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LATEX SAMPLES FOR RAMSES ELECTROPHORESIS

EXPERIMENT ON IML 2

submitted by

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3. INTRODUCTION AND STATEMENT OF WORK

This report describes activities at the Western Biomedical Research Institute through a one year period in support of work at MSFC on microgravity studies of electrokinetic phenomena with the RAMSES flight hardware. These activities comprised support consultations on the selection and handling of appropriate sample materials, surveying candidate sample materials, and development of sample preparation procedures for preparing flight samples. This contract ended prior to the final stages of the ground based developments and the efforts described here were continued under a new contract.

Electrophoretic studies under microgravity conditions were pursued during the '70's with the promise that the microgravity environment lent itself to electrophoretic processing which was impractical or virtually impossible under terrestrial conditions. As a result of these efforts, Snyder and his group (1) showed that electrohydrodynamic distortion of sample streams occurs in continuous flow electrophoresis as a result of mismatch in conductivity and dielectric properties of the sample stream and the surrounding flowing buffer curtain. Follow up fundamental studies by Snyder are designed to dissect out the role of conductivity and dielectric properties in the process under conditions of reduced gravity where the confounding effects of convection and sedimentation have been eliminated. These studies will require characterization of latex particles to constitute the sample streams.

It was anticipated that theoretical considerations provided by Professor Dudley Saville of Princeton University would determine the choice of latex. Latex diameter should be in the 0.3 to 0.8 μm range to avoid significant sedimentation in ground-based studies and yet large enough to visualize by microscopy and be detected in the electrokinetic analyzers. The particle population should be monodisperse as regards both size and electrophoretic properties with a particle density close to that of the suspending buffer. Ultraclean latexes stabilized by means of charged sulfate groups were attractive candidates since the very low pK of sulfate groups ~ 1.2 ensures that the particles maintain a constant electrophoretic mobility and surface charge over a wide range of pH.

The studies which follow address the following general issues:

- Selection of candidate commercially available latexes with the desired properties for the experiment and examination of their electrophoretic properties in the candidate flight buffer;
- Development of sample preparation methods which are compatible with the constraints of the flight hardware; and
- Pilot testing of chemical surface modifications of latexes to produce particles with more desirable properties for the flight experiments.

4. METHODS

4.1. Electrophoresis media and latex particles

Chemicals are analytical reagent grade or better unless noted otherwise. Media were made in Type I water purified in a Barnstead E-Pure system. Generally, salt solutions with pH's in the 4-8 range were stored at 4°C when not in use.

2.0 M NaCl stock. 116.9 g of Baker Analyzed Reagent grade NaCl was made up to 1.00 liter in a glass volumetric flask with fresh E-Pure H₂O. The stock was stored in a Pyrex no.1395 borosilicate reagent bottle at 4°C.

0.10 M NaCl, pH 7. 2.0 M NaCl stock (stored at 4°C) is diluted 20 fold with fresh E-Pure H₂O and mixed with 0.10 M NaHCO₃ to give 300 µM NaHCO₃ and adjusted to pH 7 with HCl.

0.010 M NaCl. 0.10 M NaCl is diluted 10 fold with fresh E-Pure H₂O. For pH 7, this stock was mixed with 0.10 M NaHCO₃ to give 300 µM NaHCO₃ and adjusted to pH 7 with HCl.

0.10 M NaOH. 2.02 g Mallinkrodt A.C.S. reagent grade NaOH is made up to 500 ml in glass and stored at room temperature in a Pyrex No. 1395 borosilicate screw cap reagent bottle.

0.10 N HCl. 5.00 g of concentrated HCl, VWR A.C.S. Reagent grade lot 33293, was made up to 500 ml in a glass volumetric flask with fresh E-Pure H₂O. The stock was stored in a polypropylene bottle.

Table 1. Polystyrene latex particles. The listed latex particle preparations were obtained from Interfacial Dynamics Corp. (Portland, Oregon).

Latex Type	Lot Number	Diameter (µm)	% Solids (w/w)
Acrylamide/acrylic acid modified latex (AAML)	415-1	0.569	3.1
Aldehyde/sulfate polystyrene latex	2-286-97	0.604	4.2
Amidine positively charged latex	10-137-81	0.600	4.6
Carboxylate modified latex (CML)	2-361-9	0.431	4.2
Carboxylate modified latex (CML)	10-236-23.230	0.833	4.2
Carboxylate modified latex (CML)	10-36-23	0.865	4.3
Sulfated polystyrene latex	10-366-20	0.468	8.4
Sulfated polystyrene latex	10-3-5	0.692	9.7
Sulfated polystyrene latex	10-238-64	0.767	8.4
Sulfated polystyrene latex	2-74-76	3.80	7.9
Zwitterionic polystyrene latex	10-62-40	0.446	4.4
Zwitterionic polystyrene latex	10-51-87	0.492	4.4

4.2. Particle Electrophoresis

Particle electrophoresis is carried out in a Coulter DELSA 440 system at 25°C. The accuracy and precision of the Coulter instrument operation will be assessed for the test series through analysis of a hydrophilic electrophoresis standard particle, carboxylate modified latex, CML, (IDC 10-36-23, 0.865 μm diameter) in 0.10 M NaCl at pH 7. This latex has been shown to have an electrophoretic mobility of -3.12 $\mu\text{m/s}$ per volt/cm in 0.10 M NaCl at pH 7.

