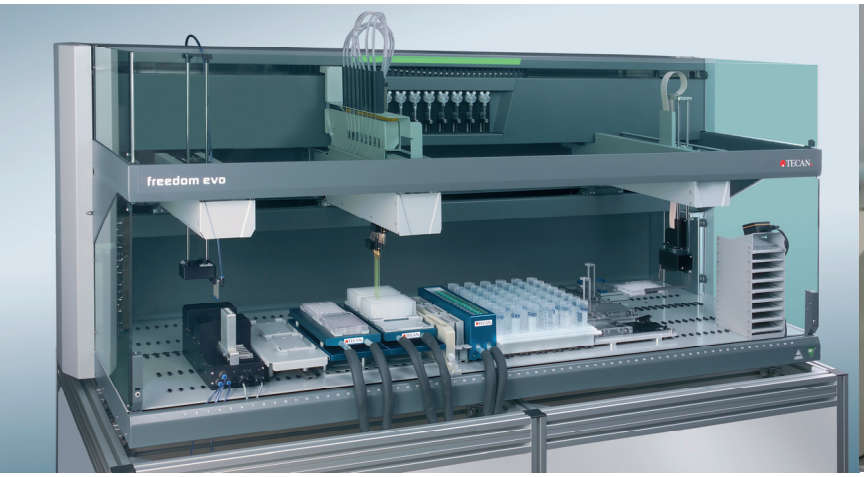
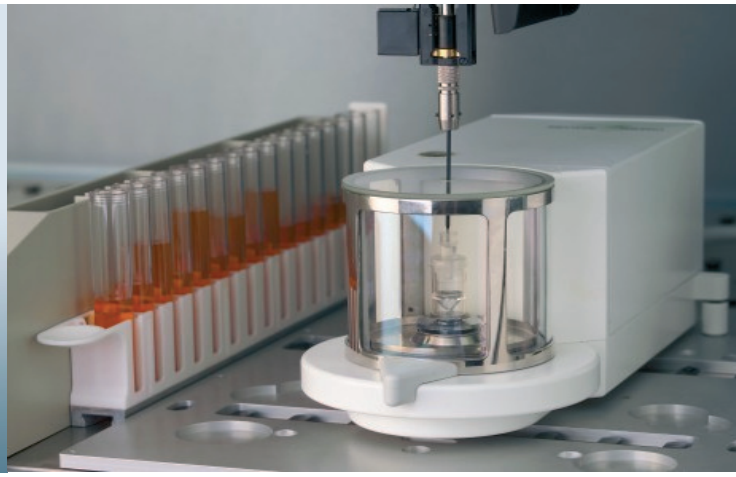
Low volume pipetting on the Tecan Freedom EVO® using Te-PS™ tips

Contact dispensing for low volume applications





Introduction

In the life science world today there is increasing demand for pipetting in the sub microliter range. One such example is protein crystallography, where the desired volume range used in experiments is commonly between 100 nl and 1 µl.
Most methods used to determine pipetting performance in this low volume range rely on indirect measurements with a dye and a diluent. This type of measurement has four potential sources of error: the pipetting of the diluent, the pipetting of the dye, the mixing of the solution in the microtiter plate, and the standard deviation of the reader. To minimize sources of experimental error, a gravimetric pipetting study was designed to determine the performance of a Freedom EVO workstation in this low volume range, under optimized conditions.

The gravimetric method was chosen because of the low error rate of the system and the possibility to measure the pipetting accuracy by calculating the absolute pipetted volume using the density of pipetted water. The experiments measured the pipetting performance of the Tecan Te-PS tips in the 0.1-1.0 µl volume range.
This study demonstrates that the Freedom EVO liquid handling workstation is capable of contact pipetting in the sub microliter range, with very good precision and accuracy.

Materials and methods

Deionized water at room temperature, degassed using helium. Aqueous polyethylene glycol (PEG 10,000), 40% (w/v) solution.

For pipetting, a Tecan Freedom EVO 200/8 liquid handling workstation was configured with eight low volume Te-PS tips, low volume Te-PS tubing and 25 µl syringes.

To weigh pipetted liquids, a Mettler Toledo UMT2 microbalance was used, which had the following specifications:

maximum capacity:	2100 mg
taring range:	2100 mg
readability:	0.0001 mg
linearity:	0.5-1 µg
repeatability:	0.25 µg
long-term stability:	± 0.0001%
stabilization time (typical):	10-16 s

Preparation

Air bubbles in the liquid handling system were removed by flushing with degassed water.
Preliminary measurements showed that the evaporation of water in the balance chamber occurred at a rate of around 3 nl/min (fig 1). This showed that it was important to saturate the balance chamber with water vapor before the experimental measurements, because the balance took 10-16 seconds to stabilize. The balance chamber was therefore saturated with water vapor, by placing cellulose beads soaked with water on the measuring cell. The tubes were left until the balance showed a stable weight.

The laboratory was air-conditioned, with stable relative humidity (17.5%) and temperature (23°C). Constant temperature was important, because the density of water and PEG solutions can be calculated from its temperature.

