An Automated Liquid Handling Strategy for Complex Matrices Based on Sample Viscosity

Keywords: Accuracy, Automated Liquid Handling, Beverages, Blood, GX-271, Liquid Handling, Liquid Handlers, Liquid Transfer, Low Volume Dispensing, Optimization, Performance, Pipetting, Polyethylene glycol, PEG, Recovery, TRILUTION® LH Software, Viscosity

This study was presented by Megan Clay, Toni Hofhine and Michael Halvorson, Ph.D. at Pittcon 2010, Orlando, FL, USA

Introduction

Performing manual liquid handling procedures in the laboratory is commonplace. When a laboratory faces an increase in sample volumes and throughput, it is often necessary to automate these liquid handling procedures. Automation of the liquid handling steps should improve day-to-day reproducibility of results.

When transitioning from a manual to an automated process, a user must optimize the operational parameters of the liquid handler. Software parameters include flow rates, rinsing parameters, air gaps, equilibration times and others. Hardware parameters may include the type or inner diameter (ID) of the probe used for the liquid transfer.

The viscosity of a liquid, defined as the resistance to a fluid to shear motion, can be especially challenging when developing an automated liquid handling protocol. Liquids with a higher or lower viscosity compared to water may require a different set of parameters to optimize the method.

This note describes an automation strategy for the scientist who is transitioning from a manual liquid method to an automated method that is based on the viscosity of the liquid matrix. Optimized automation conditions are described for liquid matrices of differing viscosities that one would typically encounter in the laboratory—from very viscous liquids like polyethylene glycol or whole blood to low viscosity liquids such as apple juice, water and methanol.

Experimental Conditions

Materials

The following liquids were employed in the study:

- Ultra-purified water (HPLC grade)
- Methanol (HPLC grade, Burdick & Jackson, Part no. 230-4)
- Polyethylene Glycol (PEG) 200 (Sigma, Part no. P3015)
- Dilutions of PEG 200
- Skim Milk
- 2% Milk
- Orange Juice (no pulp)
- Apple Juice
- Whole Blood (Biological Specialty Corporation, Colmar, PA)

The following equipment was used for the study:

- Cannon-Fenske Viscometer Tube, size 75 (Sigma, Part no. Z275298)

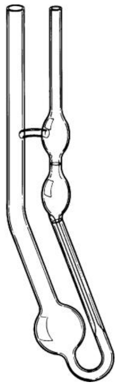


Figure 1. Cannon-Fenske Viscometer (image from www.sigmaaldrich.com)

- AND HM-202 Analytical Balance (A&D Weighing, San Jose, CA)
- Gilson GX-271 ASPEC™ with 406 Dual Syringe Pump (see Figure 2)

Figure 2. Gilson GX-271 ASPEC with 406 Dual Syringe pump (Part no. 2614008)

The Gilson GX-271 System was configured as follows:

Description	Part numbers
GX-271 ASPEC w/Dual 406 Syringe Pump	2614008
10 mL Syringes	(2) 25025345
406 Dual Adaption Kit for ASPEC and Two 10 mL Plumbing Packages	2644708 and 2644701
221 x 1.5 x 1.1mm BV Constricted Probe and 221 x 1.5 x 0.4 mm Beveled Probe	27067373 and 27067383
Rinse Stations	26034551 and 26034555
Guide Assemblies for 1.5 mm probes	2604611 and 26046228
Locator Tray for five 20-Series Racks, GX-274	26041032
Rack Code 345 for 44 – 16 x 150mm tubes	260440041
Rack Code 334 for 14 – 40 mL scintillation vials	260440081
Rack Code 343 for 80 – 13 x 100 mm tubes	260440025
Safety Shield Assembly, GX27X	2604706
TRILUTION® LH Software	21063020, 210630R20 and ORACLE10GXE

Measuring Sample Viscosity

Sample viscosity was measured in centistokes (cSt) using a Cannon-Fenske Viscometer according to established procedures (ASTM D445-09 Standard Test Method, Shugar and Ballinger, 1990). Samples were allowed to equilibrate for 10 minutes in a water bath with a temperature of 25°C before a measurement was taken. Measurements were taken in triplicate.

Liquid Transfer Using TRILUTION® LH Software

One milliliter of sample was aspirated and dispensed at different flow rates using an air gap setting of 50 µL. In each case, the probe was rinsed with an Outside Rinse with 2 mL of water at a flow rate of 20 mL/min and an Inside Rinse with 3 mL of water at a flow rate of 20 mL/min. An Equilibration Time was added in some cases to allow for pressure to equalize before aspirating or dispensing a sample. See software details in Figures 3–5.

