

### 14.10.1: Foods- Acid Value and the Quality of Fats and Oils

The acid value (AV) is a common parameter in the specification of fats and oils. It is defined as the weight of KOH in mg needed to neutralize the organic acids present in 1g of fat and it is a measure of the free fatty acids (FFA) present in the fat or oil. An increment in the amount of FFA in a sample of oil or fat indicates hydrolysis of triglycerides (Structure on the left). Such reaction occurs by the action of lipase enzyme and it is an indicator of inadequate processing and storage conditions (i.e., high temperature and relative humidity, tissue damage). The source of the enzyme can be the tissue from which the oil or fat was extracted or it can be a contaminant from other cells including microorganisms. Besides FFA, hydrolysis of triglycerides produces glycerol. The table below shows the acid value of some common oils and bee's wax.

Oil	Acid Value (AV) <sup>[1]</sup>
Canola	0.071
Maize	0.223
Soya	0.60
Virgin olive oil	6.6 <sup>[2]</sup>
Used frying oil	31 <sup>[3]</sup>
Bee's wax	17-36 <sup>[4]</sup>

FFA are a source of flavors and aromas. On one side, we have short chain FFA which tend to be water soluble and volatile with characteristic smell. On the other side, we have long chain saturated and unsaturated fatty acids. The later are more prone to oxidation in their free form and their breakdown products (aldehydes, ketones, alcohols, and organic acids) provide characteristic flavors and aromas. In most cases these flavors and aromas are considered a defect in oils, fats, and foods that contain them. However, there are instances where hydrolysis of triglycerides and oxidation of FFA are key in the development of desirable flavor and aroma in foods. This is the case of aged cheeses and some processed meats.

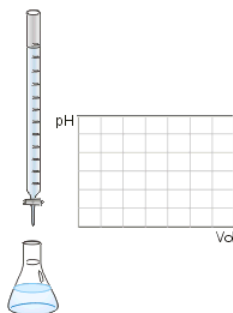


*Sunflower seed oil*

The AOAC method to determine AV in fats and oils is based on a titration in ethanol using phenolphthalein as indicator. Disadvantages of this and similar methods are the use of organic solvents (volume and toxicity), the need for heating the reaction media, incomplete solubility of the oil/fat, the need to pre-neutralize the solvents, use of large amounts of sample, and the possibility of error to detect the color change of the indicator when analyzing colored samples. There are some non-titration methods designed to overcome these disadvantages.<sup>[1]</sup> However, in spite its drawbacks, the titration method is still the most used due to the fact that it does not require expensive equipment.

## Acid-Base Titrations

A titration is a volumetric technique in which a solution of one reactant (the titrant) is added to a solution of a second reactant (the analyte) until the equivalence point is reached. The equivalence point is the point at which the titrant has been added in exactly the right quantity to react stoichiometrically with the analyte.



When an acid is titrated with a base, there is typically a sudden change in the pH of the solution at the equivalence point. If a few drops of indicator solution have been added, this sharp increase in pH causes an abrupt change in color, which is called the *endpoint* of the indicator (See the animation on the left). The actual magnitude of the jump in pH, and the pH range which it covers depend on the strength of both the acid and the base involved, and so the choice of indicator can vary from one titration to another. To learn how to choose an appropriate indicator, we need to study in some detail the variation of pH during a titration.

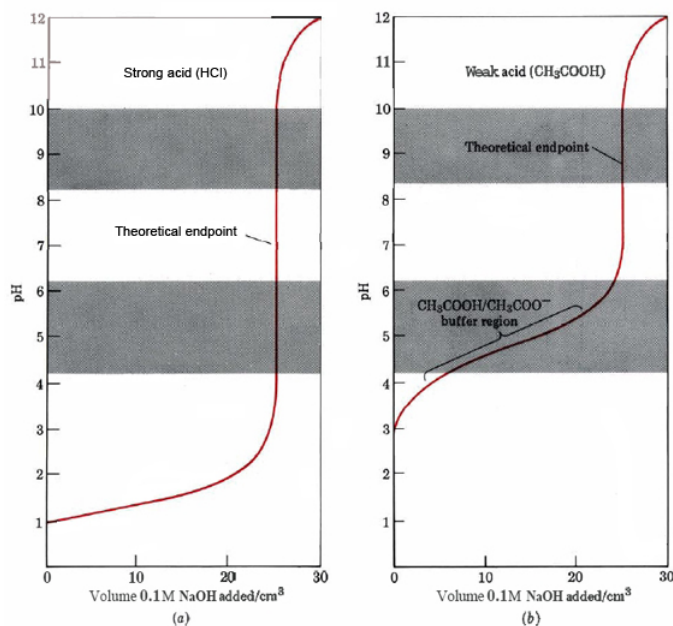
To simplify calculations we want to work with acid-base reactions that go to completion. For such reason, the most common cases we will encounter are:

