

Report on the International Workshop Dispersion Analysis and Materials Testing 2015

Latest developments of LUM

With the on-going development of LUM analytic systems for dispersion analysis and characterization of micro- and nano-particles new application fields emerge, and as a consequence, product and process development is becoming easier and faster for customers. About 80 registered participants from all over Europe, Israel, USA and Japan learned this by means of various examples presented from the wide field of applications during the workshop hosted in Berlin, Germany, in January. Besides the scientific lessons, practical courses held in three languages gave deeper insight into the opportunities offered by LUM analytical systems and provided information about how to make best use of it.

For the second time four candidates, out of 10 applicants from 7 countries, nominated for the Young Scientist Award were invited to present their research work.

In his opening lecture, Prof. Lerche introduced the latest developments within the company and the portfolio of analytical systems. Besides the improvement of LUMiSizer functionality by installing a multi-wavelength device which made the system suitable for nanoparticle characterization, the adhesion analyser LUMiFrac found new applications for flexible systems like coated fibre glass and coated needle felts. With a new designed accessories the measurement of shear stresses, as asked by customers, is also possible now. The LUMiReader X-Ray is the latest development, which allows for the characterization of highly concentrated – from transparent to even opaque nano- or micro-dispersions, extending the proprietary STEP-technology to X-ray.

Determination of adhesion forces

At the workshop 2014, the new adhesion analyser LUMiFrac was introduced to a greater audience [1]. This year Hendrik Behm from RWTH Aachen, Germany, showed results obtained with this system in the quality assessment of thin films on polypropylene. For instance, plastic bottles are coated with such films by using plasma enhanced chemical vapour deposition processes to achieve a gas barrier and enhance scratch resistance. Unfortunately, the adhesion forces between the film and polyolefin surfaces are quite low. To increase the adhesion strength and thus ensure the functionality of the coating, plasma pre-treatment of the plastic surfaces was tested. In this research project, PP films and moulded plates were coated with a silicon organic coating after pre-treatment with oxygen and argon plasmas. The adhesion of the coatings was measured by a tensile stress testing machine and by the LUMiFrac system.

Sebastian Moeller, Karlsruhe Institute of Technology, Germany, used a different testing procedure, which is also based on centrifugation, to quantify adhesion forces of wheat flour particles on smooth rigid surfaces like stainless steel as well as on structured surfaces of textiles. Among other things, wheat flour is used as a separator between raw bread rolls and surfaces of devices and trays. The particle adhesion determines the efforts and costs for cleaning these surfaces. For the determination of adhesion forces, a sample of the considered substrate covered with wheat flour was placed at the top of the measuring cuvette, and a substrate covered with an adhesive film to collect the separated particles was placed at the bottom. As the particle size distribution is quite wide and the particle shape is not uniform, a great value range for the adhesive film was determined visually by means of a microscope. The centrifugal acceleration was used to calculate the force acting on the particles. With this measuring procedure the influence of the different substrate materials and their surface roughness could be analysed. For instance fabrics from Polyester (PET) exhibited lower adhesion forces on flour particles than cotton fabrics.

Evaluation of Hansen Parameters (HSP) of particles

Already in 2014, Shin-ichi Takeda, Takeda Colloid Techno-Consulting, reported for the first time on the measurement of Hansen Parameters of micro/nano-particles by means of LUMiSizer or



LUMiReader. Originally introduced to describe the solubility of molecules with three dimensions based on dispersion forces between structural units, interactions between polar groups and hydrogen bonding, this concept was transferred to particles in dispersions. In 2015 Takeda pointed out that due to the small particle sizes in nano-particle suspensions the measurement of wetting characteristics (contact angles) does not make sense to characterize dispersibility. HSP can help to characterize dispersibility of micro- and especially nano-particles in different solvent systems and matrices. In addition to previous experiments with silica and alumina, new results were presented for various materials including carbon based materials and different aqueous and non-aqueous liquids. Furthermore Takeda attempted to define new scaling approaches for HSP calculation

