# Formulating and stabilizing greener formulations

## *Turbiscan Publication Review*

PUBLICATION REVIEW

### Introduction

Global ecosystems are under great strain due to environmental pollution. The implementation and expansion of "green" chemicals and preparation methods for everyday products is essential for a sustainable future. Creating more eco-friendly formulations often relies on replacing toxic or high carbon footprint ingredients, and it has become a topic of interest to the scientific community. However, such work requires a long process of reformulating obsolete formulas to retain product quality and efficacy. The Turbiscan technology has high potential in the development of such alternatives. This review summerizes some of the recent scientific publications to demonstrate Turbiscan's ability to rapidy and efficiently characterize new formulation processes and alternatives in industries and applications like cosmetics, agrochemicals, surfactants, encapsulation…

### Green formulations

The concept of "green chemistry", also known as sustainable chemistry, can encompass numerous fields. It can be broadly defined as "minimizing or eliminating the use of hazardous chemicals, whether they are detrimental to human health or the environment". The twelve principles of green chemistry, as defined by the American Chemical Society (ACS)[1], have been widely accepted by chemists:

1. **Prevention of waste** rather that treat or clean-up
2. **Atom Economy –** use it all
3. **Less Hazardous Chemical Syntheses**
4. **Designing Safer Chemicals**
5. **Safer Solvents and Auxiliaries**
6. **Energy Efficiency**
7. **Use of Renewable Feedstocks**
8. **Reduce Derivatives**
9. **Catalysis vs Stoichiometric Reagents**
10. **Designed for Degradation**
11. **Real-time Analysis for Pollution Prevention**
12. **Inherently Safer Chemistry for Accident Prevention**

Particularly, the high demand for novel, environmentally friendly chemicals requires dispersions and emulsions to be re-formulated and the stability of new "green" formulations must be determined. The Turbiscan® technology, widely used for characterization of dispersions (emulsions, suspensions, and foams), can help select best suited candidates for greener alternatives. Turbiscan® technology has been used for decades by formulators for stability measurements and is now recognized as a standard technique for direct physical stability analysis. Thus, the Turbiscan® with its dedicated Stability Index (TSI) are 'must-have' tools for formulation scientists for fast dispersion screening, quantitative stability evaluation and rapid product development.

### Reminder on the technique

Turbiscan® technology, based on Static Multiple Light Scattering (SMLS), consists of sending light pluses (880 nm) into a sample along its height. The instrument enables the formulation chemist to monitor changes in physical stability (coalescence, creaming, sedimentation, phase separation, etc...). The signal is directly linked to the particle concentration (φ) and size (d) by the Mie theory:


$$BS = f(\varphi, d, np, nf)$$

### 1. Natural extracts : Compatibility study

***Reference**: Nizioł-Łukaszewska Z et al. (2018), Cornus mas L. extract as a multifunctional material for manufacturing cosmetic emulsions.*

Natural extracts are highly demanded by consumers as they contain a number of functional ingredients that are difficult to achieve by synthesis. These ingredients are also perceived as a "better-for-you" option when compared to analogous synthetic products. Functional ingredients contained in natural plant extracts can include vitamin C, phenolics, flavonoids, and anthocyanins which have significant antioxidant effects. However, incorporation of complex natural extracts in consumer products brings a new set of challenges related to stability, as it is often difficult to pinpoint culprit ingredients that may cause destabilization. In addition, the extract content may vary considerably depending on the plant source, harvest time, or climatic conditions, which may produce batch-to-batch variations.

[1] Anastas, P. T.; Warner, J. C. Green Chemistry: Theory and Practice, Oxford University Press: New York, 1998, p.30.

The Turbiscan is not only beneficial for the initial formulation R&D, but can be used to monitor raw material quality and batch-to-batch variability.
Niziol-Lukeszewska et al. incorporated *Cornus mas L.* extract into a cosmetic emulsion and used the Turbiscan to assess the stability of the base O/W emulsion.