- strong base titrated with strong acid
- strong acid titrated with strong base
- weak base titrated with strong acid
- weak acid titrated with strong base

However, we rarely titrate a weak base with a strong acid and vice versa.

### Titration of a strong acid with a strong base

Suppose we place  $25.00\text{ cm}^3$  of  $0.10\text{ M}$  HCl solution in a flask and add  $0.10\text{ M}$  NaOH from a buret. The pH of the solution in the flask varies with added NaOH, as shown in Figure 1a. The pH changes quite slowly at the start of the titration, and almost all the increase in pH takes place in the immediate vicinity of the endpoint.



The pH change during this titration is caused by the proton-transfer reaction

$\text{H}_3\text{O}^+ + \text{OH}^- \rightarrow \text{H}_2\text{O} + \text{H}_2\text{O}$  (1) which occurs as hydroxide ions are added from the buret. Though hydronium ions are being consumed by hydroxide ions in the early stages of the titration, the hydronium-ion concentration remains in the vicinity of  $10^{-1}$  or  $10^{-2} \text{ mol dm}^{-3}$ . As a result, the pH remains in the range 1 to 2. As an example of this behavior let us consider the situation halfway to the endpoint, i.e., when exactly  $12.50 \text{ cm}^3$  of  $0.10 \text{ M}$  NaOH have been added to  $25.00 \text{ cm}^3$  of  $0.10 \text{ M}$  HCl in the flask. The amount of hydronium ion has been reduced at this point from an original  $2.5 \text{ mmol}$  to half this value,  $1.25 \text{ mmol}$ . At the same time the volume of solution has increased from  $25 \text{ cm}^3$  to  $(25 + 12.50) \text{ cm}^3 = 37.50 \text{ cm}^3$ . Therefore, the hydronium-ion concentration is  $1.25 \text{ mmol}/37.50 \text{ cm}^3 = 0.0333 \text{ mol dm}^{-3}$ , and the resultant pH is 1.48. Though the titration is half completed, this is not very different from the initial pH of 1.00.

The pH of the solution in the flask will only change drastically when we reach that point in the titration when only a minute fraction of the hydronium ions remain unconsumed, i.e., as we approach the endpoint. Only then we will have reduced the hydronium-ion concentration by several powers of 10, and consequently increased the pH by several units. When  $24.95 \text{ cm}^3$  of base have been added, we are only  $0.05 \text{ cm}^3$  (approximately one drop) short of the endpoint. At this point  $24.95 \text{ cm}^3 \times 0.10 \text{ mmol cm}^{-3} = 2.495 \text{ mmol}$  hydroxide ions have been added. These will have consumed  $2.495 \text{ mmol}$  hydronium ions, leaving  $(2.5 - 2.495) \text{ mmol} = 0.005 \text{ mmol}$  hydronium ions in a volume of  $49.95 \text{ cm}^3$ . The hydronium-ion concentration will now be

$[\text{H}_3\text{O}^+] = \frac{0.005 \text{ mmol}}{49.95 \text{ cm}^3} = 1.00 \times 10^{-4} \text{ mol dm}^{-3}$  giving a pH of 4.00. Because almost all the hydronium ions have been consumed, only a small fraction (one five-hundredth) remains and the volume of solution has nearly doubled. This reduces the hydronium-ion concentration by a factor of  $10^{-3}$ , and the pH increases by three units from its original value of 1.00.

