等电点的计算

周华瑞

化学平衡与化学平衡常数

可逆反应 $\mathrm{A} + \mathrm{B} \rightleftharpoons \mathrm{C} + \mathrm{D}$

$$K_{\mathrm{eq}} = \frac{[\mathrm{C}]_{\mathrm{eq}}[\mathrm{D}]_{\mathrm{eq}}}{[\mathrm{A}]_{\mathrm{eq}}[\mathrm{B}]_{\mathrm{eq}}}$$

化学平衡常数只与温度、气压有关

水的解离与pH值

$$H_2O \rightleftarrows H^+ + OH^-$$

$$K_{eq} = \frac{[H^+]\bullet[OH^-]}{[H_2O]}$$

在25℃时， 水解离反应的平衡常数为$1.8\bullet10^{-16}$ M，请你计算纯水中氢离子浓度为多少？

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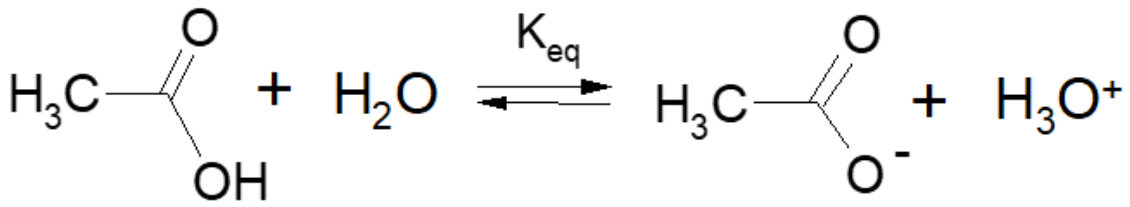
$[H_2O]$ =55.5 M.

定义离子积 $Kw=[H^+]\cdot[OH^-] = 10^{-14}$ M

弱酸弱碱的解离——以醋酸为例



$$K_a = K_{eq} \cdot [H_2O]$$

$$K_a = \frac{[H^+][CH_3COO^-]}{[CH_3COOH]} = 1.74 \cdot 10^{-5} M$$

$$[H^+] = K_a \cdot \frac{[CH_3COOH]}{[CH_3COO^-]}$$

思考

- 将0.1 mol的醋酸倒入1 L的水中，请问混合均匀后溶液的pH值为多少？

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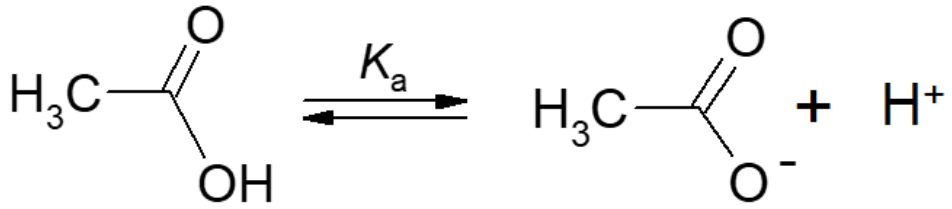


