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Advanced Techniques in Nano-crystal Engineering: Synthesis, Instrumentation, and Spectroscopic Characterization

Mr. S.D Paralkar[1], Dr. Y.S Thorat[2], Dr. B.D Tiwari[3], Ms. K.V Divate[4], Ms. A.S Dhore[5]

[1]Assistant Professor, Pharmaceutical, Amepurva Forum's Nirant Institute Of Pharmacy, Solapur
[2]Professor, DSTS Mandal's College of Pharmacy, Solapur
[3]Vice-Principle, Amepurva Forum's Nirant Institute Of Pharmacy, Solapur
[4,5]Student, Amepurva Forum's Nirant Institute Of Pharmacy, Solapur

Abstract:
The main purpose of the current study was to formulate and evaluate Nano-Crystals of clarithromycin. Drugs with weak water solubility have poor bioavailability, which impacts their therapeutic effectiveness. Nano-Crystals offer a promising solution by enhancing drug solubility, and targeted delivery to the site of requirement. The creation of Nano-scale drug formulations has assisted in resolving the bioavailability issues associated with certain poorly water-soluble BCS class 2 and class 4 medications. One type of these Nano-sized formulations are called "Nano-Crystals," which are composed of drug particles at the Nano-scale that have been stabilized by an appropriate stabilizer or surfactant. Crystals in the Nano-meters range, typically between a few Nano-meters and 1000 nm, are known as drug Nano-Crystals. They are ultra-small crystalline drug particles designed to enhance the solubility and efficacy of drugs. These Nano-Crystals improve drug delivery by increasing dissolution rates, allowing for lower doses and reducing resistance. They can be administered orally, intravenously, or topically, providing targeted and sustained medication release. This method aids in overcoming obstacles such as poor water solubility. Along with a brief overview about Nano-Crystals, this article provides making methodology of Nano-Crystals and studies the evaluation parameters along with overview of their results and discussion.

Keywords: Nano-Crystals, Clarithromycin, Low- Precipitate Method, UV Absorbance, FTIR, XRD, Functional Group, Crystalline Nature.

Introduction:

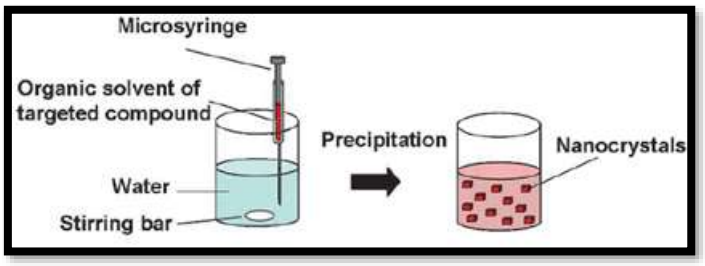
Nearly 40% of newly discovered medicinal moieties have limited clinical utility due to poor bioavailability, which is connected to their low solubility. Alternative formulation solutions for such pharmaceuticals must be studied due to their limited solubility, high log p value, high melting point, and high dose. The formulation of pharmaceuticals as "Nano-Crystals" is one such revolutionary strategy. The bulk of Nano-Crystals, which are generated using either "top-down" or "bottom-up" processes, are composed of drugs and surfactants/stabilizers. Nano-Crystals improve the therapeutic efficacy of pharmaceuticals by enhancing drug bioavailability, lowering dosage requirements, and promoting

sustained release. Nano-Crystals features such as particle size, saturation solubility, and dissolving velocity all have an impact on their enhanced performance and are required for this effect. Several advanced approaches have been developed to assess these attribute. (1, 2)

Technology Used in making Nano-Crystals:

