The use of confocal laser scanning microscopy (CLSM) to quantify microcapsule film coating quality

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Introduction

As final microcapsule functionality is primarily dependent on the quality of the microcapsule coating, quantitative assessment of the film coating thickness, homogeneity and imperfections is of utmost importance. The external and internal structure of microcapsules is often characterised by optical, scanning electron, transmission electron and atomic force microscopy. None of these methods, however, allow a quantitative determination of coating quality of small microcapsules (< 250 µm) with very thin (< 5 µm) coatings.

Our objective was to develop a protocol for determining film coating thickness and homogeneity, based on the use of a novel technique, confocal laser scanning microscopy (CLSM).

Materials and methods

<u>CLSM</u>. The key feature of confocal microscopy is the imaging aperture or pinhole, which is responsible for the majority of advantages of CLSM over other microscopy techniques (Ferrando, 2000). Using the pinhole, fluorescent light emitted from outside the focal region is excluded from forming the image; only in-focus light is transmitted to the photomultiplier. This is schematically illustrated in Figure 1.
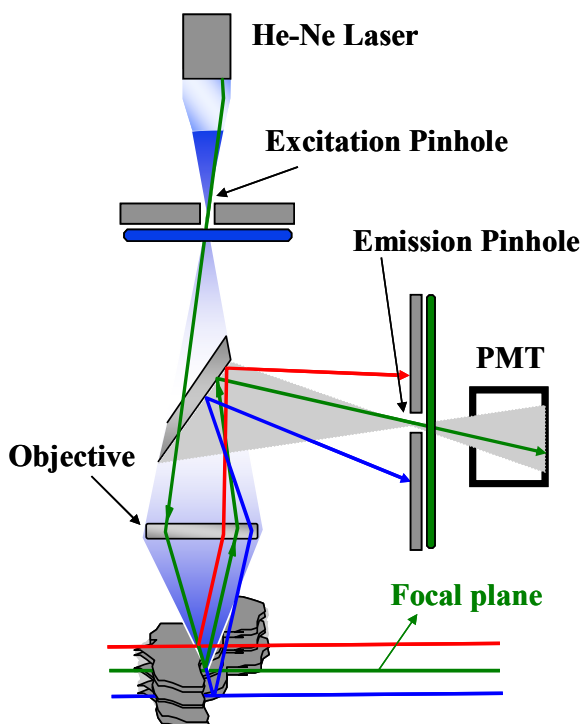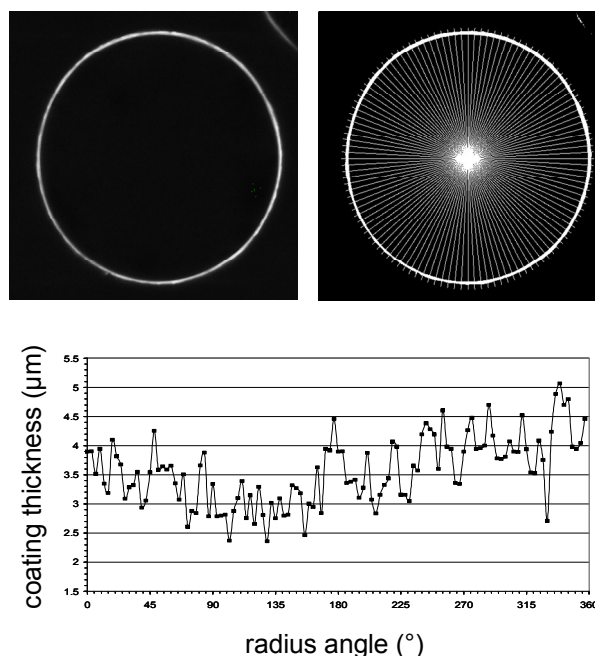

Fig. 1. CLSM principle.

Fig. 2. CLSM protocol: raw and segmented image and derived coating thickness distribution.

In fluorescence microscopy, the coating can be distinguished from the core particle using a fluorochrome. In the example shown in Figure 2, the coating material is labelled fluorescently before it is applied onto the (non-labelled) core particles. As a result, the regions visualised by CLSM originate from the coating only. Through the use of CLSM, the operator is allowed to optically section the microparticle at any desired plane. Figure 2 summarises the image processing steps for a single microcapsule focussing at the equatorial plane of the particle. After reducing the background noise, the original raw CLSM image was segmented and a coating thickness distribution of each single microcapsule was obtained. From this, a number of coating distribution parameters can be obtained, e.g.,

- The average, as a measure of coating content;
- The standard deviation, as a measure of coating variability and thus quality;
- The minimal recorded value, as a measure of the occurrence of imperfections.