The Coulter DELSA 440 (Doppler Electrophoretic Light Scattering Analyzer) consists of a rectangular capillary electrophoresis system with laser illumination of particles at desired measurement positions in the capillary. The velocities of the particles are assessed employing the Doppler effect. When light is scattered from a moving particle, its frequency is altered slightly. When this light is mixed with an unaltered beam, the resultant light beam oscillates at a frequency which is proportional to the Doppler shift. The analyzer of the instrument processes the signal from its photo diode light detectors and by Fourier analysis transforms it into its component frequencies which are translated into an signal intensity versus shift frequency. The data can be displayed as intensity (scatter intensity) versus frequency shift, electrophoretic mobility, or zeta potential at the user's discretion.

The instrument monitors the light scattered from the moving particles at four angles, 7.5, 15.0, 22.5 and 30.0° (uncorrected for the sample refractive index). The signal intensity at the different angles is affected to differing degrees by particle size.

Generally, aliquots of each sample are analyzed at both the top (TSL) and bottom (BSL) stationary levels of the sample chamber. Since the walls of the electrophoresis chamber are charged, when the electric field is on, the fluid in the chamber flows past the walls with a return flow through the center of the chamber. At a theoretically definable distance from the chamber wall, the fluid flow velocity is zero. This stationary fluid layer, or stationary level, is where particle velocities are to be measured so that the electrophoretic mobility of the particle is determined without contributions from electroosmotic flows of the suspending medium. Analyses of samples at both stationary levels minimizes the influence of setting errors for the stationary level locations, which are the most common source of systematic error in electrophoretic mobility measurements.

One sample analysis yields a mobility distribution for each of the four scattering angles. The region of the frequency-mobility spectrum containing the mobility profiles is marked for statistical analysis and the DELSA software computes a mean and standard deviation for the electrophoretic mobility. For particle populations which are monodisperse with regard to their electrophoretic mobilities, the means should be the same for each scatter angle.

The acquisition of accurate electrophoretic mobility data requires minimization of systematic errors from two principal sources. The first involves the precision and accuracy of the measurement instrument, a major determinant of which is contribution of electroosmosis, or fluid flow, to the measured particle velocity. The second is the exposure of the particles under test to materials in the system which alter the measured property, i.e. particle surface charge. Vanishingly small concentrations of materials which are active at particle surfaces can adsorb to the particle surface and dramatically alter the surface charge and hence the particle electrophoretic mobility. In aqueous media this behavior is particularly true for hydrophobic molecules and surfaces of many polystyrene latex particle preparations which are also hydrophobic.

We generally use a two step standardization technique which first employs standard hydrophilic and second hydrophobic latex particles in order to monitor and minimize the influence of these most common of systematic errors. A hydrophilic carboxylate modified latex (IDC 10-36-23, 0.87 μm diameter) was chosen for calibration of the DELSA 440 and for monitoring its accuracy and precision. Previous testing has established the mean electrophoretic mobility of -3.12 microns/sec per volt/cm for 10-36-23 in 0.10 M at pH 7. The hydrophilic surface of this particle renders it less sensitive to alteration by hydrophobic materials which if present as trace contaminants in the system would tend to adsorb to hydrophobic surfaces thereby altering their electrophoretic mobilities. This calibration check is performed at the beginning of each measurement series to confirm that the DELSA 440 has been setup properly and is operating satisfactorily. Typically, a mean mobility within 0.1 mobility units of the specified value is interpreted as indicating that the instrument is operating correctly. Measurements at each of the two stationary levels should agree to within 10%. A small error in setting the position of the chamber wall influences both of the stationary level settings, but the errors are usually offsetting, i.e. a small error will produce a positive error in mean mobility at one stationary level and a negative error of comparable magnitude at the other stationary level.

The second test procedure consists of measuring the electrophoretic mobility of a more hydrophobic sulfated polystyrene latex. In contrast to the carboxylate modified latex, the hydrophobic sulfated latex test particle, 2-74-76, has large hydrophobic regions on its surface which serve as "docking" areas in the adsorption of large and small molecular species. Previous tests indicate that the mobility of this particle should be about -5.2 ± 0.2 and -8.0 ± 0.3 microns/sec per volt/cm in 0.10 M and 0.01 M NaCl, respectively, at about pH 7 if no media contamination is present. This media test is performed once at the beginning of test series to establish that the test media are not contaminated with surfactant species which can alter the electrophoretic mobility of sulfated polystyrene and similar particles. Such agents would include heavy metal ions which can complex the sulfate group, and negative and neutral surfactants which can adsorb to the latex surface and either mask or neutralize the surface charge. Negative surfactants, such as sodium dodecyl sulfate would increase the mobility of the latex. It may be appropriate in the future to incorporate such a test to control for trace contaminants in the test media.