Dispersions in industrial processes

In deep water crude oil production, asphaltene depositions on inner surfaces of pumps, pipes, and other components cause severe problems accompanied by product losses and costly failure of process units. The main reason for asphaltene precipitation is the pressure decrease setting in, when the crude oil leaves the reservoir. This makes the crude oil become instable, due to the evaporation of low boiling components and changes in the density and composition of the oil. To avoid asphaltene precipitation, chemical inhibitors are added. There exists a standardized test procedure (ASTM D7827-12 [2]) to evaluate the effect of inhibitors. As standardized test conditions do not fully imitate the processing conditions for crude oil in the deep sea production plant, destabilizing agents like heptane or pentane have to be added at rather high amounts. Thereafter the ASTM method measures the separation behaviour of the precipitated asphaltene under gravity by means of STEP-Technology. and with and with no additives (precipitation inhibitors). Baker Hughes has recently applied LUMiSizer as new alternative method and launched the so-called Asphaltene-Analytical Centrifuge Stability Analysis method (Asphaltene-ACSA) [3]. Robert Cable presented results obtained with both methods. He showed that using the analytical photo centrifuge helps to create test conditions which are considerably more similar to the submarine conditions, because the amount of de-stabilizing agent could be reduced. Separation in centrifugal fields allows for a better differentiation between different crude oil samples. Tests can be applied even on the most insoluble high molecular asphaltenes. Thus the development and improvement of inhibitors can be carried out more efficiently. Starting in 2014, Prof. Simon Biggs from University of Leeds reported on research activities in characterizing the sedimentation behaviour of precipitations occurring in highly active nuclear waste (HAL) [1]. This liquid waste have to be stored in cooled tanks for many years. This year, Timothy Hunter reported about new results of this research group to improve understanding for safely emptying these tanks and convert the liquor into a vitrified glass for long term disposal. Fundamental research on model suspensions was carried out to characterize the separation behaviour of major precipitants, caesium phosphomolybdate (CPM) and zirconium molybdate (ZM), their interactions with the liquid and the influence of shape factors. To this end, spherical CPM and cuboidal or rod-like ZM particles made of stable isotopes were synthesized as simulants for the radioactive species. Sedimentation characteristics of model suspensions prepared at different pH-values (nitric acid) were determined by means of a LUMiSizer, especially applomeration tendency was described by a newly defined coefficient. Furthermore the compressive yield stresses of the different sediments were measured by varying excess pressure by means of the centrifugal field. It could be proofed that by pH changes vield stress of the sediment (dewaterability) can be optimized.

Dispersions of ceramic scintillating particles (Y_2O_3 : EU = red phosphor) are used for applying optical coatings, e.g. in order to enhance the light yield of lamps. Jens Adam, INM –Leibniz Institute for New Materials in Saarbrücken (Germany), focuses on the synthesis and subsequent dispersion of scintillating particles. For synthesis two different precipitation routes were applied, which led to particles in the range of 7...50/100 nm for the Conventional Basic Precipitation route (CBP, in the presence of NaOH or NH₄) and 86...326 nm for the urea based homogenous precipitation (UBHP, in the presence of urea). The particle size distribution (as a consequence of agglomeration) and the resulting luminous efficiency depend on the calcination temperature and dispersion method and solvent. Ultrasonification and soft milling that only breaks down agglomerates were considered as suitable dispersion methods. As quality criteria for the dispersion the ratio of dp₅₀/d_{SSA} was measured. The LUMiSizer proved to be fully applicable to characterize the dispersions of ceramic nano- and sub-um particles. Soft milling turned out to be an appropriate dispersion method for CBP particles calcined



at lower temperatures and UBHP particles. Monomodal dispersions with low ratio $d_{50}/d_{SSA} \le 1.8$ could be achieved.