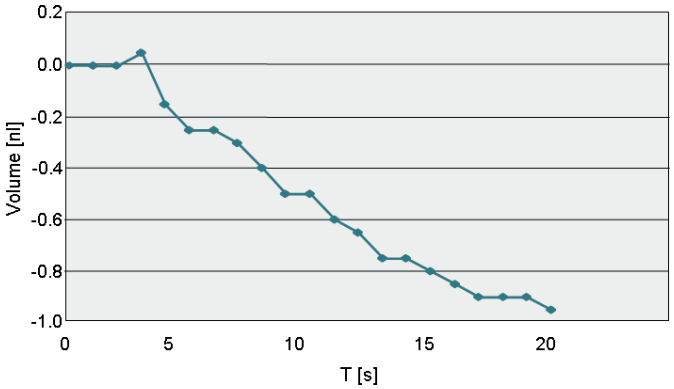


Figure 1 Evaporation of water in balance chamber. The rate of evaporation of water being weighed was sufficiently rapid to affect weight readings, so the balance chamber was saturated with water vapor before experimental readings were taken. Data shown represent the mean of two series of measurements.

Experimental procedure

The desired volume was aspirated prior to taring the balance. The pipetted liquid was dispensed onto a liquid surface for wet contact dispensing, or onto a sheet of Parafilm® for dry contact dispensing. The weight was recorded when the balance displayed a stable weight.

The results for water represent data from six independent experiments, performed on different days over a period of several weeks. Each experiment consisted of 10 measurements for each of eight tips, except for the experiments to evaluate pipetting on dry and wet surfaces, for which 15 data points were measured with one tip.

The results for 40% PEG 10,000 solution represent 40 measurements from each of four tips (160 data points in total) for each volume except 100 nl, for which 10 measurements from each of eight tips (80 data points in total) were made. The results obtained for precision and accuracy were compared to the specifications of a standard manual pipette, adjustable in the 0.1-2.5 µl volume range.

Determination of precision and accuracy

Coefficient of variation (CV) was determined to indicate imprecision (random error):

$$CV[\%] = \frac{stdev}{\overline{vol}} \times 100$$

Inaccuracy (systematic error) indicates the difference of the measured mean to the specified value:

$$Acc[\%] = \frac{\overline{vol} - vol_{specified}}{vol_{specified}} \times 100$$

Results

Pipetting of water using the Freedom EVO and Te-PS tips resulted in precision ranging from CV of 2.1% at 1.0 µl, to 5.2% at 100 nl (fig 2). Specifications for a standard manual pipette in this volume range were CV=1.5% at 1.25 µl and 6.0% at 250 nl.

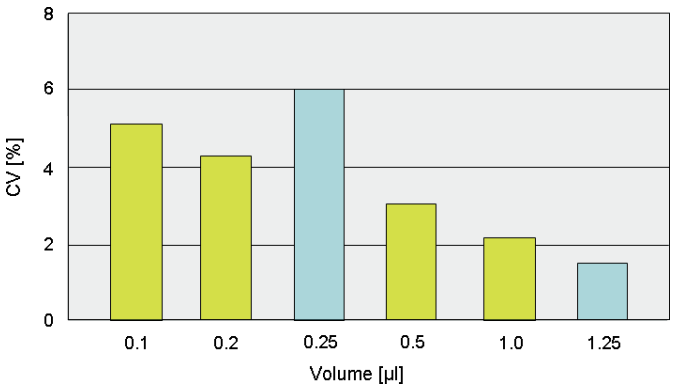


Figure 2 Precision of pipetting water with Freedom EVO workstation (green bars) and a standard manual pipette (blue bars). Data for the Freedom EVO workstation was obtained from gravimetric experiments using the Freedom EVO workstation with Te-PS tips. Data represent 480 data points derived from 10 measurements with eight tips in each of six separate experiments. Data for manual pipetting are specified performance parameters quoted for an adjustable manual pipette.

Pipetting using the Freedom EVO workstation yielded figures of inaccuracy of less than 1% at all volumes tested (fig 3).

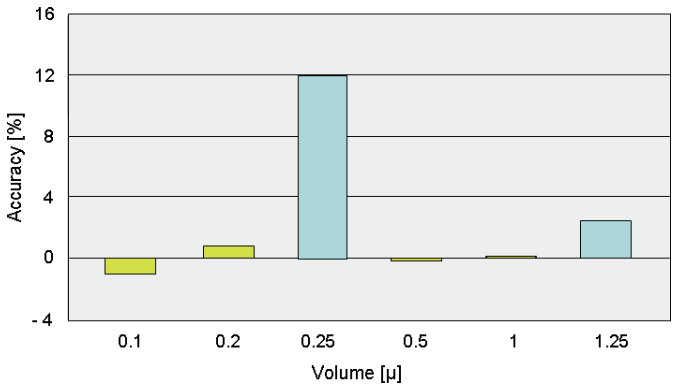


Figure 3 Accuracy of pipetting water with Freedom EVO (green bars) and a standard manual pipette (blue bars). Data for the Freedom EVO workstation was obtained from gravimetric experiments using the Freedom EVO workstation with Te-PS tips. Data represent 480 data points derived from 10 measurements with eight tips in each of six separate experiments. Data for manual pipetting are specified performance parameters quoted for an adjustable manual pipette in this volume range.

