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FORMULATION & EVALUATION OF IMIPENEM LOADED CALCIUM CARBONATE NANOCRYSTAL HYDROGEL

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ABSTRACT

The objective of this study was basically to increase the solubility as well as the permeability of the low water soluble & low permeable drug Imipenem. This is done by converting the drug into nanocrystal formulation. This type of formulation comes under the category of having many dimensions of size 100 nm or less than it because nanocrystals have the ability to alter the physical & chemical properties of the drug. The method of preparation of calcium carbonate nanocrystals used was precipitation method and they synthesized by scanning electron microscopy. Drug release study of final product done by using HPLC method and imipenem calcium carbonate nanocrystals finally loaded in Acrylamide hydrogel to make it unique formulation respectively.

KEYWORDS: Imipenem, Calcium Carbonate, Nanocrystals, Hydrogel.

INTRODUCTION

Nanocrystals are small particles, with diameters in the nanometre range (typically below 1μm) which are more soluble than the amorphous substances. Transfer of materials into the nanometre dimension changes their physical properties which are used in pharmaceutics to develop a new innovative formulation principle for poorly soluble drugs are the drug nanocrystals. There has been a considerable research interest in the area of nanocrystal drug delivery. If the drug has low solubility or low permeability its bioavailability is also low. To improve the bioavailability the drug solubility or drug permeability should be enhanced which can be done by reducing the drug size into the nanometre range. Thus, the physical and chemical approach to alter the pharmacokinetic and pharmacodynamics properties of active pharmaceutical ingredients (API) is the particulate drug delivery systems (nanometre range

particles). In addition, for drug delivery not only engineered particles may be used as carrier, but also the drug itself may be formulated at a nanoscale, and then function as its own "carrier". Nanocrystals have excellent tissue penetration and persistence.

MATERIALS AND METHODS

Imipenem was purchased from CIPLA LTD. Mumbai, Cockle shells was obtained as a gift by Temple, Chitosan & other ingredients purchased from Apex Lifesciences Pvt Ltd. All ingredients used were of analytical grades respectively.

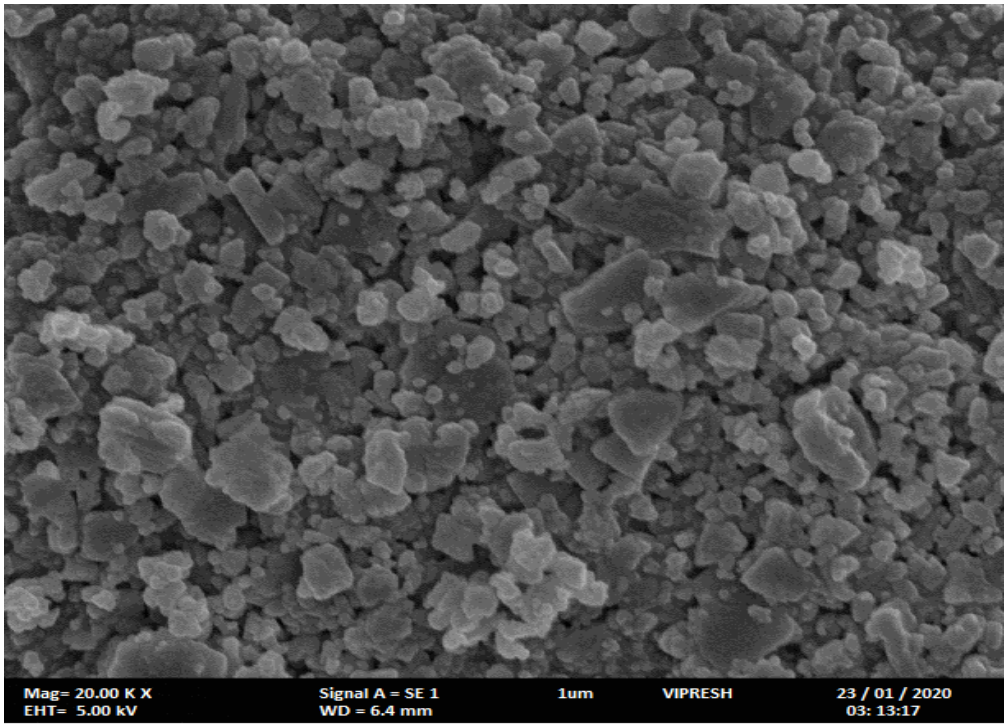
Preparation of Calcium Carbonate Nanoparticle

The cockle shells were grinded in a heavy grinder to obtain a very fine powder of the same which comprises of nanoparticles of a humungous size range. The powder was then sieved in a mechanical sieve that gave sizes of nanoparticles ranging from 43 – 150 microns. Nanoparticles of the size below 50 were taken. This powder was stored in a contamination free environment.