Microcapsule production. Top- and bottom-spray fluidised bed coating were applied to microencapsulate glass beads with an average particle diameter of 204 µm. In all experiments, 750 g of core particles were processed in a Glatt GPCG-1 fluidised bed. These core particles were coated by atomising 20 g of coating dry matter, which was sodium caseinate labelled with Rhodamine B. More information on the equipment and process specifications is given elsewhere (Depypere, 2005).

Case study: influence of the spray nozzle position in fluidised bed processes on the coating quality. Keeping all other fluidised bed process parameters constant, it was investigated to which extent the position of the spray nozzle relative to the powder bed influenced the amount of coating deposited on the core particles and the coating quality. It is well known that, in order to minimise process losses and achieve coating reproducibility, both the evaporation speed of the coating liquid and the actual physical state of the droplets are highly determinant. Therefore, controlling the distance that droplets have to travel before impinging onto a particle is of major importance (Jones, 1994). In this study, four different spray nozzle positions were tested, whereby three tests were performed in the top-spray configuration and a fourth in the bottom-spray configuration. In the top-spray configuration, the nozzle could be installed at three different heights, referred to as top low (TL), top middle (TM) and top high (TH). For these nozzle positions, the distance between the distributor and the downwards facing nozzle tip was 121, 168 and 270 mm, respectively.

Results and Discussion

In Figure 3, the microcapsules obtained in the different fluidised bed coating experiments are illustrated. Typical SEM images as well as corresponding CLSM images are shown for each experiment.

In Table 1, the statistics of the coating thickness distributions, obtained by CLSM, are reported. In addition to a value for mean coating thickness, values for mean heterogeneity as an indicator of non-uniformity of the coating thickness and mean values for the smallest thickness observed per particle are reported. Both the coating thickness variability between particles from a same experiment (inter-particle heterogeneity) and the variability within individual particles (intra-particle heterogeneity) are given. The data reported in Table 1 are based on 50 microcapsules per experiment. It was found that analysis results of 50 beads were representative of a much larger population of microcapsules obtained in the fluidised bed.
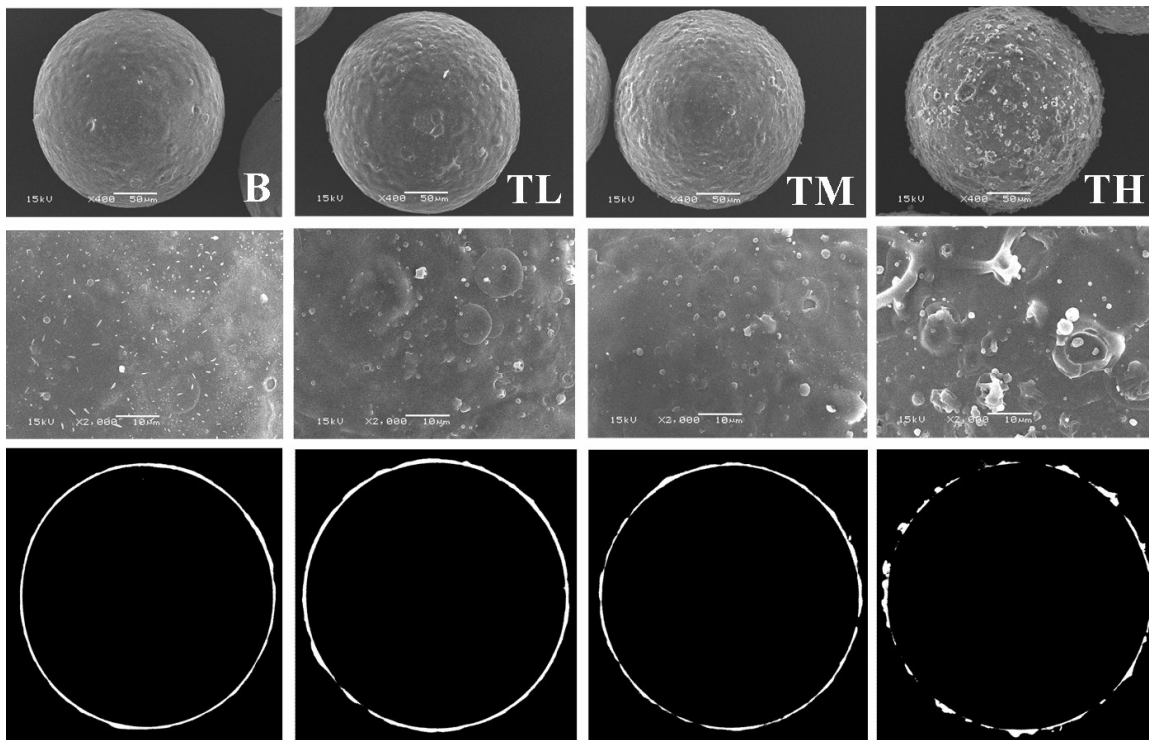
Fig. 3. Typical SEM and CLSM (bottom row) images of microcapsules from the bottom-(B) and top-spray (TL,TM,TH) fluidised bed experiments