When exactly  $25.00 \text{ cm}^3$  of base have been added, we have reached the theoretical equivalence point, and the flask will contain  $2.5 \text{ mmol}$  of both sodium and chloride ions in  $50 \text{ cm}^3$  of solution; i.e., the solution is  $0.05 \text{ M}$  NaCl. Furthermore its pH will be *exactly* 7.00, since neither the sodium ion nor the chloride ion exhibits any appreciable acid-base properties.

Immediately after this equivalence point the addition of further NaOH to the flask results in a sudden increase in the concentration of hydroxide ions, since there are now virtually no hydronium ions left to consume them. Thus even one drop ( $0.05 \text{ cm}^3$ ) of base added to the equivalence point solution adds  $0.005 \text{ mmol}$  hydroxide ions and produces a hydroxide-ion concentration of  $0.005 \text{ mmol}/50.05 \text{ cm}^3 = 1.00 \times 10^{-4} \text{ mol dm}^{-3}$ . The resultant  $\text{pOH} = 4.00$ , and the  $\text{pH} = 10.00$ . The addition of just two drops of base results in a pH jump from 4.00 to 7.00 to 10.00. This rapid rise causes the indicator to change color, so the *endpoint* matches the *equivalence point* if the indicator is chosen properly.

A Titration of a strong base with a strong acid can be handled in essentially the same way as the strong acid-strong base situation we have just described. Strong acids and bases are not naturally found in food, however, they are often used as food processing aids and analysis methods.

### ✓ Example 14.10.1.1:

Suppose that 40.00 cm<sup>3</sup> of 0.020 M KOH is titrated with 0.030 M HNO<sub>3</sub>. Find the pH of the solution in the flask (a) before any HNO<sub>3</sub> is added; (b) halfway to the endpoint; (c) one drop (0.05 cm<sup>3</sup>) before the endpoint; (d) one drop after the endpoint.

#### Solution

a) Since KOH is a strong base, [OH<sup>-</sup>] = 0.020 = 2 × 10<sup>-2</sup>. Thus  
pOH = 1.7 and pH = 14 - 2 = 12.3

b) Halfway to the endpoint means that half the OH<sup>-</sup> has been consumed. The original amount of OH<sup>-</sup> was

$$n_{\text{OH}^-} = V_{\text{NaOH}} \times c_{\text{NaOH}} \quad (14.10.1.1)$$

$$= 40.00 \text{ cm}^3 \times \frac{0.020 \text{ mmol}}{1 \text{ cm}^3} = 0.8 \text{ mmol} \quad (14.10.1.2)$$

so the amount remaining is 0.4 mmol OH<sup>-</sup>. It must have required 0.4 mmol HNO<sub>3</sub> to consume the other 0.4 mmol OH<sup>-</sup>, and so the volume of HNO<sub>3</sub> added is

$$V_{\text{HNO}_3} = \frac{n_{\text{HNO}_3}}{c_{\text{HNO}_3}} \quad (14.10.1.3)$$

$$= \frac{0.4 \text{ mmol}}{0.030 \text{ mmol cm}^{-3}} = 13.33 \text{ cm}^3 \quad (14.10.1.4)$$

The total volume of solution at this point is thus (13.33 + 40.00) cm<sup>3</sup> = 53.33 cm<sup>3</sup>, and

$$[\text{OH}^-] = \frac{0.4 \text{ mmol}}{53.33 \text{ cm}^3} \quad (14.10.1.5)$$

$$= 7.50 \times 10^{-3} \text{ mmol cm}^{-3} = 7.50 \times 10^{-3} \text{ mol dm}^{-3} \quad (14.10.1.6)$$

pOH = -log (7.50 × 10<sup>-3</sup>) = 2.12 and

pH = 14 - 2.12 = 11.88 c) If the total volume of HNO<sub>3</sub> required to react with all the OH<sup>-</sup> in solution is 26.66 cm<sup>3</sup>, when 26.61 cm<sup>3</sup> of HNO<sub>3</sub> solution have been added, the amount of H<sub>3</sub>O<sup>+</sup> added is

$$n_{\text{H}_3\text{O}^+} = 26.61 \text{ cm}^3 \times \frac{0.030 \text{ mmol}}{1 \text{ cm}^3} = 0.798 \text{ mmol} \quad (14.10.1.7)$$

