

Advanced Pharmaceutical Analysis

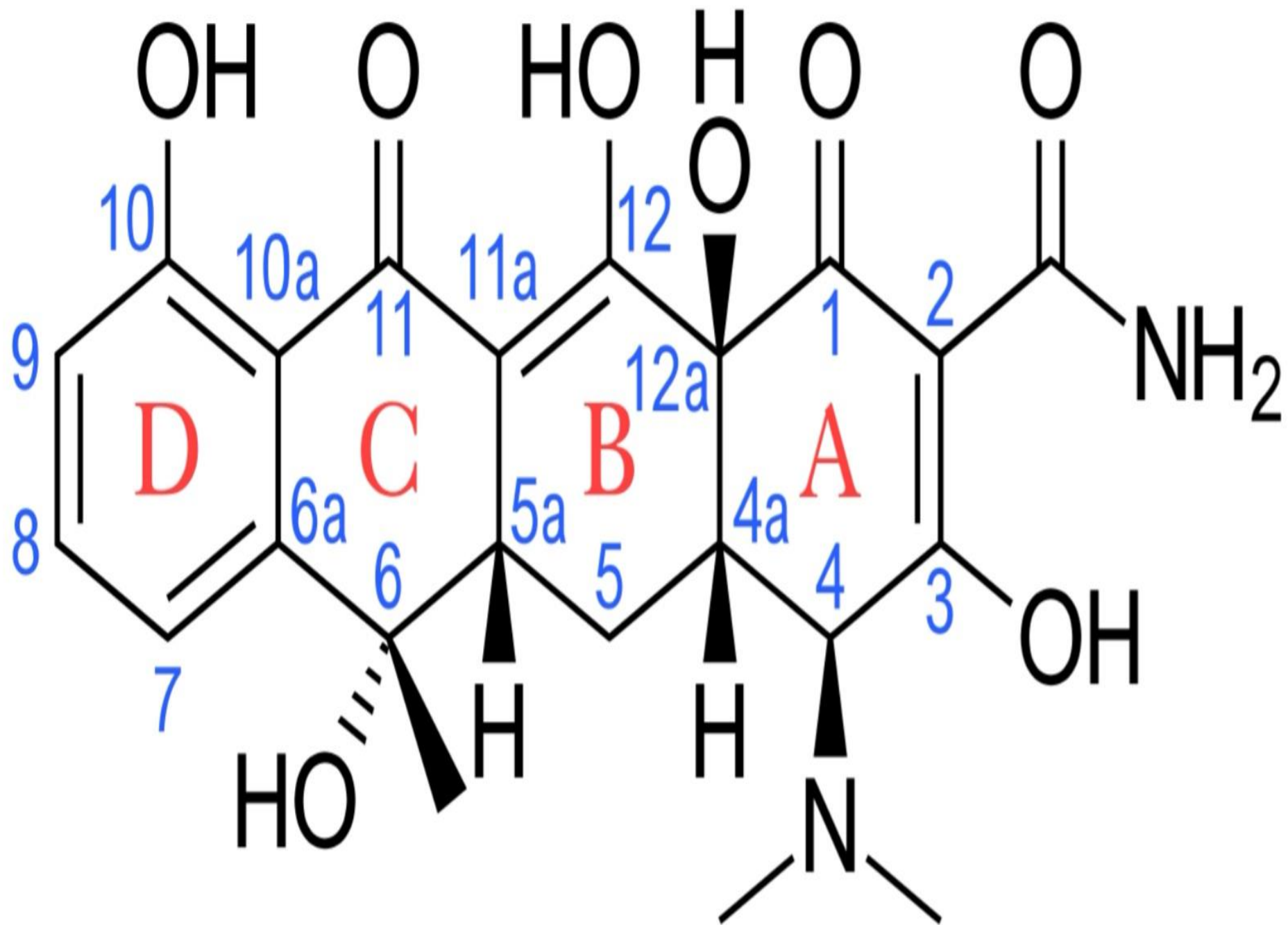
5th Stage

Lab 3

Colorimetric Assay
Methods
For
Tetracycline

Colorimetric Assay is a technique used to determine the concentration of colored compounds in solution by measuring its absorbance at a specific wavelength of light.

Colorimetric assays use reagents that undergo a measurable color change in the presence of the analyte.