4.3. *pH measurements*

Unless noted otherwise, all pH measurements were obtained with a Fisher Accumet Model 620 pH Meter equipped with a Corning combination X-EL glass electrode. Measurements were routinely made at room temperature, about 22-26°C. The apparatus was calibrated with three commercially obtained pH buffers at pH's of 4, 7 and 9 or 10. Generally, errors in pH for the extreme pH buffers were less than 0.1 unit.

5. RESULTS

5.1. Definition and characterization of flight experiment sample components

The objectives of the initial studies were to determine the electrophoretic mobility of various well characterized, surfactant-free, commercially available latex particles and to prepare a test sample for pilot tests of ground-based RAMSES equipment. These studies would provide a walk through of the processing steps for a flight sample and point up unanticipated problems and the electrophoretic characterizations would provide data for estimations of sample behavior during the flight experiment.

5.1.1. Electrophoresis Sample Buffer

The candidate electrophoresis buffer formulation for the RAMSES experiment at the time of these tests was specified by Dr. Robert Snyder. The Tris/Borate buffer concentrated stock was formulated as follows:

Constituent	Mol. Wt.	g/liter	mM
Boric Acid, H_3BO_3	61.84	10.00	162
TRIS, $C_4H_{11}NO_3$	121.14	8.237	68

This buffer concentrate was prepared with analytical reagent grade constituents and was made up to one liter with reagent grade water provided by a Barnstead E-Pure cartridge system equipped with a 0.2 μm output filter. The pH of the concentrate was 8.2.

It was expected that the buffer for the flight experiment would to be prepared by diluting the concentrated buffer to provide a conductivity of 140 $\mu Mho/cm$ at 10°C, the approximate temperature of the flight experiment. The buffer obtained by diluting the concentrate 100 fold with CO_2 -free water had a pH of 8.7 at room temperature ($23 \pm 2^\circ C$). In order to identify the buffer concentration which provide these specifications, a series of dilutions were prepared from the concentrated buffer stock and conductivities of these preparations were measured for four replicate fillings at 25°C in the sample chamber of the DELSA 440 electrophoretic analyzer to provide the data in Table 2. (The DELSA 440 was standardized with a YSI 1000 $\mu S/cm$ calibration standard).

Table 2. Conductivities of TRIS/Borate buffer at different concentrations at 25°C.

Sample	Dilution in Water	Rel. Conc.	Mean Conductivity \pm SD ($\mu Mho/cm$)
1000 \pm 1% $\mu Mho/cm$ Standard	-	NA	1005 \pm 0
TRIS/Borate Buffer Concentrate	None	1.000	623 \pm 2
TRIS/Borate Buffer	1/5	0.200	187 \pm 2
TRIS/Borate Buffer	1/7.5	0.150	149 \pm 1
TRIS/Borate Buffer	1/10	0.100	116 \pm 4
TRIS/Borate Buffer	1/20	0.050	63 \pm 2

It was recognized that the conductivities of the TRIS/Borate buffer would be significantly lower at about 10°C, the anticipated temperature of the flight experiment, than at the standard reference temperature of 25°C used for Table 2 and for electrophoretic analyses to follow. Two factors should account largely for this reduction in conductivity, namely the viscosity of the medium and the degree of ionization of the buffer salts. Based upon dynamic viscosities of 1.307 and 0.8904 centipoise for water at 10 and 25 °C, respectively, the viscosity component of the conductivity decrease was estimated to be $0.8904/1.307 = 0.68$. So for the 1/5 diluted TRIS/Borate the estimated conductivity at 10°C would be $0.68 \times 187 = 127 \mu\text{Mho/cm}$.

Attempts were made to measure the conductivities of these diluted media at 10°C in the DELSA 440. A value of 387 $\mu\text{Mho/cm}$ was obtained for the buffer concentrate, which is 0.62 x the conductivity at 25. Efforts to obtain corresponding data for the diluted buffer preparations were initially unsuccessful since condensation of water vapor on the exterior surfaces of the DELSA sample chamber produced falsely high readings. A determination of the conductivity of the diluted buffer at about the concentration to be used for the flight experiment should be made to establish the accuracy of the estimates above.

The 1/5 dilution of the TRIS/Borate buffer was chosen for the initial screening of candidate latex particles to be used as the suspending medium for the initial ground based testing in the flight hardware. The pH of this dilution was 8.5 at room temperature.

Subsequently, the conductivity of the buffer and the electrophoretic mobility of one of the latexes used for the preparation of hardware test samples were examined as a function of temperature over the range from 25 to 10°C. The data in Table 3 agree well with the estimations above based upon the changes in the viscosity of water in this temperature range.

Table 3. Electrical conductivity($\mu\text{S/cm}$) and latex electrophoretic mobility($\mu\text{m/s}$ per volt/cm) (2-361-9, CML) as a function of temperature in TRIS/borate buffer, relative concentration 0.200, pH 8.5.

