White Paper

RAININ
Pipetting 360°

Overcoming Liquid Retention Problems

Rainin Low Retention Tips

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Introduction

Pipette tips are made from polypropylene because it has many desirable properties as a life science research material:

- Chemically inert
- Good clarity
- Hydrophobic
- Autoclavable
- Moldable into finely tapered structures
- Can be sterilized by ionizing radiation
- Supple and flexible

Although hydrophobic in nature, polypropylene does exhibit varying amounts of "adhesion" with a wide variety of liquids. Certain liquids exhibit an affinity for polypropylene, allowing them to coat or "sheet" polypropylene surfaces. Liquids with high surface tension (e.g., water) will exhibit minimal adhesion to a polypropylene surface and be easily dispensed from a standard polypropylene tip. However, liquids with low surface tension, such as surfactant solutions, are more difficult to dispense completely due to greater surface area contact with the polypropylene surface. Organic liquids exhibit a diverse range of physical and chemical properties, such as hydrophibicity and surface tension. Solutions containing surfactants, alcohols and other organic additives are routinely used in life science research laboratories.

Figure 1 – Interaction of high surface tension liquid with polyprolylene surface[1]

Figure 2 – Interaction of low surface tension liquid with polyprolylene surface[1]

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Viscous liquids also pose a challenge to accurate pipetting, since their physical properties resist the relatively low forces of air displacement pipettes, making aspiration and dispensing difficult. Enzyme preparations and PCR master mixes contain glycerol as an additive that increases the viscosity of these solutions. Biological solutions containing protein and high molecular weight nucleic acids are also notoriously viscous and difficult to pipette. Accurate pipetting of these low surface tension and viscous samples becomes extremely important in highly sensitive applications, such as quantitative PCR (qPCR), genomics and proteomics. Thus, retention of residual fluid in the tip can have a substantial adverse impact upon the reliability and reproducibility of experimental results and data fidelity.

In order to overcome the effects of reduced surface tension and viscosity, and restore accuracy, the surface of pipette tips can be modified to render them "superhydrophobic." Rainin Low Retention (LR) tips are molded with an advanced fluoropolymer that places a dense lawn of fluorines on the surface, preventing direct interaction between the sample liquid and the polypropylene surface. This prevents liquid adhesion and overcomes the reluctance of tactile, viscous and low surface tension liquids to move within the pipette tip. As a result, this substantially enhances aspiration and dispensing of sample liquids and "normalizes" pipetting efficiency across a range of liquids with widely differing compositions, viscosities and surface tensions. As depicted in Figure 3, the presence of a superhydrophobic surface reduces adhesion, allowing the surface tension and cohesion of aqueous liquids to form the characteristic liquid "bead."

Figure 3 – Interaction of low surface tension fluid with super hydrophobic tip surface[1]

Superhydrophobic tip surfaces

In this paper we compare the performance of Rainin LR LTS tips to standard polypropylene LTS tips using samples of varying viscosities, reduced surface tension, varying organic composition and high protein concentration.

Figure 4 – A visual comparison of liquid sheeting between a standard tip (left) and a superhydrophobic low retention tip (right), using blue food colorant containing propylene glycol.

Methodology

Rainin Low Retention LTS tips were evaluated using a number of different solution types with diverse compositions and physical properties. To evaluate the Rainin LR LTS tip performance with respect to varying viscosities, the test solutions consisted of increasing concentrations of glycerol (5%, 10%, 20%, 30%, 40% and 50%). To evaluate the performance in the presence of low surface tension, surfactant-containing liquids, the test solutions consisted of 0.1% Triton-X-100, 5% Tween 20 and 10% sodium dodecyl sulfate (SDS). The performance of Rainin LR LTS tips was also evaluated with the following commonly used organic liquids: 50% acetonitrile (ACN), 50% dimethyl sulfoxide (DMSO) and 50% isopropanol (IPA). The LR LTS tip performance was also evaluated with protein samples using 20mg/mL of bovine serum albumin (BSA) dissolved in 50mM Tris-Cl pH 7.6 and 1mM NaCl. All of the test solutions described were present in buffer 50mM Tris-Cl pH 7.6, 1mM NaCl and 12.5mM cibacron blue dye.