Physical Properties:

- Yellow Powder
- Bitter Taste
- Odourless
- Light Sensitive
- Sparingly soluble in water

Chemical Properties:

- Amphoteric Property: (H.W.)
- Zwitter Ion: (H.W.)
- Epimerization: (H.W.)

H.W.:

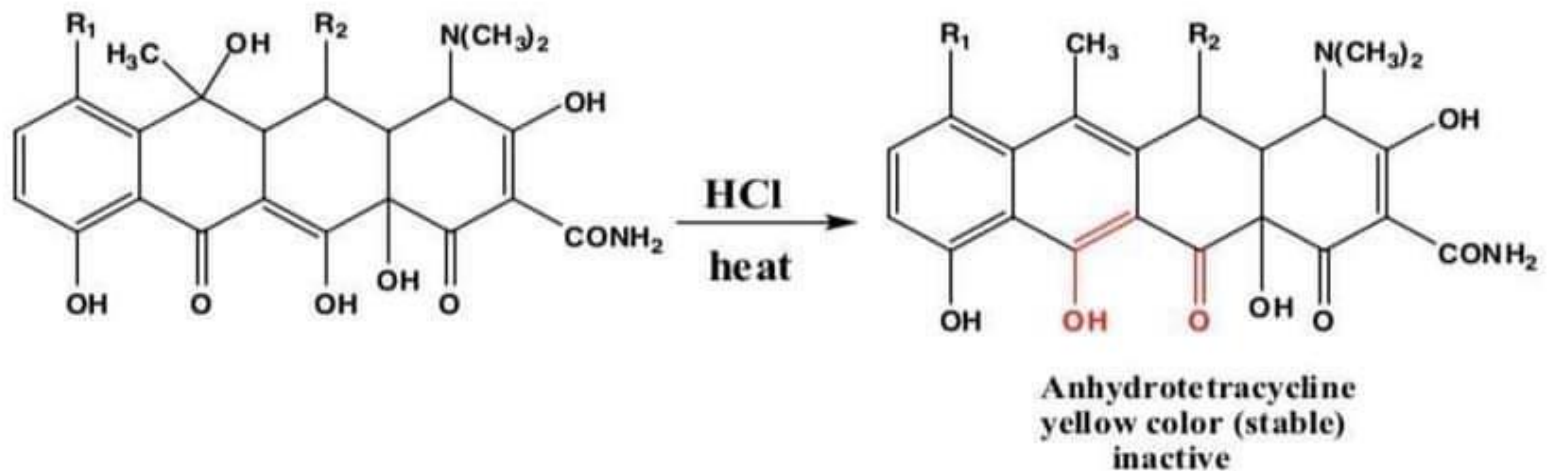
How many pka that Tetracycline has ? Why ?

Acid Colorimetric Method

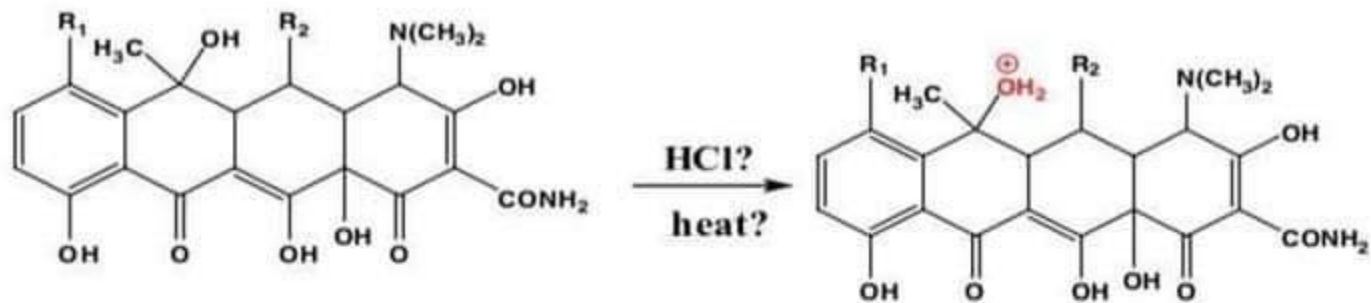
Chemical Principle

Strong acids attack –OH group at Position 6

At pH less than 2, TC eliminates a molecule of water with concomitant aromatization of ring C forming the more energetically favored resonant system of the naphthalene group found in the inactive anhydrotetracyclines.