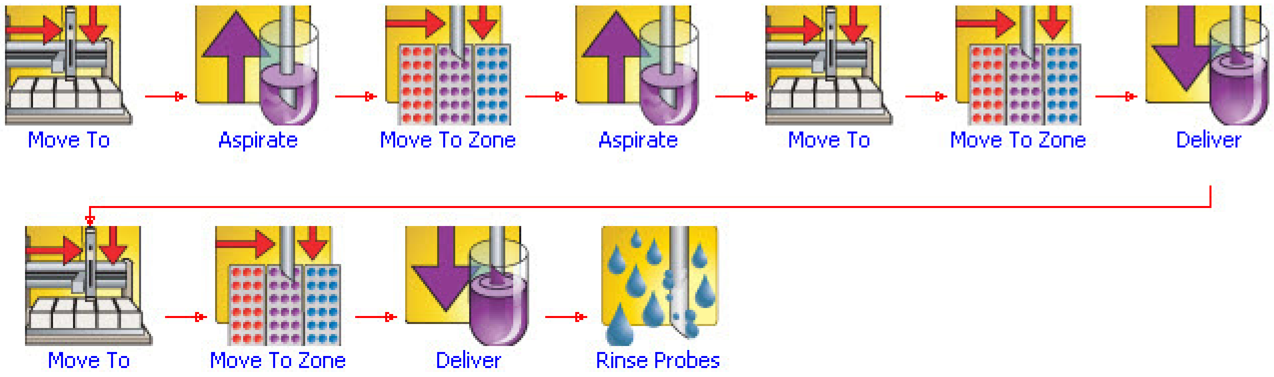
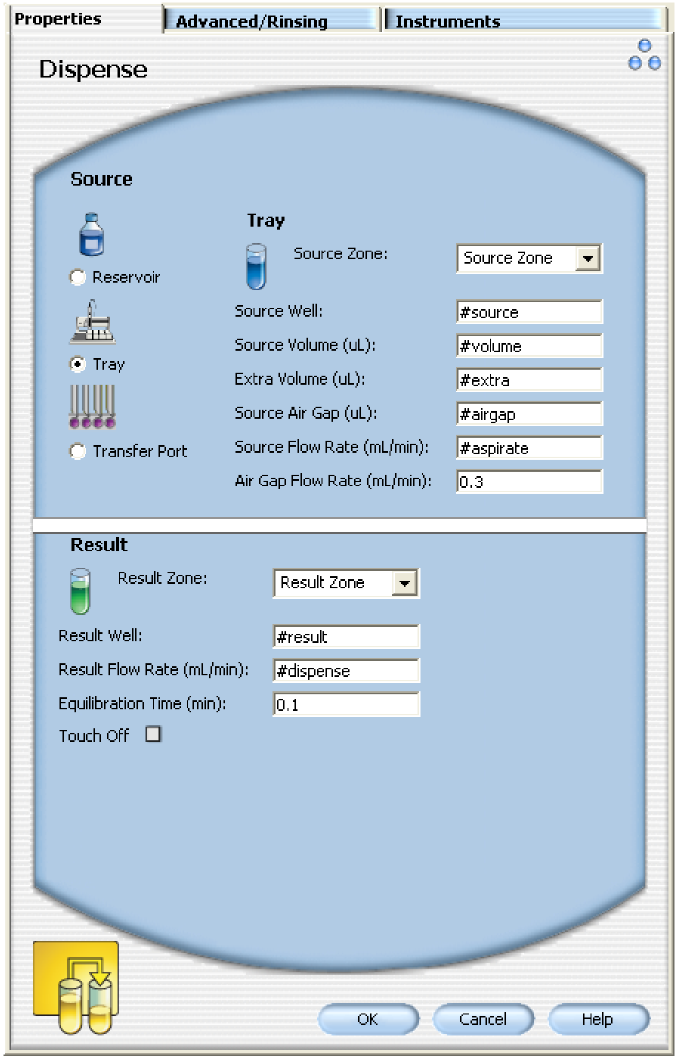


Figure 3. Transfer Method Tasks for aspirating and dispensing samples (includes air gap steps)