This would consume an equal amount of OH<sup>-</sup>, and so the amount of OH<sup>-</sup> remaining is (0.8 - 0.798) mmol = 0.002 mmol. Thus

$$[\text{OH}^-] = \frac{0.002 \text{ mmol}}{(40 + 26.61) \text{ cm}^3} \quad (14.10.1.8)$$

$$= 3.00 \times 10^{-5} \text{ mmol cm}^{-3} = 3.00 \times 10^{-5} \text{ mol dm}^{-3} \quad (14.10.1.9)$$

pOH = 4.52 and pH = 9.48 d) An excess of 0.05 cm<sup>3</sup> of acid will add

$$n_{\text{H}_3\text{O}^+} = 0.05 \text{ cm}^3 \times \frac{0.030 \text{ mmol}}{1 \text{ cm}^3} = 1.5 \times 10^{-3} \text{ mmol} \quad (14.10.1.10)$$

of H<sub>3</sub>O<sup>+</sup> to a neutral solution whose volume is (40.00 + 26.71) cm<sup>3</sup> = 66.71 cm<sup>3</sup>.

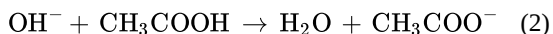
Thus

$$[\text{H}_3\text{O}^+] = \frac{1.5 \times 10^{-3} \text{ mmol}}{66.71 \text{ cm}^3} = 2.25 \times 10^{-5} \text{ mol dm}^{-3}$$

pH = 4.64

#### Titration of a weak acid with a strong base

When 25.00 cm<sup>3</sup> of 0.10M CH<sub>3</sub>COOH is titrated with 0.10M NaOH, for instance, there is a very much smaller change in pH at the equivalence point, as shown in Figure 1b, and the choice of indicators is correspondingly narrowed. The behavior of the pH in this case is very different from that of the titration of HCl with NaOH, because the acid-base reaction is different. When CH<sub>3</sub>COOH is titrated with NaOH, the OH<sup>-</sup> ions consume CH<sub>3</sub>COOH molecules according to the equation



As a result, the solution in the titration flask soon becomes a buffer mixture with appreciable concentrations of the CH<sub>3</sub>COO<sup>-</sup> ion as well as its conjugate acid. The [H<sub>3</sub>O<sup>+</sup>] and the pH are then controlled by the ratio of acid to conjugate base (equations 2 and 3 in the section on buffer solutions). When we are halfway to the endpoint, for example, [CH<sub>3</sub>COOH] will be essentially the same as [CH<sub>3</sub>COO<sup>-</sup>], and

$$[\text{H}_3\text{O}^+] = K_a \times \frac{[\text{CH}_3\text{COOH}]}{[\text{CH}_3\text{COO}^-]} \quad (14.10.1.11)$$

$$\approx K_a = 1.8 \times 10^{-5} \text{ mol dm}^{-3} \quad (14.10.1.12)$$

while the pH will be given by the Henderson-Hasselbalch equation as

$$\text{pH} = \text{p}K_a + \log \frac{[\text{CH}_3\text{COOH}]}{[\text{CH}_3\text{COO}^-]} \quad (14.10.1.13)$$

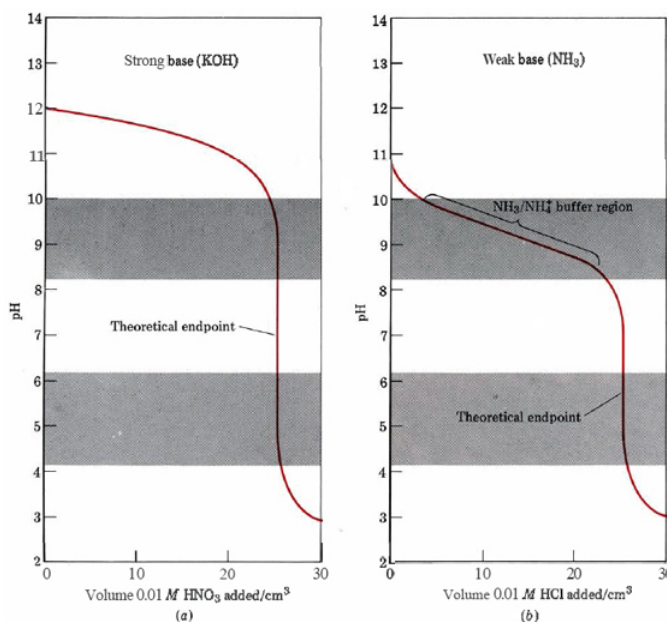
$$\approx \text{p}K_a = 4.74 \quad (14.10.1.14)$$