$0.1 - x \qquad x \qquad x$

$$K_a = \frac{[x][x]}{[0.1-x]} = 1.74 \cdot 10^{-5} \text{M}$$

$$x^2 = 1.74 \cdot 10^{-6} - 1.74 \cdot 10^{-5} x$$

$$x^2 + 1.74 \cdot 10^{-5} x - 1.74 \cdot 10^{-6} = 0$$

$x = 0.001310$, $pH = 2.883$

Henderson-Hasselbalch方程

$$HA \rightleftarrows H^+ + A^-$$

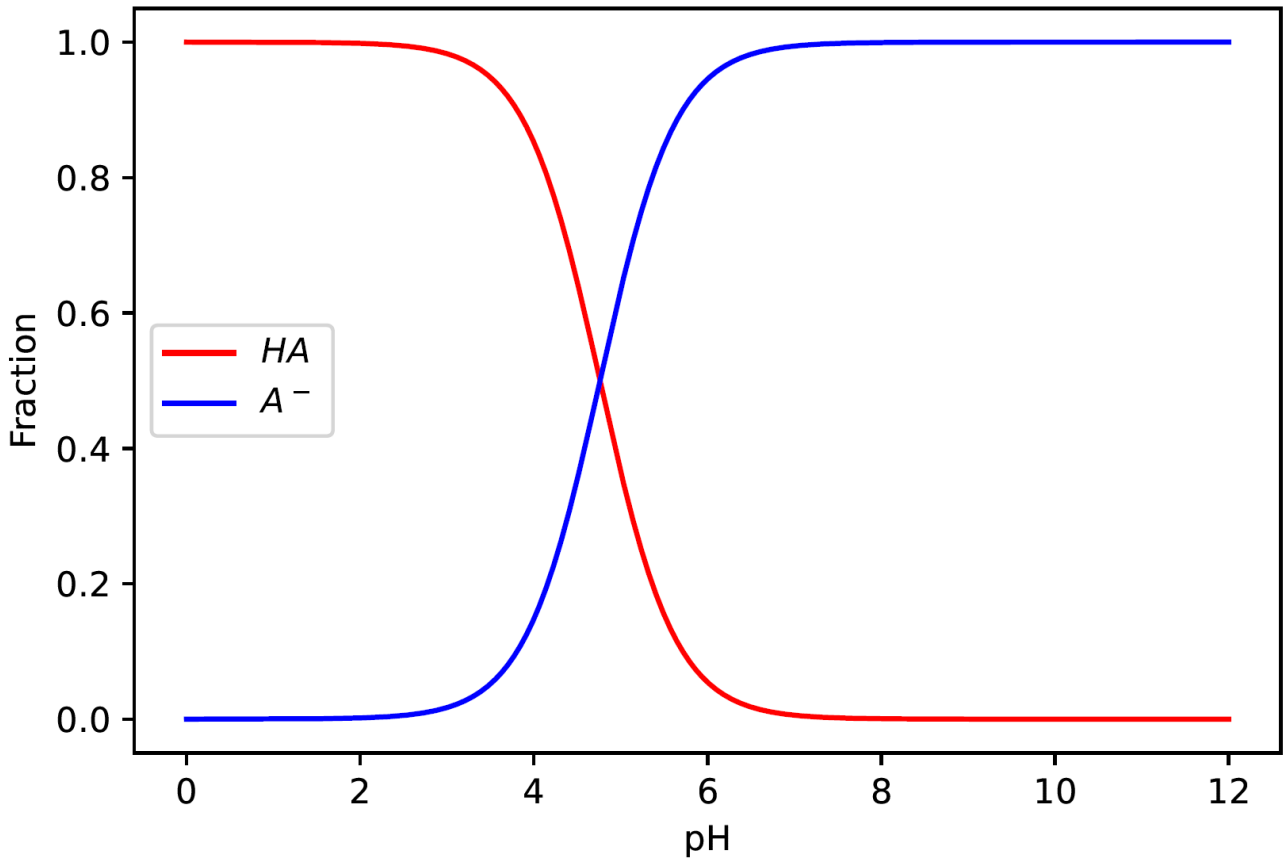
$$K_a = \frac{[H^+][A^-]}{[HA]}$$

两边取以10为底的对数，并且定义 $pK_a = -\log K_a$ ， $pH = -\log [H^+]$