Source

○ Reservoir

● Tray

○ Transfer Port

Tray

Source Zone: Source Zone

Source Well: #source

Source Volume (uL): #volume

Extra Volume (uL): #extra

Source Air Gap (uL): #airgap

Source Flow Rate (mL/min): #aspirate

Air Gap Flow Rate (mL/min): 0.3

Result

Result Zone: Result Zone

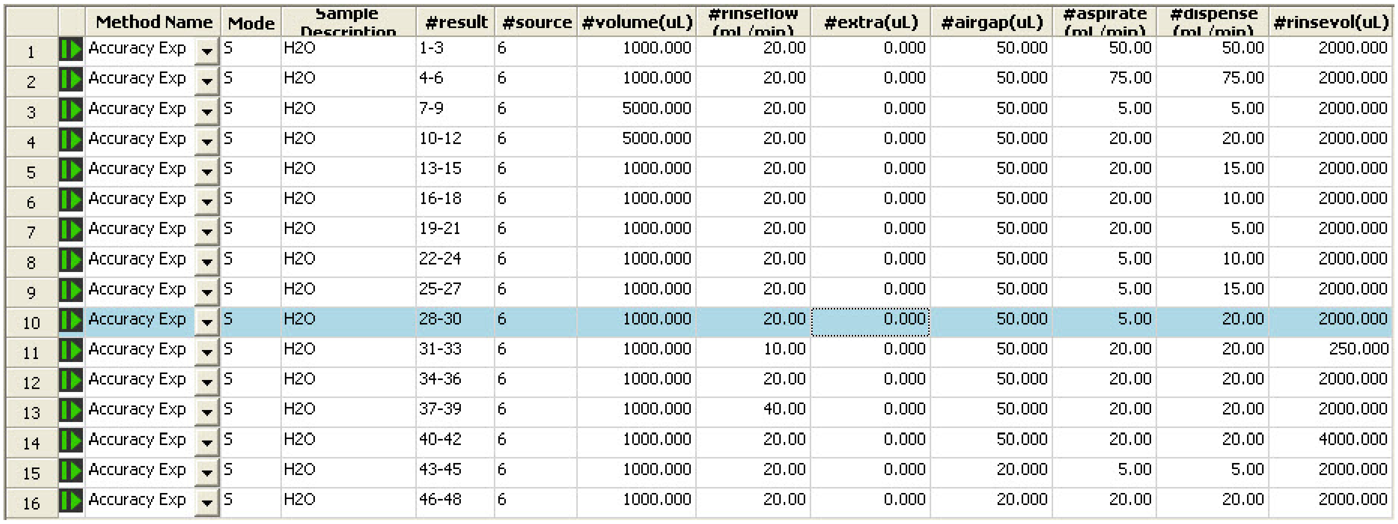
Result Well: #result

Result Flow Rate (mL/min): #dispense

Equilibration Time (min): 0.1

Touch Off ☐

Figure 4. Dispense Task Property Page From TRILUTION® LH Method. The # sign indicates that these properties are set as variables in the Sample List (See Figure 5). The variables used allow for flexibility when running samples with different viscosities within the same Sample List.



	Method Name	Mode	Sample Description	#result	#source	#volume(uL)	#rinseflow (mL/min)	#extra(uL)	#airgap(uL)	#aspirate (mL/min)	#dispense (mL/min)	#rinsevol(uL)
1	Accuracy Exp	S	H2O	1-3	6	1000.000	20.00	0.000	50.000	50.00	50.00	2000.000
2	Accuracy Exp	S	H2O	4-6	6	1000.000	20.00	0.000	50.000	75.00	75.00	2000.000
3	Accuracy Exp	S	H2O	7-9	6	5000.000	20.00	0.000	50.000	5.00	5.00	2000.000
4	Accuracy Exp	S	H2O	10-12	6	5000.000	20.00	0.000	50.000	20.00	20.00	2000.000
5	Accuracy Exp	S	H2O	13-15	6	1000.000	20.00	0.000	50.000	20.00	15.00	2000.000
6	Accuracy Exp	S	H2O	16-18	6	1000.000	20.00	0.000	50.000	20.00	10.00	2000.000
7	Accuracy Exp	S	H2O	19-21	6	1000.000	20.00	0.000	50.000	20.00	5.00	2000.000
8	Accuracy Exp	S	H2O	22-24	6	1000.000	20.00	0.000	50.000	5.00	10.00	2000.000
9	Accuracy Exp	S	H2O	25-27	6	1000.000	20.00	0.000	50.000	5.00	15.00	2000.000
10	Accuracy Exp	S	H2O	28-30	6	1000.000	20.00	0.000	50.000	5.00	20.00	2000.000
11	Accuracy Exp	S	H2O	31-33	6	1000.000	10.00	0.000	50.000	20.00	20.00	250.000
12	Accuracy Exp	S	H2O	34-36	6	1000.000	20.00	0.000	50.000	20.00	20.00	2000.000
13	Accuracy Exp	S	H2O	37-39	6	1000.000	40.00	0.000	50.000	20.00	20.00	2000.000
14	Accuracy Exp	S	H2O	40-42	6	1000.000	20.00	0.000	50.000	20.00	20.00	4000.000
15	Accuracy Exp	S	H2O	43-45	6	1000.000	20.00	0.000	20.000	5.00	5.00	2000.000
16	Accuracy Exp	S	H2O	46-48	6	1000.000	20.00	0.000	20.000	20.00	20.00	2000.000

Figure 5. An example Sample List from TRILUTION LH showing Variables. See also Figure 4.

Measurements of Precision and Recovery

All measurements were performed in triplicate. Clean and empty 13 x 100 mm test tubes were weighed and weights recorded. Transfer 1 mL of sample to the tube using the GX-271 and TRILUTION LH Transfer Method illustrated above. Weigh the test tube containing the sample and record the weight. Convert the sample weight to sample volume based on the density of the sample.

Results

Table 2. Viscosity and Density of Samples Tested. The density value was used in the % recovery calculations.

Sample Name	Viscosity (cSt)	Density (g/mL)
PEG 200	45	1.127
Whole Blood	21.39	1.0553
2% Milk	4.05	1.0497
1:2 (PEG 200:Water)	3.64	1.0601
2:5 (PEG 200:Water)	2.87	1.0503
Skim Milk	2.62	1.042
Orange Juice	2.60	1.0576
1:3 (PEG 200:Water)	2.60	1.0364
Apple Juice	1.90	1.0533
1:5 (PEG 200:Water)	1.85	1.0211
1:6 (PEG 200:Water)	1.62	1.0204
1:7 (PEG 200:Water)	1.52	1.0214
1:20 (PEG 200:Water)	1.15	1.0056
Water	1.06	0.9952
Methanol	0.6	0.7913

Figures 6 and 7 show the observed relationship between sample flow rate and viscosity over a flow rate range of 5–20 mL/min. Figure 6 clearly shows that when using the small diameter probe, the recovery drops off significantly as the flow rate increases. Figures 8 and 9 further expand upon this by taking three of the study samples and expanding the flow rate up to 75 mL/min. The recovery values and the coefficient of variation are found in Tables 3 and 4.