To eliminate any manual effect during pipetting, the pipette used for the study was a Rainin electronic E4-200XLS+. The E4-200XLS+ was used at 200 µL setting with the aspiration and dispense speeds set at 5. The tips were pre-rinsed with respective test solutions but without cibacron blue dye. The 200 µL test solutions were cycled five times in the tip followed by complete blowout following final dispense. The residual fluid remaining in the tip was recovered with 200 µL of fresh buffer. Replicate groups consisted of 10 tips. The absorbance of the cibachron blue-containing rinsates was measured at 615 nm using ThermoScientific Multiskan Sky plate reader. The volume of retained residual fluid was determined from the linear regression of respective standard curves for the each of the test solutions. Rinsates containing BSA were quantified using Pierce BCA Protein Assay Kit (Cat. No. - 23227). The standard curve for BSA was generated as per the manufacturer's instruction.

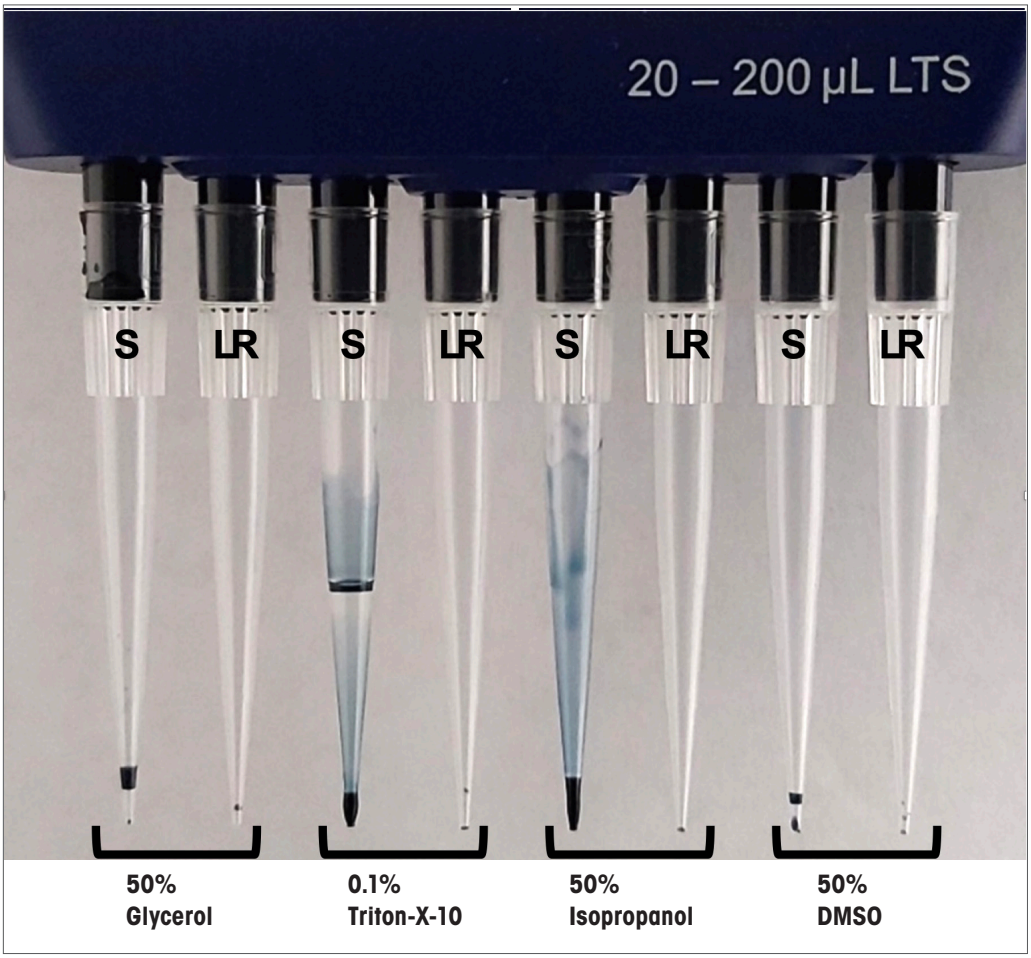

Figure 5 – Comparative liquid retentions of Standard (S) and Low Retention (LR) 200 µL tips tips with different solution types.