$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

A^- 和 HA 在溶液中的占比与pH值的关系

以醋酸为例， pK = 4.76

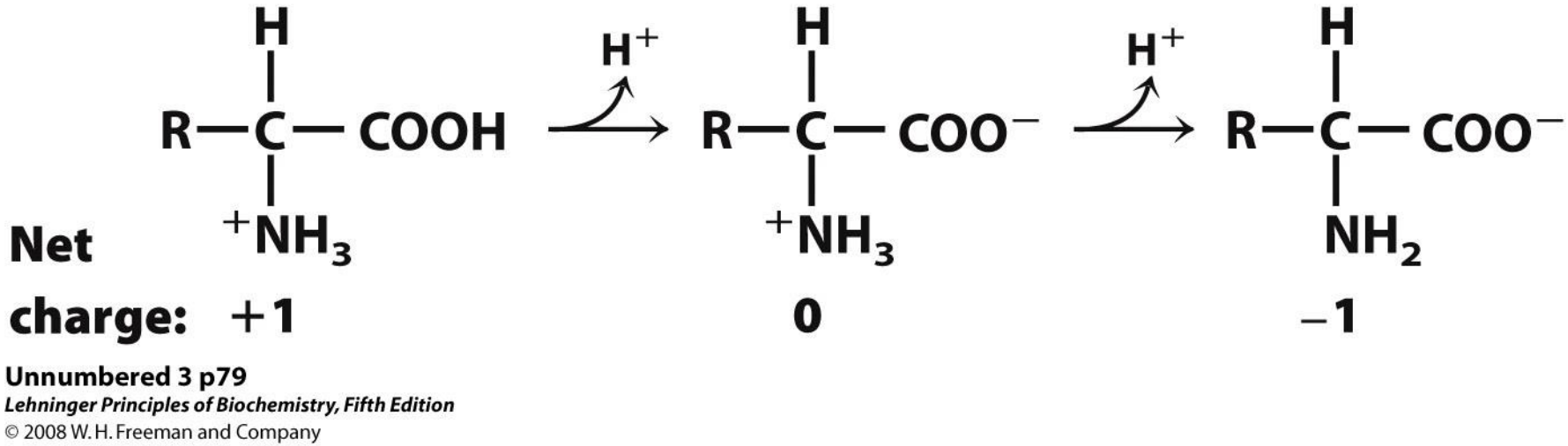


A- 占比 $f_1(\mathrm{pH}) = \frac{10^{\mathrm{pH}-\mathrm{p}K}}{10^{\mathrm{pH}-\mathrm{p}K}+1}$,

HA占比 $f_2(\mathrm{pH}) = \frac{1}{10^{\mathrm{pH}-\mathrm{pK}}+1}$

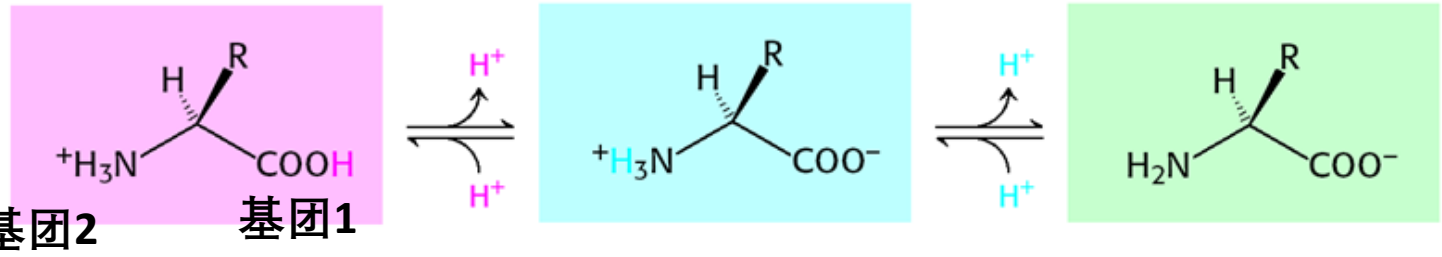
等电点计算

- 下面考虑同时带弱酸和弱碱性基团的分子，如氨基酸、蛋白质等
- 如果在某pH值下，溶液中某物质的电中性的两性离子所占比例最大(或者平均所带电荷为0)，我们称此时的pH值为该物质的等电点
- 记作 pI