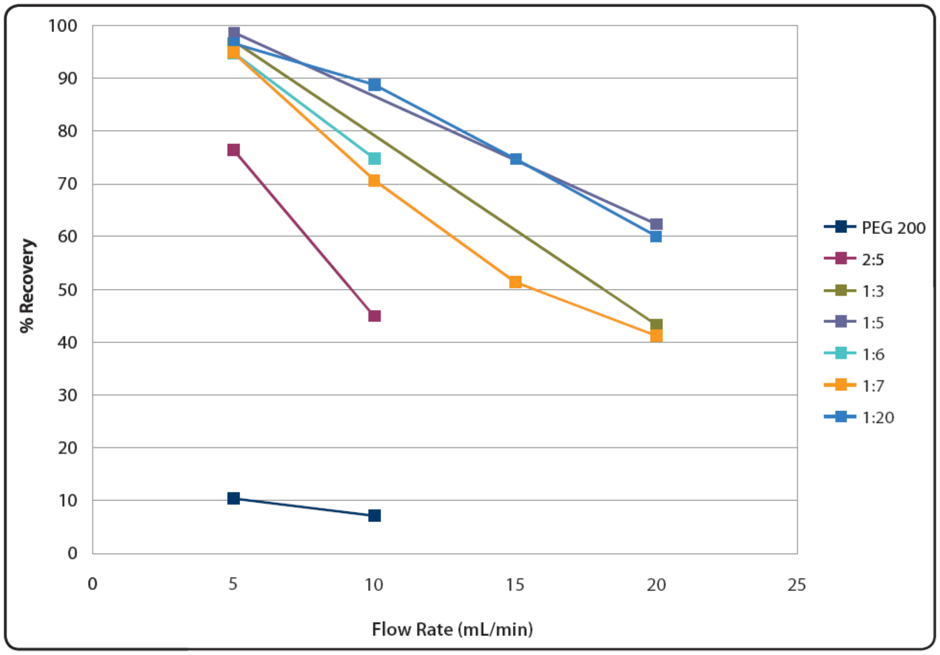


Figure 6. Recovery when transferring 1000 µL of sample at flow rates from 5–20 mL/min using a 0.4 mm ID probe.

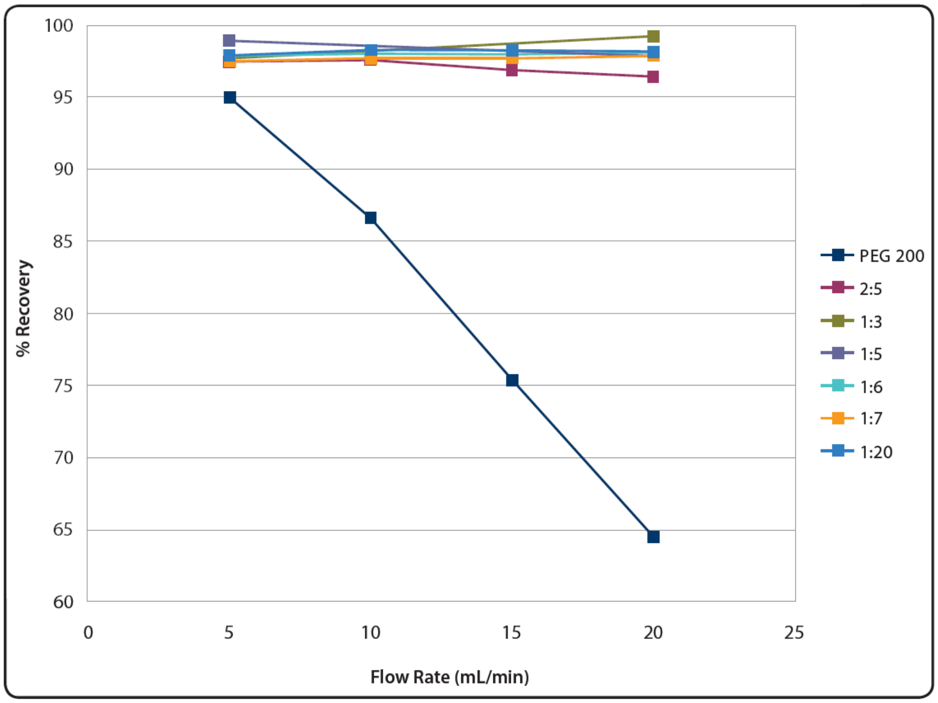


Figure 7. Recovery when transferring 1000 µL of sample at flow rates from 5–20 mL/min using a 1.1 mm ID probe.

