MICROTRAC
PARTICLE CHARACTERIZATION

Synergistic approaches in Zeta Potential and Stability Analysis for advanced research

Case study on a plant-protein based formulation

Context

Formulators encounter various challenges daily, such as the impact of switching suppliers on product quality, the risk of formula destabilization when substituting a component, or the need to incorporate a new ingredient. To meet customers' demands and formulators' expectations for the final product, it is crucial to employ cutting-edge technologies that comprehensively characterize systems. MICROTRAC provides a wide range of technologies and tools for characterization, supporting formulators in the product development process for fast decision-making based on facts. This application note presents a case study that combines zeta potential, particle size, and stability analysis to optimize protein-stabilized emulsions.

Two parameters, ONE conclusion

MICROTRAC provides solutions with unique features:

STABINO ZETA

For zeta potential measurements with **online titration** and **no dilution**.

TURBISCAN

For up to **1000x faster** of formulation destabilization and quantification.

By combining all these technologies, the time needed to introduce products to the market is reduced and the optimal formula is quickly identified with the highest level of accuracy.

How it works?

- **STABINO ZETA**

STABINO ZETA offers a **reliable** zeta potential measurement based on streaming potential. Its smart **mix-and-measure** operation prevents sedimentation, ensuring **accurate results**. Additionally, the **built-in titration function** enables fast determination of the isoelectric point (IEP) or the charge density for samples with particle size from **0.3 nm to 300 µm** and concentration from **0.01 to 40 vol%.**

- **TURBISCAN**

TURBISCAN is the world reference stability analyzer based on Static Multiple Light Scattering (SMLS). This advanced technology enables fast detection of any kind of destabilization **without dilution 1000x faster** than the naked eye. It accurately **detects and quantifies** sedimentation, creaming, and flocculation in product ranges of **0.0001 to 95 wt%.**

More about Microtrac's instruments

Case Study

Understanding these two parameters simplifies decision-making to overcome formulation issues.

For this note, protein-stabilized emulsions are studied as an example. Zeta Potential measurements enable improvement in raw material extraction, (in this case: proteins), and formulation additives selection.

It is very important for formulators to know these causes to be able to overcome them and get a stable product. The study followed three steps:

1. **Improvement on Raw material extraction** based on **zeta potential** measurements

2. **Impact of Salt Addition on Different Proteins,** measured with online titration

3. **Stability analysis** and **emulsion's optimization**

1. Improvement on Raw material extraction

Several methods exist for protein extraction, one of them is the alkaline method. This method adjusts the pH level to alter protein solubility.

At an alkaline pH, proteins are fully solubilized in the medium, whereas in acidic conditions, proteins become insoluble and precipitate. The isoelectric point (IEP) is important for identifying the precise pH at which precipitation begins, and the protein is no longer solubilized.

The built-in titration function of STABINO ZETA enables acid titration without diluting the sample. Figure 1 shows an example of IEP measurement with the STABINO for the extraction of rice bran protein. The first extraction was performed using a protocol found in the literature. The second one was enhanced based on the results obtained with the STABINO ZETA, with adjusting the pH to be more compatible with our protein in this case.

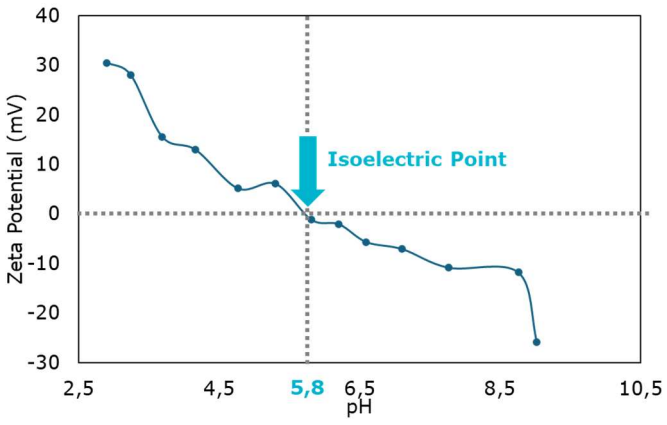

Figure 1: Isoelectric Point measurements

	Protocol	Improved Protocol
Protein Extracted (g)	0,8	1,263
pH	4,5	5,8
	Without STABINO ZETA	With STABINO ZETA

