Calculation problems

Most calculations in a biochemistry lab end up as an amount of solid or liquid to be measured. You have to realize that the tools available for measuring have both upper and lower limits. For the calculations below assume that the measuring tools available in the lab are:

- Balances with a range from 1 mg to 200 g
- Four kinds of pipettes with ranges of: 1 – 10 µL, 10 – 100 µL, 0.1 – 1 mL and 1 – 5 mL
- A spectrophotometer with a range from 0.001 to 2.0 units of absorbance
- Cuvettes for spectrophotometer: 1 mL and 3 mL capacity with 1 cm optic path

NOTE: The calculations below are not just mathematical problems, they demonstrate practical aspects of working with solutions and you may find more than one way of solving your problem.

Calculation problems <u>with answers</u>

1. Express the concentration of a 0.2 M solution of H_2SO_4 in units of N (equivalents/litre), g/L, mg/L, µg/L, ng/L, pg/L, µg/mL, mg/mL and %. (Assume density of solution = 1 g/mL). Reminder: MW of H_2SO_4 = 98.

   Answer:
   0.2 M H_2SO_4 = 0.4 N H_2SO_4 = 19.6 g/L H_2SO_4 = 19.6x10^3 mg/L H_2SO_4 = 19.6x10^6 µg/L H_2SO_4 = 19.6x10^9 ng/L H_2SO_4 = 19.6x10^{12} pg/L H_2SO_4 = 19.6x10^3 µg/mL H_2SO_4 = 19.6 mg/mL H_2SO_4 = 1.96 % H_2SO_4.

2. Prepare 5 mL of 0.01 mg/mL ATP in water starting from the solid product.

   Answer:
   That requires 50 µg of product, which is below the sensitivity of a regular analytical balance. You could prepare about 2 mL of a 1 mg/mL solution. Let say that you put in the balance 2.36 mg of ATP; you would dissolve that in 2.36 mL with a 5 mL pipet, then you could dissolve 50 µL of this solution into 4.95 mL with a 5 mL pipet.

3. Prepare 1 mL of a 1.0 ng/µL DNA solution in water from a 2.14 mg/mL DNA solution.

   Answer:
   You would need to transfer 0.467 µL of the stock solution into 1 mL of water, and you do not have a pipette to measure accurately 0.467 µL.
   From 2140 µg/mL to 1 ng/µL you need a 1:2140 dilution. You could prepare first a more concentrated solution (100 µg/mL) and from that solution prepare the other solution (dilution factor not higher than 100).

For the 100 μg/mL solution: add 46.7 μL ((100/2140)1000 μL) of the 2.14 mg/mL stock solution to 953 μL of water (for a final 1 mL solution).
For the 1 ng/μL solution, perform a 1:100 dilution from the 100 ng/μL solution: add 10 μL of the 100 ng/μL solution to 990 μL of water.

4. You have a 0.2 mL stock solution of an enzyme (5000 units of activity). You want to perform enzymatic assays of substrate concentration in 100 samples. The assay requires a concentration of enzyme of 20 units/mL and is performed in a total incubation volume of 0.1 mL. How would you deliver the enzyme to the incubation mixture of your assay?

   Answer:
   If you want to use the enzyme stock as it is (25000 units/mL), you will need to transfer aliquots of 0.08 μL (2 units) to the 100 μL incubation mixtures, but there is no pipette that can deliver that volume. You have to prepare a diluted enzyme solution. Since the total incubation volume is 100 μL, a convenient volume for enzyme delivery would be 10 μL. That means an enzyme concentration of 2 units/10 μL = 200 units/mL. The stock solution has to be diluted 25000/200 = 125 fold. You will need to prepare more than 1 mL of that solution (100 assays x 10 μL/assay). You can add 10 μL of the enzyme stock solution to 1.24 mL of buffer and use aliquots of 10 μL for the assay.

5. How would you measure the absorption at 260 nm of a 5 mg/mL DNA solution, in buffer, with a regular 3 mL cuvette. (Reminder: 1 mg/mL DNA absorbs about 20 units of absorbance at 260 nm)?

   Answer:
   The expected absorbance will be close to 100. But, the absorbance scale limit of most spectrophotometers is about 2.0, with optimal precision between 0.3 and 0.7. One has to prepare about a 1:100 dilution with a final volume not less than 3 mL. One could add 50 μL of the 5 mg/mL solution to 4.95 mL of buffer for a final concentration of 50 μg/mL and an absorbance of 1.0.

6. A 50 mL rat adipose tissue homogenate is centrifuged at 16000 g. The resulting pellet (mitochondria) is resuspended in 4 mL buffer. The homogenate (before centrifugation) and the mitochondria solutions are assayed for protein content. A 40 µL aliquot of homogenate contained 80 µg of protein whereas a 10 µL of mitochondria solution contained 45 µg of protein. Calculate the percentage of adipose tissue protein corresponding to the mitochondrial fraction.

   Answer:
   Homogenate: 80 μg in 40 μL out of 50 mL (aliquot factor of 50000/40 =1250), so the total amount of protein is 100 mg (80 μg x 1250).

Mitochondria: 45 μg in 10 μL out of 4 mL (aliquot factor of 4000/10 = 400), so the total amount of protein is 18 mg (45 μg x 400) which is equivalent to 18 % of the total protein in the tissue.

7. A 20 mL tissue homogenate containing lactate dehydrogenase (LDH) activity was analyzed to determine the amount of enzyme present. The assay was performed by incubating 20 µL of homogenate with 50 mM pyruvate and 60 mM NADH in a final volume of 1 mL. The reaction was stopped after 10 min incubation by adding 1 mL of TCA 30%, and the amount of utilized NADH was determined by diluting 50 µL of the mixture into 4 mL of water followed by measurement of the absorption at 340 nm in a 3 mL cuvette. The absorbance of the diluted solution was 0.75. Calculate the amount, in units, of LDH in the homogenate (1 LDH unit = 1 µmol of NADH utilized/min, absorptivity of NADH at 340 nm = 6220 $M^{-1}cm^{-1}$).

   Answer:
   The amount of NADH in the final solution is: (0.75/6220)x4 mL = 0.48 μmol. This corresponds to 50 μL out of 2 mL (aliquot factor of 2000/50 = 40), so the amount in the assay incubation would be 19.2 μmol (0.48x40). But the initial amount in the incubation mixture was 60 μmol (60 mM, 1 mL). The amount of NADH utilised after 10 min incubation is thus 40.8 μmol. So the LDH activity in the assay is 4.08 μmol/min or 4.08 units. But this corresponds to the 20 μL aliquot used for the assay from the total of 20 mL homogenate sample (aliquot factor of 20000/20 = 1000), so the total LDH in the homogenate was 4080 units (4.08x1000).