

## High throughput workflow for coacervate formation and characterization in shampoo systems

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### Synopsis

Cationic cellulosic polymers find wide utility as benefit agents in shampoo. Deposition of these polymers onto hair has been shown to mend split-ends, improve appearance and wet combing, as well as provide controlled delivery of insoluble actives. The deposition is thought to be enhanced by the formation of a polymer/surfactant complex that phase-separates from the bulk solution upon dilution. A standard characterization method has been developed to characterize the coacervate formation upon dilution, but the test is time and material prohibitive.

We have developed a semi-automated high throughput workflow to characterize the coacervate-forming behavior of different shampoo formulations. A procedure that allows testing of real use shampoo dilutions without first formulating a complete shampoo was identified. This procedure was adapted to a Tecan liquid handler by optimizing the parameters for liquid dispensing as well as for mixing. The high throughput workflow enabled preparation and testing of hundreds of formulations with different types and levels of cationic cellulosic polymers and surfactants, and for each formulation a haze diagram was constructed. Optimal formulations and their dilutions that give substantial coacervate formation (determined by haze measurements) were identified. Results from this high throughput workflow were shown to reproduce standard haze and bench-top turbidity measurements, and this workflow has the advantages of using less material and allowing more variables to be tested with significant time savings.

### BACKGROUND

Coacervation is a critical phenomenon in shampoo applications (1). In shampoo formulations with polycationic polymers and anionic surfactants, different types of polymer/surfactant aggregates can form depending on concentration of a formulation (2). In formulations with high concentration of surfactants (above the surfactant CMC), the anionic micelles shield electrostatically the cationic polymer and it is solubilized. Upon dilution (~10-fold) of the shampoo formulations, two types of complex polymer/

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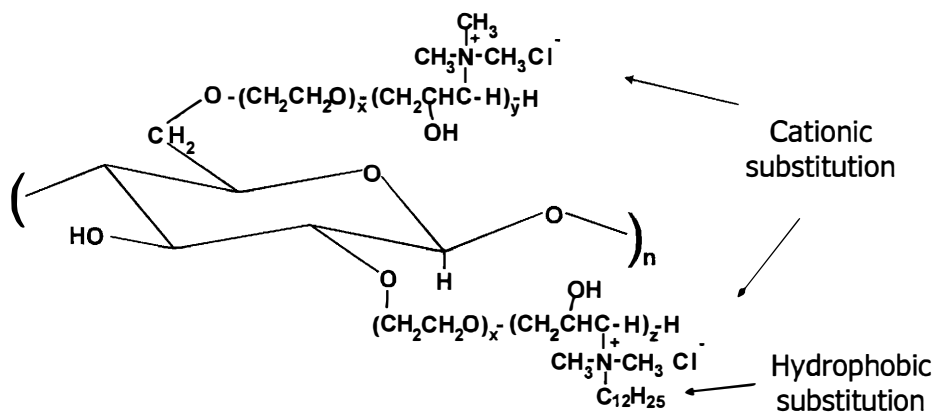


Figure 1. DOW SoftCAT™ polymers.

surfactant structures can form: a coacervate phase rich in polycation molecules and a co-phase that is lean in polycation molecules. The resulting coacervates have low solubility in water, resulting in increased haze of formulations (3). The profile of a coacervate haze curve is characteristic of a polymer type and indicative of properties imparted to the hair. Polymers with high cationic charge give coacervation at lower dilution and over a narrow range of dilutions, resulting in gel-like coacervates. Polymers with low cationic charge give coacervation at higher dilution and over a broader range of dilutions, resulting in liquid-like coacervates (3). Coacervates can effectively deposit on the hair surface, providing multiple benefits in the hair care: reduction of combing friction of hair, mending of split-ends, controlled delivery of insoluble actives, improved feel and appearance, etc. Gel-like coacervates provide more hair body, while liquid-like coacervates give soft feel and volume enhancement of hair (3).

