A Test Reaction for Titration with a Thermal Activity Monitor

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The Thermal Activity Monitor is normally calibrated electrically but for the purpose of making an overall check of the instrument and its operation it is of interest to have available well documented test reactions. The titration of "hepes" (2-[4-(2-hydroxyethyl)-1-piperazinyl] ethansulfonic acid) buffer with a dilute solution of sulphuric acid is useful in this connection.

SOLUTIONS

1. Prepare 0.20 M aqueous hepes buffer solution adjusted to pH = 7.0-7 5 (e.g. with NaOH).
2. Prepare 0.0100 M aqueous H_2SO_4 solution using a standardized ampoule (Titrisol, Merck).

In all cases, freshly boiled, distilled or Milli-Q filtrated water should be used.

EXPERIMENTAL DESIGN

The described procedure is suited for the 30 μW range.

Load the ampoule of the titration vessel with 0.9 ml (2.7 ml if a 3 ml vessel is used) of the hepes solution. Fill the annular space from the O-ring seal on the stirrer shaft to the beginning of the cone with the same solution. Vapour phase problems will the n be minimized. Seal the vessel as usual and transfer it stepwise to the measuring position of the calorimeter. Establish a good baseline while loading the syringe.

Load the syringe with the sulphuric acid and avoid introducing air bubbles (important!). Put the syringe in its holder and let the motor make the plunger move until some droplets can be seen at the tip of the syringe. Remove the droplets with a piece of plastic (or any material that does not adsorb the liquid).

Insert the syringe into the vessel and let a stable baseline be established. Make a series of 5-6 injections, each with a volume of 5-15 μl. The time between the injections should be 40- 60 minutes.

CALCULATIONS

Integrate the areas and take the average value, Q. Calculate the energy per mole H_2SO_4 injected, Qm:

$$Q_m = Q / (V \bullet c) \qquad (1)$$

where V is the volume of acid injected in each step and c is the acid concentration. (The positive Q here denotes an exothermic process).

RESULTS AND DISCUSSION

The results cannot be directly compared with literature values of enthalpy of protonization for hepes, since the process investigated contains contribution from other processes as well. The values should instead agree with the following results:

At 25 °C: ΔH_m = -54.3 ±0.5 kJ/mol H_2SO_4
At 37 °C: ΔH_m = -59.8 ±0.4 kJ/mol H_2SO_4

The uncertainties given are twice the overall standard deviation of the mean for 6 consecutive injections.

Measurement performed according to this procedure are not significantly changed by uptake of atmospheric CO_2 which will influence experiments done with lower concentration.

A more general discussion about calibrations of microcalorimeters and the use of test substances has been prepared. (Briggner, L.E; Wadso, I. J.Biochem.Biophys. Methods - in press **(1990)**