Low-Energy Precipitation Method - It seems that the bottom-up reprecipitation method of creating Nano-Crystals is not widely used. Technically speaking, one perceived drawback of the strategy is that it does not work with a large variety of compounds. Through a methodical investigation of three different molecules—ibuprofen, glyburude, and artemsinin—(2,3) we have demonstrated that basic anti-solvent precipitation may be used to create stable Nano-Crystals of uniform size for each of the three medications. Finding suitable stabilizers, which rely on the drug molecule selection, seems to be the technical difficulty. The impacts of temperature, stirring rate, infusion rate, and process factors were also examined. The local supersaturation that occurs during the antisolvent precipitation process is thought to be influenced by each of these factors. We tried, but failed, to justify the selection of optimal stabilizers in terms of chemical interactions between the stabilizer molecules and crystal surfaces. (2, 3, 4)

FIG.1 LOW ENERGY PERCIPITATE METHOD OF MAKING NANO-CRYSTALS



Clarithromycin:

Clarithromycin belongs to BCS Class 2, which is Low solubility & high permeability. It belongs to the class of macrolide antibiotic, chemically related to erythromycin and azithromycin used to treat various bacterial infections. Its IUPAC name is 6- O- Methylerythromycin A, it is a semi-synthetic derivative of erythromycin, and the major difference is the methylation of hydroxyl group at position 6 of lactone ring, which improves its stability in acidic condition. Clarithromycin works by inhibiting bacterial proteins synthesis; binds to 50s ribosomal subunit of bacteria preventing translocation of peptides. Halts bacterial growth and replication. Primarily bacteriostatic (inhibits bacterial growth) but at higher concentration against certain bacteria it can be bactericidal (kills bacteria)

Pharmacokinetics: Absorption – Clarithromycin is well absorbed orally. It is acid-stable which allows it to be administration without enteric coating unlike Erythromycin.

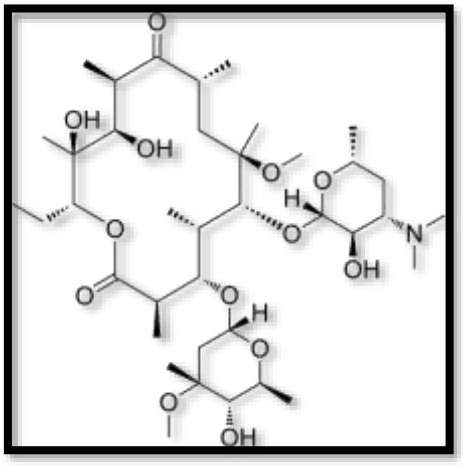
Bioavailability- Approximately 50-55% after oral administration.

Metabolism- It undergoes 1st pass metabolism in liver to form its active metabolite, 14- Hydroxyl Clarithromycin which has similar or stronger activity against certain pathogens.

Half-life- Approximately 3-4 hours for clarithromycin but 14 Hydroxy Clarithromycin has longer half-life 5-7 hours.

Excretion- Primarily excreted through urine (20 – 30 %), feces (4%) (5)

FIG.2 CLARITHROMYCIN



Materials:

Clarithromycin (Amepurva Forum Nirant Institute of Pharmacy), HPMC (Amepurva Forum Nirant institute of pharmacy), Acetone (Amepurva Forum Nirant institute of pharmacy).

1) Clarithromycin
2) Acetone
3) HPMC
4) Distilled Water

1) Clarithromycin:

Mol.Formula: $C_{38}H_{69}NO_{13}$

Mol. Weight: 748.0

Clarithromycin is: (3R,4S,5S,6R,7R,9R,11R,12R,13S,14R)-4-[(2,6-Dideoxy-3-C-methyl-3-O-methyl-α-L-ribo-hexopyranosyl)oxy]-14-ethyl-12,13-dihydroxy-7-methoxy-3,5,7,9,11,13-hexamethyl-6-[[3,4,6-trideoxy-3-(dimethylamino)-β-D-xylo-hexopyranosyl]oxy]oxacyclotetradecane-2,10-dione (6-O-methylerythromycin A) Clarithromycin contains not less than 96.0 per cent and not more than 102.0 per cent of $C_{38}H_{69}NO_{13}$, calculated on the anhydrous basis.(6)

Category: Antibacterial.

Storage: Store protected from moisture.