The objective of this work was to study coacervation in shampoo formulations prepared with cationic cellulosic polymers and a variety of surfactants, using haze as an indicator of coacervation. Evaluation of multiple composition variables required preparation and evaluation of a large number of samples (>10,000), and with standard (bench-top) methods this would be a tedious, time-consuming work. Therefore, in our approach, a high throughput workflow was selected, with a Tecan liquid handler used for preparation of samples and a nephelometer used for measurements of the haze index.

## MATERIALS

Four DOW SoftCAT™ SL cellulosic cationic polymers (4) were evaluated in this study: SL-5, SL-30, SL-60, and SL-100 (Figure 1).

All four SL polymers have trimethylammonium and dimethyldodecyl-ammonium groups, with the degree of cationic substitution fixed at ~0.2. The numbers in code names of SL polymers correspond to the degree of hydrophobic substitution, with SL-100 having the highest level of hydrophobe. However, in all four polymers the level

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of hydrophobic substitution is low, providing hydrophobic character similar to that of the PQ-10 polymer (UCARE™ LR-30M). Solutions of those polymers in water at 1 wt % level have viscosities of 2500–2800 cP (4).

Ionic surfactants ALSCOAP\* NS-230 [sodium lauryl ether (laureth) sulfate, 2 mol; SLES-2 mol;  $\text{H}(\text{CH}_2)_{12}-(\text{OCH}_2\text{CH}_2)_2-\text{O}-\text{SO}_3^- \text{Na}^+$ ], ALSCOAP\* TH-330 [sodium lauryl ether sulfate, 3 mol; SLES-3 mol;  $\text{H}(\text{CH}_2)_{12}-(\text{OCH}_2\text{CH}_2)_3-\text{O}-\text{SO}_3^- \text{Na}^+$ ], OBAZORINE\* CAB-30 (cocoamido-propyl betaine; CAPB;  $\text{H}(\text{CH}_2)_n-\text{CO}-\text{NH}-(\text{CH}_2)_3-\text{N}^+(\text{CH}_3)_2-\text{CH}_2-\text{CO}_2^-$ ), NEOSCOAP\* CN-30-SF (methylcocoyletaurate–desalinated), OBAZORINE\* 662N (sodium cocoamphoacetate–non-desalinated), and OBAZORINE\* 662N-SF (sodium cocoamphoacetate–desalinated) were obtained from TOHO Chemical Industry Co., Ltd.

## METHODS

A Tecan 200 liquid handler (Tecan Systems, Inc.) was used to make formulations in 96-well micro-titer plates. The Tecan is fitted with a robotic arm with eight syringe tips, and each pipette is controlled by an individual syringe drive. Haze values were measured using a Nepheloskan Ascent (Thermo Labsystems) with an integration time of 20 ms, and the output data were in relative nephelometer units (RNU). The light scattering of each cell in the microtiter plate was corrected by subtracting the background reading for an empty plate. For 3D visualization of data, Miner 3D software from Tableau Software was used.

*Preparation of formulations.* Each formulation was prepared with one of the SL polymers, one of the SLES surfactants (2 mol or 3 mol), CAPB, and optionally with one of the remaining surfactants labeled as “surfactant 3” (CN-30-SF, 662N-Sf, or 662N). In formulations with the highest surfactant + polymer concentration (at dilution 1) the level of polymer was either 0.20 wt % or 0.40 wt %, the level of SLES was 6.00 wt %, 8.00 wt %, or 10.00 wt %, the level of CAPB was from 1.25 wt % to 5.00 wt %, while the level of “surfactant 3” was from 0 to 3.75 wt %. The maximum level of CAPB and “surfactant 3” was limited to 5.00 wt %, while the total level of surfactants was limited to 15.00 wt %. Sixteen “surfactant packages” with different combinations of surfactants were chosen for this study (Table I).

In our high throughput workflow water was placed in a source trough, while polymer and surfactant solutions were placed in mother plates. Those components were added to destination plates (in 8×8 arrangement) to form formulations with the desired composition. Figure 2 shows schematically the composition of 64 formulations in a destination plate prepared at dilution 1.