Table 1: Comparison with protein extracted with IEP

As seen in Table 1, the IEP (Isoelectric Point) method resulted in a more efficient protein extraction compared to the protocol described in the literature (+57% weight extracted). For the protein used in this study, the optimal pH is 5.8, rather than the pH of 4.5 suggested.

2. Impact of salt addition on different proteins

The previously extracted protein is compared to different proteins from the market to evaluate their behavior to salt (NaCl) addition.

This comparison enables the identification of the protein which is the most resistant to salt variations and determine whether salt is the best additive to use with the chosen protein.

The proteins were suspended in water and a salt titration was **automatically** performed in **less than 5 minutes** with STABINO ZETA, as shown in Figure 2.

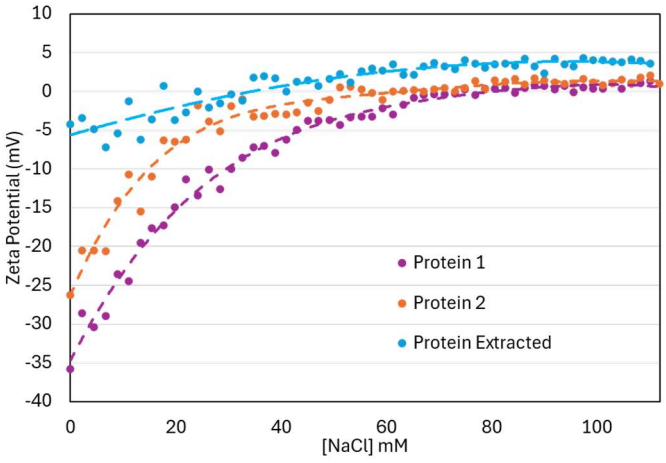

Figure 2: Salt Titration with different protein suspensions

Figure 2 shows that when more salt (NaCl) is added, the negative zeta potential goes up until it reaches out zero. The protein extracted has the highest zeta potential compared to its alternative industrially available, and the value gets close to zero with a lower amount of salt. Therefore, this protein has a low resistance to salt variation which can impact the final stability.

On the other hand, Protein 1 and Protein 2 start with a stronger zeta potential value and need more salt to reach the neutral value of zeta potential. These proteins respond better to the addition of salt (NaCl), making them more suitable for formulations with NaCl.

Based on these results, Protein 1 was selected to formulate the emulsions.

3. Stability analysis and emulsion optimization

After the identification of the best Protein, in this section, different concentrations of the protein (Protein 1), were screened to identify the formulation with the optimal stability.

The ratio of oil fraction was the same for the different emulsions (60% vol.) with Protein 1 concentration going from 2, 4, 6, 8 up to12% wt.

▪ Stability analysis

The destabilization detected by TURBISCAN are represented as a function of sample height (in mm) over time. The bottom of the sample is represented on the left part of the graphic and the top of the sample is on the right part. The color gradient on the time scale corresponds to each scan time lapse with the first scan in blue and the last scan in red.

As we can see in the figure, a decrease of the backscattering (BS) signal over time is observed the top and the bottom of the sample. The destabilizations are identified within the **first minutes** of measurement. This phase separation is less important in emulsions with high protein concentrations (Figure 4).