Description. White or almost white crystalline powder (6)

Clarithromycin is an acid-stable macrolide antibiotic that is taken orally and shares structural similarities with erythromycin. It inhibits a variety of Gram-positive and Gram-negative organisms, atypical pathogens, and certain anaerobes. Its broad spectrum of antimicrobial activity is comparable to that of erythromycin. Clarithromycin has been shown to be clinically effective in treating infections of the skin and soft tissues, the lower and upper respiratory tracts (including those caused by atypical bacteria), and pediatric patients. Class II BCS antibiotics like clarithromycin have a lot of solubility problems. To increase the solubility of the medication. Clarithromycin is practically insoluble in water, soluble in acetone and dichloromethane, slightly soluble in ethanol. (7)

2) Acetone:

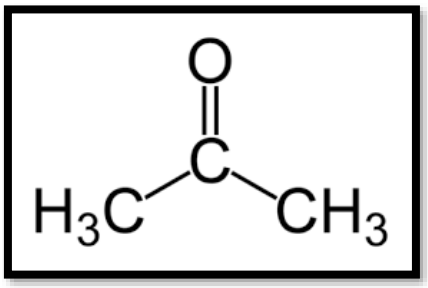
Acetone: 2-Propanone; $(CH_3)_2CO$ = 58.08

Analytical reagent grade of commerce.

Clear, colorless, volatile liquid; odor, characteristic;

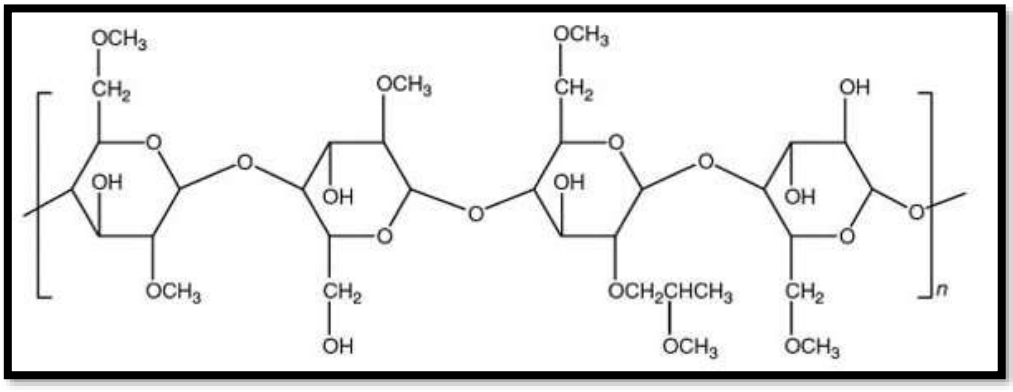
Flammable; b.p. about 56°; wt. per ml, about 0.79 g.
Acetone, Dry. Acetone which contains not more than 0.3 percent w/w of water. (6)
Acetone, C3H6O, is a transparent, colorless, volatile liquid with an aromatic smell. It is also referred to as dimethyl form-aldehyde, dimethyl ketone, ketone propane, methyl ketone, 2-propanone, and pyroacetic ether. Because it can dissolve a wide range of compounds, acetone is a commonly used organic solvent. It is well-known for having a potent solvating capacity and dissolving both inorganic and organic substances. Paints, nail polish removers, and medicinal medicines are just a few of the industrial and consumer items that employ acetone as a solvent. (7)

FIG.3 ACETONE



3) HPMC: (Hydroxy Propyl Methyl Cellulose)
Cellulose, 2-Hydroxypropylmethyl Ether; Hypromellose
Hydroxy Propyl Methyl Cellulose is a cellulose having some of the hydroxyl groups in the form of the methyl ether and some in the form of the 2-hydroxypropyl ether. The various grades commercially available are distinguished by a number indicative of the apparent viscosity in millipascal seconds of a 2 per cent w/v solution measured at 20°.
Category: Treatment of tear deficiency; pharmaceutical aid (tablet excipient; suspending agent).
Description: A white or yellowish white, fibrous or granular powder; almost odorless; hygroscopic after drying. Hydroxy Propyl Methyl Cellulose. Practically insoluble in hot Water, in acetone, in ethanol, in ether and in toluene. It swells in water forming an opalescent, viscous colloidal solution. (6)