All formulations were prepared in 800 mg amounts. In the preparation of formulations shown in the Figure 2, 160 mg of 1.0 wt % aqueous solution of SL-5 was added into eight vials in column 1, and therefore final formulations had 0.2 wt % of this polymer. The same amount of 1.0 wt % solutions of polymers SL-30, SL-60, and SL-100 was added into the vials in columns 2, 3, and 4, respectively. The same procedure was repeated for the vials in columns 5 through 8, but in this case 320 mg of 1.0 wt %

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Table I  
Surfactant Levels in Formulations at Highest Concentration (Dilution 1)

Surf pack <sup>a</sup>	The surfactant level (wt %)				
	SLES <sup>b</sup>	CAPB	S3 <sup>c</sup>	CAPB+S3	Total
1	10.00	5.00	0.00	5.00	15.00
2	10.00	3.75	1.25	5.00	15.00
3	10.00	2.50	1.25	3.75	13.75
4	10.00	2.50	0.00	2.50	12.50
5	10.00	1.25	1.25	2.50	12.50
6	10.00	1.25	0.00	1.25	11.25
7	8.00	5.00	0.00	5.00	13.00
8	8.00	3.75	1.25	5.00	13.00
9	8.00	2.50	2.50	5.00	13.00
10	8.00	1.25	3.75	5.00	13.00
11	8.00	3.75	0.00	3.75	11.75
12	8.00	2.50	1.25	3.75	11.75
13	6.00	5.00	0.00	5.00	11.00
14	6.00	3.75	1.25	5.00	11.00
15	6.00	2.50	2.5	5.00	11.00
16	6.00	1.25	3.75	5.00	11.00

<sup>a</sup> Surfactant packages.  
<sup>b</sup> SLES-2 mol or SLES-3 mol.  
<sup>c</sup> The “surfactant 3” (CN-30-SF, 662N-Sf, or 662N).

	1	2	3	4	5	6	7	8
A	SL-5, 0.2% Surf Pack 1	SL-30, 0.2% Surf Pack 1	SL-60, 0.2% Surf Pack 1	SL-100, 0.2% Surf Pack 1	SL-5, 0.4% Surf Pack 1	SL-30, 0.4% Surf Pack 1	SL-60, 0.4% Surf Pack 1	SL-100, 0.4% Surf Pack 1
B	SL-5, 0.2% Surf Pack 2	SL-30, 0.2% Surf Pack 2	SL-60, 0.2% Surf Pack 2	SL-100, 0.2% Surf Pack 2	SL-5, 0.4% Surf Pack 2	SL-30, 0.4% Surf Pack 2	SL-60, 0.4% Surf Pack 2	SL-100, 0.4% Surf Pack 2
C	SL-5, 0.2% Surf Pack 3	SL-30, 0.2% Surf Pack 3	SL-60, 0.2% Surf Pack 3	SL-100, 0.2% Surf Pack 3	SL-5, 0.4% Surf Pack 3	SL-30, 0.4% Surf Pack 3	SL-60, 0.4% Surf Pack 3	SL-100, 0.4% Surf Pack 3
D	SL-5, 0.2% Surf Pack 4	SL-30, 0.2% Surf Pack 4	SL-60, 0.2% Surf Pack 4	SL-100, 0.2% Surf Pack 4	SL-5, 0.4% Surf Pack 4	SL-30, 0.4% Surf Pack 4	SL-60, 0.4% Surf Pack 4	SL-100, 0.4% Surf Pack 4
E	SL-5, 0.2% Surf Pack 5	SL-30, 0.2% Surf Pack 5	SL-60, 0.2% Surf Pack 5	SL-100, 0.2% Surf Pack 5	SL-5, 0.4% Surf Pack 5	SL-30, 0.4% Surf Pack 5	SL-60, 0.4% Surf Pack 5	SL-100, 0.4% Surf Pack 5
F	SL-5, 0.2% Surf Pack 6	SL-30, 0.2% Surf Pack 6	SL-60, 0.2% Surf Pack 6	SL-100, 0.2% Surf Pack 6	SL-5, 0.4% Surf Pack 6	SL-30, 0.4% Surf Pack 6	SL-60, 0.4% Surf Pack 6	SL-100, 0.4% Surf Pack 6
G	SL-5, 0.2% Surf Pack 7	SL-30, 0.2% Surf Pack 7	SL-60, 0.2% Surf Pack 7	SL-100, 0.2% Surf Pack 7	SL-5, 0.4% Surf Pack 7	SL-30, 0.4% Surf Pack 7	SL-60, 0.4% Surf Pack 7	SL-100, 0.4% Surf Pack 7
H	SL-5, 0.2% Surf Pack 8	SL-30, 0.2% Surf Pack 8	SL-60, 0.2% Surf Pack 8	SL-100, 0.2% Surf Pack 8	SL-5, 0.4% Surf Pack 8	SL-30, 0.4% Surf Pack 8	SL-60, 0.4% Surf Pack 8	SL-100, 0.4% Surf Pack 8