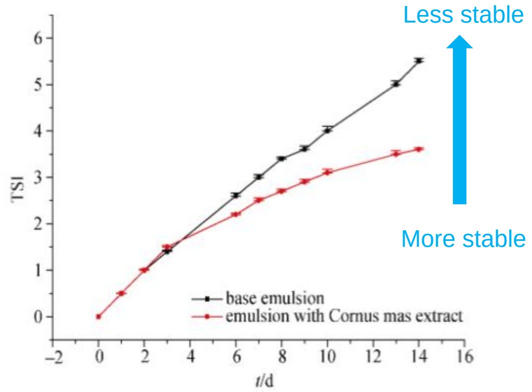


*Figure 1: Evaluation of the stability of the base emulsion (black) and the emulsion containing the C. mas extract (red) over 16 days*

Incorporation of the extract initially showed the same stability as the base formulation, but the TSI values diverged after approximately 3 days (Fig. 1). Importantly, the extract-containing formulation showed better stability (lower TSI) than the base formulation, which was attributed to the antioxidant and also the effect of other component like amino acids and proteins which can act as co-surfactants or additional stabilizing agents. Experiments performed under centrifugation (3 000 r.min$^{-1}$ for 30 min) did not provide any valuable information, showing the value of using the non-invasive and non-destructive Turbiscan® technology.

## 2. Green delivery of hydrophobic pesticides

***Reference**: Zhang B et al. (2020), Optimization of extraction technology of poly-mannuronic acid to a green delivery system for the water-insoluble pesticide, λ-Cyhalothrin*

Many pesticides exhibit poor water solubility and require surfactants to create semi-stable dispersions. Surfactants are generally synthesized from non-renewable resources, so Zhang et al. sought to extract poly-mannuronic acid (PM) from a plant source to use as a green stabilizer of the pesticide λ-cyhalothrin. PM was first treated to be amphiphilic via a Ugi multi-component reaction (Ugi-PM). Clear differences were visible between the control O/W emulsion (no Ugi-PM) and the other emulsions with different concentrations of Ugi-PM. Detecting differences between emulsions with varing Ugi-PM concentrations is more challenging and subject to the ability of the operator to clearly see the difference even after 30 days. Figure 2 shows pictures of the emulsion with different amounts of UGI-PM at 0, 14, 21 and 30 days.

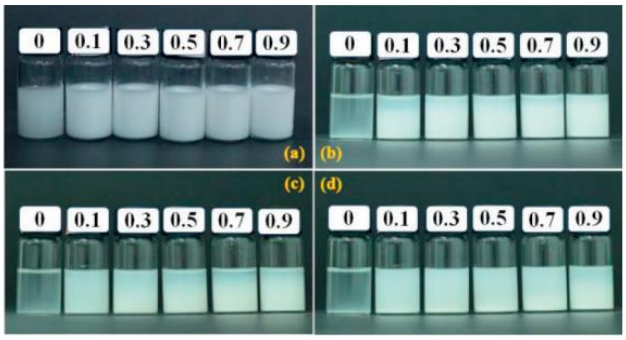


*Figure 2: Photos of the λ-cyhalothrin emulsions prepared with different concentrations of Ugi-PM after aging for (a) 0 d, (b) 14 d, (c) 21 d, (d) 30 d.*

The average particle size and the zeta potential were determined (via DLS, Figure 3). Particle size and zeta potential reached an optimum at a >0.5 wt% concentration. The Turbiscan measurement was also performed for **4 hours** and the stability comparison was pe via the Global TSI (entire sample height) and Bottom TSI (focus on the bottom precipitates) - Figure 4.

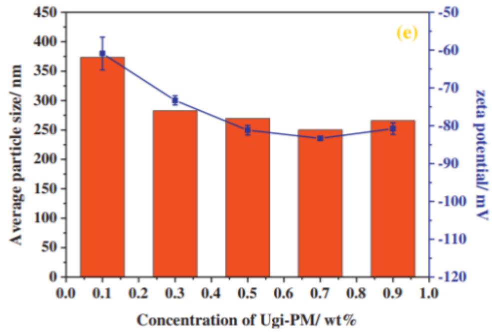


*Figure 3. Average particle size and zeta potential (DLS) of the emulsions with different Ugi-PM concentrations.*