FIG.4 HYDROXY PROPYL METHYL CELLULOSE



3) Distilled water:
Vaporizing water and condensing in a pure state. It is mainly used for preparing reagents but may also be required for other laboratory operations such as rinsing an analyte, transferring a test material as a slurry,

as a calibration standard or analytical blank and for cleaning of apparatus. Unless specifically indicated, water meeting the requirements for Purified Water derived by other means of purification could be equally suitable where the use of distilled water is recommended.(6)

Method:

Nano-Crystals is prepared by using Precipitation method. This method contain mainly three step. First step is preparation of organic phase, second is preparation of aqueous phase, Third phase is drop wise addition of organic phase into aqueous Phase. To prepare the first phase, Drug is dissolved in suitable organic solvent (in which drug is very soluble). And that Solvent must be water miscible. Then add the solvent in it & properly dissolve the drug in it. Afterwards add required Excipients. In second phase water is mixed with stabilizers & properly mixed. The third phase have drop-wise addition of organic phase into aqueous phase , drug with solvent is drop-wise added Into water & stabilizer mixture with the continuous stirring on the magnetic stirrer at 1500 rpm, like that way Nano-Crystals Was prepared. To obtain Nano-Crystals form evaporate the organic solvent at some extend using magnetic stirrer at 350rpm then centrifuge ,from which layers get separate .Remove the supernant & get the sample . Kept the sample 24hr in Desiccator & get dried Nano-Crystals. (4)

Instrumentation:

Magnetic Stirrer:

A magnetic stirrer is a piece of equipment used in laboratories to mix or stir fluids in order to create a homogenous mixture. The only feature of the current magnetic stirrers is an analog control knob that regulates the mixing speed. Since science and technology have advanced, a magnetic stirrer based on a microcontroller was created for this investigation. Errors in reading the speed and time of mixing the samples are to be expected because this tool is created with digital speed and timing settings. A homogeneous mixture of two or more compounds that dissolve one another and make it impossible to physically differentiate the constituent substances is called a solution. A solute and a solvent make up the solution. There are two types of solutions, such as electrolyte solutions and non-electrolyte solutions, depending on the electrical conductivity (ionization power). A solution that has the ability to conduct electricity is called an electrolyte solution. There are two types of electrolytes in this solution: strong and weak. (7, 8)

FIG 5: MINI MAGNETIC STIRRER

Centrifuge:

The first commercial centrifuge was introduced in 1864, marking the beginning of the lengthy history of centrifuges. A dairy centrifuge was created by Antonin Prandtl to separate milk from cream. Friedrich Miescher was the first to isolate a cell organelle in a laboratory setting using a centrifuge five years later, in 1869. In many labs, centrifuges are used to separate liquids, gasses, or fluids according to their densities. (9)

FIG.6 NANO-CRYSTALS KEPT IN THE CENTRIFUGE

Working of Centrifuge:

Particles suspended in a liquid can be separated using a centrifuge based on their size, density, medium viscosity, and rotor speed. Particles that are less dense than the solvent will float to the top of a solution due to gravity, whereas those that are denser would sink. Centrifugation separates particles in a solution by utilizing even the smallest variations in density. A centrifugal force is created as the rotor revolves around a central axis, pushing particles away from the rotational axis. The particles will sediment if the centrifugal force is greater than the buoyant forces of the liquid media and the frictional force the particle creates. (9)

How to balance Centrifuge tube:

Three tubes can be balanced in two different ways. Putting three sample tubes adjacent to one another and making three balancing tubes to be placed exactly across from the sample tubes is the first alternative. An alternative would be to uniformly distribute three sample tubes around the rotor. One balancing tube should be made, and two sets of three tubes should be positioned across from one another to balance five tubes. Make a single balancing tube and position two sets of four tubes across from one another to balance seven tubes.(9)