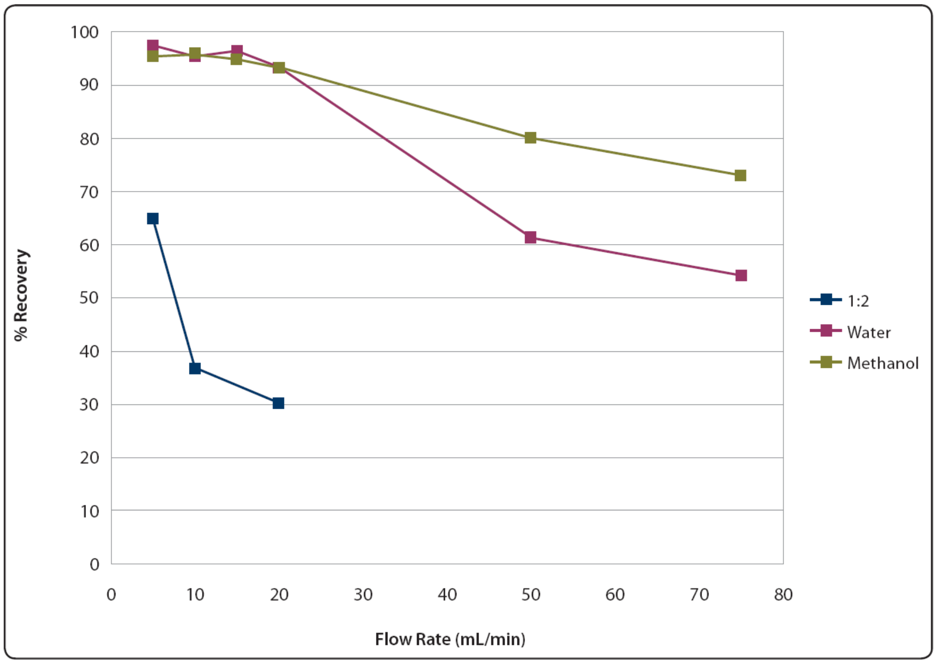


Figure 8. Recovery when transferring 1000 µL of selected samples at flow rates up to 75 mL/min using a 0.4 mm ID probe.

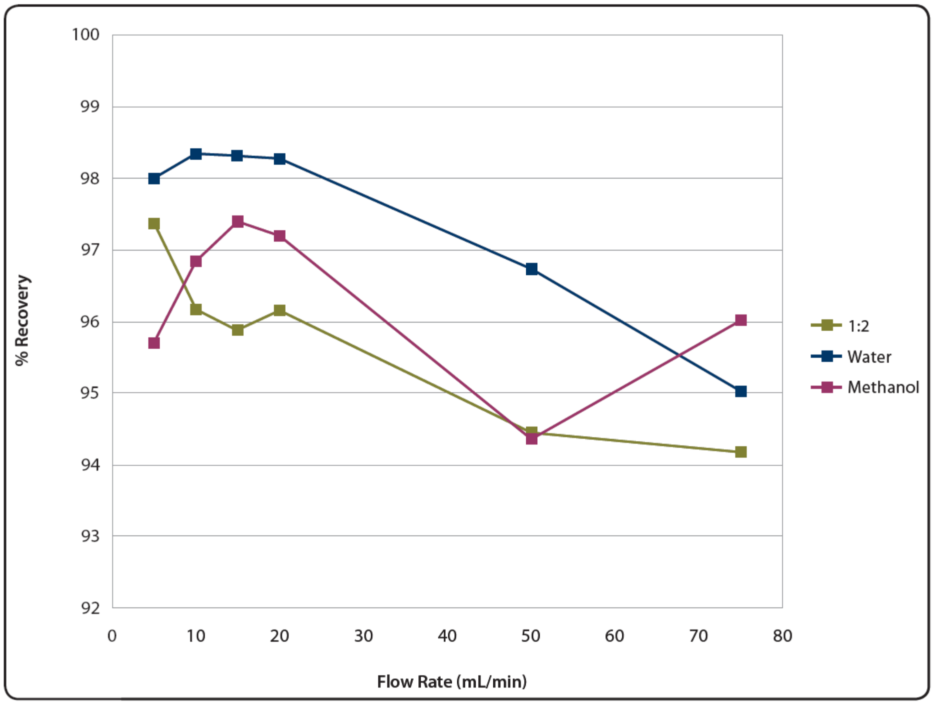


Figure 9. Recovery when transferring 1000 µL of selected samples at flow rates up to 75 mL/min using a 1.1 mm ID probe.