Another application for preparing dispersions from ceramic particles was presented by Sonia Sales, member of the Instituto de Tecnología Cerámica (ITC) at the University Jaume I. Castellón (Spain). She and her colleagues produce ceramic inkjet inks for application in the industrial decoration of ceramic tiles. The tiles are thermally treated after printing. In contrast to organic pigments the colouring effect of ceramic pigments is weaker, thus the particle concentration in the ink must be increased. The quality of the printing strongly depends on the stability of the ink. Due to the high particle concentration, sedimentation and clogging may cause major operational problems like blockage of printer nozzles and changes in the ink viscosity. The LUMiFuge was applied to measure colloidal stability and sedimentation characteristics of 13 different ink formulations. For some of them transmission fingerprints revealed flocculation behaviour which were quantified by front tracking. In addition, viscosity profiles were measured. With centrifugal analysis even differences in the sedimentation behaviour of apparently stable inks were detected. Flocculated and non-flocculated ink samples could be clearly distinguished by the viscosity curves (as the flocculated samples showed pseudo plastic behaviour) and graphs of sedimentation velocity plotted versus relative centrifugal acceleration. Non-flocculated samples showed linear behaviour.

Characterization of highly concentrated dispersions

Prof. Giora Rytwo from Migal and TelHai College Academic Institutes, Israel, was the first to present results obtained with the new baby of LUM, the LUMiReader X-Ray. He investigated clay mineral suspensions for waste water treatment using the combination of LUMiSizer and LUMiReader X-Ray. In detail, two plate-like smectites (SHCa-1 hectorite and SWy-1 montmorrillonite) and acicular Paligorskites from the Negev desert were dispersed. Besides the shape the particles differed with respect to surface charges. The acicular mineral particles sedimented like a monodisperse material: Pictures of the cuvettes after accelerated separation in a LUMiSizer with an acceleration of 1800 g revealed a clearly visible boundary between a sediment layer and the clear supernatant From the thickness of the sediment layer and the volume concentration determined by X-ray transmission it could be seen that these sediments were not highly consolidated. The plate-like minerals showed completely different sedimentation behaviour. In visual inspection of the cells after accelerated separation the larger part of the cuvette volume was turbid due to suspended particles; furthermore a very thin layer of denser sediment could be seen on the bottom. X-ray analysis revealed that this sediment layer at the bottom was highly concentrated, while the larger turbid volume above contained only a low volume concentration of minerals.

Food emulsion stability

In food production, emulsifying processes lead to creamy products like mayonnaise and cream cheese. Very fine and stable dispersions of the fat/oil fraction in an aqueous phase result in a good mouth feeling.

Egg volk is well known to be an excellent natural emulsifier. It consists of a plasma fraction (EY-P), which is soluble in water and contains high fractions of low density lipoproteins (LDL), and a granule fraction (EY-G). Prof. Ulrich Kulozik and co-workers at the Chair for Food Process Engineering and Dairy Technology (Technical University of Munich), Germany, worked several years on the development of an industrial process to separate both fractions with the aim to utilize egg yolk in food production more efficiently. Thus the plasma fraction can be used as emulsifier and the granule fraction as stabilizer [4]. The two fractions were separately pre-treated by the enzyme phospholipase A2 in variable milieu conditions (pH and ionic strength). The determination of droplet sizes in the emulsions prepared from the plasma or the granule fraction under different pre-treatment conditions led to the conclusion, that pre-treatment and environmental conditions affect the emulsifying properties of LDL from plasma less than those of the granule fraction. With the latter, a clear influence of spray drying and enzymatic treatment on the contact angle and the oil droplet sizes could be verified. Nevertheless, SDS-Polyacrylamide gel electrophoresis provided effects in the interfacial composition of oil droplets with enzymatic pre-treatment of LDL. Results of LUMiFuge experiments obtained during accelerated separation within a time period of two hours at 1800 q (20 °C) confirmed the differences in the emulsifying behaviour of both yolk fractions.



