

A Test Reaction for Titration with a Thermal Activity Monitor

L.E. Briggner
G. Olofsson
A. Schön

*University of Lund
Department of Thermochemistry
LUND, Sweden*

Field of application: *Chemistry, biochemistry.*

Instrument: *Thermometric 2277
Thermal Activity Monitor*

Date: *11 December 1989*

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 H₂SO₄ 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 μ W 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 then 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 new stable baseline be established. Make a series of 5-6 injections, each with a volume of 5-15 μ l. 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 H₂SO₄ injected, Q_m:

$$Q_m = Q / (V \cdot c) \quad (1)$$

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:

$$\text{At } 25\text{ }^{\circ}\text{C: } \Delta H_m = -54.3 \pm 0.5 \text{ kJ/mol H}_2\text{SO}_4$$

$$\text{At } 37\text{ }^{\circ}\text{C: } \Delta H_m = -59.8 \pm 0.4 \text{ kJ/mol H}_2\text{SO}_4$$

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 CO₂ 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; Wadsö, I. J.Biochem.Biophys. Methods - in press (1990))