Table of Ingredients:

TABLE 1: TABLE OF INGREDIENTS FOR THE FORMULATION OF NANOCRYTALS

Sr.no	Ingredients	Role	F1
1.	Clarithromycin	API	1.5mg
2.	HPMC	Stabilizer	0.7mg
3.	Acetone	Solvent	10ml
4.	Distilled water	Anti-solvent	20ml

Solid State Characterization: Nano-Crystals of Clarithromycin Characterization:

The solid state characterization of the Nano-Crystals can be done by using Powdered X-Ray Diffraction (P-XRD) and Fourier Transform Infrared Spectroscopy (FTIR). (12)

1.) UV Analysis of Clarithromycin:

UV spectroscopy: Solubility measurements were examined using UV absorbance measurements at 210 nm using a UV spectrophotometer.

Also to find out about the unknown structures of the organic groups. (15)

FIG 7: UV GRAPH OF CLARITHROMYCIN

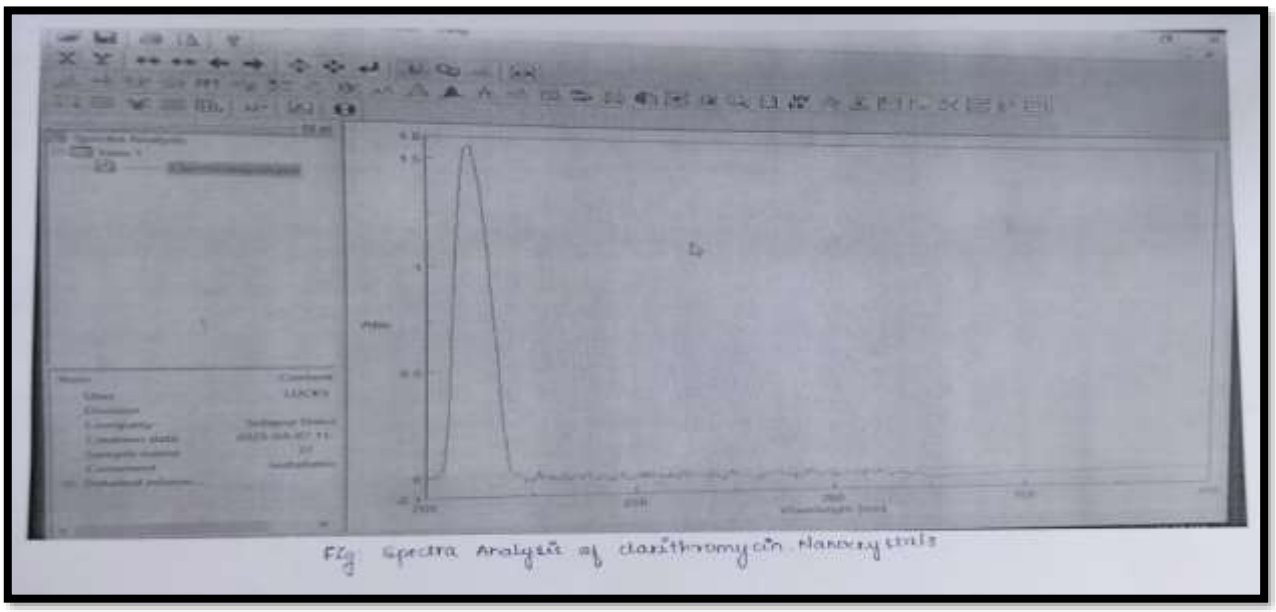


TABLE 2: UV INTERPRETATION OF CLARITHROMYCIN

Recorded Range (nm)	**Observed Peak (λmax)**	**Functional Group**	**Organic Group**	**Interpretation**
200–400	~210–220	-OH, C=O (lactone), -O-	Macrolide (macrocyclic	Strong absorption due to **n → σ*** transitions from lone pair electrons

			lactone), ether, alcohol	on **O/N atoms**; no extended conjugation is evident.
>230	None	—	—	No significant absorption in this region confirms **absence of conjugated π-systems** or **aromatic rings**.

