AMINOKISLOTALARNI TITRAMETRIK USULDA TAXLIL QILISH.

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Annotatsiya. Titirlash usuli yordamida noma'lum aminokislotani yoki aniq aminokislotaning noma'lum konsentratsiyasi aniqlanadi. Bu jarayonda tomchilatgan holda H^+ yoki OH^- ionlari berish orqali amalga oshiriladi. Titrlashni amalga oshirish uchun egri chiziqlari, pKa qiymatlari, bufer hududlarini aniqlash, protonlanish holatlari, zaryadlar va izoelektrik nuqtalarni bilish zarur. Bu ishda biz aminokislotalarni titirlashning nazariy asoslari va biokiyodan masalalar yechimiga to'xtalamiz.

Kalit so'zlar: titrlash egri chizig'i, aminokislotalar, pH, biokimyo, pKa, izoelektrik nuqta.

Аннотация. Метод титрования используется для определения неизвестной аминокислоты или неизвестной концентрации конкретной аминокислоты. Этот процесс осуществляется путем капельного добавления ионов H+ или OH-. Для проведения титрования необходимо знать кривые, значения pKa, буферные области, состояния протонирования, заряды и изоэлектрические точки. В данной работе мы сосредоточимся на теоретических основах титрования аминокислот и решении задач из биохимии.

Ключевые слова: кривая титрования, аминокислоты, pH, биохимия, pKa, изоэлектрическая точка.

Abstract. The titration method is used to determine the unknown amino acid or the unknown concentration of a specific amino acid. This process is carried out by adding H+ or OH- ions dropwise. To perform the titration, it is necessary to know the curves, pKa values, buffer regions, protonation states, charges and isoelectric points. In this work, we will focus on the theoretical foundations of amino acid titration and the solution of problems from biochemistry.

Keywords: titration curve, amino acids, pH, biochemistry, pKa, isoelectric point.

Kirish.

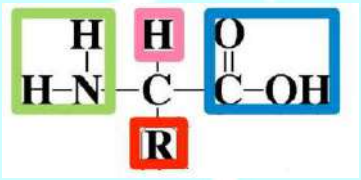
Aminokislotalar kimyosi, shu jumladan pKa qiymatlari, pI qiymatlari va protonlashgan holatlarini yoritb bergan va kuchsiz kislota uchun titrlash egri chizig'i o'rganilgan[1,2,3,4].

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Aminokislotalarning umumiy formulasi va tasnifi. *Aminokislotalar* (amino acids) -NH_2 va –C(O)OH guruhlar tutgan bifunktsional birikmalar bo‘lib, ularning dastlabki vakili aminosirka (glitsin, glikokol H_2NCH_2COOH) kislotadir.

Aminokislotalar quyidagilardan iborat:

- **Amino guruh (-NH_2)**
- **Karboksil guruh (-COOH)**
- **Vodorod atomi (-H)**
- **Yon zanjir (-R)**



Aminokislotalarni ularning -R ning umumiy kimyoviy xarakteristikalari asosida quyidagicha sinflash mumkin.

1. Neytral yon zanjir: qutbsiz yon zanjirga ega bo‘lib, ion yoki vodorod bog‘ hosil qilmaydigan va kimyoviy jarayonda ajralmaydigan (ta’sirlashmaydigan) aminokislotalar. Masalan: alanin, glitsin va bosh.

2. Qutbli zayradsiz yon zanjir: **R guruhlari suvda ionlashtirilmaydi, lekin u vodorod bog‘lanishida ishtirok etishi mumkin. Masalan: serin, sistein va bosh.**

3. **Kislotali yon zanjir (manfiy zaryadli): -R** tarkibida $–COO^-$ saqlagan aminokislotalar. **Masalan:** glutamat va aspartat kislota.

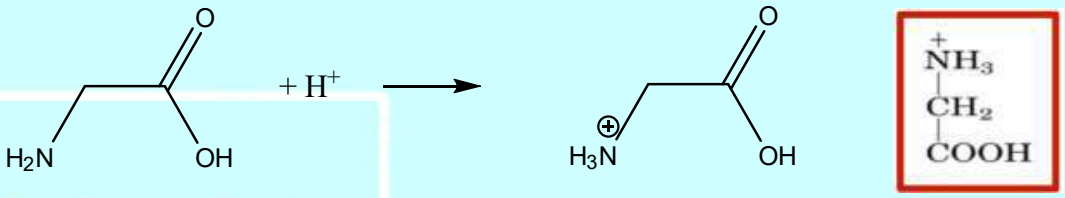
4. **Asosli yon zanjir (musbat zaryadli): -R** tarkibida tarkibida -NH_3^+ saqlagan aminokislotalar. **Masalan:** arginin, gistidin va lizin.

Aminokislotalarning xossalari.

