)
HAp A	0.7245
HAp B	0.9631

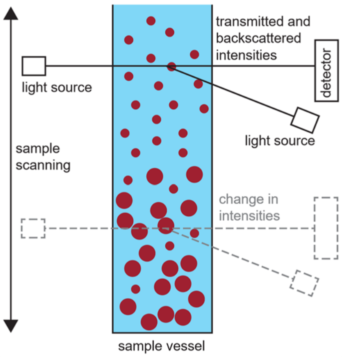
Fig. 2 *Left: sedimentation rates of the HAp particles in 40% WPI solution and distilled water. Right: the average diameter of two different HAp particles.*

Besides, as shown in **Fig. 2** right, the calculated average diameters of HAp A and HAp B are 0.7245 µm and 0.9631 µm, respectively, which is consistent with the result of the sedimentation rate. The sedimentation rate of HAp particles in water is much higher than that in WPI solution. The reason is, that the sedimentation rate is inversely proportional to the liquid viscosity. Therefore, polymer solution with higher viscosity, like the WPI solution provide the possibility to obtain a more stable and homogeneous system. Importantly, the sedimentation rate is slow enough to allow a reaction time of 7 min.

The Stability Analysis System MS 20

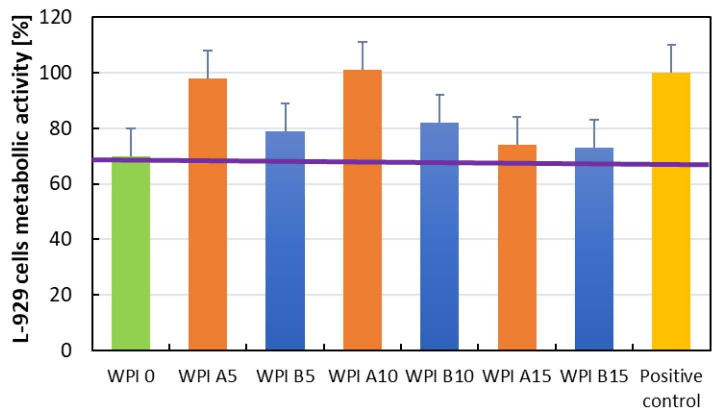
The MultiScan MS 20 is a measuring device for the automatic optical stability and aging analysis of a variety of multi-phase dispersions, in particular suspensions and emulsions, and the comprehensive characterization of **time- and temperature-dependent destabilization mechanisms**.

The **transmission** and **backscattering intensity** is scanned and recorded position dependently. This allows to detect processes such as **agglomeration, coalescence, sedimentation, creaming and the clouding** which is reported in the current article.



Due to its modular design the MS 20 can be operated with up to six scan towers. The scan towers are independently controllable, which allows to perform **several measurements simultaneously under individual conditions**.

To further understand the performance of these composite hydrogels, the viability of cells in WPI-based hydrogels with different HAp content was tested. **Fig. 3** shows that the viabilities of cells exposed to WPI A5 and WPI A10 were similar to the positive control, indicating that HAp A-modified WPI composites could keep a very good metabolic activity for L-929 cells.



Sample	HAp (%)
WPI 0	-
WPI A5	5
WPI A10	10
WPI A15	15
WPI B5	5
WPI B10	10
WPI B15	15

Fig. 3 *Left: viability of cells in WPI-based hydrogels with different HAp content (A: HAp A; B: HAp B). Right: percentage share of HAp in WPI based hydrogels.*

Overall, the authors could show that HAp modified WPI biocomposites exhibit an excellent performance in the viability and monocyte activity test of cells, giving this material a great potential for bone regeneration applications. Particularly, the author studied the stability of WPI-HAp solution with an optical stability analyzer, which is important to learn the sedimentation rate and diameter of the particles and helped to find the perfect formulation and reaction time.

The optical stability analysis system MultiScan MS 20 (DataPhysics Instruments GmbH, Germany) was used in this research.

For more information, please refer to the following article:

Composites Based on Hydroxyapatite and Whey Protein Isolate for Applications in Bone Regeneration; Dagmara Słota, Magdalena Głab, Bożena Tyliszczak, Timothy E. L. Douglas, Karolina Rudnicka, Krzysztof Miernik, Mateusz M. Urbaniak, Paulina Rusek-Wala and Agnieszka Sobczak-Kupiec; *Materials, **2021**, 14, 2371*; DOI: 10.3390/ma14092317