Comparing this to the pH of 1.78 calculated above for the halfway stage in the titration of HCl, we find a difference of roughly three pH units. The effect of the buffering action of the  $\text{CH}_3\text{COOH}/\text{CH}_3\text{COO}^-$  conjugate pair is thus to keep the pH some three units higher than before and hence to cut the jump in pH at the endpoint by approximately this amount.

Exactly at the equivalence point we no longer have a buffer mixture but a 0.05-M solution of sodium acetate. This solution is slightly basic, and its pH of 8.72 can be calculated from equation 4 on the section covering the pH of weak base solutions. Beyond this equivalence point, the story is much the same as in the strong-acid case. Addition of even a drop ( $0.05\text{cm}^3$ ) of excess base raises the  $\text{OH}^-$  concentration to  $10^{-4} \text{ mol dm}^{-3}$  and the pH to 10. Of the three indicators which could be used in the titration of HCl, only one is useful for acetic acid. This is phenolphthalein, which changes color in the pH range 8.3 to 10.0. This endpoint lies within the pH jump from 7 to 10 which occurs  $\pm 0.05 \text{ cm}^3$  (one drop) from the endpoint. Both the other two indicators would give a color change *before* the true equivalence point. As shown in Figure 1b, the color of methyl red would start changing after only about  $4 \text{ cm}^3$  of base had been added!

The titration of a weak base with a strong acid also involves a buffer solution and consequently requires a more careful choice of indicator.

The pH variation during titrations of strong and weak bases with a strong acid are shown in Figure 2. In the case of the titration of  $0.010 \text{ M NH}_3$  with  $0.010 \text{ M HCl}$ , methyl red, but not phenolphthalein, would be a suitable indicator. In general the best indicator for a given titration is the one whose  $\text{p}K_a$  most nearly matches the pH calculated at the theoretical endpoint.



#### ✓ Example 14.10.1.2:

Ten grams of olive oil are dispersed in  $50 \text{ cm}^3$  ethanol for a final volume of  $60.86 \text{ cm}^3$ . The mixture is titrated with  $50 \text{ cm}^3$  KOH  $0.1 \text{ M}$ . a) What is the AV of this sample of olive oil? Assuming that the only free fatty acid present in the sample is oleic acid and that we are working with an aqueous solution at all times, what is the pH of the mixture b) before any KOH is added?, when c)  $10 \text{ cm}^3$ , d)  $25 \text{ cm}^3$ , e)  $35 \text{ cm}^3$  e) an excess of  $1.5 \text{ cm}^3$  KOH  $0.1 \text{ M}$  have been added?

Note: Oleic acid (c-9-octadecenoic)  $\text{p}K_a = 9.85$  [5]

## Solution

a) If  $50 \text{ cm}^3$  of  $0.1 \text{ M KOH}$  were used to neutralize the sample, the number of moles of  $\text{KOH}$  is

$$n_{\text{KOH}} = V_{\text{KOH}} \times c_{\text{KOH}} \quad (14.10.1.15)$$

$$= 50.00 \text{ cm}^3 \times \frac{0.1 \text{ mmol}}{1 \text{ cm}^3} = 5.0 \text{ mmol} \quad (14.10.1.16)$$

The molar mass of  $\text{KOH}$  is  $56.10 \text{ g/mol}$  and the mass of  $\text{KOH}$  employed in  $\text{mg}$  is

$$m_{\text{KOH}} = n_{\text{KOH}} \times M_{\text{KOH}} \quad (14.10.1.17)$$

$$= 5.0 \text{ mmol} \times \frac{56.10 \text{ mg}}{\text{mmol}} = 280.5 \text{ mg} \quad (14.10.1.18)$$

this amount of  $\text{KOH}$  was used to neutralize  $10 \text{ g}$  of olive oil. The acid value is then

$$\text{AV} = \frac{\text{mg}_{\text{KOH}}}{\text{g}_{\text{sample}}} \quad (14.10.1.19)$$

$$= \frac{280.5 \text{ mg}_{\text{KOH}}}{10 \text{ g}_{\text{sample}}} = 28.05 \quad (14.10.1.20)$$

This AV is considerable higher than the one shown in the table above. What does this tell you about the oil?