Table 3. General Recovery and Precision with a 1.1 mm ID Probe

	1.1 mm ID probe (P/N 27067373) with Water as Reservoir Solvent											
Sample	**5 mL/min**	**%CV**	**10 mL/min**	**%CV**	**15 mL/min**	**%CV**	**20 mL/min**	**%CV**	**50 mL/min**	**%CV**	**75 mL/min**	**%CV**
PEG 200	95.02	2.00	86.65	0.44	75.39	1.89	64.57	2.05	N/A		N/A	
1:2	97.37	0.20	96.18	0.62	95.88	0.43	96.15	0.55	94.44	0.10	94.18	0.07
2:5	97.50	0.02	97.56	0.05	96.87	0.57	96.42	0.58	N/A		N/A	
1:3	97.73	0.16	N/A		N/A		99.25	0.26	N/A		N/A	
1:5	98.95	0.15	N/A		N/A		97.92	0.02	N/A		N/A	
1:6	97.91	0.03	97.99	0.03	97.96	0.01	98.08	0.08	N/A		N/A	
1:7	97.52	0.01	97.67	0.04	97.67	0.03	97.84	0.04	N/A		N/A	
1:20	97.90	0.02	98.30	0.09	98.28	0.01	98.20	0.01	N/A		N/A	
Water	98.00	0.09	98.34	0.07	98.31	0.09	98.28	0.03	96.73	0.07	95.02	0.12
Methanol	95.69	0.33	96.84	0.57	97.39	0.12	97.19	0.29	94.36	1.49	96.01	0.22

Table 4. General Recovery and Precision with a 0.4 mm ID Probe

	0.4 mm ID probe (P/N 27067383) with Water as Reservoir Solvent											
Sample	**5 mL/min**	**%CV**	**10 mL/min**	**%CV**	**15 mL/min**	**%CV**	**20 mL/min**	**%CV**	**50 mL/min**	**%CV**	**75 mL/min**	**%CV**
PEG 200	10.47	6.29	7.24	26.10	N/A		N/A		N/A		N/A	
1:2	64.74	2.77	36.93	2.61	N/A		30.31	7.31	N/A		N/A	
2:5	76.44	0.42	45.02	2.64	N/A		N/A		N/A		N/A	
1:3	97.12	0.64	N/A		N/A		43.23	2.91	N/A		N/A	
1:5	98.73	0.04	N/A		N/A		62.44	0.72	N/A		N/A	
1:6	94.88	0.29	74.86	4.31	N/A		N/A		N/A		N/A	
1:7	94.92	0.43	70.74	3.03	51.40	1.51	41.30	3.76	N/A		N/A	
1:20	96.66	0.15	88.83	1.30	74.79	2.43	60.15	0.60	N/A		N/A	
Water	97.47	0.05	95.36	0.48	96.41	0.40	93.38	2.09	61.29	3.14	54.23	2.88
Methanol	95.37	0.67	95.72	0.62	94.83	0.06	93.26	0.77	80.05	8.13	73.05	8.85

Figure 10 demonstrates the effect of equilibrium time (leaving the probe in the sample for a pre-determined amount of time after syringe aspiration) on sample recovery. All three data points for each sample were performed at a flow rate of 10 mL/min. The first data point has no equilibration times and demonstrates that highly viscous samples cannot be accurately dispensed at a flow rate of 10 mL/min with a 0.4 mm ID probe. The second and third data points add 15 and 30 seconds of equilibration time respectively. One can observe the significant increase in recovery, especially for the more viscous samples. The recovery and precision data for Figure 10 are shown in Table 5.

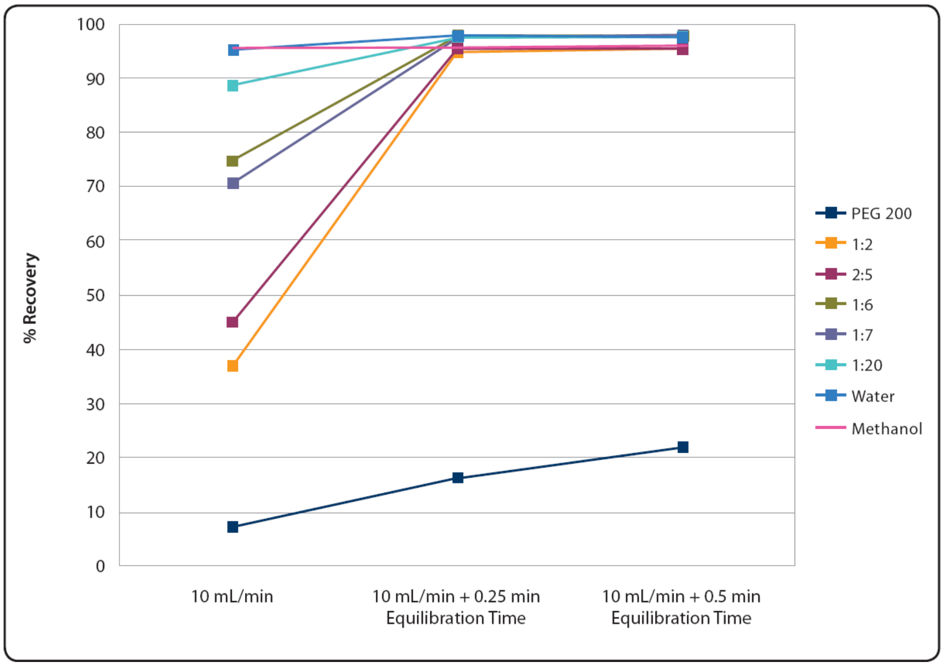


Figure 10. Effect of equilibration time on recovery when 1000 µL of sample is transferred using a 0.4 mm ID probe at a flow rate of 10 mL/min