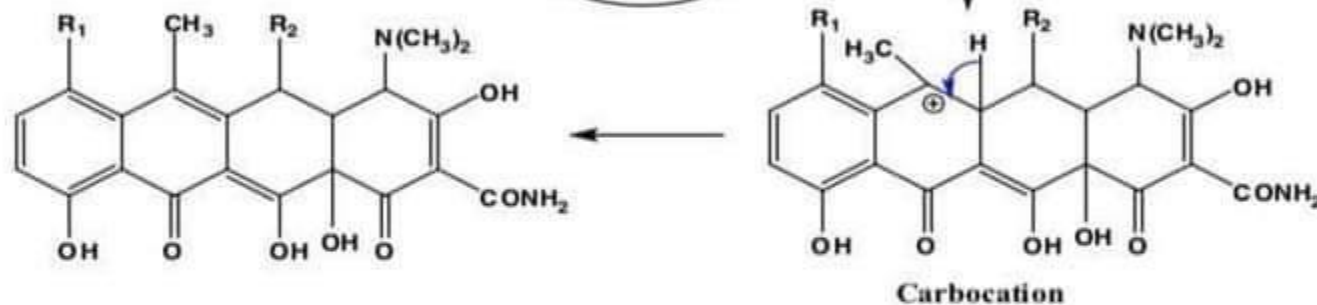


λ_{max} : 440 nm

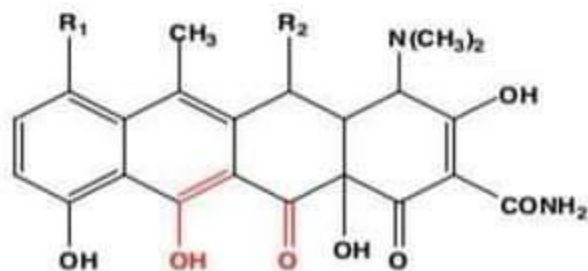


The hydroxyl group is a bad leaving group but in presence of proton leads to

Formation of oxonium ion



tautomerism why?



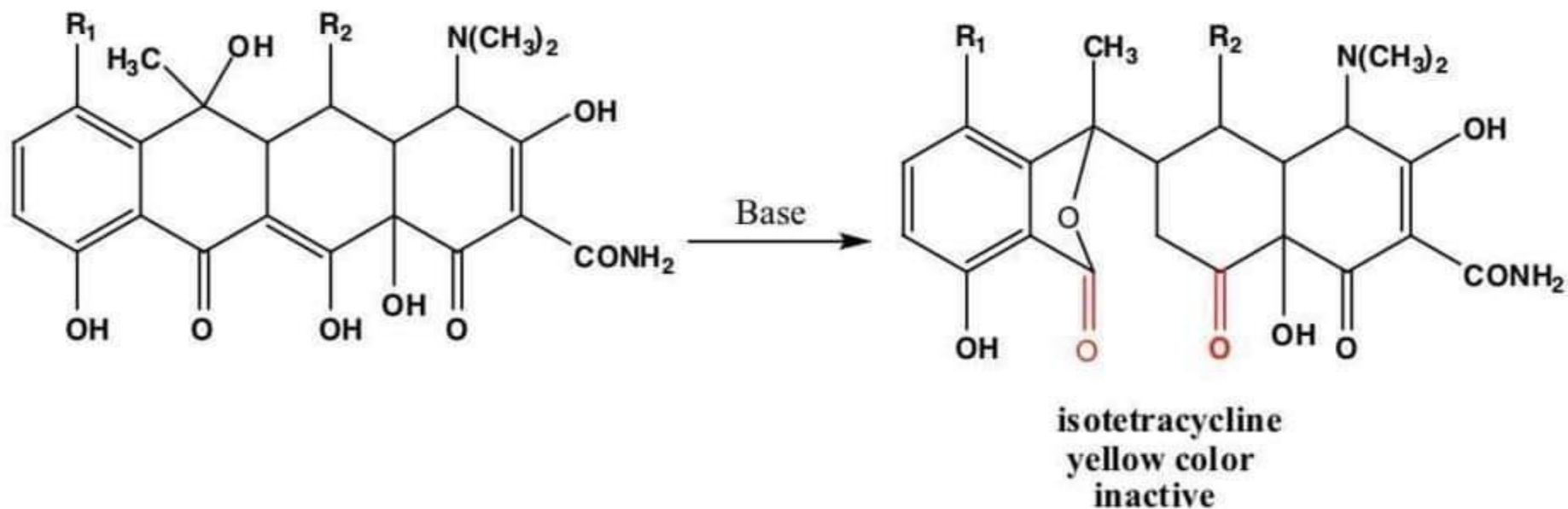
Anhydrotetracycline
deep yellow color (stable) why?
inactive

