Chemistry 201

Phosphate buffers

NC State University

Phosphate buffer

Phosphate buffers are widely used for laboratory biochemical studies because of they form the basis of the buffer in the cytosol of cells. Phosphate buffers are practical since there are three possible buffer ranges due to the fact that it is a polyprotic acid. There is a pKa at 7.2, which is very near physiological pH as well. In addition to the features we must consider the disadvantage that many phosphate salts are sparingly soluble. For example, calcium phosphate has quite low solubility. Calcium normally has a low concentration in cytosol.

Phosphate buffer

How many grams of Na_2HPO_4 (M_m = 142 g/mol) would you add to 100 mL of 0.5 M NaH_2PO_4 to make a pH = 7.40 “phosphate buffer”?

Phosphate buffer

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Solution: use Hendersen-Hasselbach

$$K_2 = \frac{[HPO_4{}^{2-}][H^+]}{[H_2PO_4{}^-]}$$

$$K_2 = 6.2 \, x \, 10^{-8}$$

$$pH = pKa + log_{10}\left(\frac{[HPO_4{}^{2-}]}{[H_2PO_4{}^-]}\right)$$

which can be written as

$$[HPO_4{}^{2-}] = [H_2PO_4{}^-]10^{pH-pKa}$$

Phosphate buffer

How many grams of Na_2HPO_4 (M_m = 142 g/mol) would you add to 100 mL?

Phosphate buffer

How many grams of Na_2HPO_4 (M_m = 142 g/mol) would you add to 100 mL?

Calculate the concentration needed

$$[H_2PO_4{}^-] = [0.5]10^{7.4-7.2} = 0.79$$

and then the mass. The volume is 100 mL so the number of moles is:

$$n = (0.79\ M)(0.1\ L) = 0.079\ moles$$

and the mass is

$$m = nM_m = (0.079\ moles)\left(142\frac{g}{mol}\right)$$

m = 11.2 grams.

two bases

equiv. pt.