Experiment	Bottom (B)	Top low (TL)	Top mid (TM)	Top high (TH)
Mean	2.64	2.59	2.37	1.76
Heterogeneity	0.70	0.88	0.94	1.58
intra-particle	0.62	0.82	0.86	1.57
inter-particle	0.32	0.33	0.37	0.23
Minimum	0.86	0.19	0.05	0

Table 1. Quantification of coating thickness and quality by CLSM (average values in µm).

With respect to the data for average (mean) coating thickness in Table 1, it can be seen that less coating material was retrieved on the core surface when the distance between the nozzle tip and the core particles was increased. Spray-drying effects are known to cause coating losses when the distance the coating droplets have to travel, is not minimised (Smith, 1983). Furthermore, CLSM allowed to quantify that with increasing distance between the nozzle tip and the powder bed, the quality of the resulting coating became poorer. The variability between particles (inter-particle heterogeneity) was quite smaller than the variability within individual particles (intra-particle heterogeneity). This is indicative of the fact that in the end, a similar amount of coating material was deposited onto each particle, but that the distribution of the coating material over the particle surface was not so homogeneous. The results in Table 1 also indicate that more and more coating imperfections occur when the distance between the nozzle and the powder bed is increased.

When the CLSM images and data are compared with classical SEM images of the coating surface (Figure 3), it can be confirmed from the latter that the coating quality was superior for the bottom-spray experiment and worsened with increasing distance between the top-spray nozzle and the powder bed. For the bottom-spray experiment, a dense homogeneous coating containing very few dispersed material was observed. In contrast, for the microcapsules obtained via top-spray coating, more coating deficiencies were detected. Whereas a relatively good coating was still obtained when

the nozzle was in the lowest available position, a very bad coating, showing deep craters and high amounts of spray-dried material in the film coating, was typically observed for microcapsules from the top-spray experiment with the nozzle in the highest position.

Adaptation of the CLSM protocol to non-spherical particles
The originally developed CLSM protocol for the quantification of the microcapsule coating thickness and quality was applicable to small-sized (< 250 µm) spherical non-porous particles, only. While the above described CLSM protocol involved image analysis, the newly developed protocol additionally involved the analysis of the pixel database of a CLSM image. A vector method was developed, which allowed to measure coating thickness perpendicular to the core particle surface. This is illustrated in Figure 4 where CLSM images of larger glass beads and salt crystals, both coated with Rhodamine B labelled sodium caseinate, are shown.
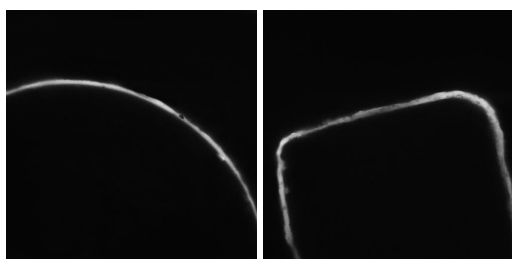

Fig. 4. CLSM images of coated glass beads (d_{43} > 300 µm) and salt crystals.

Conclusions

In this research, confocal laser scanning microscopy (CLSM) was found to be an adequate non-destructive technique for the quantification of the coating thickness and quality of thin-coated small inert spherical particles. Combined with image analysis, it was found to be possible to relatively quickly quantify the microcapsule quality with high accuracy, provided the coating material could be labelled fluorescently.

Compared to other techniques where a single value for coating thickness as an average for the whole sample population is obtained, CLSM generates a coating thickness distribution for each individual particle. It is clear that in this distribution, much more information such as coating thickness heterogeneity, coating deficiencies, etc., is contained.

The originally developed CLSM protocol showed to be valuable in the quantification of coating thickness and quality of inert spherical particles. As it is clear that food particles are far from perfect spheres without porosity, steps have been taken to adapt the methodology for particles of all shapes and sizes, as well as for porous particles. The ultimate research goal is the development of an integrated coating quality evaluation system based on CLSM visualisation.

References

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