- Aminokislotalar amfoterdir, ya'ni u kislota sifatida (proton berishi) yoki asos sifatida (proton biriktira olish)reaksiyaga kirisha oladi.
- **Aminokislotalarning amfoter xossalari** ularning ionlanuvchi α-amino va α-karboksilik guruhining mavjudligi bilan bog‘liq bo‘lib, ular muhitining pH darajasiga qarab ba'zan kislotalar, ba'zan esa asoslar sifatida xossalarni namoyon qilishi mumkin.
- Har bir aminokislota turli xil **izoelektrik nuqtaga (pI)** ega.
- pI: Bu musbat zaryad manfiy zaryadga teng bo‘lgan pH qiymati (ya'ni bu molekulaning sof zaryadi nolga teng) (zwitter iondir).

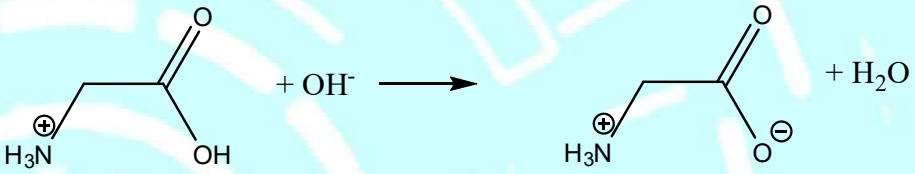
❖ **Neytral aminokislota glitsinni ishqoriy titrlash.**

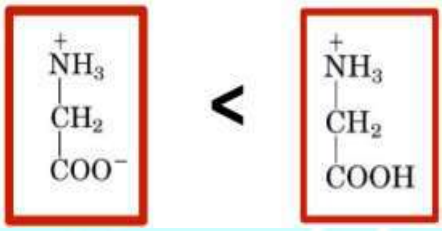
1. Aminokislotani ishqoriy titrlash uchun muhit kislotali qilib olinadi, bunda kislota solinganda pH≈0 ga yaqinlashadi. Natijada aminoguruh protonlanadi.



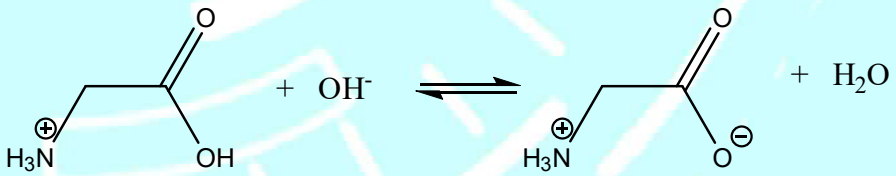
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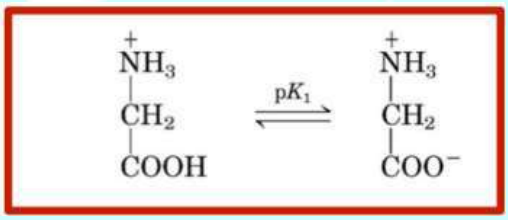
2. pH ko‘tarilganda, ya’ni ishqor qo‘shilganda -COOH guruhi ham protonlana boshlaydi va nisbat quyidagicha bo‘ladi:





3. Ishqor qo‘shish davom ettirilganda shunday payt kelganda C_A (kation) va C_B (Zwitter ion) konstratsiyalari tenglashadi. Bunda pH = pKa_1 bo‘ladi, bu holda sistema bufer vazifasini bajaradi.





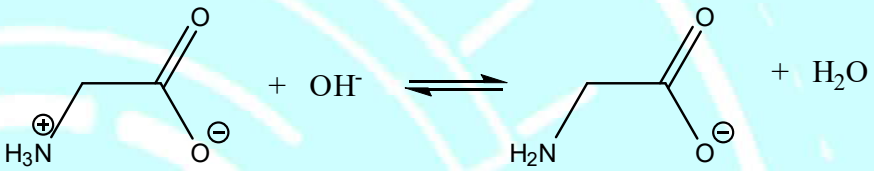
4. pH ning yanada oshishi, eritmada asosan zwitter ioni bo‘ladi va bu nuqtada pH = pI(izoelektrik nuqta) ga teng. Zwitter ioni – zaryadi 0 ga teng ion bo‘lib, u katoga yoki anodga tortilmaydi. Zwitter ion konsetratsiyasi eng yuqori bo‘lgan holat – izoelektrik nuqta deyiladi. Izoelektrik nuqta quyidagicha topiladi.

$$pI = \frac{pKa1+pKa2}{2}$$



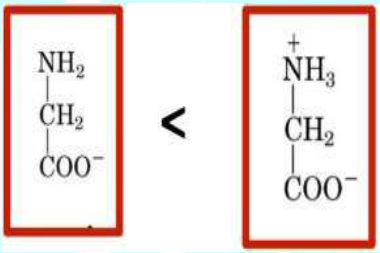
5. pH ortishi bilan ikkinchi guruh - NH_3^+ ham deprotonlana boshlaydi.