Figure 2. Schematic representation of a destination plate with formulations at dilution 1.

aqueous solution of SL polymer was added, and final formulations in those vials had 0.4 wt % of polymer. Surfactants were added into eight vials in the row A in amounts that gave formulations with final concentrations required for surfactant package 1 (10.0 wt

% SLES and 5.0 wt % CAPB; see Table I). Similarly, surfactants were added into vials in the rows B through H in amounts that gave formulations with final concentrations required for surfactant packages 2 through 8. Water was added in amounts required to bring the total formulation amount to 800 mg. The same procedure was used to prepare a second destination plate with another set of 64 formulations at dilution 1. In that case the same polymers in the same amounts were used as in the first plate, but surfactants were added in amounts that gave formulations with final concentrations required for surfactant packages 9 through 16. Therefore, one set of two plates with all sixteen surfactant packages contains 128 formulations at dilution 1. Two SLES surfactants (SLES-2 mol and SLES-3 mol) were evaluated in this study, and with each of them three sets of surfactant packages were prepared with three different "surfactants 3" (CN-30-SF, 662N-Sf, or 662N). This increased the number of plates by a factor of six.

In order to generate coacervation curves, formulations were prepared over a broad range of dilution levels. There are two different approaches to the preparation of diluted formulations for coacervation study. In a standard, "bench-top" approach, a set of formulations at the highest concentration of components (dilution 1) is prepared first. Those formulations are then diluted by mixing with water at different ratios. However, in our study the viscosity of formulations at dilution 1 was too high for the desired precision in automated transfer of aliquots into another set of vials (where water would be added). Therefore, an alternative approach was used where each formulation was independently prepared by mixing polymer solutions, surfactant solutions, and water at reduced levels relative to dilution 1. For example, in preparation of formulations at dilution 2 concentrations of a polymer and surfactants were reduced to one-half relative to their concentration at dilution 1. Those formulations were prepared by adding polymer and surfactants in amounts that were 50% of their amounts in dilution 1, and the amount of added water was increased to keep the total amount of formulations at 800 mg level. The same approach was used to prepare formulations at six additional dilutions: 4, 6, 8, 10, 13, and 20 (Table II).

Preparation of formulations at those eight dilutions increased the number of plates by factor of eight. Therefore, the number of formulations is 128 (in one set of two plates)  $\times$  6 (a factor for different SLES and "surfactant 3" types)  $\times$  8 (for eight dilutions) = 6,144. All formulations were prepared in duplicates, increasing the number of formu-

**Table II**  
Composition of Formulations at Different Dilutions

Dilution	(P+S)/Add water ratio <sup>a</sup>	P+S <sup>a</sup> (wt %)	Add water <sup>b</sup> (wt %)
1	1/0	100.0	0.0
2	1/1	50.0	50.0
4	1/3	25.0	75.0
6	1/5	16.7	83.3
8	1/7	12.5	87.5
10	1/9	10.0	90.0
13	1/12	7.7	92.3
20	1/19	5.0	95.0

<sup>a</sup> P+S = The amount of polymer and surfactants relative to the level at dilution 1.