The UV-Vis spectrum of Clarithromycin in acetone shows a strong absorption peak at **~210–220 nm**, attributed to **n → σ*** electronic transitions from lone pair electrons on oxygen or nitrogen atoms within the macrolide ring. The absence of any significant absorption above 230 nm confirms the lack of extended conjugation or aromatic chromophores in the molecule. The use of acetone as a solvent, while generally appropriate, may contribute minor baseline noise near the far-UV range.

Clarithromycin in Acetone

- **λmax ≈ 210–220 nm**
- Transition: Likely **n → σ*** (from non-bonding electrons on O/N)
- **No conjugation or aromaticity**, hence no peaks in the 250–400 nm region
- Acetone is suitable but may slightly affect clarity near the far-UV range (10, 15)

2.) FTIR Analysis of Clarithromycin:

FTIR: FTIR can be used to investigate the molecular interactions between the medicine and excipients in nanocrystal compositions. When two components interact, the FTIR detects the changes in the molecules' vibrational frequencies to determine the interactions. (12, 14)

FIG 8: FTIR GRAPH OF CLARITHROMYCIN

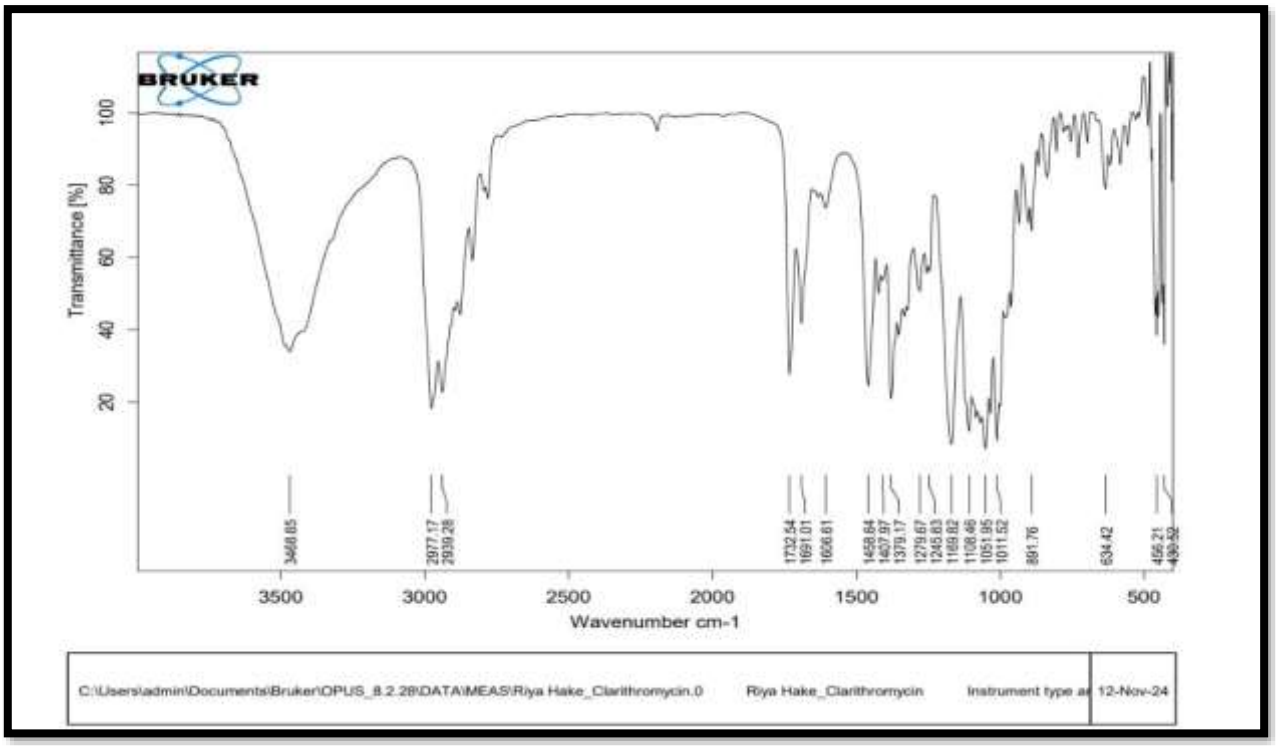


TABLE 3: FTIR INTERPRETATION OF CLARITHROMYCIN

Wavenumber (cm^{-1})	Functional Group	Type of Vibration	Interpretation
3448.65	O–H (alcohol/phenol)	Stretching (broad)	Indicates presence of **hydroxyl groups**, typical in macrolides like Clarithromycin.
2977.17, 2939.28	C–H (alkane)	Asymmetric/symmetric stretching	Aliphatic **–CH_3/–CH_2–** groups, consistent with macrolide ring.