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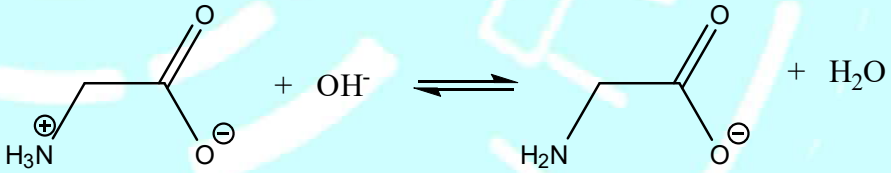


umumiy zaryadi: 0 umumiy zaryadi: -1

C_B C_C

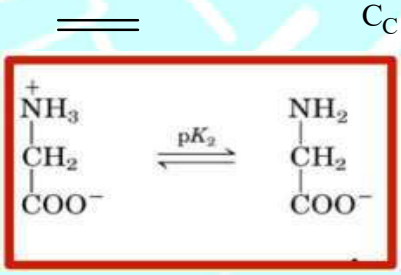


6. Shundan so‘ng, C_B va C_C larning konsentratsiyalari o‘zro tenglashadi. Bunda pH = pKa_2 bo‘ladi, bu holda sistema bufer vazifasini bajaradi.

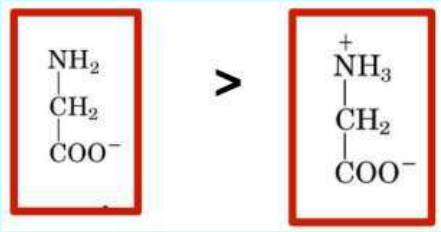


umumiy zaryadi: 0 umumiy zaryadi: -1

C_B C_C



7. $C_B < C_C$ kata bo‘lgan holat, bu zonada ham buffer tizim yuzaga keladi.

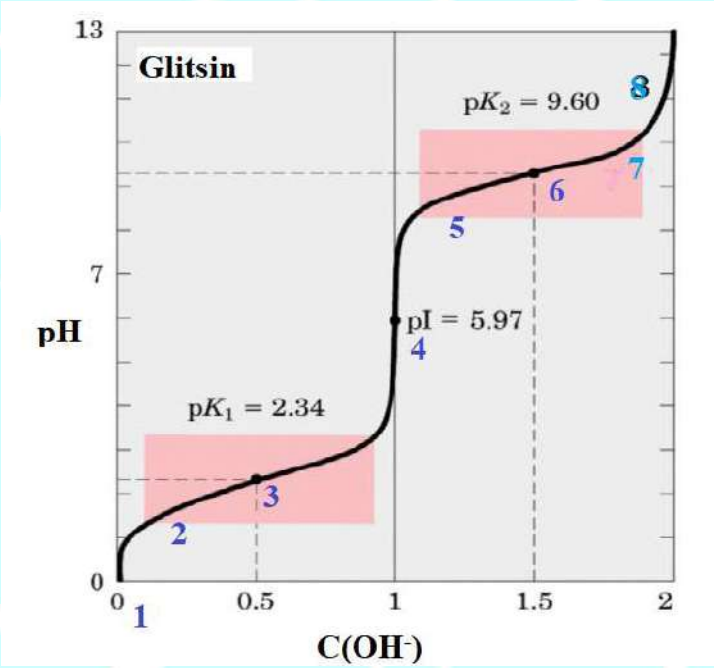


8. NH_3^+ guruhi ajraladi va shu bilan birga oxirgi nuqtada glitsin to‘liq dissotsilanadi.



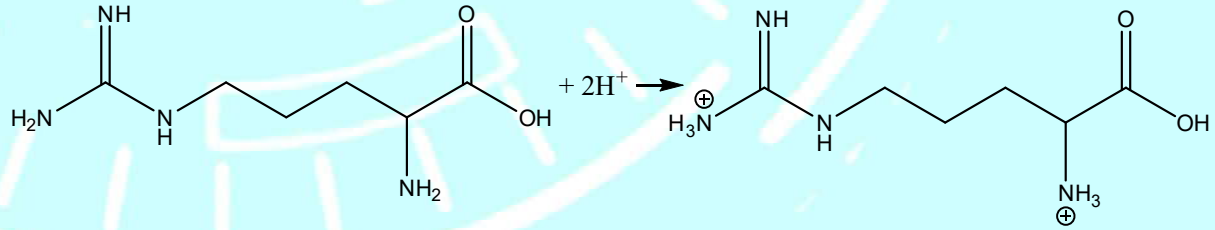
Glitsinni ishqoriy titrlash egrisi quyidagicha(yuqoridagi bosqichlar to‘liq ko‘rsatilgan).