Temp	Mobility	Cond.	Rel. Mob.	Rel. Cond.
25.2	-3.66	195	100	100
20.5	-3.40	178	93	91
16.0	-3.13	162	86	83
10.3	-2.69	138	73	71

5.1.2. Initial specifications for latex sample properties

For the initiation of ground based testing, latex particle batches were to be identified from which mixtures of two electrophoretically separable particle populations could be prepared. The particle diameters were specified to fall in the 0.4 to 1.0 μm range. Three sample preparations were to be used for testing, the first two each containing one of the latex particle batches at a solids concentration of about 15% in Tris-borate buffer, and a third containing a total latex solids concentration of 30% comprised of 15% of each of the two particle batches.

5.2. Latex Samples for Ground-based Separation Experiments

5.2.1. Electrophoretic analysis of latex particle candidates

Five latex particle preparations with different surface chemical groups and sizes in the 0.4 to 1.0 μm diameter range were screened from the Interfacial Dynamics inventory. The mean electrophoretic mobility of each preparation was measured in the 1/5 dilution of the TRIS/Borate buffer, pH 8.5, at 25°C in the Coulter DELSA 440. The results are summarized in Table 4.

The mobility of the media test particle, 2-74-76, was measured in order to provide a test of possible trace component variability in future batches of the buffer. The mobilities of the other latexes ranged from a low value of -2.32 for one of the carboxylate modified latexes to -6.83 for one of the sulfate latexes. Two latexes, 10-238-64 and 2-361-9, were chosen as the pair for the initial hardware testing since they fell within the specified size range and provided a mobility difference which should be resolvable with the equipment. The mobility difference between the two of 3.6 mobility units at 25°C corresponded to a calculated difference of 2.6 units at 10°C.

Table 4. Electrophoretic survey of latexes in 1/5 TRIS/Borate buffer at 25°C. Mean electrophoretic mobilities are given in units of $\mu\text{m}/\text{s}$ per volt/cm with the indicated standard error of the mean (SEM).

Sample	Diam. (μm)	Surface Type	Mean Mobility \pm SEM	Comment
10-36-23/0.1 M NaCl	0.865	CML	-3.22 \pm 0.07	Cal. Check: Exp. Value - 3.12
TRIS/Borate:				
2-74-76	3.80	Sulfate	-8.82 \pm 0.16	Medium Quality Check
10-36-23.230	0.833	CML	-2.32 \pm 0.16	
10-238-64	0.767	Sulfate	-6.83 \pm 0.26	
10-366-20	0.468	Sulfate	-5.82 \pm 0.25	
2-361-9	0.431	CML	-3.22 \pm 0.10	
2-286-97	0.604	Ald./Sulfate	-5.67 \pm 0.07	Reactive aldehyde groups
10-36-23/0.1 M NaCl	0.865	CML	-3.14 \pm 0.03	Reconfirm calibration

5.2.2. Preparation of latex test samples for initial testing

The initial ground based testing of latex in the RAMSES apparatus aimed to demonstrate that the samples could be loaded and visualized in the apparatus without problems. Table 5 describes the five latex preparations which were prepared for the June, 1993 ground based testing of the RAMSES apparatus in France. Each latex sample contained TRIS/Borate buffer at a relative

concentration of about 0.20, which was anticipated to approximate the concentration of the curtain buffer, and 10 ppm iodine as a bactericide.

The preparation of the listed latex samples at low solids concentration entailed simple dilution of commercially available stock with the appropriate quantities of TRIS/Borate buffer and iodine reagent (1000 ppm reagent grade iodine crystals in 34% w/w absolute ethanol in water). In contrast, the preparation procedure was more involved for the concentrated latex samples since the targetted solids concentrations were four to eight times the commercially available solids concentrations.

The general features of the preparation procedure were as follows. All the materials in the process were tracked on a weight basis to provide the data for computing material recoveries and final compositions of the preparations. First, the latex stock was mixed with sufficient TRIS/Borate buffer concentrate as defined above to give a relative concentration of about 0.20. Then the latex samples were centrifuged (12,000 x g for 2-361-9 and 4,800 x g for 10-238-64) at 35°C to sediment the latex. An amount of supernatant fluid was removed which was calculated to raise the solids concentration of the remaining latex and fluid to 35%. The latex pellets were then resuspended by swirling the tube contents and by trituration with a pipet. There were minimal losses of latex and fluid throughout the procedure with total mass recoveries of 98 and 99% for the two batches of material. The final calculated solids concentrations (w/w) were 36 % for the sulfate latex, 10-238-64, and 38 % for the CML, 2-361-9. These stock suspensions were stored in screw cap polyethylene bottles at 4°C.

It was noted during the resuspension of the latex pellets that the CML latex, 2-361-9, resuspended more easily than the sulfated latex. Small aggregates could occasionally be seen in the sulfate latex stock. Vigorous vortexing was used to attempt dispersion of the aggregates, but the high solids content of the suspensions made difficult the observation of aggregates. During the procedures to make test samples from the concentrated stocks for the June testing, no further measures were taken to eliminate aggregates. This proved to be unsatisfactory as will be discussed below.

Table 5. Description; of latex samples provided for ground based testing of the RAMSES apparatus in May, 1993.