With spreadable cream cheese, the envisaged soft texture is achieved by cutting cheese and cooking it with added water and emulsifying salt (ES). Wolfgang Hoffmann from the Department of Safety and Quality of Milk and Fish Products at Max Rubner-Institute in Kiel, Germany, described how emulsifying salt modifies casein by exchanging Ca-ions with Na-ions. Thus the emulsifying quality of the casein is significantly increased. The emulsion stability depends on the ES concentration (varied between 0.9% and 1.7%) the cutter speed, the cooking time and temperature. For the lowest ES concentrations an insufficient fat emulsification and a heterogeneous casein matrix could be detected by electron microscopy as well as by analytical photo centrifugation of the processed cheese at 60°C. With higher ES concentrations, higher cutting speeds and processing times, emulsions turned out to be stable at centrifugation conditions corresponding to more than 290 days under gravity. The effects of the considered processing parameters could clearly be distinguished by means of the instability index [5]. The analytical centrifugation showed effects like zone sedimentation, polydisperse creaming and sedimentation and turned out to be a sensitive tool for the characterization of emulsion stability.

Emulsion stability and particle characterization in cosmetics and nano-medicine

Structural lamellar emulsions are important for cosmetic products, because they exhibit similarity to the skin and thus active molecules are expected to penetrate well. Emeline Aviman from University of Barcelona, Spain, examined the stability of such emulsions with anti-aging properties, which had been prepared with different types and amounts of preserving agents. Traditionally used preserving agents like parabene, MIT, or formaldehyde-releasing systems are nowadays prohibited according to new EU regulations on safety of cosmetic products. New formulations are to be found that fulfil the requirements on effectiveness and shelf life. The stability analysis had to be carried out according to ISO/TR 13097 [6]. The considered cosmetic products consists of miscellaneous ingredients, among which are only few active substances like antioxidants or the anti-age component, the others are emulsifiers, moisteners, emollients and preserving agents. Destabilization alterations are common, like creaming, and coalescence of oil droplets, sedimentation and of and the degradation of a chemical property (pH, rancidity) may occur. The accelerated stability analysis by the LUMiSizer was compared to conventional analysis under the influence of gravity, and the centrifugal method clearly revealed the advantage of a fast delivery of results. With the LUMiSizer different standard operating procedures (SOP) were defined, corresponding to different storage times under gravity (1, 3 and 51 months) and carried out at different temperatures. In addition to the performed accelerated separation stability analysis [6], texture or chemical stability had to be assessed by corresponding established methods to get the whole picture of such complex formulations.

Magnetic nanoparticles that are covered with functional layers are in the focus of interest for biomedical applications, e.g. for gene delivery in the frame of cancer therapy [7]. Olga Mykhaylyk from University Clinic of Technical University Munich (Klinikum rechts der Isar, Germany) reported about optimization of polymer decoration of magnetic nanoparticles to form associated nucleic acids vectors for gene delivery under the influence of a magnetic field (Magneto infection) These viral complexes can be intravascularly administered and accumulated in target tissues by magnetic force Magnetization is an important feature of such nanoparticles but also magnetic liposomes, micro bubbles etc. Particle mobility under the influence of a magnetic force can be used to quantify it. Mobility was quantified by a customized LUMiReader PSA equipped with a set of NdFeB magnets positioned at variable distance and/or positions relative to the optical cell in a way that the parallel light beam passes the cell perpendicularly to the direction of the magnetic force. The influence of both gravity and magnetic field on the motions of magnetic objects can be analysed. Different magnetic fields and field gradients can be applied by strength and number of magnets, respectively, as well as their distance to the measuring sample. Magnetic mobility and mobility distributions were determined for a variety of magnetic nano- and micro particles, nanoparticle loaded cells, liposomes and micro bubbles as well as of viral magnetic vectors which amount from a few to nearly a thousand µm/s. At the Infectious Disease Research Institute (IDRI) in Seattle (USA) vaccine adjuvants are produced. As Chris Fox explained, adjuvants are added to vaccines to enhance or shape immune responses and thus result in an improved antigene efficacy. There exist various different types of adjuvants acting on cells according to different mechanisms (cf. [8]). Most widely used adjuvants are aluminium salt microparticles and oil-in-water nano-emulsions. Here two case studies were presented performed in real time by a LUMiReader. One was carried out with oil-in-water emulsions from squalene prepared with different phospholipids as emulsifiers. The curves of integral transmission over the separation time



provided clear differences between the different phospholipids and helped choosing the best suited alternative. In the second case study dispersion analysis was applied among other analytical devices to investigate the effect of the adsorption of TLR ligands (toll-like receptors) on aluminium hydroxide particles with respect to their sedimentation behaviour. Besides the type and concentration of the ligand the size of alumina particles was considered. Again, dispersion analysis was evaluated to be an important tool in a complementary suit of techniques for the stability analysis of adjuvant particulate formulations.