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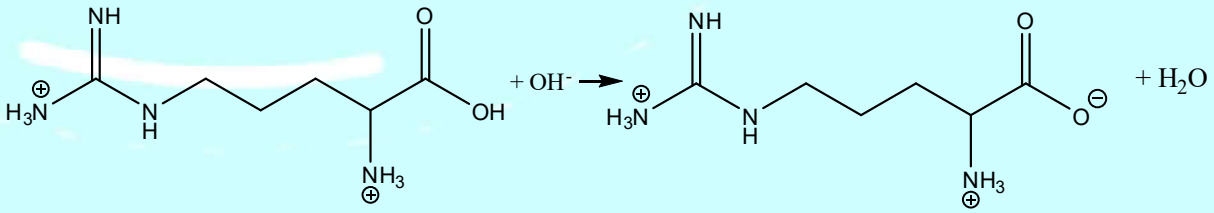


❖ **Asosli aminokislota argininni ishqoriy titrlash.**

Ishqoriy titrlash uchun muhit kislotali qilib olinadi, bunda kislota solinganda pH≈0 ga yaqinlashadi. Natijada aminoguruh protonlanadi.



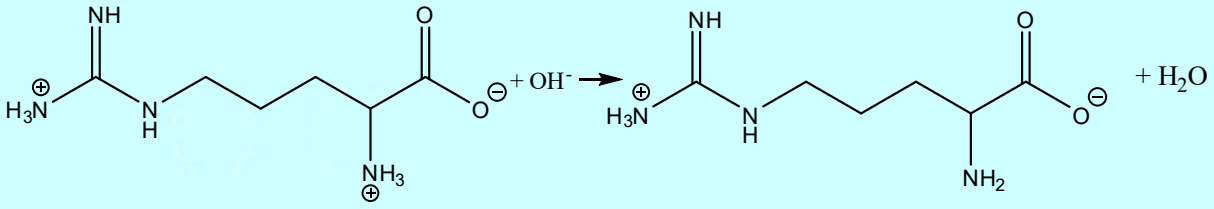
I-bosqich.



umumiy zaryadi: +2
C_A

umumiy zaryadi: +1
C_B

II-bosqich.

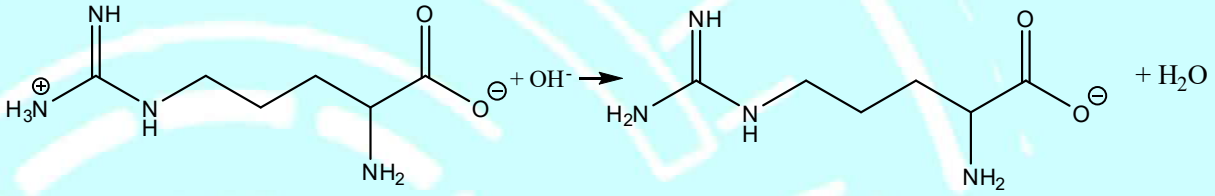


umumiy zaryadi: +1
C_B

umumiy zaryadi: 0
C_C

III-bosqich.

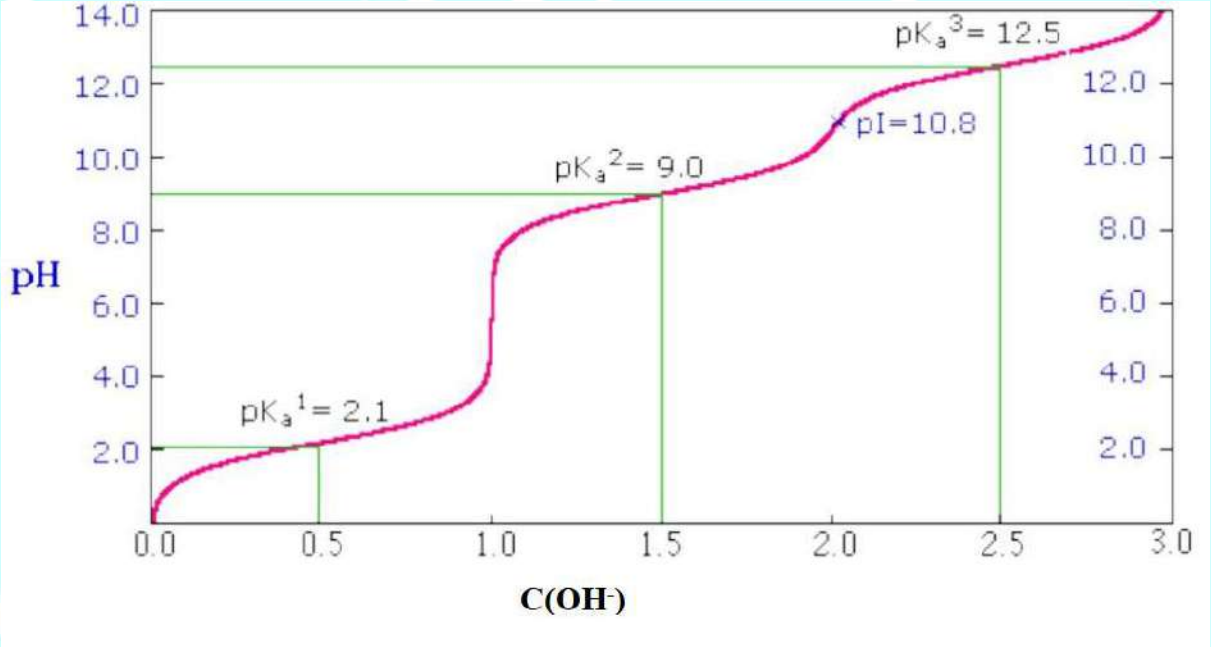
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umumiy zaryadi: 0
C_C