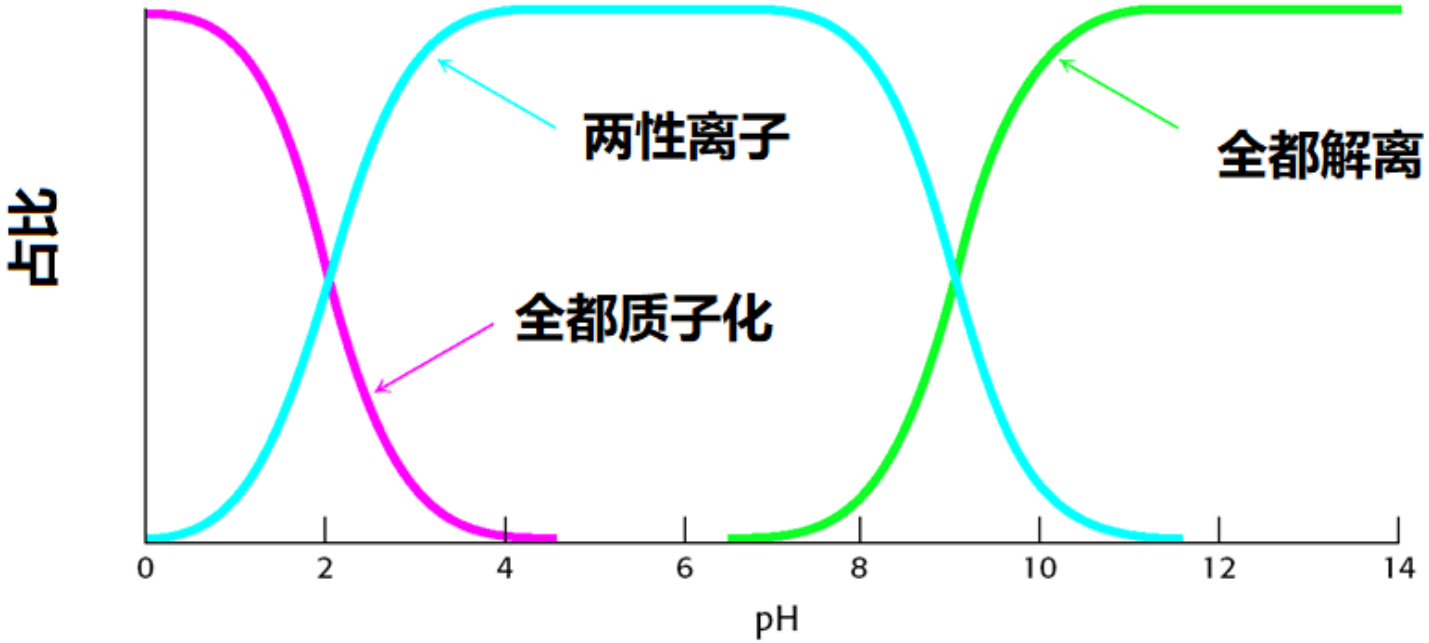


Unnumbered 3 p79
Lehninger Principles of Biochemistry, Fifth Edition

等电点的推导——方法一



基团2　　基团1



对于两性离子的占比 f，我们可以用函数

$$f(\mathbf{pH}) = f_{1\text{解离}}(\mathbf{pH}) \cdot f_{2\text{未解离}}(\mathbf{pH}) = \frac{1}{10^{\mathbf{pK_1}-\mathbf{pH}}+1} \cdot \frac{1}{10^{\mathbf{pH}-\mathbf{pK_2}}+1}$$