1723.54	C=O (ester/lactone)	Stretching	Sharp **carbonyl stretch** – confirms **lactone ring** (macrolide core).
1631.01, 1606.61	C=C or C=O (conjugated or amide)	Stretching	Possibly minor **amide/olefinic stretches**; consistent with possible side chains.
1456.84 – 1379.77	C–H bending (CH_3, CH_2)	Bending	**Alkyl group deformations**, confirming aliphatic character.
1265.63, 1206.48, 1160.23	C–O (ether/ester)	Stretching	Indicates **ether or ester** linkages – multiple C–O stretches confirm macrolide ring and sugar moiety.
1011.52 – 891.76	C–O or C–C	Various bending/stretching	Fingerprint region – complex vibrations of **macrocyclic** and sugar structures.
634.42, 456.21, 436.52	Miscellaneous bends	Bending	Out-of-plane deformations or ring puckering modes – usually ignored in functional analysis.

Functional Group Summary:

TABLE 4: SUMMARY OF FUCTIONAL GROUPS IN CLARITHROMYCIN

Functional Group	Confirmed?	Evidence
Hydroxyl (–OH)	Yes	Broad band at 3448 cm^{-1}
Ester/Lactone (C=O)	Yes	Strong peak at 1723 cm^{-1}
Ether (C–O–C)	Yes	Multiple bands in 1200–1000 cm^{-1}
Alkane (C–H)	yes	Peaks at 2977, 2939 cm^{-1}
Amide/C=C	Possibly	Weak bands around 1600 cm^{-1}

3.) XRD of Clarithromycin:

P-XRD: Another method for assessing the nanocrystals' crystallinity is P-XRD. The partial or total amorphization of the drug in nanocrystals as a result of homogenization is indicated by the strong peaks

with decreased intensity or, in certain situations, by the absence of peaks in the nanocrystals' X-ray diffractogram.(12, 13)

