

## Microencapsulation Process Analysis Using FlowCAM®

### Objective

Microencapsulation is a pervasively used technique for delivering particles in a wide range of applications, ranging from pharmaceuticals to foods to detergents. Microencapsulation is a process by which small amounts of a substance (active ingredient) are packaged inside a second substance in order to shield the active ingredient from the surrounding environment.

The first large-scale application of microencapsulation dates back to the 1950's, when National Cash Register (NCR) used complex coacervation to produce carbonless copy paper. However, despite the long history and myriad uses of microencapsulation, the technique remains both a science *and* an art due to the fact that each microencapsulation application can require new materials and methods to be used. Because of this, the ability to study microencapsulation at a microscopic level is critical for the understanding and development of new processes.

Imaging particle analysis using FlowCAM® offers an ability to gain unique insights into the microencapsulation process by being able to dynamically monitor capsule formation over time while studying the effects of temperature, concentration, pH and other variables that affect the process. Complex coacervation, whereby microcapsules are formed by combining two hydrocolloids to produce a shell around droplets of the active ingredient (usually in an emulsion) is a common technique which can greatly benefit from FlowCAM monitoring. This is a complex process, and the insight from FlowCAM can be invaluable (Figure 1).

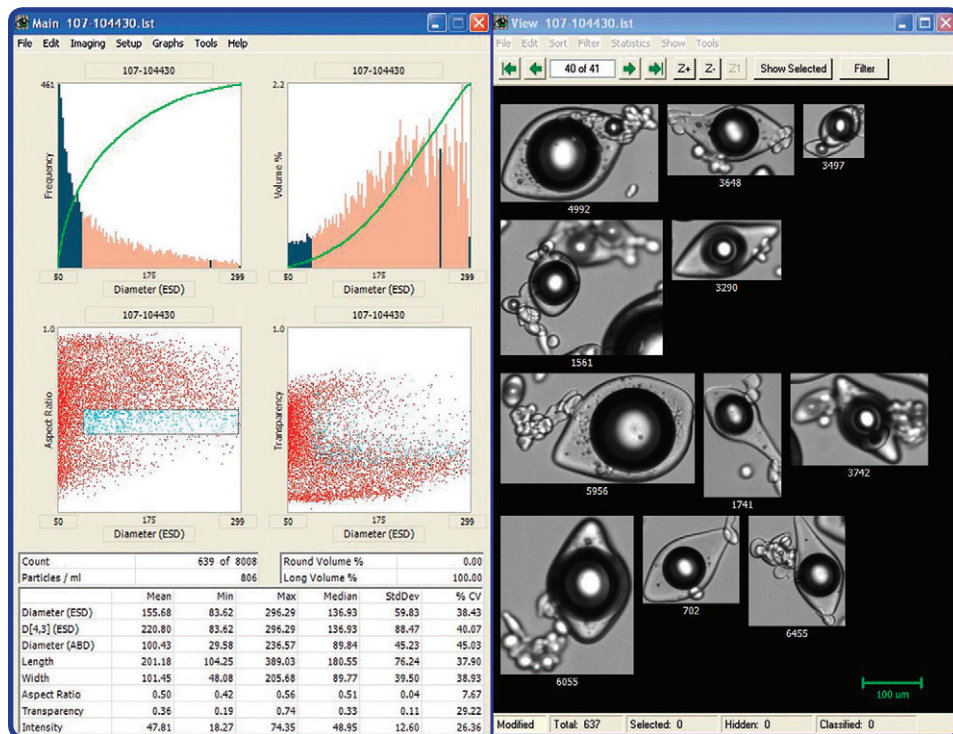


Figure 1: Screenshot of FlowCAM analysis of coacervates

### Method

FlowCAM was used to monitor coacervate formation in a test vat over a period of time as the sample cooled under constant agitation. The sample was being pumped up into the FlowCAM directly from the reaction vessel in a continuous fashion.

The view of the flow cell was continuously monitored by eye, and data runs were collected every 15-30 minutes or when a significant change was observed by eye. The expected size range for the coacervates was in the 80µm to 140µm diameter range, so the instrument was set up with the 4X objective (approximately 40X overall magnification) and a 300µm (depth) flow cell. Measurements were also made during each run for correlating temperature and pH data to the particle images.

### Results and Conclusions

Figures 2 through 5 show particle images obtained through the FlowCAM for different times during the experiment. From visual examination only, it seems fairly apparent the run done at  $t_0 + 39$  minutes shows the most clean coacervate formation. After this, the particles began to agglomerate as more gelatin began to attach itself to the capsules.

To check the qualitative observations made, the VisualSpreadsheet™ software was used to perform a statistical pattern recognition on each run, in order to quantify the number of coacervates found in each run. To do this, the first step was to build an image “library” containing coacervate particles of varying size and orientation. The library built is shown in Figure 6.