Table 5. Effect of Equilibration Time on Recovery

	0.4 mm ID probe (P/N 27067383) with Water as Reservoir Solvent					
Sample	**10 mL/min**	**%CV**	**10 mL/min + 0.25 min Equilibration Time**	**%CV**	**10 mL/min + 0.5 min Equilibration Time**	**%CV**
PEG 200	7.24	26.10	16.21	3.07	21.93	2.81
1:2	36.93	2.61	94.94	0.14	95.58	0.22
2:5	45.02	2.64	95.53	0.09	95.53	0.28
1:6	74.86	4.31	98.01	0.32	97.73	0.06
1:7	70.74	3.03	97.58	0.31	97.96	0.04
1:20	88.83	1.30	97.58	0.08	97.63	0.05
Water	95.36	0.48	97.87	0.02	97.70	0.07
Methanol	95.72	0.62	95.75	0.13	96.26	0.09

Figure 11 (below) relates the generalized PEG 200:Water samples used for the majority of the study back to actual ("real world") samples used in the laboratory. The samples included various beverages and whole blood. It demonstrates how the recoveries found for the generic samples can be related back to all samples with a similar viscosity. The recovery values and %CV data for the beverage and blood samples compared in the study can be found below in Table 6.

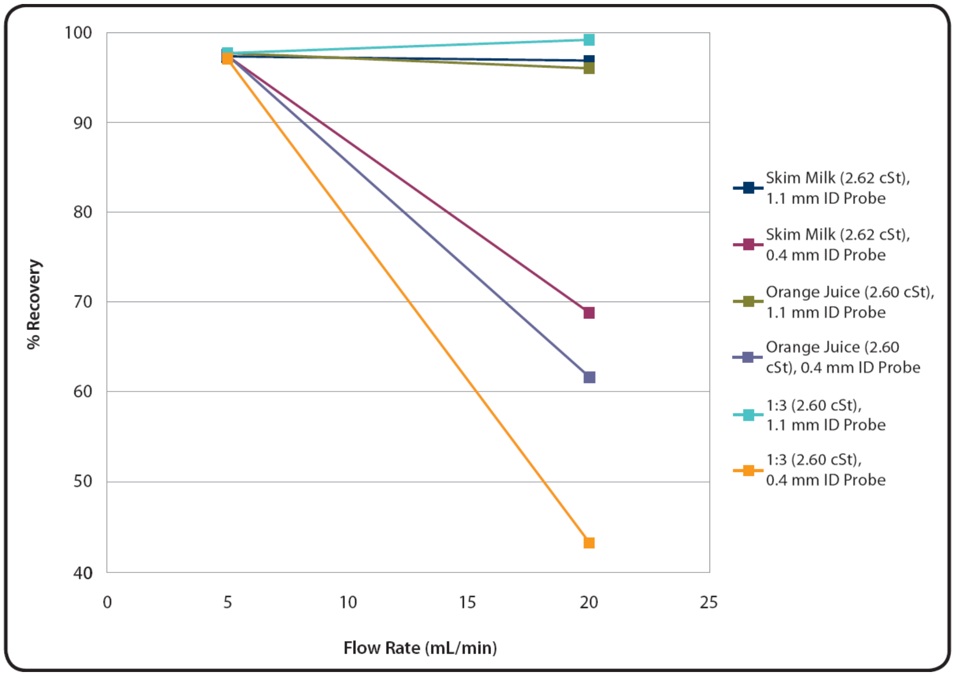


Figure 11. Effects of flow rate on the recovery of selected beverage samples

Table 6. Recovery and Precision when transferring beverage and whole blood samples using different ID probes and flow rates

	1.1 mm ID probe (P/N 27067373) with Water as Reservoir Solvent				0.4 mm ID probe (P/N 27067383) with Water as Reservoir Solvent			
Sample	**5 mL/min**	**%CV**	**20 mL/min**	**%CV**	**5 mL/min**	**%CV**	**20 mL/min**	**%CV**
Whole Blood	95.24	0.15	94.92	0.22	29.50	1.66	8.31	6.86
2% Milk	96.87	0.04	95.44	0.07	96.88	0.06	61.66	1.74
Skim Milk	97.39	0.13	96.90	0.03	97.46	0.32	68.85	4.28
Orange Juice	97.76	0.05	96.14	0.12	97.48	0.09	61.69	4.39
Apple Juice	97.70	0.27	97.67	0.10	97.69	0.24	81.16	3.92

Conclusion

Based on the experimental data, an automation strategy was developed which recommends the appropriate probe diameters and flow rate to use with liquids of varying viscosities. A **Sample Viscosity Flow Chart** is provided on the last page of this document. This should assist the researcher in making decisions about flow rates and appropriate probe size when transferring a manual protocol to an automated liquid handling protocol.