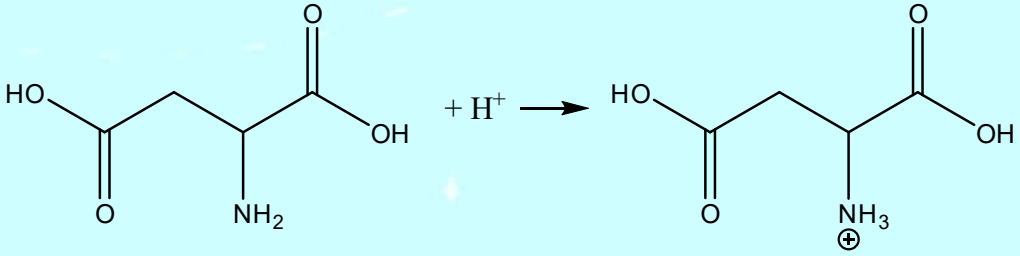
umumiy zaryadi: -1
C_D

Argininni ishqoriy titrlash egrisi quyidagicha(yuqoridagi bosqichlar to‘liq ko‘rsatilgan).

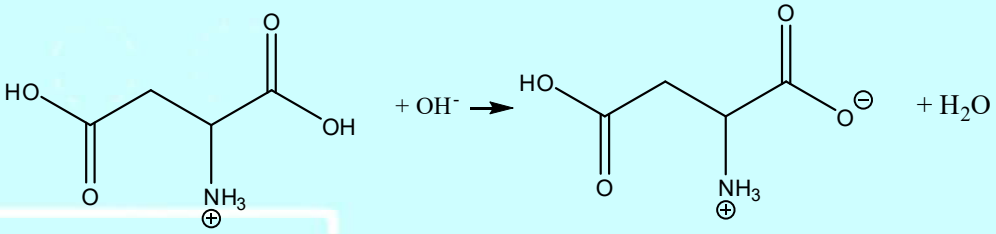


❖ **Kislotali aminokislota asparta kislotasini ishqoriy titrlash.**

Ishqoriy titrlash uchun muhit kislotali qilib olinadi, bunda kislota solinganda pH≈0 ga yaqinlashadi. Natijada aminoguruh protonlanadi.



I-bosqich.

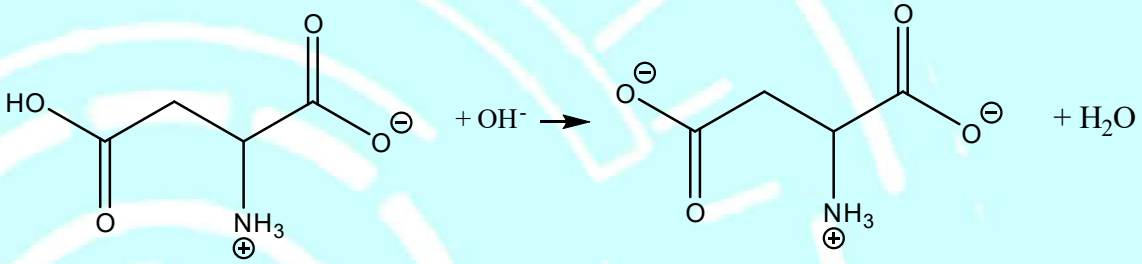


umumiy zaryadi: +1
C_A

umumiy zaryadi: 0
C_B

II-bosqich.

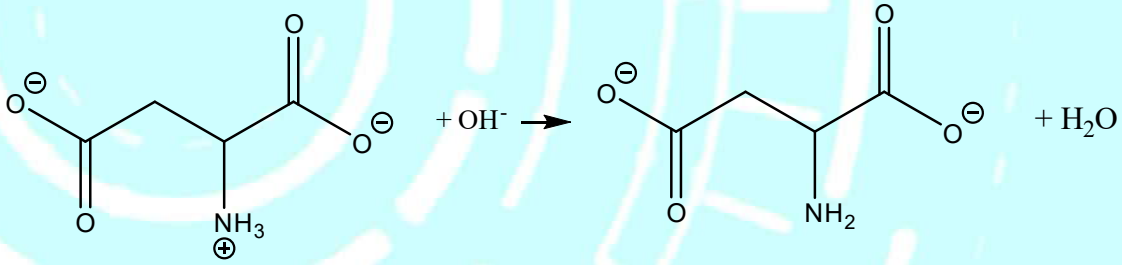
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umumiy zaryadi: 0
C_B