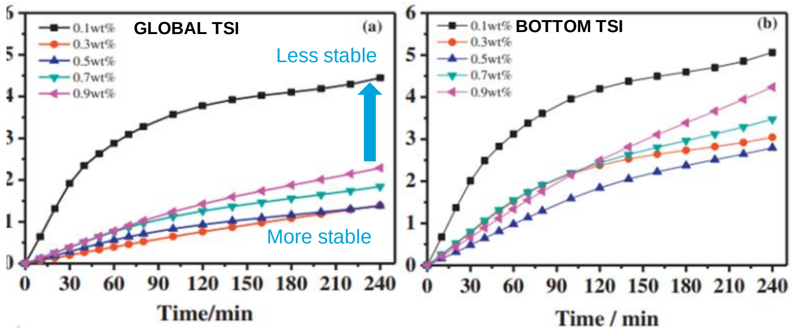


*Figure 4: Emulsion stability over time at different Ugi-PM concentrations. (a) overall kinetic instability; (b) kinetic instability at the bottom.*

The TSI values provide similar information where 0.1 wt% UGI-PM showed the highest destabilization and an optimum UGI-PM concentration was achieved between 0.3% and 0.5% (lowest TSI and slowest kinetics). For the >0.5% emulsions, the TSI value increased significantly and samples presented lower stability. Indeed, at higher concentrations of UGI-PM, flocculation by depletion caused extra destabilization.

The stability measurement done with the Turbiscan provides an extra level of understanding. While DLS data (particle size and zeta potential) indicates an optimal concentration of 0.5%-0.7%, Turbiscan shows the optimum stability between 0.3% and 0.5% and in only 4 hours of measurement (no destabilization visible after 30 days, Fig. 2). It was determined that 0.3 wt% Ugi-PM would be optimal to reduce costs and maximize stability of the pesticide formulation.

## 3. Transforming a byproduct into a green alternative for emulsion stabilizer: Lignin nanoparticles

***Reference****: Agustin M.B et al.(2019), Rapid and Direct Preparation of Lignin Nanoparticles from Alkaline Pulping Liquor by Mild Ultrasonication.*

Utilization of waste products of common industrial processes has the potential to satisfy many of the green chemistry principles related to waste minimization, efficiency, and pollution reduction. Lignin is the most abundant natural aromatic polymer on Earth and a major byproduct of the pulp and paper industry, with 50+ million tons produced every year. Currently, this biomass is burned to power paper mills in an attempt to recover the chemical energy and minimize solid waste. However, this generates significant greenhouse gas (GHG) emissions. A promising alternative is the utilization of lignin in value-added processes to produce functional chemicals.

Agustin et al. recently developed a rapid and direct preparation method of lignin nanoparticles (NPs) that show great promise as emulsion stabilizers. The authors used the Turbiscan to assess the quality of NPs, produced by their novel process, for emulsion stability.

Fig. 5, shows the emulsions prepared with the NPs at different concentrations (0.15, 0.3, 0.45 and 0.6 wt%) and without any additional stabilizers. Emulsion droplet size decreased with increasing NP concentration and the sample displayed a growing opacity and brown color.

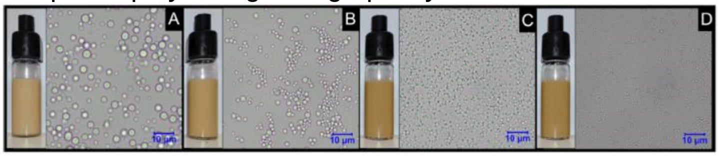


*Figure 5: O/W emulsions with (a) 0.15, (b) 0.3, (c) 0.45, and (d) 0.6 wt% of lignin NPs*

Turbiscan stability data can be obtained regardless of the sample opacity/transparency and without any dilution, thus preventing misinterpretation due to the sample perturbation. The TSI (Figure 6) and raw backscattering (BS) values were used to evaluate the effectiveness of the NPs to create an emulsion and to determine the optimum concentration for emulsion stabilization.