buffer

weak acid

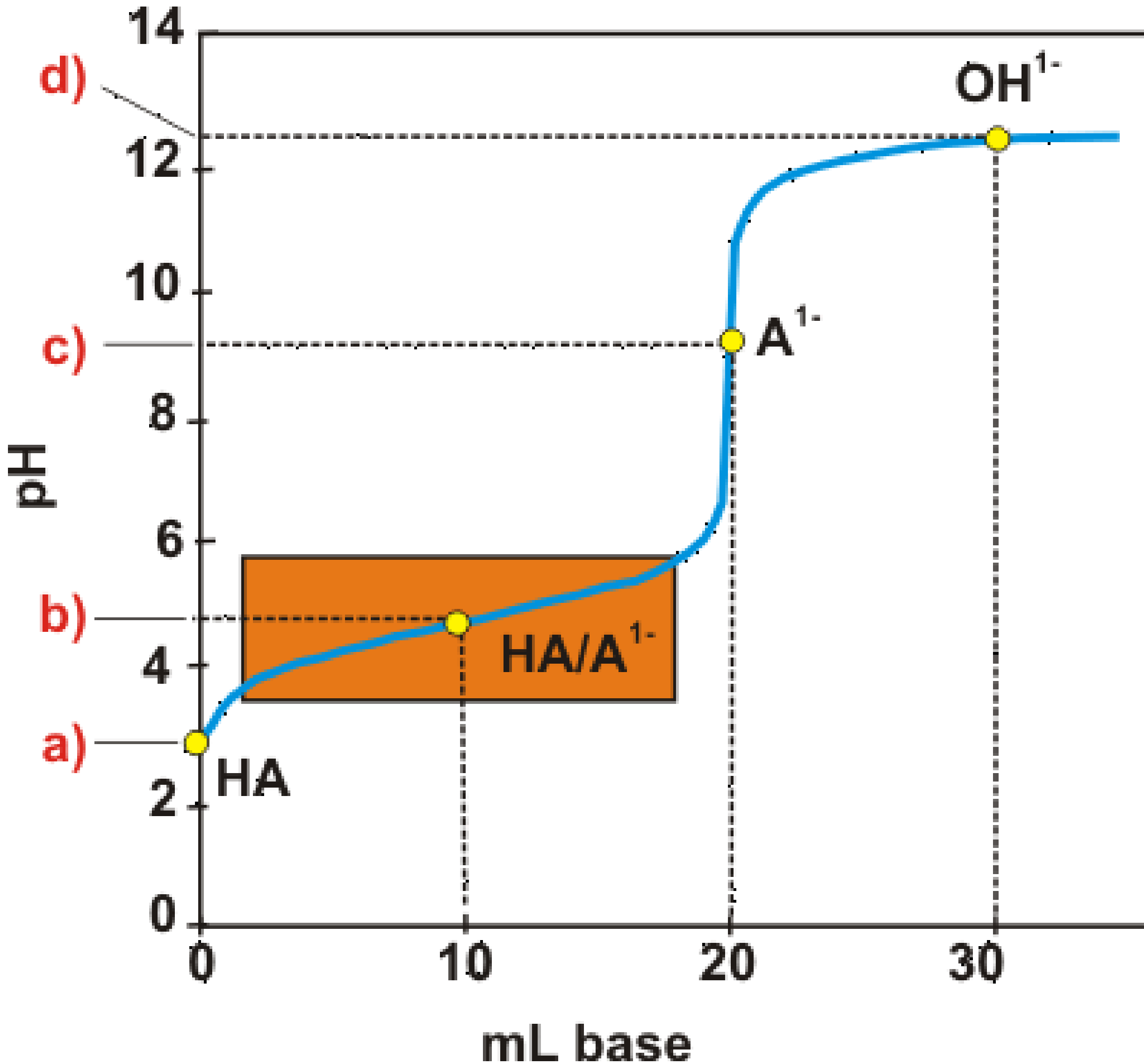