Sample Id.	Latex Components	% Solids	Comment
0.42 mm Crimson	L-5269, lot 6711	1.67	Crimson, excitation at 625 nm and emission at 645 nm (1.7 ml in 5 ml bottle)
0.43 mm CML	2-361-9 Carboxylate modified latex (CML)	1.67	Non fluorescent control for crimson latex (2.2 ml in 5 ml bottle)
0.43 mm CML	2-361-9 Carboxylate modified latex (CML)	about 17%	10.5 ml in 20 ml bottle
0.77 mm Sulfate	10-238-64 Sulfated latex	about 17%	10.5 ml in 20 ml bottle
0.77 mm Sulfate + 0.43 mm CML	10-238-64 Sulfated latex 2-361-9 Carboxylate modified latex (CML)	about 17% about 17%	10.5 ml in 20 ml bottle

5.2.3. Problems encountered during initial ground based testing and remedial measures

Problems were encountered during the initial ground based testing as described in Appendix 1. These included:

- Fouling of the quartz surfaces in the optical detection cell by latex particles;

Clogging of sample fluid lines into the sample storage vessels by the concentrated latex suspensions;

Computations of the migration behavior of the latex samples (Appendix 4) for the anticipated operation parameters for the flight experiment indicated that the fastest latex sample should have a mobility of about $-0.8 \mu\text{m/s}$ per volt/cm or less in order to prevent its running to the edge of the sample cell.

After discussion of these issues (see letters in Appendices 2 and 3) with Dr. Snyder, the following action plan was devised:

- Test the electrophoretic mobilities of promising commercially available alternative latex particle types which have more hydrophilic surfaces, to minimize aggregation problems, and which might have electrophoretic mobilities in the range of $-1 \mu\text{m/s}$ per volt/cm so that it could be used with the slower particle used in the June 1993 experiments to produce a two particle mixture for separation in the flight experiment;
- Test promising chemical modification methods for reducing the mobility of sulfate latexes while increasing the hydrophilicity of the particle surface, thereby reducing its tendency to aggregate; and
- Modify the latex suspension preparation scheme in order to eliminate all aggregate particles which could occlude a 0.5 mm diameter tube;

5.2.4. Tests of other commercially available latexes

One class of latex which had not been tested was the zwitterionic latexes which carried both positively and negatively charged surface groups. These were attractive since they do not adsorb various materials as readily as do sulfate latexes or CML's. However, it was not known what their mobility properties would be in the TRIS/Borate buffer. As can be seen in Table 6, both particles had large negative mobilities at 25°C. These particles were ruled out as promising candidates.

Also shown in Table 6 is a comparison of the mobilities of CML's in the 0.4 to 0.7 μm diameter range. Included in the comparison is an aliquot of the stored concentrated CML, 2-361-9, which was prepared for the May 1993 testing. It is clear that even if the flight experiment is run at about 10°C so that the mobilities of the particles are reduced by about one third, they still will have mobilities of about -3 . Thus other measures appeared necessary to obtain suitably low mobilities.

Table 6. Electrophoretic survey of zwitterionic latexes and CML's in 1/5 TRIS/Borate buffer at 25°C. Mean electrophoretic mobilities are given in units of $\mu\text{m/s}$ per volt/cm with the indicated standard error of the mean (SEM).

Sample	Diam. (μm)	Surface Groups	Mean Mobility \pm SEM	Comment
10-36-23/0.1 M NaCl	0.865	CML	-3.09 ± 0.04	Cal. Check: Exp. Value -3.12
TRIS/Borate:				
10-51-87	0.492	Zwitterionic	-6.16 ± 0.06	
10-62-40	0.446	Zwitterionic	-6.09 ± 0.16	
10-237-62	0.715	CML	-4.20 ± 0.35	
2-361-9	0.431	CML	-3.92 ± 0.24	From 5/94 Conc. Stock
2-322-97	0.557	CML	-3.82 ± 0.22	
10-236-60	0.641	CML	-4.05 ± 0.24	
10-36-23/0.1 M NaCl	0.865	CML	-3.05 ± 0.04	Reconfirm calibration

5.2.5. Latex modifications to reduce electrophoretic mobility

Two approaches were tested to determine whether either could substantially reduce the electrophoretic mobility of commercially available latexes. The first was to coat the latex surface with a molecule which reduced the electrophoretic mobility. The second was to chemically modify the latex surface to reduce the number of ionizable groups.

For the coating experiments a variety of agents could be used. For example, methylcellulose had been shown to virtually eliminate the surface charge of hydrophobic sulfate polystyrene latex by Herren et al. (2). However, earlier attempts in this laboratory to work with particles coated with such materials which were very strongly adsorbed to the latex surface displayed a tendency to make the particles adhesive to other uncoated particles or surfaces. For example, methylcellulose coated particles tended to adhere to uncoated CML particles in mixtures during storage or to uncoated polystyrene Coulter Accuvette II surfaces following sedimentation of the coated particles onto the uncoated polystyrene surface. Thus, while many agents such as methyl cellulose, polylysine, or other polymers appeared at first glance to be candidates for treating the particles, the constraints of minimal interaction between particles in two particle mixtures and between the particles and the walls of the test devices severely limited the range of possibilities.