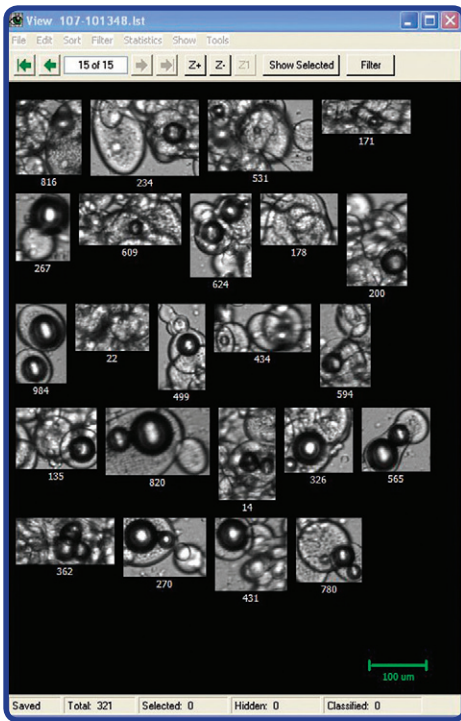


Figure 2: Coacervate Images  
 $T = t_0 + 9$  minutes  
 Active ingredients are the dark circles

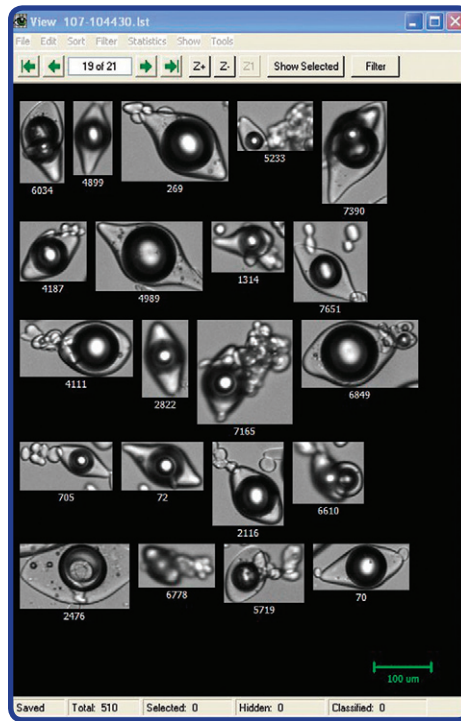


Figure 4: Coacervate Images  
 $T = t_0 + 39$  minutes  
 Coacervates are fully formed

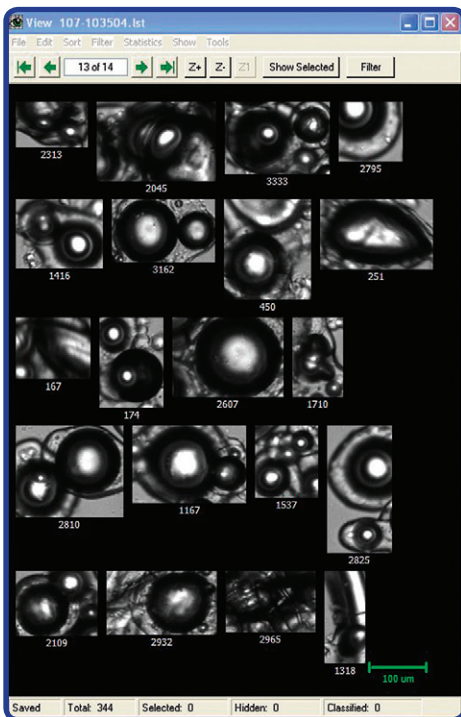


Figure 3: Coacervate Images  
 $T = t_0 + 30$  minutes  
 Gelatin casing begins to form around active ingredients

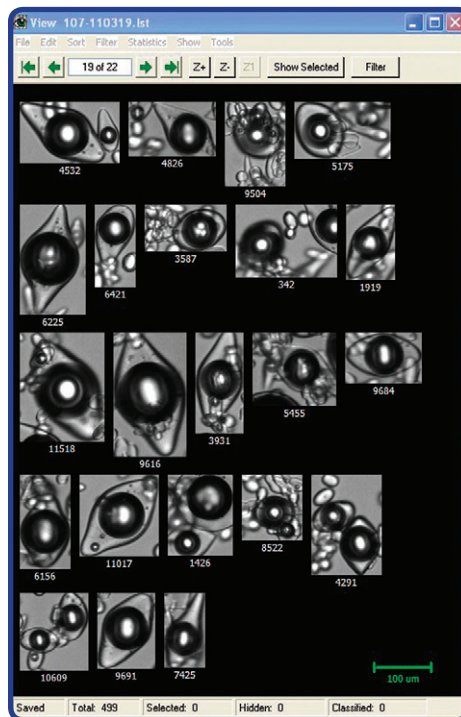


Figure 5: Coacervate Images  
 $T = t_0 + 58$  minutes  
 Coacervates still visible, but agglomeration beginning to occur

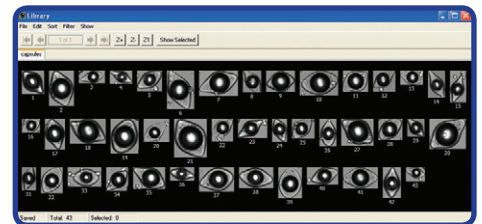


Figure 6: Coacervate Images  
 Stored in "library" for statistical pattern recognition

The results of the statistical pattern recognition operation are shown in a table in Figure 7. These results give quantitative confirmation of the earlier subjective results: namely that at  $t_0 + 39$  minutes the most clean coacervates have been formed. After this point, the gelatin begins to attach itself to the capsule walls, causing agglomeration, and eventually disintegration, of the coacervates.

Time	Number of particles matched to Library	Percent Matched
$t_0$	0	0%
$t_0 + 9$ min	0	0%
$t_0 + 30$ min	0	0%
$t_0 + 39$ min	1,199	15%
$t_0 + 58$ min	895	8%
$t_0 + 83$ min	572	7%

Figure 7: Table showing matching statistics after statistical pattern recognition is run against coacervate library

In summary, the FlowCAM yields tremendous insight into the process of coacervate formation, and can be an indispensable tool for microencapsulation R&D and QC applications.