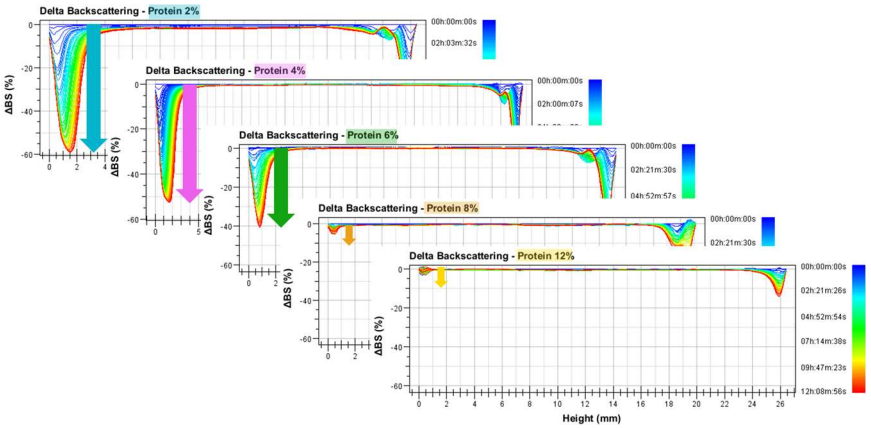

Figure 4: Destabilization detected by TURBISCAN for emulsions with different protein concentrations

- **Optimal formulation selection**

The Turbiscan Stability Index (TSI) is an automatic calculation that sums all destabilizations into a single number for easy, one-click ranking and comparison. Therefore, the HIGHER the TSI value, the LOWER the Stability is.

As shown in Figure 5, the emulsions with 8% wt. protein and 12 % wt protein are the most stable. This ranking is done within **the first 20 minutes**. The increase of protein concentration up to 12% wt does not have impact on the stability compared to 8%wt. The optimal concentration of protein in this case is 8%wt.

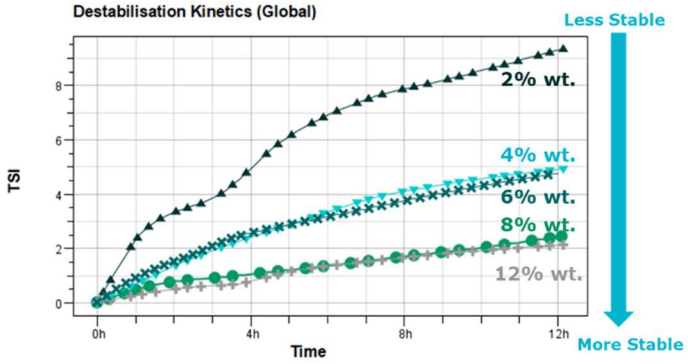

Figure 5: Destabilization detected by TURBISCAN for emulsions with different protein concentration

CONCLUSION

Based on the previous results, the STABINO ZETA measurements were valuable to optimize raw material extraction, such as proteins in this case. Additionally, the online salt titration provided insight into how salt affects this protein. The stability measurements with the TURBISCAN provided a deeper understanding of protein concentration impact on the emulsion stability without any sample dilution.

With the STABINO ZETA and the TURBISCAN measurements the whole process from the extraction of the raw material to the optimization of the final was done **without dilution**, **faster and reliably.**

Abd Rahim, F. N., Wan Ibadullah, W. Z., Saari, N., Brishti, F. H., Mustapha, N. A., Ahmad, N., & Arulrajah, B. (2023). The effect of alkaline extraction and drying techniques on the physicochemical, structural properties and functionality of rice bran protein concentrates. International Journal of Biological Macromolecules, 242. https://doi.org/10.1016/j.ijbiomac.2023.124908

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Li, D., Zhao, Y., Wang, X., Tang, H., Wu, N., Wu, F., Yu, D., & Elfalleh, W. (2020). Effects of (+)-catechin on a rice bran protein oil-in-water emulsion: Droplet size, zeta-potential, emulsifying properties, and rheological behavior. Food Hydrocolloids, 98. https://doi.org/10.1016/j.foodhyd.2019.105306