A new concept of data analysis for the LUMiSizer

The focus of the research by Johannes Walter, University of Erlangen-Nürnberg, Germany, is of on analytical ultracentrifuges (AUC). Analytical ultracentrifuges were designed to characterize macromolecules, like proteins, about 100 years ago. The rotor operates in a vacuum chamber at very high gravity and sedimentation distance is a few millimetres. The point sensor scans the sample along its height. Despite these operational differences between AUC and the analytical centrifuges (AC) produced by LUM, Walter suggested transferring the principle of data analysis from AUC to the LUMiSizer. Compared to traditional data analysis for AC, AUC utilize the so-called direct boundary model to fit the experimental transmission profiles. It s allows for meniscus correction and improves the signal-to-noise-ratio. Even optical artefacts (scratches) of the measuring cell and hydrodynamic instabilities may be detected. It was reported that experimental transmission profiles obtained by STEP-technology of LUM (no scanning sensor) can be loaded into AUC software and analysed correspondingly. In this case, particle sedimentation velocity is characterized (expressed) by a sedimentation coefficient (often expressed in Svedbergs). It was shown that sedimentation coefficients and particle size derived from it obtained from AC data equal results from AUC experiments for different micro- and nano-particles.

Young Scientist Award: finalists' presentations

A highlight of the 2015 workshop was again the session of LUM Young Scientist Award finalists. Four candidates were nominated and invited to present their recent scientific achievements. Nomination was based on evaluation of application report and workshop abstract, letter of recommendation of supervisor as well as list of papers and talks, CV. Year 2015 nominees were David J. Growney (Supervisor Prof. St. P. Armes), Mads K. Jorgensen (Supervisor Prof. M.L. Christensen), Johannes Knoll (Supervisor Prof. H. Nirschl), Jos van Rijssel (Supervisor Prof. B.H. Erné). David J. Growney (Department of Chemistry, University of Sheffield, UK) reported on stabilizing carbon black dispersions in unipolar solvents by adding diblock copolymers [9]. These copolymers are adsorbed to the particle surface, either as single molecules or as micelles. Size and also average density of dispersed particles are shifted due to thickness and density of outer polymer layer on the particles. It was in detail elaborated how to determine Stokes relevant particle density by LUMiSizer technique. In addition the density of the outer polymer layer was calculated by means of a core shell model. It was proofed that accurate particle size distribution is obtained by using the effective particle density and analytical photo centrifugation becomes the preferred technique for characterization of non-aqueous dispersions. In addition, LUMiSizer results were reported, that the stabilizing effect of diblock polymer depends on polymer concentration. It acts as an effective flocculant at low copolymer concentration and as steric stabilizer above a critical concentration. Among other things, it was shown that the polymer concentration that marks the boundary between the two mechanisms, is nearly temperature independent, but varies with the chosen solvent.