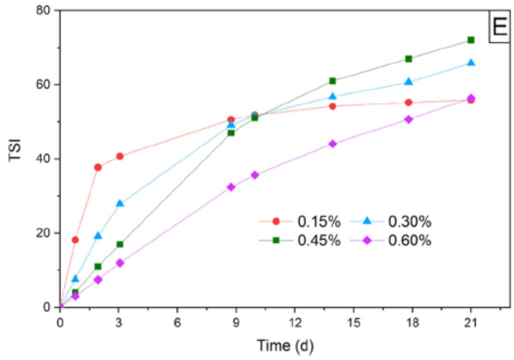


*Figure 6: TSI value in function of time for O/W emulsions with 0.15, 0.3, 0.45 and 0.6 wt% lignin NP*

Figure 6 shows the different stability kinetics of the formulations, where higher TSI values indicate lower stability. From the microscopy pictures, we could expect the best stability for the highest concentration of NPs (hence smallest particle size). The Turbiscan analysis provides another level of understanding thanks to its global approach and no dilution. The sample with the lowest NP concentration (large particle size) showed a very fast destabilization in the first days due to fast creaming. However, when the creaming was complete, the stability remained similar and certainly shows a good redispersion ability. At higher NP concentrations, the destabilization kinetics slowed due to slower migration speed. However, the destabilization continued on a longer run and particle size variation may be expected.

## 4. Encapsulation : Multiple emulsion using Green solvents

***Reference****: García M.C et al.(2019), Formulation variables influencing the properties and physical stability of green multiple emulsions stabilized with a copolymer*

Formulations can be designed to release the material in a specific and controlled environment (temperature, pH or ionic strength). Multiple emulsions (ex. water in oil in water, W/O/W) are known for their encapsulating abilities and thus, have large range of applications in the pharmaceutical, food, agrochemical, and cosmetic industries. Garcia et al. recently studied the use of a green and eco-sourced solvent, 2-ethylhexylactate, to create multiple emulsions and fulfill European regulatory requirements. The formulation also contained a stabilizing agent (A-B Block copolymer Atlas$^{TM}$ G-5000) and an antifoamer.

*Table 1. Compositions of the studied emulsions.*

| | E15/1.5 | E15/3 | E15/6 | E30/3 | E40/4 | E50/5 |
|---|---|---|---|---|---|---|
| **2-ethylhexyl lactate (wt%)** | 15 | 15 | 15 | 30 | 40 | 50 |
| **Atlas G-5000 (wt%)** | 1.5 | 3 | 6 | 3 | 4 | 5 |
| **Antifoamer (wt%)** | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |

The multiple emulsions were tested for 4 days at 25°C. The Turbiscan data obtained from the E15 formulations listed above are shown in Fig. 7.

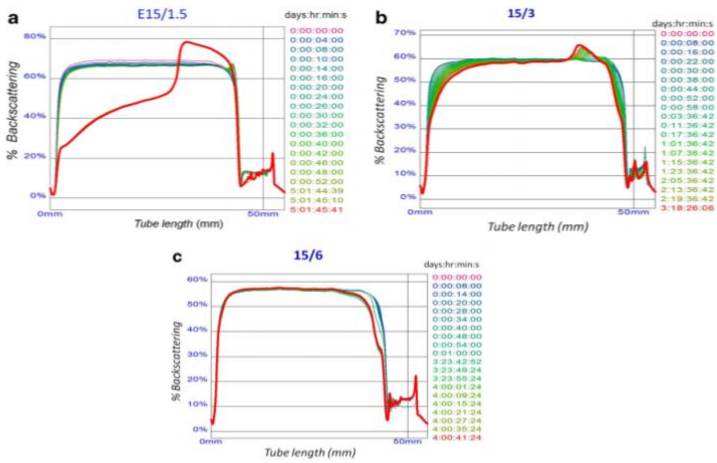


*Figure 7: Backscattering intensity of various emulsion formulations at 25 °C (a–c).*

It is clear from the backscattering intensity plots that drastic phase separation was observed for the E15/1.5 sample, indicating by the dramatic increase from ~20% BS at the bottom to ~80% BS at the top of the vial. The other formulations did not exhibit such destabilization, and only slight clarification was observed at the bottom of the E15/3 sample. The E15/6 sample was largely unchanged.

Various criteria can be used to assess acceptable stability and in this study, the sample was considered destabilized after a backscattering intensity change of approximately 5%.

| | E15/1.5 | E15/3 | E15/6 |
|---|---|---|---|
| **Time to reach threshold** | <1 h | 3 days | +4 days |

The authors also tested higher oil-phase concentrations with polymer stabilizer concentrations ranging from 3 to 5 wt% (Figure 8).