Since we had little data on coatings in a TRIS/Borate buffer like that chosen for the RAMSES apparatus, we decided to do a quick test of two coating agents in order to determine the magnitude of charge reduction which might be obtainable with such a technique. The coating agents chosen were Tween 20, a neutral surfactant, and bovine serum albumin (BSA), a serum protein which has an isoelectric point of about 4.8 and displays a modest electrophoretic mobility in other media.

The coating procedure consisted of mixing 100 μl of latex with 150 μl of TRIS/Borate buffer concentrate, and then 500 μl of reagent grade water + coating agent at a stock concentration of 100 mg/ml. The mixtures in 2 ml polypropylene centrifuge tubes were allowed to stand overnight at room

temperature, about 17 hours. The samples were centrifuged to pellet the latex which was resuspended in 1 ml of 1/5 TRIS/Borate buffer at room temperature. This wash process was repeated four times followed by suspension of the final pellet in 300 μ l of 1/5 TRIS/Borate buffer. Aliquots of these suspensions were analyzed in the 1/5 TRIS/Borate buffer to give the results in Table 7.

Three levels of coating agent were tested in order to ensure that a fully coated surface was obtained. The washing procedure was extensive enough to ensure that weakly bound material would be removed from the system. As can be seen from the Table, the maximal reduction in electrophoretic mobility obtained with either BSA or Tween 20 was about 25 to 30%.

Table 7. Analyses of the effects of coating with BSA or Tween 20 on the electrophoretic mobility of the sulfated latex, 10-3-5, in 1/5 TRIS/Borate buffer at 25°C. Mean electrophoretic mobilities are given in units of μ m/s per volt/cm with the indicated standard error of the mean (SEM).

Sample	Coating Agent	Coating Conc. mg/ml	Mean Mobility \pm SEM	% Reduction	Comment
10-36-23/0.1 M NaCl	None		-3.11 \pm 0.06		Cal. Check: Exp. Value -3.12
TRIS/Borate: 10-3-5	None		-6.27 \pm 0.12	0	
"	BSA	0.67	-5.74 \pm 0.13	8	
"	"	6.7	-4.44 \pm 0.19	28	
"	"	67	-4.58 \pm 0.30	26	
"	Tween 20	0.67	-4.63 \pm 0.37	25	
"	"	6.7	-5.31 \pm 0.37	14	
"	"	67	-5.07 \pm 0.21	18	
10-36-23/0.1 M NaCl	None		-3.06 \pm 0.10		Reconfirm calibration

The second latex modification approach tested was the acid hydrolysis of sulfate latex which is reported to remove the charged sulfate group and leave a hydroxyl group (3). It was reasoned that this could produce two desirable results, i.e. the reduction of the electrophoretic mobility of the latex while maintaining a moderately hydrophilic surface with the polar hydroxyl groups. It was not known, however, how such particles would behave in the RAMSES buffer.

Samples of two sulfate latexes were incubated at elevated temperature and were analyzed throughout the incubation in order to monitor the progress of the hydrolysis. Since the hydrolysis resulted in the replacement of a sulfate group with a hydroxyl group, the negative charge of the particle was expected to decrease as the reaction proceeded. It was not known how extensive a charge reduction could be achieved before the particles might aggregate.

Latex samples were incubated in a temperature controlled vacuum oven, but without the vacuum applied. The temperature control was rather crude, with the temperature ranging from 90 to about 105°C, with the suspension temperatures typically in the ninety degree range. Each sample was contained in a No. 9826 Pyrex screw cap tube with a teflon liner. Each tube contained 17 to 23 g. of

latex suspension at the latex stock solids concentration as indicated in Table 1. The smaller latex, 10-3-5, had a diameter of 0.692 μm , and the second, 2-74-76, had a mean diameter of 3.80 μm . The tubes were capped and placed in a rack upright in the oven. Periodically the tubes were inspected and at the intervals shown in Table 8, the samples were mixed to resuspend sediments and aliquots were drawn and diluted in 10 mM NaCl buffered to pH 7 with sodium bicarbonate. The samples were analyzed with the DELSA 440.

A third sample was prepared with 10-3-5 in which 8 microliters of concentrated HCl was mixed with 3.3 g. of latex stock to give an HCl concentration of approximately 0.03 M or a pH of about 1.5. The sample was contained and incubated as indicated above.

Table 8 shows the progress of the hydrolysis reactions. Several features of the sample behavior were notable:

- The decreases in mobility as a function of time were approximately colinear for the two latexes without added HCl up to about 45 hr;
- The larger diameter latex, 2-74-76, sedimented between samplings and by 45 hours was substantially aggregated and would not easily resuspend. Thus, close particle contact during the hydrolysis was indicated as a problem in maintaining colloidal stability;
- Addition of HCl to the hydrolysis to reduce the pH significantly increased the rate of hydrolysis but by about 4 hours after the time point in Table 8, the suspension aggregated;

Table 8. Hydrolysis kinetics for sulfated latexes (10-3-5 and 2-74-76) heated at about 90 °C. Mean electrophoretic mobilities are listed in units of $\mu\text{m/s}$ per volt/cm for the indicated latex preparations suspended in 0.01 M NaCl and analyzed at 25 °C.

