

**RESEARCH ARTICLE**

# STUDY THE PROTONATION POINT FOR NITROGLYCERINE AND EFFECT OF NITROGLYCERINE ON ISOELECTRIC POINT OF VARIOUS AMINO ACIDS.

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**Abstract:** Nitroglycerin, which is also commonly referred to as GTN, is an extremely powerful secondary explosive. It is particularly more dangerous than other traditional explosions because it is extremely unstable. Being unstable means that any unnecessary movement can cause the substance to detonate, making unstable substances extremely hazardous and dangerous to work with or around. Nitroglycerin ointment (Nitro-Bid) is used to prevent episodes of angina (chest pain) in people who have coronary artery disease (narrowing of the blood vessels that supply blood to the heart). Nitroglycerin ointment can only be used to prevent attacks of angina; it cannot be used to treat an attack of angina once it has begun. Nitroglycerin ointment (Rectiv) is used in adults to treat pain from anal fissures (a split or tear in the tissue near the rectal area). Nitroglycerin is in a class of medications called vasodilators. Nitroglycerin ointment prevents angina by relaxing the blood vessels so that the heart does not need to work as hard and therefore does not need as much oxygen.

Each amino acid has a pH at which almost all of the molecules are present in the zwitterionic form (0 net charge). At a pH below the isoelectric point, the cation concentration increases (net (+) charge: protonation occurs, excess H<sup>+</sup>). At a pH above the isoelectric point, the anion concentration increases (net (-) charge: deprotonation occurs, excess OH<sup>-</sup>).

In the present study we proposed to observe protonation for nitroglycerine and effect of nitroglycerine on isoelectric point of amino acid. This Study is found to provide interesting and significant data, which possibly may have correlation with significant protonation point by addition of various amount nitroglycerine on these amino acids.

**Keywords:** Nitroglycerine, angina, isoelectric point.

## Introduction:

The 1<sup>st</sup> some amino acids were discovered in the early 19th century. In 1806, French chemists Louis-Nicolas Vauquelin and Pierre Jean Robiquet isolated a compound in asparagus that was subsequently named asparagine, the first amino acid to be discovered [1,2]. Amino acids are biologically important organic compounds containing amine (-NH<sub>2</sub>) and carboxylic acid (-COOH) functional groups, usually along with a side-chain specific to each amino acid [3,4,5]. Amino acids are the building blocks of proteins, and also play a central role as intermediates in metabolism [6]. Amino acid molecules are bonded together (through peptide linkages) to form proteins. The kind of protein that results is managed by the types of amino acids involved and the sequence in which the amino acids are arranged. The twenty-eight main amino acids in the human body combine in a number of ways to account for more than 40,000 proteins known to us [7].

The word isoelectric comes from 'iso,' which means the same, and 'electric,' which implies charge. The isoelectric point or pI of an amino acid is the pH at which an amino acid has a net charge of zero. The isoelectric point (pI, pH (I), IEP), is the pH at which a particular molecule carries no net electrical charge. The net charge on the molecule is affected by pH of its surrounding environment and can become more positively or negatively charged due to the gain or loss, respectively, of protons (H<sup>+</sup>). The isoelectric point, pI, is the pH of an aqueous solution of an amino acid at which the molecules have no net charge. In other words, the positively charged groups are exactly balanced by the negatively charged groups. When this dissolved amino acid is titrated with acid, it acts as a base, and with base, it acts as an acid which makes them an amphoteric molecule.

These ionizations follow the Henderson-Hasselbalch equation:

$$pH = pK_a + \log \frac{[\text{unprotonated form (base)}]}{[\text{Protonated form (acid)}]}$$

When the concentration of the unprotonated form equals that of the unprotonated form, the ratio of their concen-

trations equals 1, and  $\log 1=0$ . Hence,  $pK_a$  can be defined as the pH at which the concentrations of the protonated and unprotonated forms of a particular ionizable species are equal. The  $pK_a$  also equals the pH at which the ionizable group is at its best buffering capacity; that is the pH at which the solution resists changes in pH most effectively.