umumiy zaryadi: -1
C_C

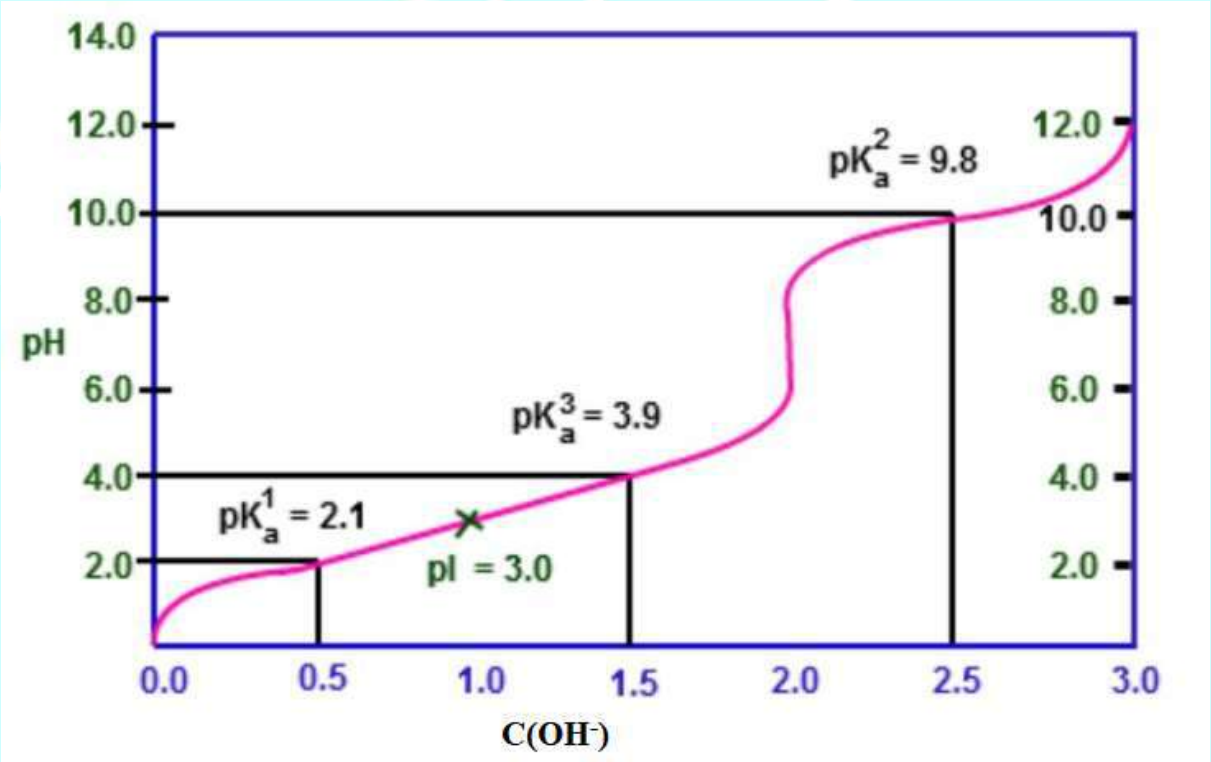
III-bosqich.



umumiy zaryadi: -1
C_C

umumiy zaryadi: -2
C_D

Aspartatning ishqoriy titrlash egrisi quyidagicha(yuqoridagi bosqichlar to‘liq ko‘rsatilgan).



Hisob-kitob uchun masala(35-IBO olimpiadasi II-bosqich saralsh savoli).

1-topshiriq:Biokimyo (12 ball) AMINOKISLOTALARNI TITRLASH.

Aminokislotalar karboksil va amino guruhlarni o‘zida saqlagan organik molekulalardir. Ma’lumki tabiiy oqsillar tarkibiga 20 xil aminokislotalar kirishi mumkin, Quyida berilgan jadvalda 20 xil aminokislotalarning formulalari ko‘rsatilgan. Aminokislotalarning aksar vakillari eritma muhitida amfoter xossani

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o‘zida namoyon qiladi, bunga sabab aminokislota molekulasidagi amino **(-NH_2)** va karboksil **-(COOH)** guruhlarning xossalaridir. Neytral aminokislotalarning radikal **(R)** guruhi zaryadga ega bo‘lmaydi.

Amino guruh **(-NH_2)**: asosli xossani namoyon qiladi, past **pH** da (kislotali muhitda) amino guruh protonlanadi **(H^+)** va musbat zaryadlangan holatga **(NH_3^+)** o‘tadi.

Karboksil guruh **(-COOH)**: kislotali xossani namoyon qiladi, yuqori pH da (ishqoriy muhitda) karboksil guruh deprotonlanadi **(H^+)** va manfiy zaryadlangan holatga **(COO^-)** o‘tadi.

Zvitterion: Oraliq **pH** da aminokislotalar zvitterion shaklida bo‘lishi mumkin, Zvitterionda amino guruh protonlangan **(NH_3^+)**, karboksil guruh deprotonlangan **(COO^-)** holatda bo‘ladi.