来表示

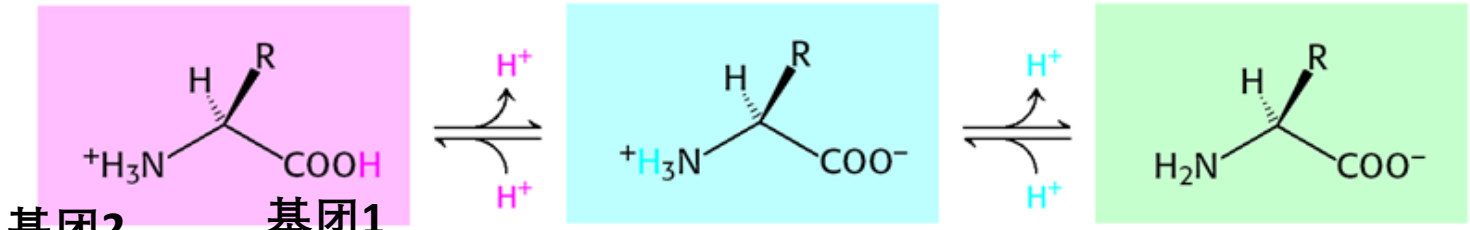
等电点的推导——方法一

问题转化为pH值为何值时，f 取得最大值

$$f(\mathbf{pH}) = f_{1\text{解离}}(\mathbf{pH}) \cdot f_{2\text{未解离}}(\mathbf{pH}) = \frac{1}{10^{\mathbf{pK_1 - pH}} + 1} \cdot \frac{1}{10^{\mathbf{pH - pK_2}} + 1}$$

实际上当pH=(pK1+pK2)/2 时, f(pH)取得最大值！

等电点的推导——方法二



基团2　　基团1

我们考虑溶液中该物质总体的平均电荷 Z，即为

$$\mathrm{Z}(pH) = (-1) \times \frac{1}{1 + 10^{pK_1 - pH}} + 1 \times \frac{1}{1 + 10^{pH - pK_2}}$$