Results

1. Pipetting viscous samples

Viscous fluids are more difficult to move with the relatively weak negative and positive pressures generated by air displacement pipettes. This provides an opportunity for insufficient volumes of liquid to be aspirated and dispensed, thereby compromising pipetting accuracy.

As demonstrated in Figure 6, the retention of glycerol-containing solutions in standard LTS tips increased, in a manner roughly equivalent to the glycerol concentration. These data indicate that at 50% glycerol, 2.7% of the original 200 µL sample volume was retained in a standard LTS tip. In contrast, the Rainin LR LTS tips retained only negligible glycerol-containing liquid in a manner that is apparently unrelated to the ascending glycerol concentrations. At the highest (50%) glycerol concentration, only ~0.04% of the original sample was retained.

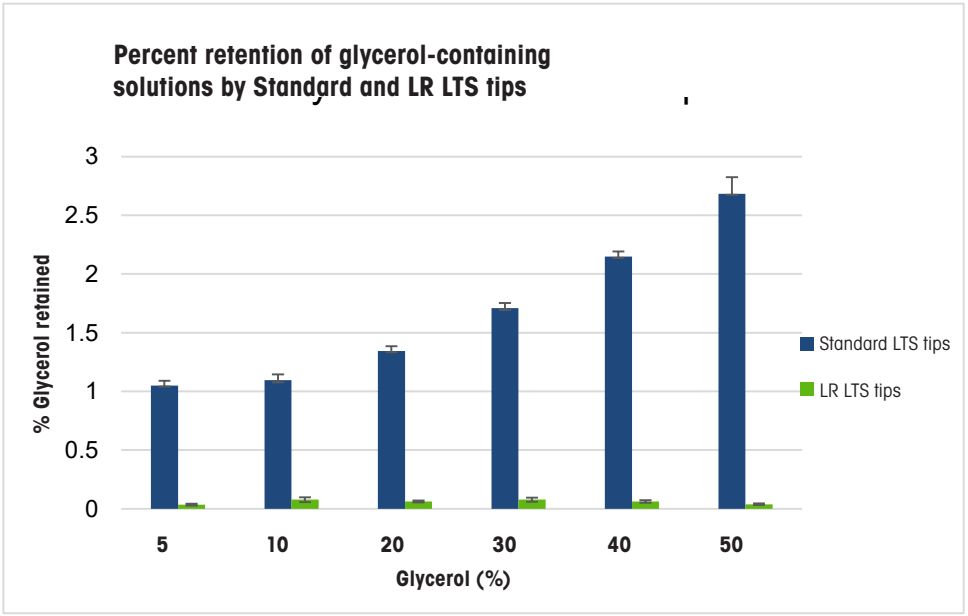

Figure 6 – Retention of glycerol-containing solutions in standard LTS and LR 200 µL tips LTS tips

2. Pipetting low surface tension liquids

The retention of low surface tension liquids in the LR LTS and standard LTS tips was compared by pipetting different surfactant solutions (Figure 7). Rainin LR LTS tips retained 0.04% of 0.1% Triton-X-100 while standard LTS tips showed 1.5% retention. With respect to 5% Tween-20 and 10% SDS, LR LTS tips retained approximately 0.4% and 1% of Tween 20 and SDS respectively, while standard LTS tips exhibited much greater retention of approximately 1.4% and 2.2%, respectively.