Time (Hr)	Electrophoretic Mobility			% of Control Mobility		
	10-3-5	10-3-5 (HCL)	2-74-76	10-3-5	10-3-5 (HCL)	2-74-76
0	-6.98	-6.98	-9.09	100	100	100
19	-5.74	-4.26	-7.85	82	61	86
45	-4.81		-7.16	69		79
68	-3.74			54		
72	-3.31			47		
91	-2.95			42		

- By 91 hours, the mobility of 10-3-5 had decreased by close to 60%. Rough estimates of mobility in 1/5 TRIS/Borate buffer at about 10°C indicated a mobility of about -1.6 which was in the desired range so the reaction was terminated.
- The hydrolyzed sample was centrifuged so that supernatant fluid could be removed in order to increase the solids content. During attempts to resuspend the latex pellet the particles began to aggregate, even when TRIS/Borate was added to the suspension. Much of the aggregation appeared reversible through sonication. However, this behavior indicated that such particles were prone to aggregation and had an increased potential for problems during their processing for the flight experiment or during the experiment.
- The electrophoretic mobility of 10-3-5 hydrolyzed for 91 hours was substantially lower than untreated latex in 10 mM NaCl at pH 7. However, in TRIS/Borate buffer the mobility was only slightly lower than that of the untreated sample (Table 9). In contrast, the mobility of the

hydrolyzed latex in TRIS buffer at nearly the same pH as that of the 1/5 TRIS/Borate approximated that observed in NaCl. This was interpreted to indicate that borate probably formed negatively charged complexes with hydroxyl groups on the latex surface. Thus any gain in charge reduction produced by the hydrolysis was offset by the acquisition of charge through interaction with the buffer anions.

Table 9. Comparative electrophoretic behavior of 10-3-5 in different buffers.

Sample	Treatment	Medium	Mean Mobility ± SEM
10-3-5	None	10 mM NaCl	-6.98 ± 0.24
"	"	1/5 TRIS/Borate	-6.27 ± 0.12
"	"	14 mM TRIS, pH 8.5	-6.11 ± 0.12
10-3-5	91 hour hyd.	10 mM NaCl	-2.95 ± 0.20
"	"	1/5 TRIS/Borate	-5.77 ± 0.07
"	"	14 mM TRIS	-3.24 ± 0.26

5.2.6. Electrophoretic mobility as a function of temperature for CML, 2-361-9, in 1/5 TRIS/Borate buffer

The temperature of the flight experiment had not been finalized at the time of these studies, but it was reckoned that it would fall in the range from 10 to 20°C. It was anticipated that over this temperature range that both the conductivity and electrophoretic mobility of latex particles would decrease with decreasing temperature by about 2% per degree due to viscosity increases of the buffer of that magnitude.

Both parameters were measured in the DELSA 440 apparatus over the temperature range from 10 to 25°C. Table 10 shows the set of measurements which confirmed the expected behavior for the 1/5 TRIS/Borate buffer and demonstrated essentially colinear decreases in both the buffer conductivity and particle mobility. The magnitudes of the effects were: -1.81 %/°C for electrophoretic mobility and -1.95 %/°C for conductivity.

Table 10. Analyses of effects of temperature on the electrophoretic mobility of the carboxylate modified latex, 2-361-9, in 1/5 TRIS/Borate buffer. Mean electrophoretic mobilities are given in units of μm/s per volt/cm.

Temp.	Mobility	Cond(uS/cm)	Rel. Mob.	Rel.Cond.
25.2	-3.66	195	100	100
20.5	-3.40	178	93	91
16.0	-3.13	162	86	83
10.3	-2.69	138	73	71

5.2.7. Preparation of candidate latexes for 12/94 ground based testing

By the time of the preparations for the groundbased testing of the RAMSES hardware in late fall of 1993, modifications to the sample preparation scheme had been identified which promised to eliminate the particle aggregation and sample line clogging reported earlier. Since a low mobility

particle had not been developed it was decided to use the best available pair with the best current preparation methods to formulate a pair with which the general operations could be tested in the apparatus, including sample loading, running the instrument and visualizing the sample.

For the sample pair, the 0.69 μm sulfated latex, 10-3-5, and the 0.43 μm CML latex, 2-361-9, were chosen. As noted for the June, 1993 sample preparations, the commercially available latex stock was centrifuged to sediment the latex, then a calculated amount of supernatant fluid was removed which was calculated to give the desired solids concentration of about 15%. The latex was then resuspended by gentle inversion mixing on an inversion mixer until there was no sign of a latex pellet remaining. The 15% suspensions (about 16 g each) were dialysed against three one liter batches of 1/5 TRIS/Borate buffer. Each sample was filtered through glass wool packed in a Pasteur pipette to remove any large aggregates in the size range which might occlude sample tubing in the RAMSES apparatus. A 9.5 g. aliquots of each suspension were shipped in polypropylene containers to MSFC for ground based testing and the remainder of the suspensions were stored in identical polypropylene containers at 4°C.