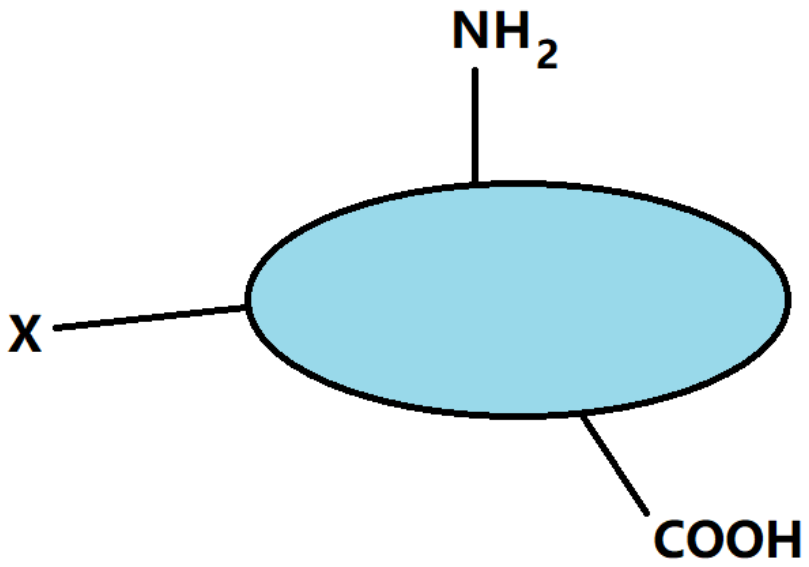
当 Z(pH)取值为0时的pH值即为等电点，此时可以计算

pH=(pK1+pK2)/2

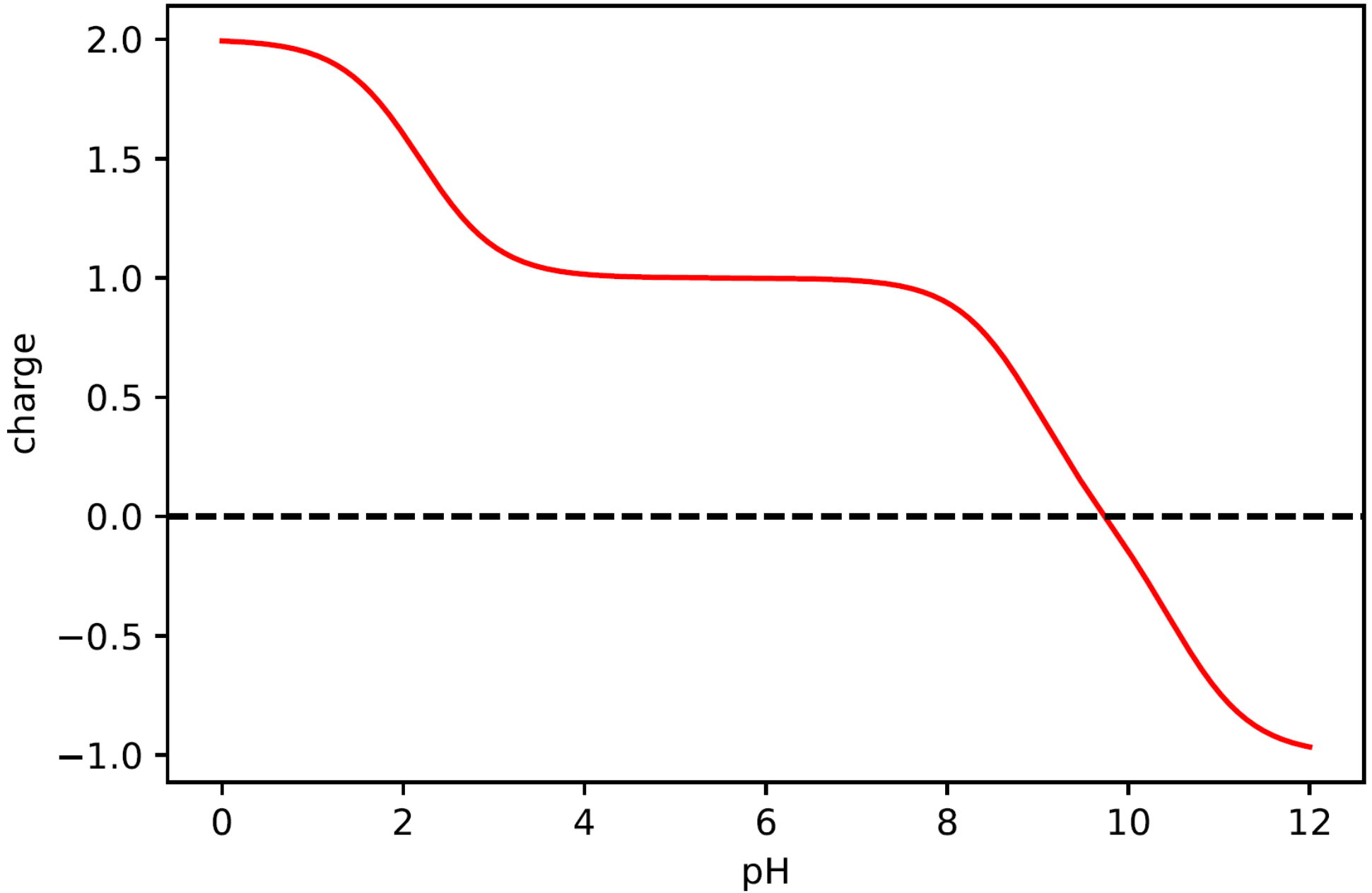
例：求Lys的等电点

pK_a Values for Common Alpha Amino Acids				
Amino Acid Type	Amino Acid	α-COOH	α-NH_3^+	RH or RH^+
Hydrophobic: Aliphatic	Glycine	2.34	9.60	
	Alanine	2.34	9.69	
	Valine	2.32	9.62	
	Leucine	2.36	9.68	
	Isoleucine	2.36	9.68	
	Proline	1.99	10.6	
	Methionine	2.28	9.21	
Hydrophobic: Aromatic	Phenylalanine	1.83	9.13	
	Tyrosine	2.2	9.11	10.07
	Tryptophan	2.38	9.39	
Hydrophilic: Polar Uncharged	Serine	2.21	9.15	
	Threonine	2.63	10.43	
	Cysteine	1.71	10.78	8.33
	Asparagine	2.02	8.8	
	Glutamine	2.17	9.13	
Hydrophilic: Acidic	Aspartic Acid	2.09	9.82	3.86
	Glutamic Acid	2.19	9.67	4.25
Hydrophilic: Basic	Arginine	2.17	9.04	12.48
	Histidine	1.82	9.17	6.00
	Lysine	2.18	8.95	10.53