#### OBSERVATION TABLE:-

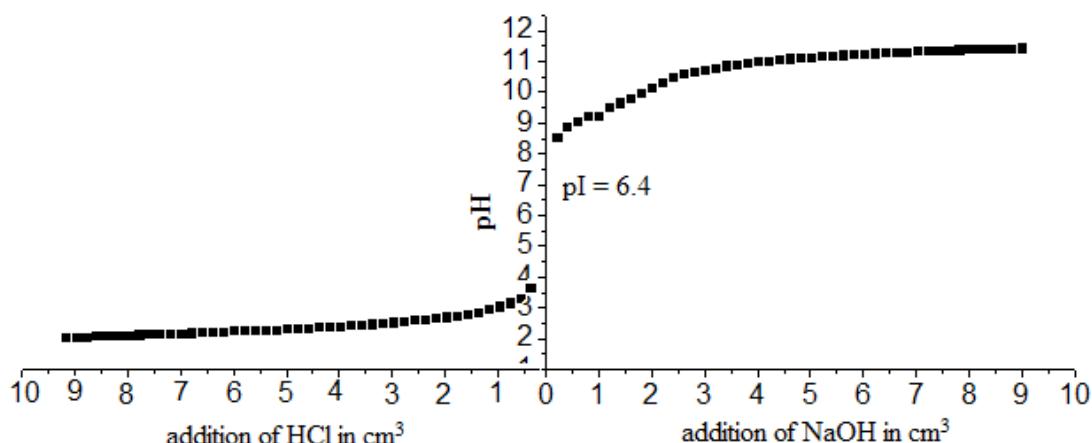
Observation Table by Calculation

		Blank	0.05 NG	0.1 NG	0.15 NG	0.2 NG
Glycine	pK <sub>1</sub>	1.45	1.45	1.67	1.42	1.6
	pK <sub>2</sub>	8.94	9.06	9.06	9.05	9.17
	pI	5.20	5.25	5.25	5.24	5.29
L-Arginine	pK <sub>1</sub>	1.48	1.48	1.48	1.47	1.46
	pK <sub>2</sub>	7.47	7.34	7.40	7.46	7.25
	pI	4.47	4.41	4.44	4.47	4.36
Alanine	pK <sub>1</sub>	1.44	1.44	1.46	1.44	1.43
	pK <sub>2</sub>	9.29	9.27	9.01	9.40	9.47
	pI	5.36	5.36	5.24	5.42	5.45
Glutamic Acid	pK <sub>1</sub>	3.96	4.23	3.71	3.72	3.60
	pK <sub>2</sub>	9.97	9.94	10.09	10.06	10.06
	pI	6.97	7.09	6.90	6.98	6.83
L-Isoleucine	pK <sub>1</sub>	1.67	1.67	1.69	1.71	1.55
	pK <sub>2</sub>	7.67	7.70	7.73	7.69	7.77
	pI	4.67	4.69	4.71	4.70	4.66

Observation table by Graph

	blank	0.05 NG	0.1 NG	0.15 NG	0.2 NG
Glycine	6.4	6.4	6.4	6.4	6.4
L-Arginine	9.8	9.7	9.7	9.7	9.8
Alanine	6.4	6.4	6.8	6.8	6.4
Glutamic Acid	3.5	3.4	3.5	3.5	3.5
L-Isoleucine	6	6.05	6.05	6.05	6.2