This oil has probably been heated (cooking/frying), it is old, or it has been stored under conditions favoring lipase activity. In the case of refined oils, a high AV indicates inappropriate or incomplete refining procedures.

b) From the number of moles of  $\text{KOH}$  employed to neutralize the oil we can conclude that there were  $5.0 \text{ mmol}$  of oleic acid in the sample and the ethanolic dispersion. Given that the volume of the dispersion was  $60.83 \text{ cm}^3$  the concentration of oleic acid is then

$$c_{\text{oleic}} = \frac{n_{\text{oleic}}}{V}$$

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$$= \frac{5.0 \text{ mmol}}{60.83 \text{ cm}^3} = 0.082 \text{ mmol cm}^{-3} = 0.082 \text{ mol dm}^{-3}$$

Using equation (5) from Weak acids in foods - pH and beyond

$$[\text{H}_3\text{O}^+] = \sqrt{K_a c_a} \quad (14.10.1.21)$$

$$= \sqrt{(1.41 \times 10^{-10} \text{ mol dm}^{-3})(0.082 \text{ mol dm}^{-3})} \quad (14.10.1.22)$$

$$= \sqrt{1.16 \times 10^{-11} \text{ mol}^2 \text{ dm}^{-6}} \quad (14.10.1.23)$$

$$[\text{H}_3\text{O}^+] = 3.4 \times 10^{-6} \text{ mol dm}^{-3} \quad (14.10.1.24)$$

$$\text{pH} = -\log(3.4 \times 10^{-6}) = 5.47 \quad (14.10.1.25)$$

c) When  $10 \text{ cm}^3$  of  $\text{KOH}$  solution had been added, the volume of the dispersion is  $60.83 + 10 = 70.83 \text{ cm}^3$  and the corresponding number of moles of  $\text{OH}^-$  is

$$n_{\text{OH}^-} = V_{\text{OH}^-} \times c_{\text{OH}^-} \quad (14.10.1.26)$$

$$= 10.00 \text{ cm}^3 \times \frac{0.1 \text{ mmol}}{1 \text{ cm}^3} = 1.0 \text{ mmol} \quad (14.10.1.27)$$

This means that  $1.0 \text{ mmol}$  of oleic acid have reacted and produced  $1.0 \text{ mmol}$  of oleate, its conjugate base. At this point, the number of moles of oleic acid and oleate are

$$n_{\text{oleic}} = 5.0 \text{ mmol} - 1.0 \text{ mmol} = 4.0 \text{ mmol oleic acid} \quad (14.10.1.28)$$

$$n_{\text{oleate}} = 1.0 \text{ mmol oleate} \quad (14.10.1.29)$$

and their concentrations

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$$c_{\text{oleic}} = \frac{4.0 \text{ mmol}}{70.83 \text{ cm}^3} = 0.056 \text{ mmol cm}^{-3} = 0.056 \text{ mol dm}^{-3}$$

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$$= \frac{1.0 \text{ mmol}}{70.83 \text{ cm}^3} = 0.014 \text{ mmol cm}^{-3} = 0.014 \text{ mol dm}^{-3}$$

Since we have both the acid and its conjugate base in the dispersion we can use the Henderson-Hasselbach equation to calculate the pH

$$\text{pH} = \text{p}K_a + \log \frac{[\text{oleate}]}{[\text{oleic}]} \quad (14.10.1.30)$$

$$= 9.85 + \log \frac{0.014 \text{ mol dm}^{-3}}{0.056 \text{ mol dm}^{-3}} \quad (14.10.1.31)$$

$$= 9.25 \quad (14.10.1.32)$$

Note that all the  $\text{OH}^-$  has reacted with oleic acid and it is not present in the dispersion. **d)** When  $25 \text{ cm}^3$  have been added, we are halfway to the equivalence point. Half of the oleic acid has been converted into oleate and their concentrations are equal.