COOH:2.18, NH2: 8.95, X:10.53



Lys平均电荷随pH变化

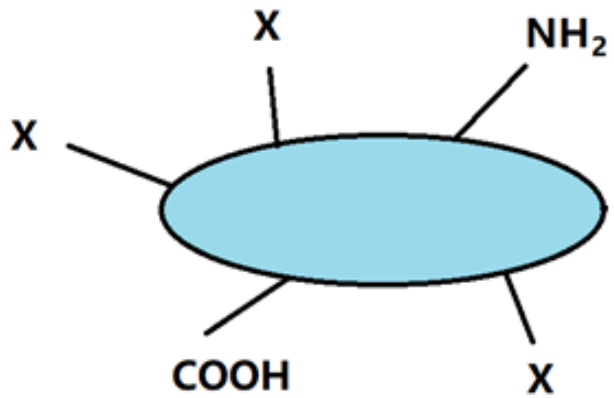


In [78]:

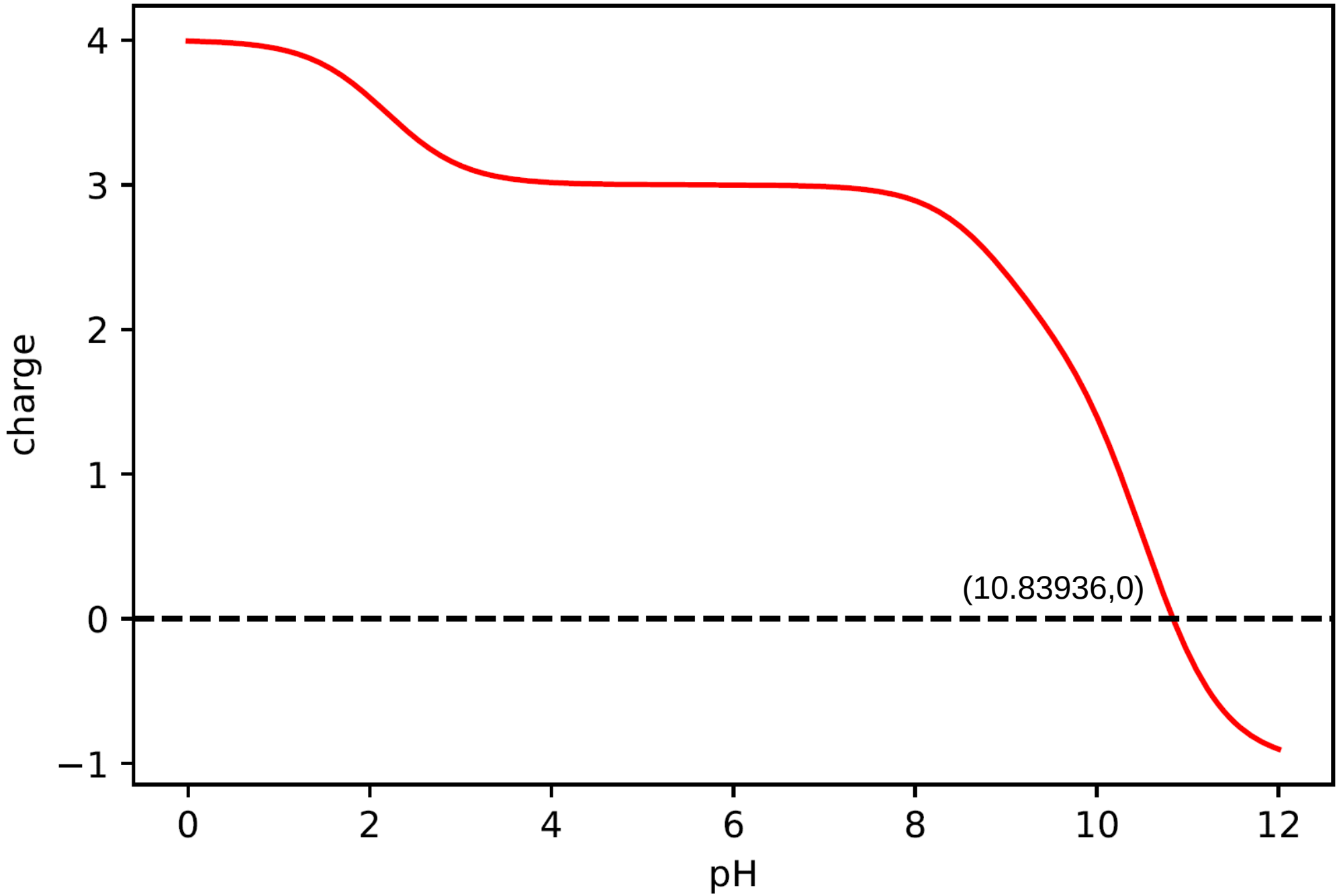
```
import numpy as np
from intersect import intersection
import matplotlib.pyplot as plt
import math

def charge(pH):
    f_lys_p = 1/(1+10**(pH-10.53))
    f_NH3_p = 1/(1+10**(pH-8.95))
    f_COO_n = 10**(pH-2.18)/(1+10**(pH-2.18))
    z = f_lys_p+f_NH3_p-f_COO_n
    return z

x = np.linspace(0.00,12.00,1000)
y = charge(x)
y_1 = [0 for i in x]
plt.plot(x,y,'r')
plt.xlabel("pH")
plt.ylabel("charge")
plt.axhline(y=0, color="black", linestyle="--")
pI= intersection(x,y,x,y_1)[0][0] # 求y与y_1的交点
print(pI)

pI1 = (10.53 + 8.95)/2 #近似算法得到的pI
print(pI1)

plt.savefig("iso.pdf")
```

```
9.740000087491314
9.739999999999998
```