Mads Koustrup Jorgensen (Department of Biotechnology, Aalborg University, Denmark), studied the compressibility and swelling characteristics of filter cakes by analytical centrifugation. The aim of the study was to develop methodology of analytical centrifugation further and to monitor cake height at different compressive pressures. Cake compression and swelling (relaxation of the compressed cake) was induced by gradually increasing and subsequently gradually decreasing the rotational speed of the LUMiSizer. Suspensions and sludges of different origin were studied. Sediments of inorganic colloids (anatase) had a low compressibility and on the other hand, sludge cakes had a high compressibility and exhibit a high degree of compression reversibility. Reversibility was also observed for particles consisting of a styrene core and hydrated PAA shells of two different thicknesses (100 k Da and 250 kDa), where higher compressibility of sediments was found with the 250 kDa particle



suspensions. By comparing different sludge samples from municipal and industrial waste water treatment plants with and with no membranes a correlation between filter cake solidosity and compressibility on the one-hand and suspension physical-chemical properties and filtration properties on the other hand was stated. In general, reversibility of compression was inversely proportional to the height of the cake. Johannes Knoll (Institute of Mechanical Engineering and Mechanics, Karlsruhe Institute of Technology (KIT), Germany) investigated the adhesion forces of functionalized magnetic particles by means of a LUMiFuge. At KIT a process for recovering target proteins from fermentation broths was developed, in which the protein is adsorbed on functionalized magnetic particles, and these particles are separated from the broth by means of a special centrifuge with a magnetic collector device. After this, the loaded particles must be completely removed from the collector device; this is why the clear determination of adhesion forces is important. The nominee developed a measuring procedure to quantify adhesion forces by analytical centrifugation. A special cell holder was used, which allows to place a permanent magnet below the sample cell. The procedure comprises as a first step the building up a sediment of the particles by centrifugation. In a second step the cell with the cell is turned by 180°, placed back on the rotor and subsequently the initial thickness of the "inverted sediment" measured at low RPM. Thereafter the rotor speed is stepwise increased. If centrifugal force acting on a particle overcomes adhesion force, the particle "dissolves" from sediment surface and settles to the cell bottom. Decrease of sediment height is measured in situ during centrifugation by the optical measuring sensor system of LUMiFuge. In this way particle-particle adhesion force distribution was determined. The centrifugal field can be superimposed by a magnetic field with different magnets and magnetization of magnet particles probed. It was shown that particle-substrate interaction depends on surface roughness, magnetic field, particle magnetization and surface decoration with proteins. Particle adhesion forces determined by analytical centrifugation were in the range from some tenth pN to some hundred nN.

The 2015 LUM Young Scientist Award winner was Jos van Rijssel (Van't-Hoff Laboratory of Physical and Colloid Chemistry, Utrecht University, The Netherland). He was awarded with the prize for his achievement to determine the strength of colloidal interactions between monodisperse nanoparticles (second virial coefficient of osmotic pressure) exploring sedimentation equilibrium concentration profiles. Suspensions of PbSe quantum dots of 4 to 10 nm size dispersed in decalin were used at volume concentrations of 2 % – 15 % v/v. To achieve equilibrium between sedimentation and back-diffusion, uninterrupted prolonged operation (up to 100 days) of a LUMiSizer was performed and local concentrations were determined based on transmission measurements across ultrathin cells of an optical path of 50 μ m. In short, the concentration of the equilibrium profile can be derived from the transmission profiles and, on the other hand, the osmotic pressure as function of state was constructed and the value of the second osmotic virial coefficient extracted by fitting the equation of state [10]. It was found that the second virial coefficient decreases with increasing quantum dot diameter. Additional Cry-TEM and SAXS experiments support results regarding thermodynamic properties of nanoparticle systems by analytical centrifugation.

Practical Course: Particle characterization

A practical course on the basics of particle characterisation, on how to measure droplet and particle size distributions by means of STEP technology, on the existing limitations of the measuring procedure and the opportunities to evaluate the data was given in three languages (German, English and Spanish). The principle of STEP technology was explained as well as how to establish a Standard Operational Procedure (SOP) for unknown samples. The different opportunities of relative and absolute analysis tools were explained by means of various examples.

Invitation to the next workshop 2016

All colleagues in the community are kindly invited to join the upcoming International Workshop Dispersion Analysis & Materials Testing 2016 in Berlin, Germany, from 26-27 September 2016. LUM instrument users please note the CALL FOR PAPERS 2016, young researchers are invited to apply for LUM Young Scientist Award 2016. Follow us on <u>www.dispersion-letters.com</u> for more details.



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