Aminokislotalarning zvitterion shaklida umumiy zaryadi neytraldir. Aminokislota zvitterion (neytral zaryad) sifatida mavjud bo‘lgan **pH** uning izoelektrik nuqtasi **(pI)** deb nomlanadi. Amino va karboksil guruhlari uchun o‘ziga xos pKa qiymatlari turli xil aminokislotalar orasida farq qiladi, bu ularning izoelektrik nuqtalarini farqlanishiga olib keladi. Titrlash egri chizig‘idan foydalanib, **pI** (izoelektrik nuqtani) ni aminokislotaning anion va kation shakllarning **pKa** qiymatlari orasidagi burilish nuqtasi sifatida empirik aniqlash mumkin.

Kislotalarning umumiy dissotsiyalanish muvozanati: **HA<->H++A-** ushbu muvozanat uchun kislota dissotsilanish konstantasi **Ka** quyidagi formula orqali topiladi:

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

Asosan kislotalarning kuchi ularning **pKa** qiymatlari orqali ifodalandi.

$$pKa = -\log_{10}(Ka)$$

Bunday ikki negizli aminokislotalarni titrlashda titrlash ikki bosqichda sodir bo‘ladi, chunki ko‘proq kislotali karboksil guruhi (pastki pKa_1) va kamroq kislotali amino guruh (pKa_2) ketma-ket protonlarini yo‘qotadi.

Ikki dissotsatsiya bosqichi uchun ko‘rinadigan pKa qiymatlarini har bir bosqichning o‘rta nuqtalariga asoslanib hisoblash mumkin.

Buning uchun Henderson-Hasselbalch tenglamasidan foydalanish mumkin.

$$\mathrm{pH} = \mathrm{pKa} + \log\left(\frac{[\mathrm{A^-}]}{[\mathrm{HA}]}\right)$$

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pKa_1 (karboksil guruh uchun pKa) kislota guruhining yarmi titrlangan nuqtadir, ya'ni eritmada anion (A^-) va kislota guruhi (HA) bir xil miqdorda bo'lgan nuqta. Shunday qilib, tenglama ushbu nuqtada quyidagicha bo'ladi:

$$pH = pKa$$

Xuddi shunday, pKa_2 (amino guruhi uchun pKa) aniqlanishi mumkin.

Quyida sizga noma'lum X aminokislotani 0.3024 M li NaOH yordamida titrlash tajribasidan olingan ma'lumotlar keltirilgan (tajribada 25 ml li byuretkadan foydalanilgan).

Qo'shilgan NaOH hajmi (ml)	**pH**
0.00	1.4
1.00	1.4
2.00	1.5
3.00	1.6
4.00	1.7
5.00	1.8
6.00	1.9
7.00	2.1
8.00	2.2
9.00	2.4
10.00	2.7
11.00	3.2
12.00	9.4
13.00	9.9
14.00	10.2
15.00	10.4
16.00	10.6
17.00	10.8
18.00	11.0
19.00	11.3
20.00	11.6
21.00	11.8
22.00	12.0
23.00	12.1
24.00	12.2
25.00	12.3

Q1.1. Yuqoridagi jadvalda keltirilgan ma'lumotlardan foydalanib, sizga taqdim etilgan millimetrovka qog'ozga titrlash jarayonining (pH ga nisbatan NaOH hajmi (ml)) grafigini chizing.

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Q1.2. Titrlash egri chiziqlaringizdan **pI** nuqtani toping va uni grafikda belgilang va **pI** nuqta nechaga teng ekanini javoblar varaqasiga yozing.

Q1.3. Titrlash egri chiziqlaringizdan **pKa_1** va **pKa_2** ni toping va ularni grafikda belgilang va **pKa_1** va **pKa_2** nuqtalarni nechaga teng ekanini javoblar varaqasiga yozing.

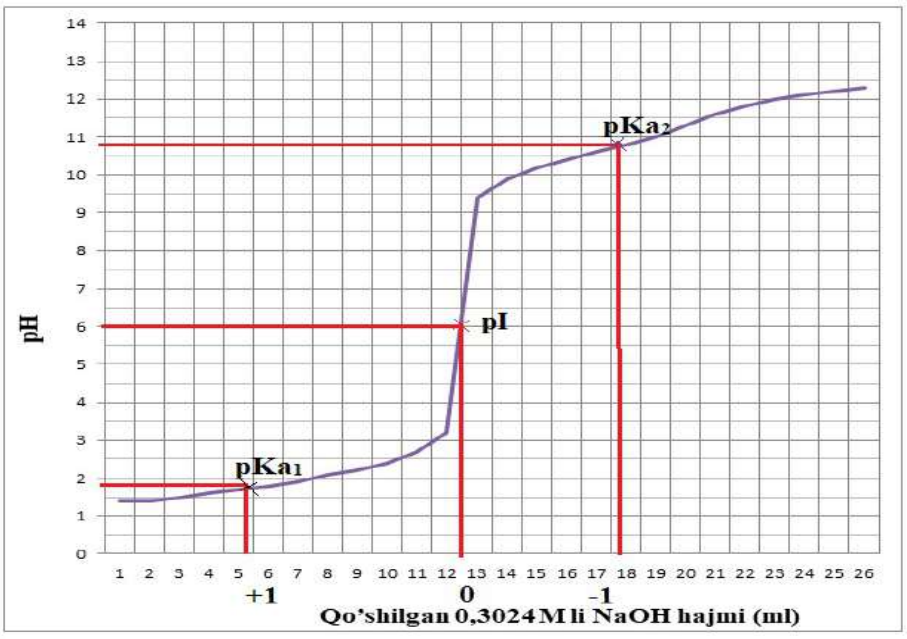
Q1.4. 0,866 g noma'lum X aminokislotasi 75 ml deionlangan suvda eritilgan. Noma'lum X aminokislotaning molekulyar og‘irligini aniqlang va quyida berilgan aminokislotalarning molekulyar og‘irliklari asosida titrlangan aminokislotaning nomini javoblar varaqasiga yozing. Eslatma: To‘liq protonlangan aminokislotadan titrlash jarayonini boshlash uchun aminokislota eritmasiga 3,2 ml 0.3024 M li HCl eritmasi qo‘shilgan. Bu 3,2 ml 0.3024 M li NaOH eritmasiga teng. pI nuqtaga yetish zarur bo‘lgan NaOH ning haqiqiy mol miqdorini aniqlash uchun pI nuqtaga yetish uchun sarflangan NaOH hajmidan 3,2 ml ayiriladi.