The Mechanism of Reaction

Base colorimetric method

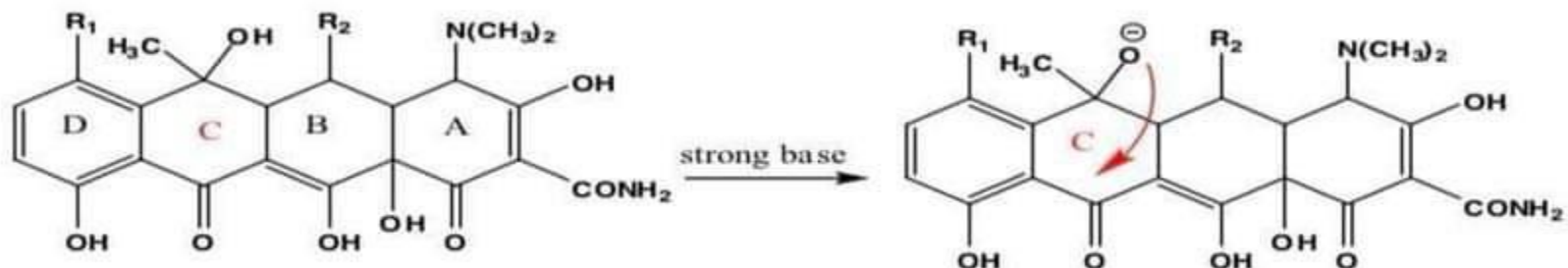
Chemical Principle

Bases promote a reaction between the 6- OH group and the ketone group at the 11-position, causing the bond between the 11 and 11a atoms to *cleave* forming the lactone ring found in the **inactive isotetracycline**.

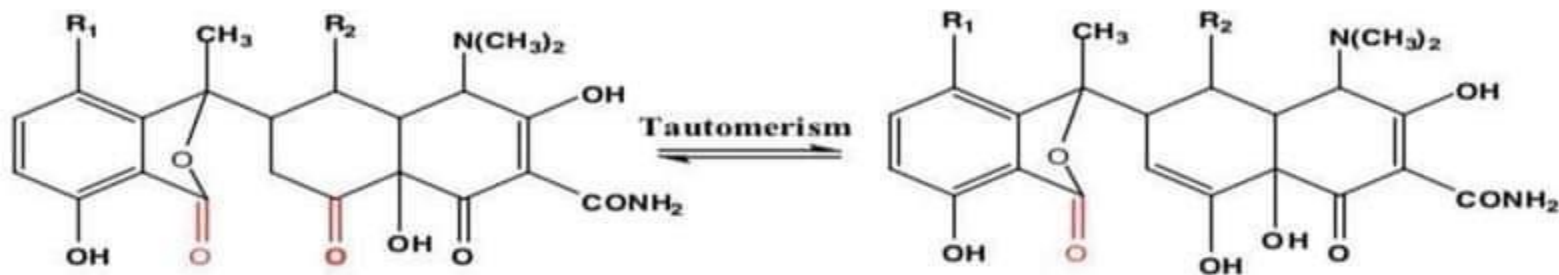


$\lambda_{max} : 380 \text{ nm}$

Mechanism of the basic method



1. Formation of alkoxide anion (strong) (unstable)
2. Attack of the carbonyl group and cleavage of 11-11a bond



isotetracycline
yellow color with absorption maximum
at 380nm
inactive as antibacterial

Formation of lactone ring
(cyclic ester)

H.W. : Difference between Resonance & Tautomerism ?

Practical Work: (Two Weeks)

I. Acidic Colorimetric Assay Of Tetracycline

II. Unknown : Acidic Colorimetric Assay Of Tetracycline