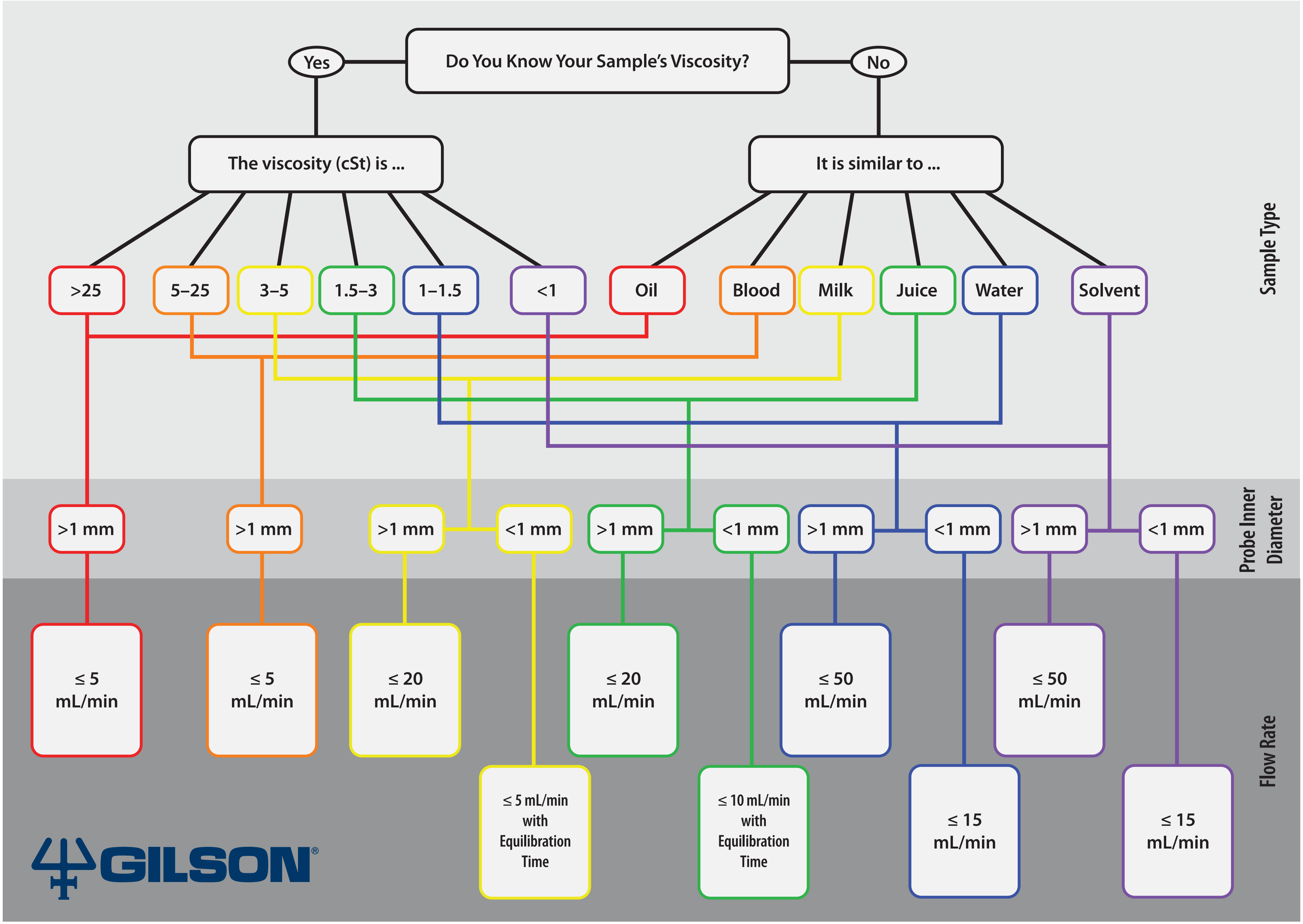
The data provided indicate that flow rates highly affect sample recoveries. Low flow rates achieved the best recovery values. The inner diameter (ID) of the probe also had an effect on recovery values. The 0.4 mm ID probe produced lower recovery values with liquids of higher viscosity. Using a 0.5 minute recovery time with this probe improved results significantly. The %CV of transfers with recovery rates of 95% or greater ranged from 0.01% to 2.0%. When automating sample transfers of highly viscous samples, using a low flow rate and a probe with a larger ID are recommended.

References

The Engineering Toolbox (2009). Fluids – kinematic Viscosities. Kinematic viscosities for some common fluids. http://www.engineeringtoolbox.com/kinematic-viscosity-d_397.html

Shugar and Balinger (1990). Viscosity. In **Chemical Technician's Ready Reference Handbook, Third edition.** McGraw-Hill, Inc. Pages 408-415.

Sample Viscosity Flow Chart



Do You Know Your Sample's Viscosity?

Yes

No

The viscosity (cSt) is ...

It is similar to ...

>25

5–25

3–5

1.5–3

1–1.5

<1

Oil

Blood

Milk

Juice

Water

Solvent

Sample Type

>1 mm

>1 mm

>1 mm

<1 mm

>1 mm

<1 mm

>1 mm

<1 mm

>1 mm

<1 mm

Probe Inner Diameter

≤ 5 mL/min

≤ 5 mL/min

≤ 20 mL/min

≤ 5 mL/min with Equilibration Time

≤ 20 mL/min

≤ 10 mL/min with Equilibration Time

≤ 50 mL/min

≤ 15 mL/min

≤ 50 mL/min

≤ 15 mL/min

Flow Rate

GILSON®