<sup>b</sup> Add water = The amount of additional water above the level at dilution 1.

lations to 12,286. In case of surfactant packages that did not contain “surfactant 3” the number of formulations was reduced, but the total number of prepared formulations was still well above 10,000.

Small aliquots (0.150-mL) of formulations were transferred to a shallow-well micro-titer plate and their haze values were measured with the Nepheloskan. All formulations prepared at dilution 1 were visually clear, and their haze values were below 2 RNU (relative nepheloskan units) which is the detectability limit for this method. Formulations that were prepared at higher dilutions showed haze over a broad range of RNU values, depending on several composition variables. Besides nephelometry measurements, each formulation was also visually inspected in order to assure that gel or precipitate was not present. Two haze measurements were taken for each formulation, and the lower value was used. Each formulation was prepared in duplicate, and the haze was calculated as an average of two lower haze values for duplicate formulations.

### DATA VISUALIZATION

In order to study the effect of individual variables, all formulations were organized in a three-dimensional framework, and their haze level was color-coded (Figure 3). The polymer type, as well as the type and level of SLES, are indicated along the X-axis, while the type and level of “surfactant 3” are indicated along the Y-axis. Formulations prepared with SLES-2 mol are shown on the left half of the XY plane (light gray), while formulations prepared with SLES-3 mol are shown on the right half (dark gray). Each half is divided into three sections showing data for formulations with 6 wt %, 8 wt %, and 10 wt %, 8 wt %, and 10 wt %, 8 wt %, and 10 wt %.

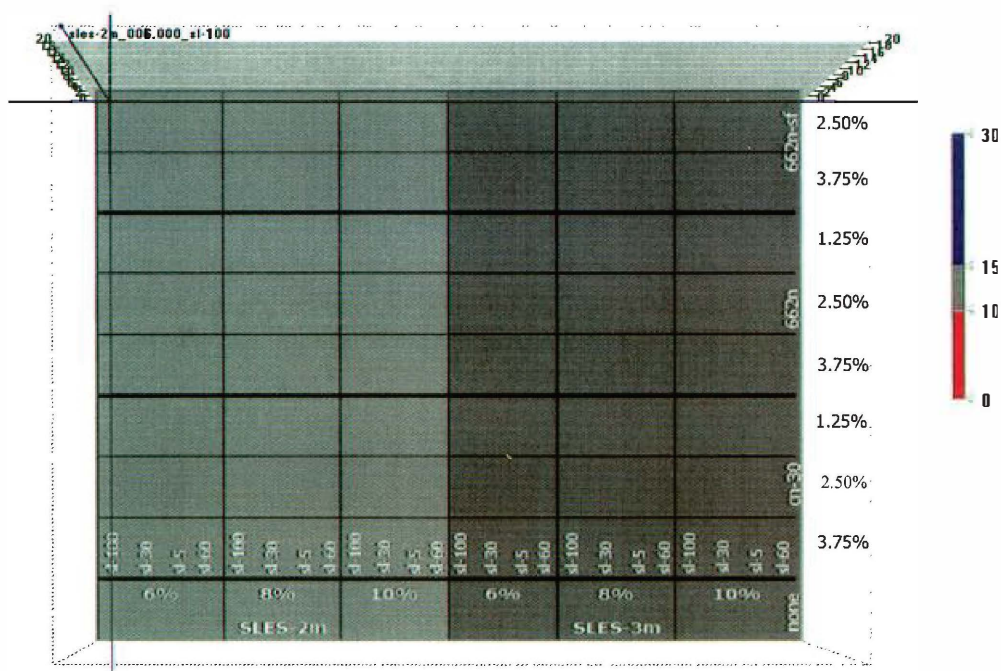


Figure 3. Data hierarchy for 3D presentation of haze values.