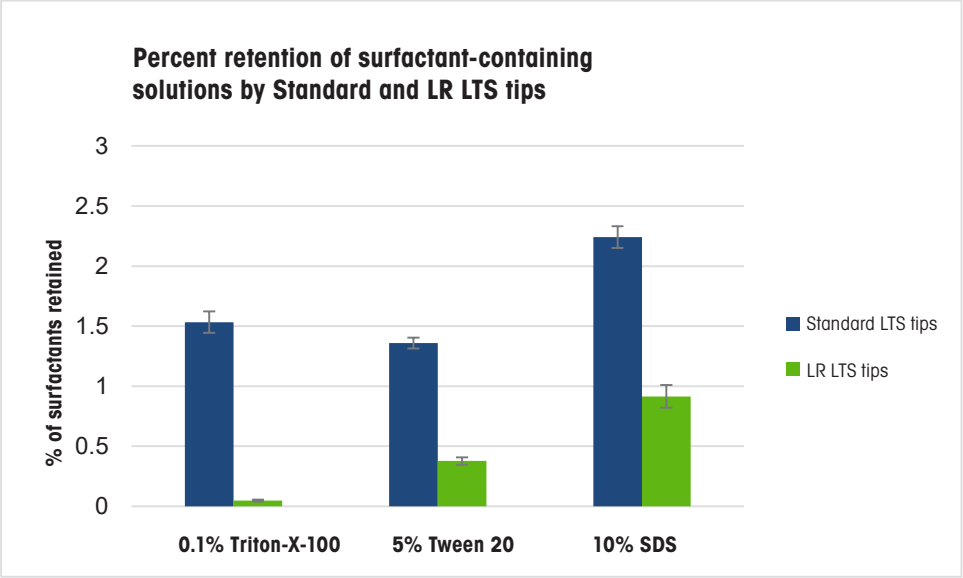

Figure 7 – Retention of 0.1% Triton-X-100, 5% Tween 20 and 10% SDS in standard LTS and LR LTS 200 µL tips.

3. Pipetting organic liquids

Various solvents like isopropanol, DMSO and acetonitrile are frequently used in biological research applications. The extent of retention of these solvents at 50% concentration in standard LTS and LR LTS tips was determined (Figure 8). Standard LTS tips retained 2.8% of 50% IPA as compared to LR LTS tips that showed 10 times less retention at 0.25%. Standard LTS tips retained four times more 50% DMSO as compared to LR LTS tips, which retained 0.1% of 50% DMSO. With respect to 50% ACN, standard LTS tips retained ~1% of solution compared to LR LTS tips that show 0.06% retention.

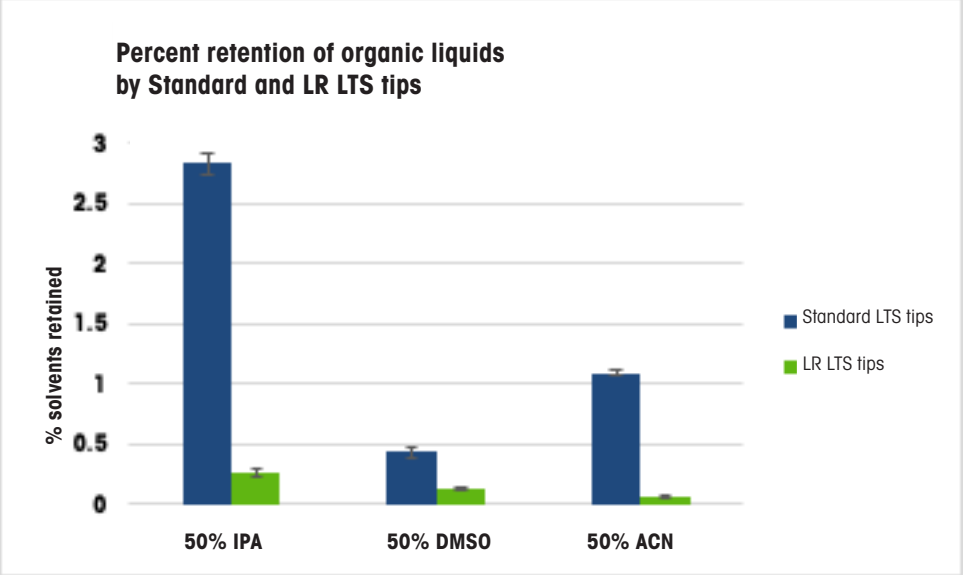

Figure 8 – Retention of 50% isopropanol (IPA), dimethyl sulfoxife (DMSO) and acetonitrile (ACN) in standard and LR LTS 200 µL tips.