$n_{\text{oleic}} = n_{\text{oleate}} = 2.5 \text{ mmol}$   $c_{\text{oleic}} = c_{\text{oleate}} = \frac{2.5 \text{ mmol}}{85.83 \text{ cm}^3} = 0.029 \text{ mmol cm}^3 = 0.029 \text{ mol dm}^3$  Again, we have both the acid and its conjugate base in dispersion and we use the Henderson-Hasselbach equation

$$\text{pH} = \text{p}K_a + \log \frac{[\text{oleate}]}{[\text{oleic}]} \quad (14.10.1.33)$$

$$= 9.85 + \log \frac{0.029 \text{ mol dm}^{-3}}{0.029 \text{ mol dm}^{-3}} \quad (14.10.1.34)$$

$$= 9.85 + 0 = 9.85 \quad (14.10.1.35)$$

So halfway the equivalence point  $\text{pH} = \text{p}K_a$  **e)** If  $35 \text{ cm}^3$   $0.1 \text{ KOH}$  have been added

$$n_{\text{OH}^-} = V_{\text{OH}^-} \times c_{\text{OH}^-} \quad (14.10.1.36)$$

$$= 35.00 \text{ cm}^3 \times \frac{0.1 \text{ mmol}}{1 \text{ cm}^3} \quad (14.10.1.37)$$

$$= 3.5 \text{ mmol} \quad (14.10.1.38)$$

So  $3.5 \text{ mmol}$  of oleic acid have been converted into oleate and  $1.5$  remain in the dispersion. With a new volume of  $95.83 \text{ cm}^3$ , their corresponding concentrations are

$$c_{\text{oleic}} = 0.016 \text{ mol dm}^{-3} \quad (14.10.1.39)$$

$$c_{\text{oleate}} = 0.036 \text{ mol dm}^{-3} \quad (14.10.1.40)$$

and

$$\text{pH} = \text{p}K_a + \log \frac{[\text{oleate}]}{[\text{oleic}]} \quad (14.10.1.41)$$

$$= 9.85 + \log \frac{0.036 \text{ mol dm}^{-3}}{0.016 \text{ mol dm}^{-3}} \quad (14.10.1.42)$$

$$= 10.20 \quad (14.10.1.43)$$

**f)** If an excess of  $1.5 \text{ cm}^3$  are added to the dispersion, its final volume is  $112.33 \text{ cm}^3$ . Since all the oleic acid has been converted into oleate, there are no more protons to react with the  $\text{OH}^-$  ions and it will remain in solution.

The number of moles of  $\text{OH}^-$  ions is

$$n_{\text{OH}^-} = V_{\text{OH}^-} \times c_{\text{OH}^-} \quad (14.10.1.44)$$

$$= 1.5 \text{ cm}^3 \times \frac{0.1 \text{ mmol}}{1 \text{ cm}^3} = 0.15 \text{ mmol} \quad (14.10.1.45)$$

for a concentration of Since the  $\text{OH}^-$  is the strongest base in solution, it will govern the pH of the reaction mixture. The concentration of  $\text{OH}^-$  generated by oleate is negligible, so we calculate the pH of the dispersion as

$$\text{pOH} = -\log [\text{OH}^-] \quad (14.10.1.46)$$

$$= -\log (1.33 \times 10^{-3}) = 2.87 \quad (14.10.1.47)$$

and

$\text{pH} = 14 - 2.82 = 11.18$  **Notice** the assumptions we made to calculate pH at different stages of this titration. Initially, the oil is dispersed in ethanol, the dissociation constants of acids change depending on the medium and strictly speaking it is probably incorrect to talk about pH in this conditions. The method is also based on the assumption that protons are in the interface ethanol-water and readily react with aqueous  $\text{NaOH}$ . These are some of the limitations of the method and the reason for the need of a titration instead of direct measurement of pH on the sample.

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