and 10 wt % SLES at dilution 1, and each of those sections was further divided into four slices showing data for different SL polymers. The dilution level is indicated along the Z-axis. In this 3D framework each formulation is shown as a small ball with the color representing the haze level. Formulations with the RNU values below 10 are shown in red, in the haze range from 10–15 in grey, while formulations with haze values above 15 are shown in blue. In order to allow better visualization of a large number of data, formulations from this study are divided into three sets where the SLES level at dilution 1 is 6 wt % (Figure 4), 8 wt % (Figure 5), or 10 wt % (Figure 6). Among formulations prepared with low level of SLES-3 mol and higher level of SL polymers (Figure 4, right figure), high haze was observed with all combinations of CAPB and “surfactant 3” and in a broad range of dilutions. The corresponding formulations prepared with SLES-2 mol also showed high haze, but it typically occurred only in the middle of the dilution range. Another series of formulations was prepared with the same low level of SLES, but at the lower level of SL polymers (Figure 4, left figure). In this series the haze was drastically reduced, and this was especially pronounced in formulations prepared with SLES-2 mol. When formulations were prepared at a moderate level of SLES-3 mol and at higher level of SL polymer (Figure 5, right figure) high haze was again observed, but the corresponding formulations prepared with SLES-2 mol showed high haze only in a narrow range of dilutions. At the same moderate level of SLES and with lower polymer level (Figure 5, left figure) high haze was observed in only a small number of formulations, and predominantly with SLES-3 mol. This effect was even more obvious when formulations were prepared at high level of SLES. Even at the higher polymer level (Figure 6, right figure) the number of formulations with high haze was reduced relative to that for the formulations with lower SLES level, and almost all of the formulations that were prepared with SLES-2 mol showed low haze in the whole range of dilutions.

Again, at the lower polymer level (Figure 6, left figure) high haze was observed in only a few formulations that were prepared with SLES-3 mol, while formulations prepared with high level of SLES-2 mol and at a lower polymer level were clear at all dilutions. In all sets of formulations studied, the haze level is essentially independent of the polymer type, indicating that the level of hydrophobic substitution has only a minor effect on haze. Therefore, in formulations from this study the type and level of SLES had a major effect on the haze index.

Other surfactants showed only moderate effects on the haze index. Formulations with

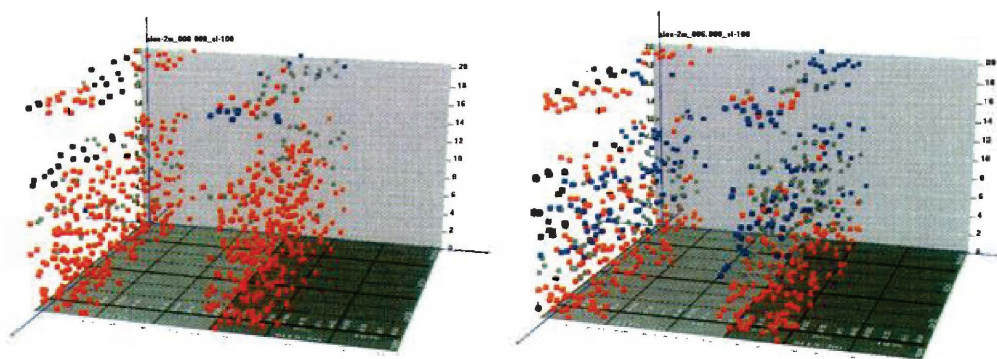
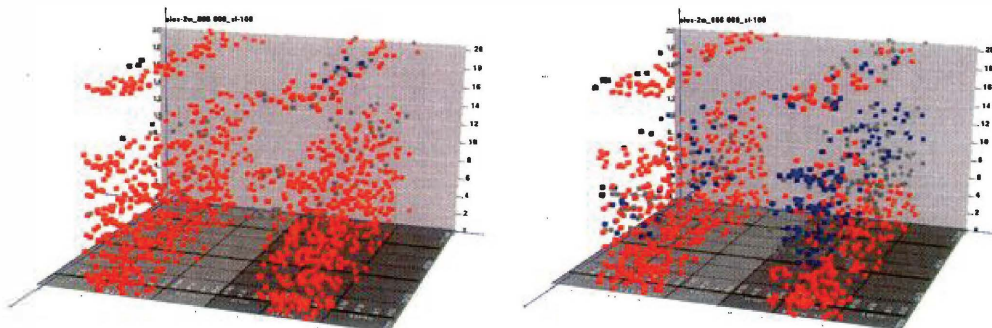
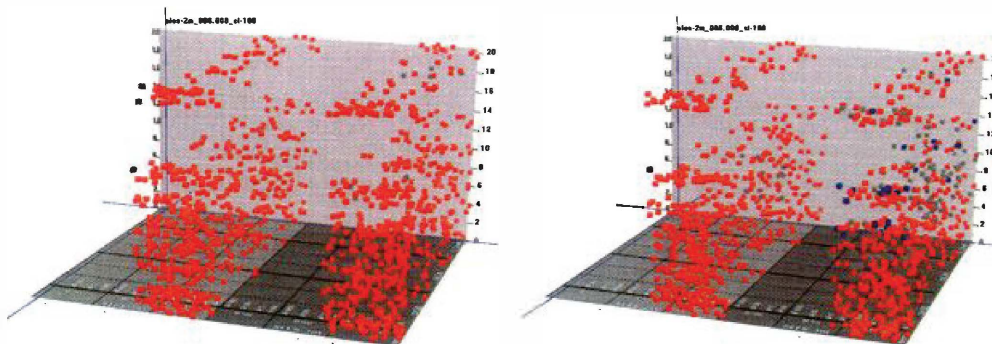