Aminokislota	Molekulyar og’irlik
Glitsin	75
Alanin	89
Valin	117
Leysin	131
Metionin	149
Izoleysin	131
Prolin	115
Sistein	121
Serin	105
Treonin	119

Aminokislota	Molekulyar og’irlik
Asparagin	132
Glutamin	146
Lizin	146
Arginin	174
Gistidin	155
Aspartat	133
Glutamat	147
Fenilalanin	165
Tirozin	181
Triptofan	204

Masalaning yechimi:

1.1. Yuqoridagi jadvalda keltirilgan ma'lumotlardan foydalanib quyidagi grafik chizildi.



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1.2. Grafikda ko‘rinadiki, 11 ml va 12 ml ishqor sarflanganda pH keskin o‘zgargan. Demak, izoelektrik nuqta taxminan 11,5 ml ishqor sarflangan vaqtga to‘g‘ri keladi. Shu vaqtda aminokislotaning H^+ ionlari to‘liq neytrallangan va hajmga mos pH ni topsak, u taxminan pH≈6 ga teng bo‘ladi.

Izoelektrik nuqta pH≈pI≈6 ga teng.

1.3. Grafikda izoelektrik nuqta aniqlangandan so‘ng pKa_1 va pKa_2 ni aniqlaymiz.

pKa_1 ni aniqlash uchun izoelektrik nuqta uchun sarflangan ishqor miqdorining yarmi sarflangandagi pH ga teng bo‘ladi. 11,5 ml/2=5,75 ml da pH =pKa_1 ga teng bo‘ladi. Bu quymat pH≈1,8, pKa_1≈1,8 ga teng.

pKa_2 ni aniqlash uchun izoelektrik nuqta uchun sarflangan ishqor miqdorining yarmicha ko‘proq sarflangandagi pH ga teng bo‘ladi. 11,5 ml + 11,5 ml/2=17,25 ml da pH =pKa_2 ga teng bo‘ladi. Bu quymat pH≈10,8, pKa_1≈10,8 ga teng.

1.4. Dastlabki aminokislotani kislotali muhitga o‘tkazish uchun 3,2 ml 0,3024 M li HCl eritmasidan solingan. Demak, ishqor va kislota(HCl) konsentratsiyalari teng bo‘lgani uchun ishqorning 3,2 ml hajmi qo‘shilgan HCl ni neytrallashga sarflangan bo‘lsa, qolgan 11,5 ml – 3,2 ml = 8,3 ml hajmi aminokislotani neytrallash uchun sarflangan. Uning tarkibida:

$n(NaOH) = C_M \cdot V = 0{,}0083\ l \cdot 0{,}3024M = 0{,}00251$ mol sarflangan.

0,866 g noma'lum X aminokislotasi 75 ml deionlangan suvga solinib va uning 25 ml hajmli olingan bo‘lsa(tajribada 25 ml li byuretkadan foydalanilgan), aminokislota 25 ml olingan idishda(75ml/25ml=3) 3 marta kam aminokislota 0,866 g/3 = 0,288666 g bor.

Grafik bir asosli aminokislota uchun mos bo‘lganligi uchun:

X(aminokislota) + NaOH = Tuz + H_2O

0,28866 g ----------0,00251 mol

Mr(X)=115 g/mol-----1 mol

Mr(X) = 115 g/ mol bo‘lsa, bu aminokislota prolindir.

Xulosa: Ushbu maqola noma'lum aminokislotalarni aniqlash, titrlash egri chizig‘ini yaratish va tahlil qilish qobiliyatini shakillantiradi. Protonlanish holatlari, buferzonalari va izoelektrik nuqtalar haqida tushunchalarini bilish va masalalar yechida amaliy ko‘nikmalarni beradi

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