Table 11 describes each suspension and provides means and standard errors of the means for the electrophoretic mobilities ($\mu\text{m/s}$ per volt/cm) measured at 25° for aliquots drawn from the final suspensions and diluted in 0.20 x TRIS-Borate buffer. The mobilities measured at 10° were obtained earlier with aliquots of the parent latex stocks diluted in TRIS-Borate.

Table 11. Description of samples prepared for 12/94 RAMSES ground based testing.

Sample Id.	Elect. Mob. (25°)	Elect. Mob. (10°)
2-361-9 Carboxylate modified latex (CML), 0.43 μm	-3.50 \pm 0.17	-2.69 \pm 0.11
10-3-5 Sulfate latex, 0.69 μm	-6.03 \pm 0.21	-4.04 \pm 0.14

5.2.8. Results of 12/94 ground based testing and development decisions

The experiments conducted at MSFC in December, 1993 included the loading and running of latex samples in the RAMSES apparatus at about 18°C. In contrast to the first attempts to handle latex samples in the May, 1993 studies, there were no problems with clogging of tubes or fouling of the optical surfaces of the equipment. The remaining problem was that the mobilities of the two latex particle populations were too high. However, when the instrument voltages were decreased, the slower of the populations could be kept within the visible portions of the chamber during its passage from the sample insertion point to the exit point.

Following discussion of alternatives for obtaining another particle to substitute for the faster latex, it was decided to request from Interfacial Dynamics Corporation the synthesis of a latex particle which contains a shell of hydrophilic polymer. Such a particle promised to provide a low electrophoretic mobility with little tendency for the particles to aggregate. It was not known, however, how low a mobility might be obtainable.

5.2.9. Electrophoretic analysis of AAM latex

Interfacial Dynamics produced an experimental lot of acrylic acid -acrylamide modified (AAM) latex, lot number 415-1. The mean particle diameter for the lot was 0.569 μm with a solids content of 3.1

% The electrophoretic properties of the lot were examined at two ionic strengths in NaCl at pH 7 and in the 1/5 dilution of the RAMSES TRIS/Borate buffer. As can be seen from table 12, the electrophoretic mobilities in NaCl are substantially lower than values from about -3 to -4 typically observed for CML particles. In the RAMSES buffer the observed electrophoretic mobility of -1.6 is about 2 units less than that of the CML, 2-361-9, which was the slower of the two latexes in the December, 1993, ground based experiments. Thus, an AAM latex appears to be a good prospect for future experiments in the RAMSES apparatus.

Table 12. Electrophoretic analysis of AAM latex in different media at 25 °C. Mean electrophoretic mobilities are given in units of $\mu\text{m/s}$ per volt/cm with the indicated standard error of the mean (SEM).

Medium	Electrophoretic Mobility \pm SEM
100 mM NaCl, pH 7	-0.63 ± 0.11
10 mM NaCl, pH 7	-1.37 ± 0.15
1/5 TRIS/Borate, pH 8.5	-1.59 ± 0.29

6. FINAL COMMENTS

The objectives of these reported studies were to provide ground based support services for the flight experiment team for the RAMSES experiment to be flown aboard IML-2. The specific areas of support included consultation on the performance of particle based electrophoresis studies, development of methods for the preparation of suitable samples for the flight hardware, the screening of particles to obtain suitable candidates for the flight experiment, and the electrophoretic characterization of sample particle preparations. The first phases of these studies were performed under this contract, while the follow on work was performed under grant number NAG8 1081, "Preparation and Characterization of Latex Samples for RAMSES Experiment on IML 2."

During this first phase of the experiment the following benchmarks were achieved:

- Methods were tested for the concentration and resuspension of latex samples in the $>0.4\mu\text{m}$ diameter range to provide moderately high solids content samples free of particle aggregation which interfered with the normal functioning of the RAMSES hardware.
- Various candidate latex preparations were screened and two candidate types of latex were identified for use in the flight experiments, carboxylate modified latex (CML) and acrylic acid-acrylamide modified latex (AAM). These latexes have relatively hydrophilic surfaces, are not prone to aggregate, and display sufficiently low electrophoretic mobilities in the flight buffer so that they can be used to make mixtures to test the resolving power of the flight hardware.

7. REFERENCES

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8. APPENDICES

8.1. Appendix 1:

9/20/93 Letter from Dr. M. Clifton to Dr. R. Snyder describing problems encountered in initial ground based tests of latexes in RAMSES apparatus and draft letter from Dr. R. Snyder to Francois Fouré regarding how to proceed

8.2. Appendix 2:

10/8/93 Response from Dr. G. Seaman to Dr. R. Snyder concerning troubleshooting problems in latex analyses in RAMSES apparatus

8.3. Appendix 3:

10/8/93 Response from Dr. R. Knox to Dr. R. Snyder concerning troubleshooting problems in latex analyses in RAMSES apparatus

8.4. Appendix 4:

9/13/93 Letter from Dr. M. Clifton to Dr. R. Snyder summarizing calculations of latex particle electrophoretic mobilities which appeared with the anticipated flight operation parameters for the RAMSES apparatus