When analyzed individually, each of the eight tips of the Te-PS demonstrated consistent performance in pipetting 100 nl (fig 4).

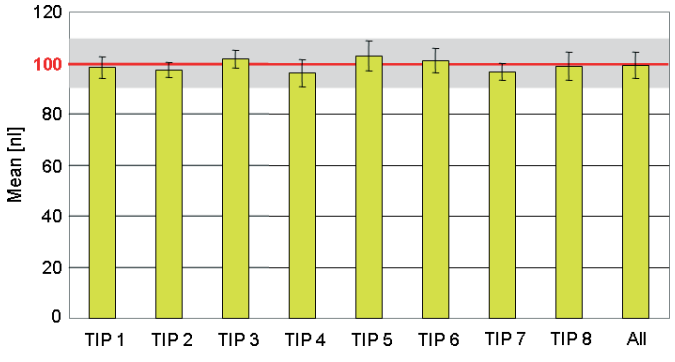


Figure 4 Precision and accuracy of individual Te-PS pipette tips. The data represent means of 60 data points, derived from 10 measurements in each of six separate experiments. Error bars represent standard deviation.

It was shown that dry contact pipetting of 100 nl had a comparable performance to wet contact pipetting for both precision and accuracy (fig 5). With CV values of 5.7% for wet contact and 6.2% for dry contact pipetting, the data offer the confidence to assume that dry contact pipetting will offer similar performance to wet contact pipetting through the volume range of 100 nl-1.0 µl.

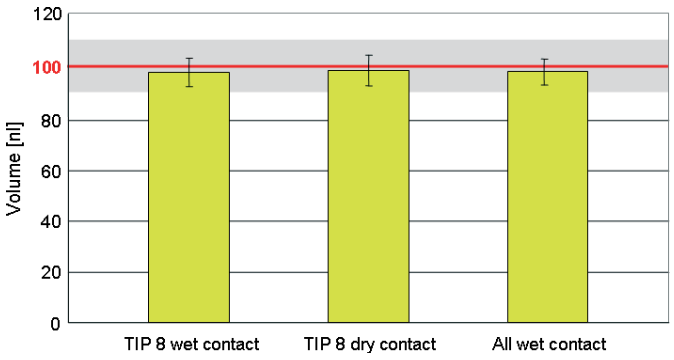


Figure 5 Wet and dry contact pipetting performance. The data represent means of 60 data points, derived from 10 measurements from one tip in each of six separate experiments. Error bars represent standard deviation.

Pipetting 40% PEG 10,000 solutions yielded even better precision than with water. CVs ranged from 0.8% at 1.0 µl, to 4.2% at 100 nl (fig 6). An accuracy of 0.013% was achieved for pipetting 500 nl.

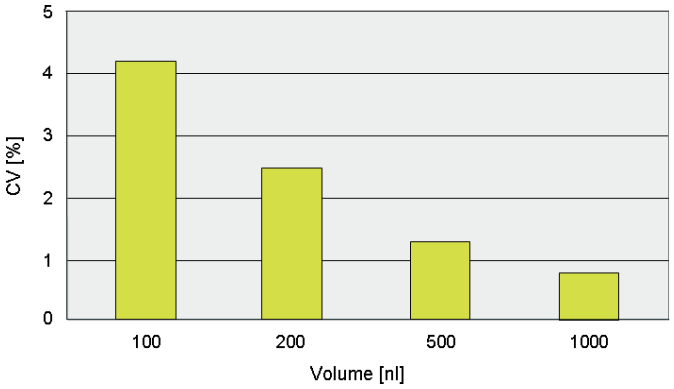


Figure 6 Precision of pipetting 40% PEG 10,000 using the Freedom EVO workstation and Te-PS tips. The data represent 480 datapoints derived from 10 measurements with eight tips in each of six separate experiments.

Conclusion

The capability of the Freedom EVO liquid handling workstation to pipette water and PEG solution in the sub microliter range, under optimized conditions, was tested using a gravimetric experimental method. The results showed that excellent precision and accuracy can be achieved for pipetting low volumes of water across the volume range of 100 nl to 1.0 µl. Pipetting 40% PEG 10,000 solution achieved even better precision and accuracy than water, demonstrating the ability of the Freedom EVO to dispense low volumes of even challenging liquids with high viscosity.

Each of the eight Te-PS tips was shown to have both good precision and accuracy. Dry contact pipetting performance was demonstrated to be of similar accuracy and precision to wet contact pipetting.

The Freedom EVO platform with Te-PS tips and 25 µl syringes demonstrated excellent performance in pipetting 100 nl of both water and 40% PEG 10,000 solution. This set-up is therefore an ideal platform for life science applications such as protein crystallography, where small volume pipetting is required.

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