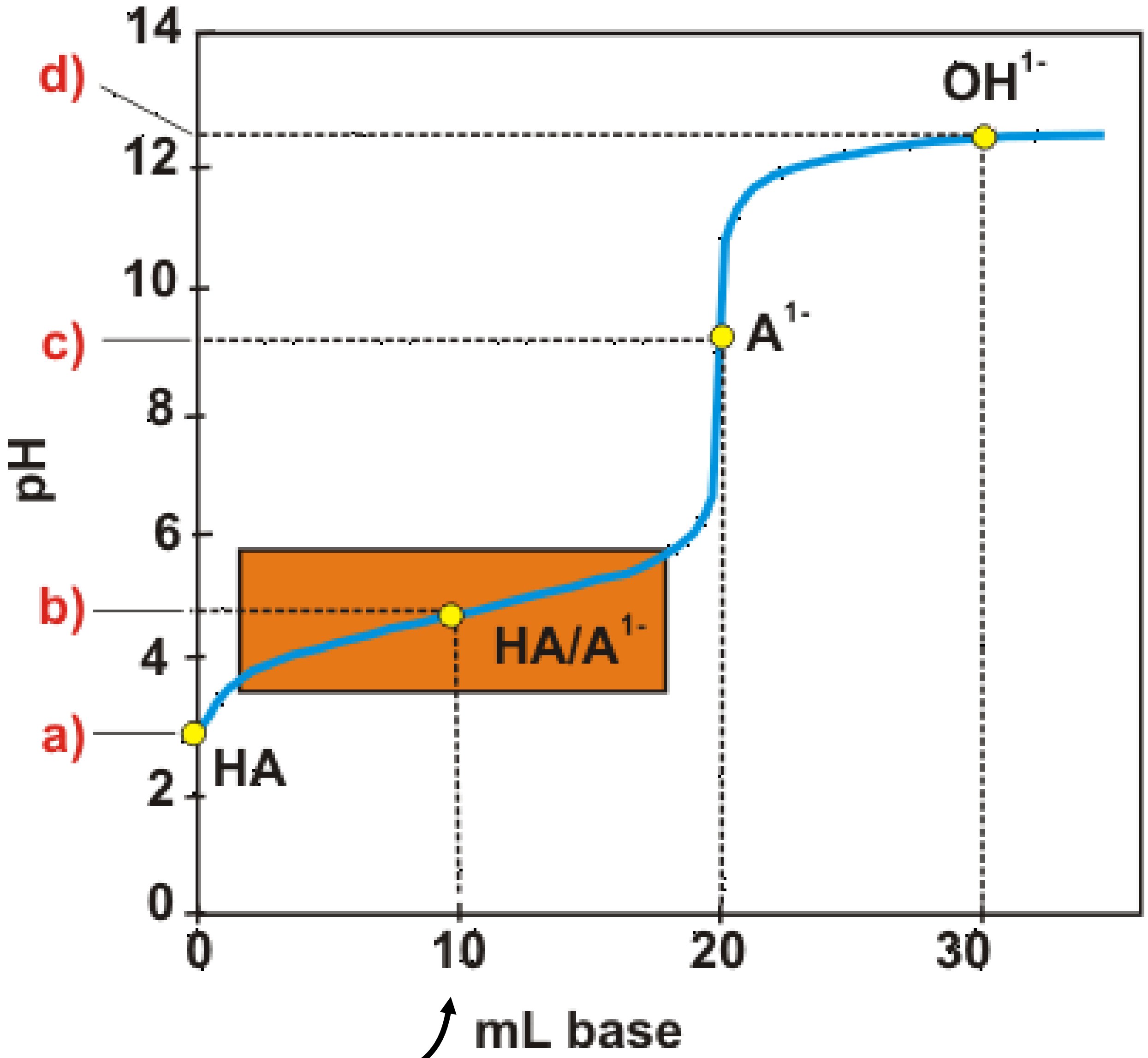
Figure 7.3