例：求Lys-Lys-Lys的等电点



Lys-Lys-Lys 平均电荷随pH变化



In [38]:
```
import numpy as np
from intersect import intersection
```

In [50]:
```
import matplotlib.pyplot as plt
import math
```

In [72]:
```
def charge(pH):
    f_lys_p = 1/(1+10**(pH-10.53))
    f_NH3_p = 1/(1+10**(pH-8.95))
    f_COO_n = 10**(pH-2.18)/(1+10**(pH-2.18))
    z = 3*f_lys_p+f_NH3_p-f_COO_n
    return z
```

In [77]:
```
x = np.linspace(0.00,12.00,1000)
y = charge(x)
y_1 = [0 for i in x]
plt.plot(x,y,'r')
plt.xlabel("pH")
plt.ylabel("charge")
plt.axhline(y=0, color="black", linestyle="--")
pI= intersection(x,y,x,y_1)[0][0] # 求y与y_1的交点
print(pI)

pI1 = 10.53 + math.log(2,10) #近似算法得到的pI
print(pI1)

plt.savefig("iso.pdf")
```

```
10.839368214799547
10.83102999566398
```

练习

- 1.现有含0.2mol/L醋酸钠和0.6mol醋酸（pK_a=4.16）的溶液，它的pH值是多少？
- 2.计算配置0.2mol/L醋酸盐缓冲液（pH=5.0）所需的醋酸和醋酸钠的浓度
- 3.对于下图蛋白质中组氨酸的咪唑基（pK_a=6.0），请问生理状态下（pH=7.3），结合质子的咪唑基所占比例为多少？