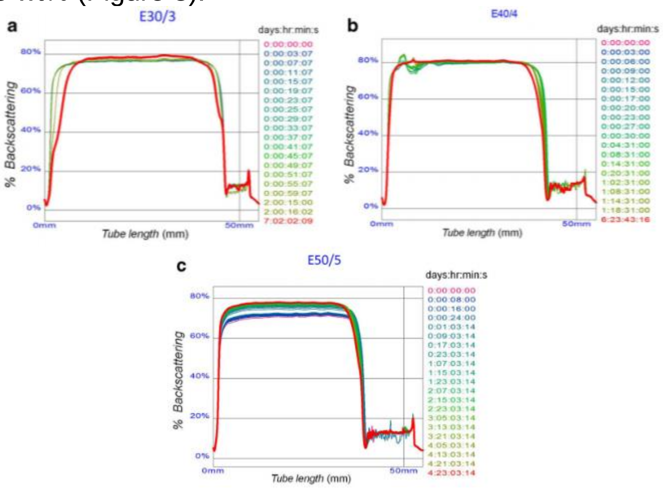


*Figure 8: Turbiscan backscattering data obtained at 25 °C for (a) E30/3, (b) E40/4, and (c) E50/5 emulsions.*

A new destabilization mechanism was observed in the E50/5, flocculation, as indicated by the global increase in BS signal over the entire height of the sample (Fig. 8c). From these data, the formulation chemist can decide which destabilization is more detrimental and work towards preventing or inhibiting this mechanism. The quantitative data provided by the Turbiscan give formulation chemists a valuable tool with which to compare stabilities and identify the mechanisms that cause the destabilization. This quantitative data enables **fast optimization** studies like the one described above, and enable design of experiments (DoE) methodology.

## 5. Stability optimization of a biocellulose mask

***Reference**: Perugini et al. (2018), Biocellulose Masks as Delivery Systems: A Novel Methodological Approach to Assure Quality and Safety.*

Bacterial cellulose (BC) exhibits excellent biocompatibility and the ability to absorb and donate moisture, making it the perfect carrier for delivering nourishing ingredients to your skin. In addition, BC can be produced without the need to harvest trees and process pulp using harmful chemicals. This makes BC a good candidate as a green alternative for cosmetic products such as microfiber masks. The authors studied a novel manufacturing route of such BC masks and provided a test method to predict their stability. Indeed, to ensure that the mask is stable, BC shouldn't transfer to the liquid surrounding the mask as it would mean that the mask is deteriorating. To characterize the interactions between the biopolymer matrix and the solution, the liquid from squeezed masks was analyzed using the Turbiscan, a fast, low volume, and non-destructive technology (6-hour tests using 20 g only). Tests were performed on 6-month old, 12-months one, and freshly made masks.

*Figure 9: Image of the bacterial cellulose cosmetic face mask (left) and its microstructure (right).*

The results showed a large difference in terms of physical stability of samples, directly related to the mask's date of manufacture. Fig. 10 presents the delta transmission in function of time for a mask manufacturer 12 months prior the analysis

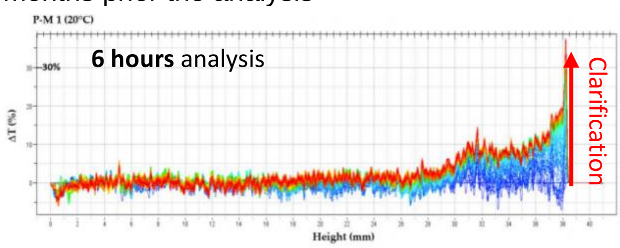


*Figure 10: Stability profile of 12-month mask solution from Turbiscan analysis*

The transmission profile revealed that some aggregation occurred on the surface of the sample, though not detectable by visual inspection. This could clearly be the sign of the interaction between the BC cellulose polymer and the solution. The results allow detection of stability differences, with less transfer of the BC matrix into the surrounding liquid for the fresh mask. To quantify the stability difference, the TSI was used. Lower TSI values mean less changes over time and indicate high dispersion stability. In Table 2, it is clear even after only **5 minutes** of analysis that the fresh sample is the most stable.