Figure 4. Haze values of formulations prepared with polymers at 0.2 wt % (left figure) or 0.4 wt % (right figure) and SLES-2 mol or SLES-3 mol at 6 wt % at dilution 1.





**Figure 5.** Haze values of formulations prepared with polymers at 0.2 wt % (left figure) or 0.4 wt % (right figure) and SLES-2 mol or SLES-3 mol at 8 wt % at dilution 1.



**Figure 6.** Haze values of formulations prepared with polymers at 0.2 wt % (left figure) or 0.4 wt % (right figure) and SLES-2 mol or SLES-3 mol at 10 wt % at dilution 1.

high level of CAPB (3.75 wt % or 5 wt %) and low level of “surfactant 3” (0 or 1.25 wt %) showed higher haze than the corresponding formulations with low level of CAPB and high level of “surfactant 3”. The level of SoftCAT™ SL polymers also showed a moderate effect on haze, with higher polymer level resulting in higher haze. However, the type of a polymer had a negligible effect on haze, suggesting that small differences in the level of hydrophobic substitution are not critical in formation of coacervates.

Selected formulations from this study were also prepared using a standard (bench-top) method. Water was added to formulations that were prepared at dilution 1 to give dilutions 6 and 8. Haze data for those samples were essentially identical (within the limits of experimental error) to haze values of formulations from the high-throughput study. This result further confirms the applicability of high throughput workflow for study of coacervate formation in shampoo systems.

## CONCLUSIONS

In this study of coacervate formation in shampoo systems, a high throughput workflow was developed for the preparation of formulations with different types and levels of cationic cellulosic polymers and surfactants, and for measurement of their haze values. Our results provide information on the effect of formulation components on the haze



index. Higher level of SoftCAT™ SL polymers (0.40 wt %) resulted in higher haze than the lower level (0.20 wt %), while the level of hydrophobic substitution had only a minor effect on haze. Among the surfactants evaluated, SLES showed major effect on the haze index. SLES-3 mol showed higher haze than SLES-2 mol, and lower levels of SLES (6 wt % and 8 wt %) showed higher haze than 10 wt % SLES. The level of other surfactants also showed effect on the haze index. Formulations with high levels of CAPB (3.75 wt % or 5 wt %) and low level of “surfactant 3” (0 wt % or 1.25 wt %) showed higher haze than the corresponding formulations with low level of CAPB and high level of “surfactant 3”. Haze data from this high throughput study showed good correlation with samples that were prepared using a standard (bench-top) method. This study illustrates that high throughput workflow is a valuable tool for study of shampoo systems.

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