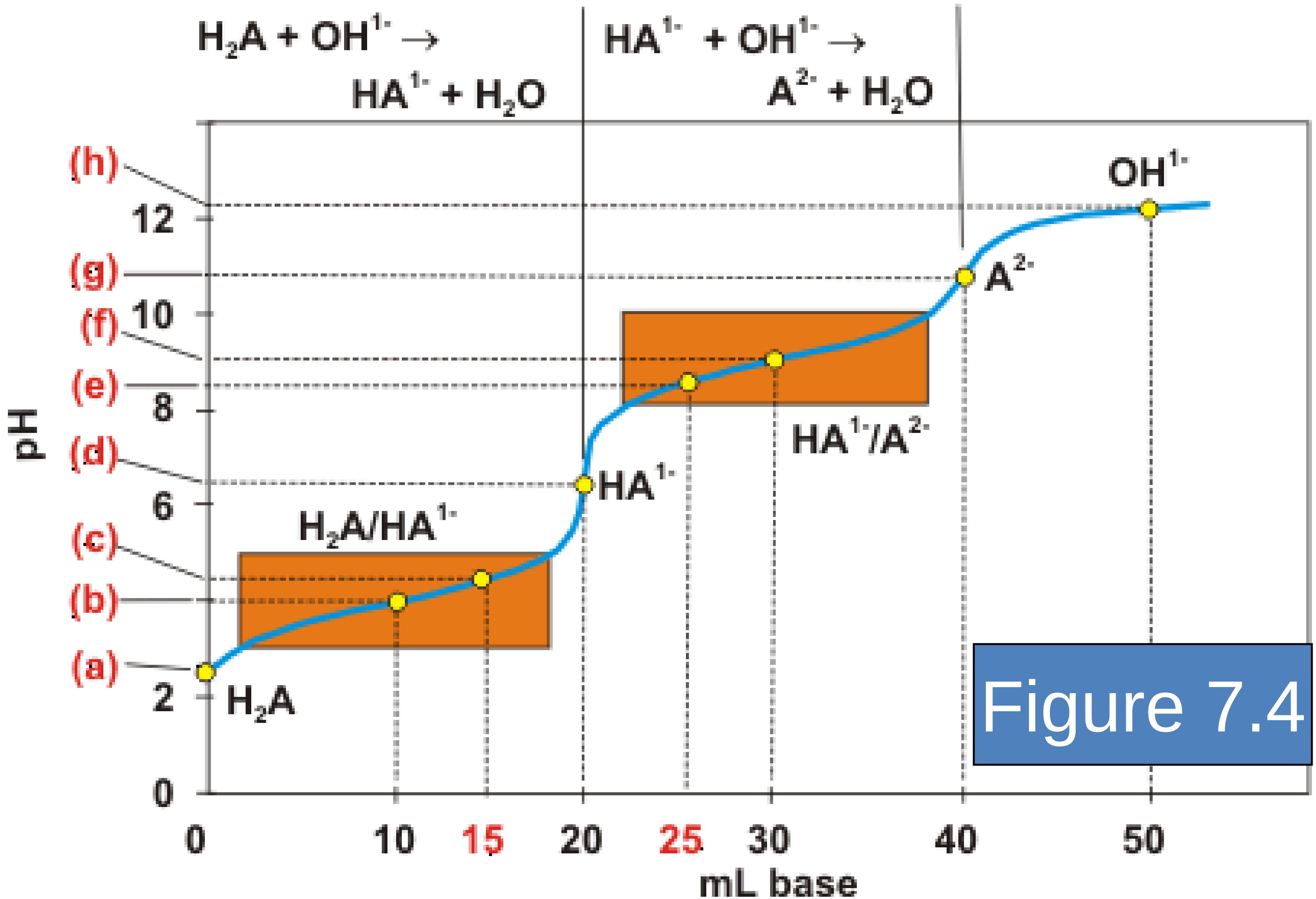
from H-H:

$pH = pK_a$

equal amts HA & A^{1-}

halfway to equiv. pt.





$H_2A + OH^{1-} \rightarrow HA^{1-} + H_2O$
$HA^{1-} + OH^{1-} \rightarrow A^{2-} + H_2O$
(h)
(g)
(f)
(e)
(d)
(c)
(b)
(a)
pH
0
2
6
8
10
12
0
10
15
20
25
30
40
50
mL base
H_2A
H_2A/HA^{1-}
HA^{1-}
HA^{1-}/A^{2-}
A^{2-}
OH^{1-}

Figure 7.4

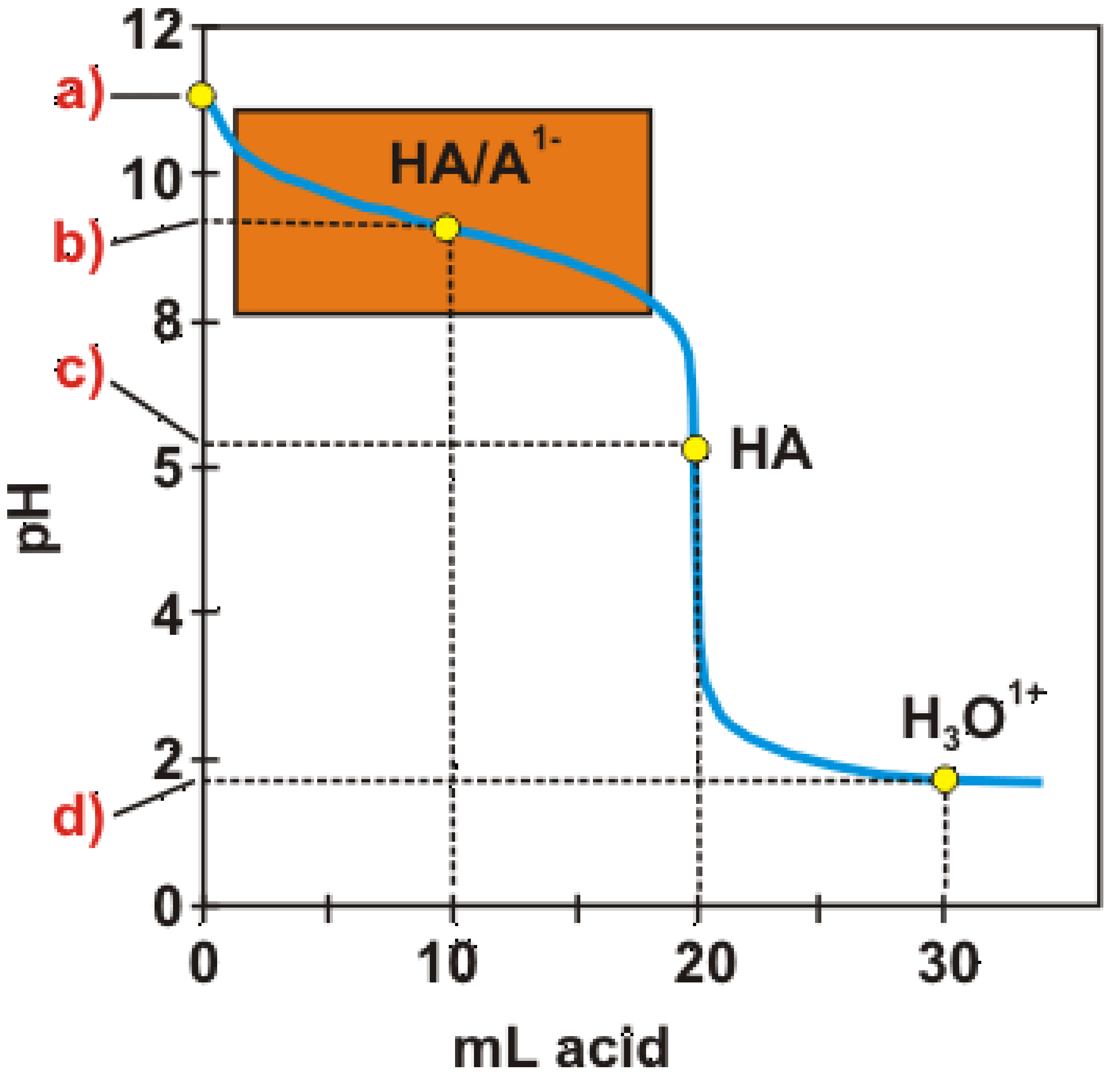
weak base

buffer

equiv. pt.

two acids

Figure 7.5



Practical phosphate buffer

How many grams of Na_2HPO_4 (M_m = 142 g/mol) qnd NaH_2PO_4 (M_m = 120 g/mol) would you need to weigh out to make 1L of a 100 mM phosphate buffer at pH = 7.00 ?

Practical phosphate buffer

How many grams of Na_2HPO_4 (M_m = 142 g/mol) qnd NaH_2PO_4 (M_m = 120 g/mol) would you need to weigh out to make 1L of a 100 mM phosphate buffer at pH = 7.00 ?

Solution: use Hendersen-Hasselbach

$$pH = pKa + log_{10}\left(\frac{[HPO_4{}^{2-}]}{[H_2PO_4{}^{-}]}\right)$$

to determine the ratio of concentrations in the final buffer.

$$\frac{[\mathrm{HPO_4^{2-}}]}{[\mathrm{H_2PO_4^{-}}]} = 10^{7.0-7.2} = 10^{-0.2} = 0.630$$

Practical phosphate buffer

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$$H_2PO_4^- + H_2O \rightarrow HPO_4^{2-} + H_3O^+$$

$$0.1 - x \quad \rightarrow \quad x \quad \quad x$$

$$\frac{[HPO_4^{2-}]}{[H_2PO_4^-]} = 0.630 \qquad \frac{0.1 - x}{x} = 0.630 \qquad 0.1 = 1.630x$$

$$x = \frac{0.1}{1.630} = 0.0613$$

Practical phosphate buffer

How many grams of Na_2HPO_4 (M_m = 142 g/mol) qnd NaH_2PO_4 (M_m = 120 g/mol) would you need to weigh out to make 1L of a 100 mM phosphate buffer at pH = 7.00 ?

$$H_2PO_4^- + H_2O \rightarrow HPO_4^{2-} + H_3O^+$$

$$NaH_2PO_4 \quad (0.0613\ \text{mole})\left(\frac{120\ \text{g}}{\text{mole}}\right) = 7.35\ \text{g}$$

$$Na_2HPO_4 \quad (0.0387\ \text{mole})\left(\frac{142\ \text{g}}{\text{mole}}\right) = 5.49\ \text{g}$$