FIG 9: XRD GRAPH OF CLARITHROMYCIN

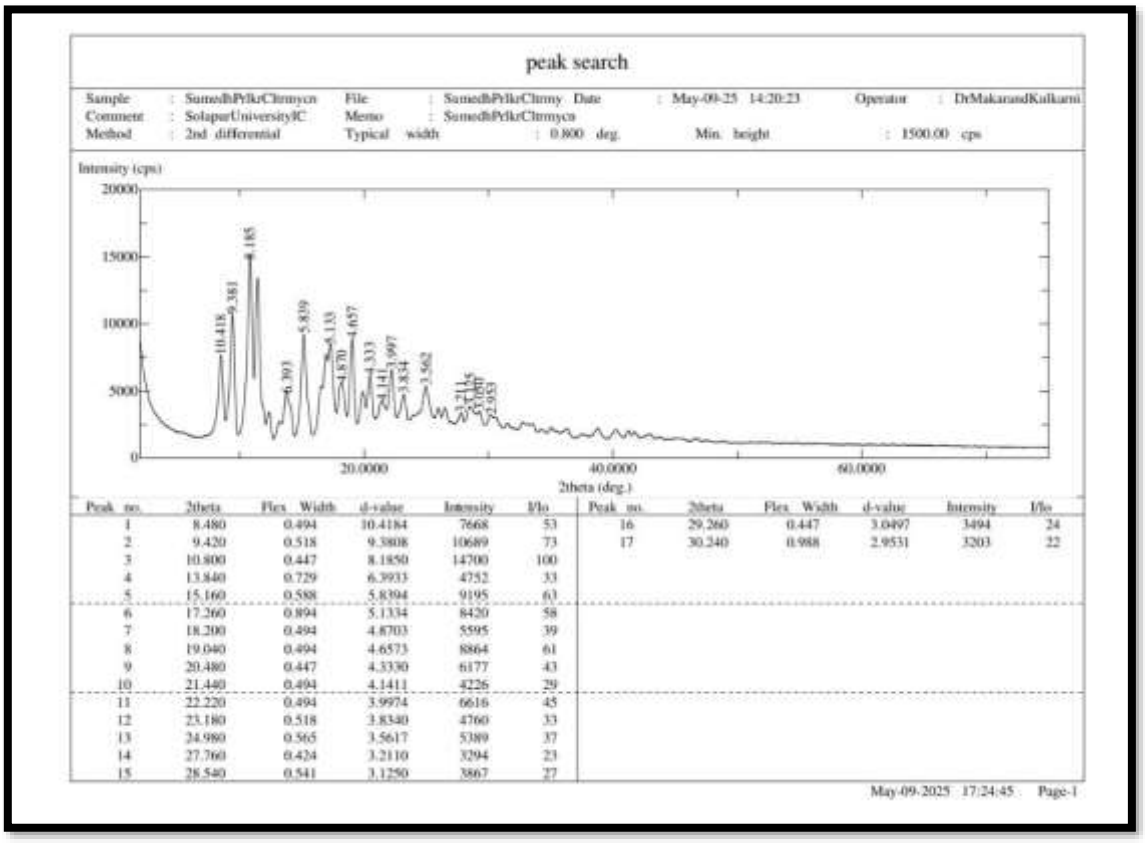


TABLE 5: XRD ANALYSIS OF CLARITHROMYCIN

Peak No.	2θ (°)	d-spacing (Å)	Intensity (cps)	Crystalline Nature
1	8.480	10.4184	7668	Highly crystalline
2	9.420	9.3808	10689	Crystalline
3	10.800	8.1850	14700	Strongest peak (100%)
4	13.840	6.3987	4752	Crystalline
5	15.160	5.8394	9195	Crystalline
17	30.240	2.9531	3203	Low intensity peak

- Multiple **sharp peaks**: Indicates **high crystallinity**, which is important for drug stability and solubility.
- Strongest **peak at 10.8° (2θ)** with 100% relative intensity shows the most dominant lattice plane.
- The presence of peaks between **8° and 30° (2θ)** range is characteristic of the **semi-crystalline or crystalline organic compounds**, typical for **macrolides**.
- d-**spacing values** (from ~10.4 to ~2.95 Å) help characterize the lattice structure. These values can be matched against reference JCPDS files for confirmation (if required). (10, 11, 15)

Final summarised analysis and interpretation combined UV, FTIR and XRD:

TABLE 6: SUMMARIED ANALYSIS AND INTERPRETATION OF CLARITHROMYCIN (UV, FTIR, XRD)

Technique	Recorded Range	Observed Peaks	Functional/Structural Group	Interpretation
UV-Vis	200–400 nm	λmax ≈ **210 nm**	π → π* (C=O, conjugation)	Electronic transition in **lactone ring**, analyzed with acetone as solvent. Use Beer-Lambert law for quantification.
FTIR	4000–400 cm^{-1}	3448, 2977, 1723, 1456, 1265, 1011 cm^{-1}	–OH, C–H, C=O, C–O	Confirmed presence of **hydroxyl**, **ester/lactone**, **ether** groups – macrolide signature.
XRD	5°–60° 2θ	Major peaks at 10.8°, 9.42°, 15.16°, etc.	Crystalline lattice planes	Indicates **crystalline nature** of Clarithromycin. Supports purity and structural integrity.

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