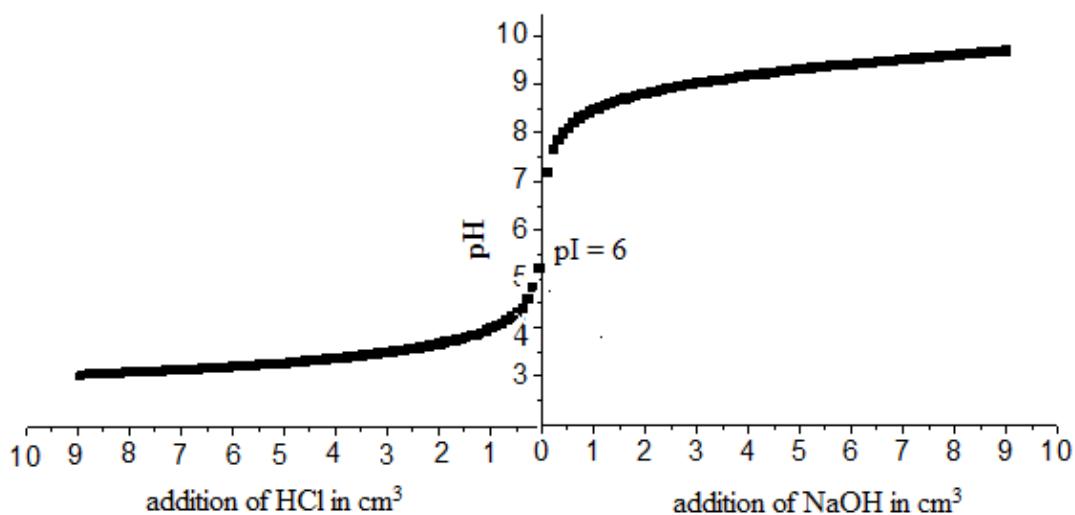
## GRAPHICAL REPRESENTATION:-



Glycine

L-Arginine

Alanine



L-Isoleucine

The pK is the pH at the midpoint of the buffering region (where the pH changes only slightly upon addition of either acid or base). The pK is the pH corresponding to the inflection point in the titration curve. The end point of a titration curve represents the observed end of the titration.

The isoelectric point is the pH at which the amino acid has a net zero charge. For a simple diprotic amino acid, the pI falls halfway between the two pK values. For acidic amino acids, the pI is given by  $\frac{1}{2} (pK_1 + pK_2)$  and for basic amino acids it's given by  $\frac{1}{2} (pK_2 + pK_3)$ <sup>[8]</sup>.

For over 130 years, nitroglycerin has been used medically as a potent vasodilator to treat heart conditions such as angina pectoris and chronic heart failure. Though it was previously known that these beneficial effects are due to nitroglycerin being converted to nitric oxide, a potent vasodilator, it was not until 2002 that the enzyme for this conversion was discovered to be mitochondrial aldehyde dehydrogenase[9]. Nitroglycerin was first used by William Murrell to treat anginal attacks in 1878, with the discovery published that same year [10,11]. Nitroglycerin belongs to a group of drugs called nitrates, which includes many other nitrates like isosorbide dinitrate (Isordil) and isosorbide

mononitrate (Imdur, Ismo, Monoket). These agents all exert their effect by being converted to nitric oxide in the body by mitochondrial aldehyde dehydrogenase.

## MATERIALS & METHODS

**Chemical and Reagents:** - All the chemicals used for the work are of A.R. grade of S. D. Fine Chem. Sisco.

**Instruments:** - pH meter (Elico) and Electrical balance (Type Citizen CY 204).

## EXPERIMENTAL

Amino Acids like Alanine, Glutamic acid, Glycine, L-Arginine, Isoleucine are selected for this work. This study is carried out to detect the protation point of this amino acid with the addition of various amount of Nitroglycerine.

### Procedure for making the solutions:-

Dilute 50 cm<sup>3</sup> of 0.02M glycine solution to 250 cm<sup>3</sup> with distilled water in a standard measuring flask. Pipette out 50 cm<sup>3</sup> of the diluted glycine solution in a 100 cm<sup>3</sup> dry beaker. Wash, rinse and fill the burette with 0.1 M HCl Solution. Insert the electrode in the glycine solution in the beaker and record the initial pH. Add 0.2 cm<sup>3</sup> of HCl Solution to the glycine solution and stir it till constant pH value is reached. Same procedure follows for NaOH. Procedure is same for other solutions.

## CONCLUSION:-

For studying the effect of NG on Isoelectric Point of some amino acid pH metrically. The study was carried out by addition of varying amount of NG (0.05 to 0.2) to 50 cm<sup>3</sup> of amino acid (0.004M). The results were compared with the blank. The study was carried out pH metrically by titrating against acid and base and determination of isoelectric point by calculation and by graph.

For all amino acids selected for the study i.e. Alanine, Glutamic Acid, Glycine, L-Arginine, and Isoleucine. The significant effect was observed for higher concentration of NG i.e. 0.2 cm<sup>3</sup> in 50 cm<sup>3</sup> of amino acid solution for lower volume i.e. 0.05 and 0.1 cm<sup>3</sup> much change on isoelectric point not observed. In case of Alanine, Glycine the + ve effect is observed i.e. Isoelectric Point is

slightly increases to 5.20 to 5.29 for Alanine and 5.36 to 5.45 for Glycine. Whereas for rest of the amino acid i.e. Glutamic acid and L-arginine slight -ve effect is observed. As isoelectric point decreases from 4.47 to 4.36 for Glutamic acid and in case of L-Arginine some significant effect is observed as isoelectric point slightly increases for 0.05 remains almost same for 0.15 but decreases for 0.2 cm<sup>3</sup> of NG. In case of L-Isoleucine not much significant effect is observed on addition of NG i.e. isoelectric point was observed to remain unaffected.

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