*Table 2. Turbiscan stability index (TSI) values of the solutions imbedded in the BC masks.*

| Mask | 5min | 30min | 1 h | 3 h | 6 h |
|---|---|---|---|---|---|
| **12 months** | 0.7 | 2.3 | 3.4 | 6.1 | 7.2 |
| **6 months** | 0.4 | 1.7 | 2.5 | 4.5 | 5.9 |
| **Fresh** | 0.2 | 1.5 | 1.7 | 3.1 | 3.9 |

Quantifying the stability enables more detailed and statistical approaches for defining mask quality and shelf life and thus allows formulators to complete their ingredient optimization in a fraction of the time compared to a subjective visual tests (authors states up to 20 times faster). *"Multiple light scatterin*g [i.e : Turbiscan] *was successful to evaluate stability, as well as possible interactions between the formulation and the cellulose matrix, leading to a more precise definition of the expiry date (shelf-life) attributable to these new delivery system."*

## 6. Essential oil as an alternative to protect food and avoid food waste via edible coatings

***Reference**: Ah Oh.Y et al.(2016), Comparison of effectiveness of edible coatings using emulsions containing lemongrass oil of different size droplets on grapy berry safety and preservation.*

Food production contributes to a significant proportion of worldwide pollution. Some environmental impacts of food production include deforestation, fertilizer run-off causing algal blooms, and a reduction in species diversity due to habitat destruction and monocultures. The USDA estimates that 30-40% of the food supply in the United States is lost to pests, spoilage, and the culling of blemished or imperfect products. Edible coatings have the potential to extend the shelf-life of various food products, reducing food waste due to spoilage and improving the appearance to reduce culling. These food coatings must be safe, anti-microbial, and ideally be natural and functional (i.e. add vitamins, minerals, antioxidants etc.).

Lemongrass oil (LO), is an effective natural antimicrobial agents and a good candidate for antimicrobiological edible coatings. These coatings (emulsions) are used in postharvest technology to improve food safety and extend the shelf life of grape berries. Ah Oh.Y et al. studied the development of a stable emulsion and investigated the impact of the particle size on the effectiveness of the protection. Indeed emulsion stability is key for fruit protection: "*coatings formed from stable emulsion demonstrate high barrier properties against gas, volatiles and moisture*".

The coating emulsion contained LO + Chitosan and was prepared by using high shear mixing and high-pressure processing. By adapting the process, the authors succeed in finding the best process to reach the optimum stability and extend shelf life.

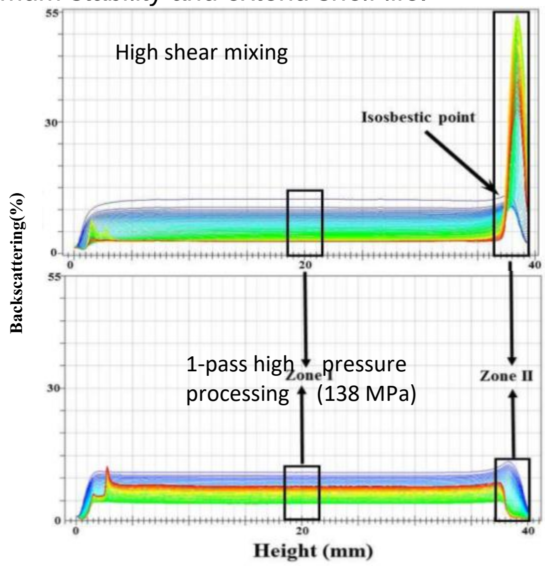


*Figure 11: Raw backscattering data of lemongrass oil-chitosan emulsions prepared via high shear mixing (top) and high-pressure processing (bottom) at 138 MPa.*

Figure 11 shows reduced flocculation (Zone I) and creaming (Zone II) of the coating produced using a 1-pass high pressure process compared the high shear mixing technique. The data obtained via the Turbiscan technology shows the clear impact of the process on the destabilization process and intensity, allowing the chemist to adjust the process to obtain the best formulation.

## Conclusions

This review has shown the capability of the Turbiscan to characterize and optimize green formulations in a variety of applications. The results provide **accurate and quantitative** data for dispersion stability analysis and remove the guesswork associated with visual stability observations. This high-resolution analysis can even **accelerate the stability testing from many days or weeks to a couple of days and sometimes hours**. This helps move projects to completion at a much faster rate compared to traditional methods. The Turbiscan has been proven to provide fast and accurate results that can be applied to all types of green formulations including in autoemulsifying concentrate forms, O/W emulsions, suspensions, microemulsions, and microcapsule formulations.