4. Pipetting protein solutions

Proteins are extensively pipetted in a wide range of biological research applications. High protein concentrations exhibit greater viscosity and reduced surface tension and, hence, are often recalcitrant to pipetting. When tested with 20 mg/mL BSA, standard LTS tips retained ~2% of BSA, while LR LTS tips retained 4 times less at 0.5%. This clearly indicates the advantage of using LR LTS tips in pipetting protein samples (Figure 9).

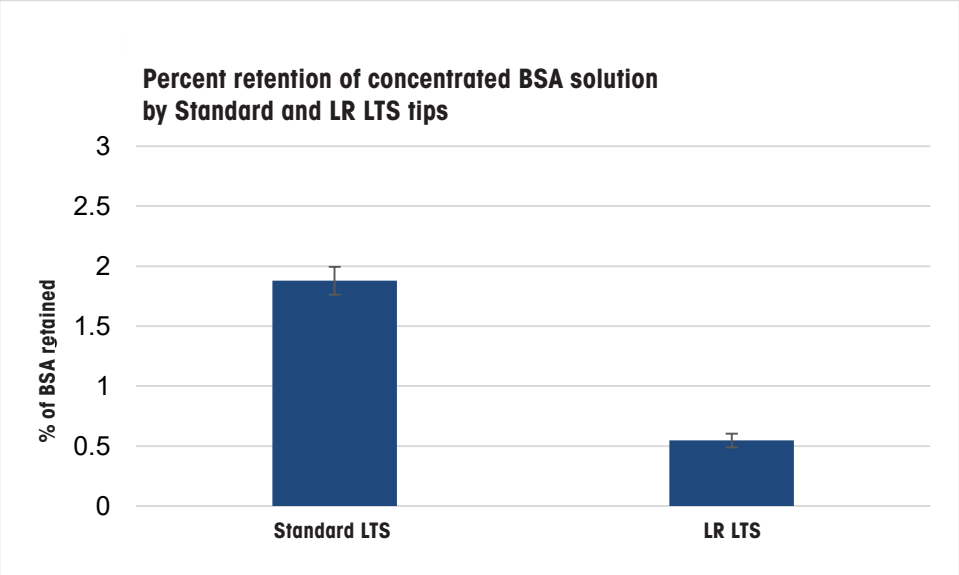

Figure 9 – Retention of 20mg/mL BSA in standard and LR 200 µL LTS tips.

Table No. 1. depicts the comparative performance of standard and LR LTS tips across the range of different liquid sample type evaluated.

Sample / Test Solution	Retention	
	Standard LTS	Low Retention Tip
5% Glycerol	1.050±0.040	0.034±0.008
10% Glycerol	1.096±0.047	0.078±0.020
20% Glycerol	1.342±0.043	0.061±0.009
30% Glycerol	1.709±0.043	0.077±0.017
40% Glycerol	2.148±0.042	0.060±0.010
50% Glycerol	2.681±0.141	0.038±0.007
0.1% Triton-X-100	1.533±0.089	0.047±0.007
5% Tween 20	1.359±0.044	0.377±0.030
10% SDS	2.241±0.091	0.915±0.094
50% IPA	2.824±0.084	0.253±0.028
50% DMSO	0.427±0.040	0.127±0.019
50% ACN	1.085±0.024	0.061±0.005
20 mg/mL BSA	1.878±0.116	0.545±0.056

Conclusions

The various liquids and solutions used in life science research labs are diverse, exhibiting an almost limitless range of physical and chemical properties. Many of these liquid types pose challenges to accurate pipetting due to their tendency to resist complete aspiration and dispensing. Incomplete dispensing (liquid retention) will adversely impact experimental outcomes and data fidelity by altering the relative amounts of various reactants in carefully crafted experiments. The data presented in this report clearly demonstrate the effectiveness of a low retention tip fluoropolymer surface in enhancing the movement of various liquid types and improving dispensing.The fluoropolymer surface of LR tips negates the effects of liquid composition, substantially reducing liquid retention, facilitating a "normalization" of pipetting accuracy across a wide range of sample types.

References

1. Counteracting Liquid Retention In Tips: Benefits Of Using Superhydrophobic Tips (2016), white paper, Rainin
2. Guide to Good Pipetting: Get Better Results (2020), pipetting handbook, Rainin

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