Fig. 1: cockle shells grinded into powder and collected.

Preparation of Active Nanoparticle

10gm of cockle shell powder was weighed in which 15 gm (35.3 ml) of concentrated HCL added slowly in small quantity at a time. After that 0.15 gm of chitosan (dissolved in 30% acetic acid) added in calcium chloride solution & mix it properly. Then this mixture blended with 7gm of Sodium carbonate slowly and slightly heated it to complete the entire reaction. Kept overnight this mixture for incubation process. Filtered out the precipitate and washed it with distilled water several time to remove salt and then dried heating process used to burn the chitosan. Finally desired nanoparticles of calcium carbonate obtained &stored in air tight container.



SEM Image of Calcium Carbonate

Preparation of PBS Buffer

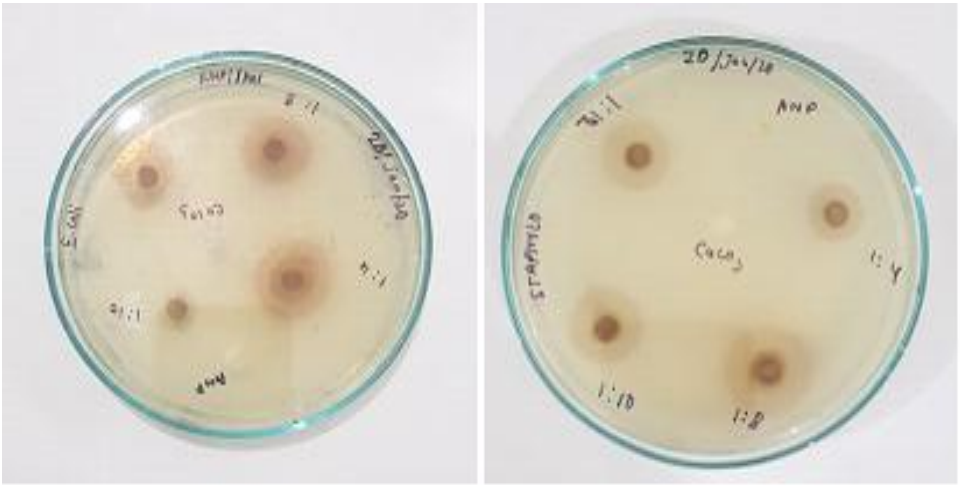
First of all absolutely clean flasks and beakers washed with dilute HCL and then with distilled water. After that measure a volume of 800 ml of distilled water of HPLC grade and transfer into a 5000 ml beaker. Add a magnetic stir bar to the beaker and place the flask on a magnetic stir plate. Adjust the speed of the magnetic stir bar so that oxygen is not introduced into the solution while it is rapidly mixed. Then transfer to the beaker: 8 g of sodium chloride, 0.2 g of potassium chloride, 1.44 g of Na2HPO4 0.25 g of KH2PO4. Allow the solutes to dissolve for 3 to 5 min. Ensure that there are no remaining particles of undissolved salts in the solution before adjusting the pH respectively. If particles are present, continue the stirring vigorously. Reduce the speed of the magnetic stir bar so that the solution will gently mixed. Ensure that the pH meter has been properly calibrated and rinse the pH probe with HPLC grade distilled water. Remove the excess water from the probe tip (without touching the probe tip) with a clean paper towel. Place the pH probe into the solution. Slowly add 1 M HCL dropwise with a pipette and allow the HCL to fully dissolve into the solution. Measure the pH with the pH metre. Repeat the Steps until the pH of the solution will comes at 7.4. Pour the solution into a fresh graduated cylinder & adjust the final volume to 1 litre with distilled water. Store the PBS solution at room temperature. The PBS Solution is sterile; when using the PBS Solution, ensure that sterile techniques are employed respectively.

Hydrogel Formation

Acrylamide solution was prepared in a 100ml flask equipped with mechanical stirrer. Acrylamide was dissolved in degassed distilled water. In general, Acrylamide (3.0 g, 42mmol) was dissolved in 30.0 ml of distilled degassed water. MBA (methylene bi acrylamide) as a crosslinker (0.050 g in 2 ml water, 0.32mmol) was added to the Acrylamide solution and the mixture was continuously stirred under nitrogen gas. 1:8 of ANP/IMP were added to the reaction mixture and allowed to stir for 10 min. Then APS (0.05 g dissolved in 2 ml water, 0.22mmol) and SMBS (Sodium metabisulphite) (0.05 g dissolved in 2 ml water, 0.26mmol) as a redox initiator were added to the solution and stirred for 30 min. Obtained hydrogels in bulk state were cut in similar pieces (~2×2×2 mm).

ELISA assay for antibiotic activity and drug release by ANP

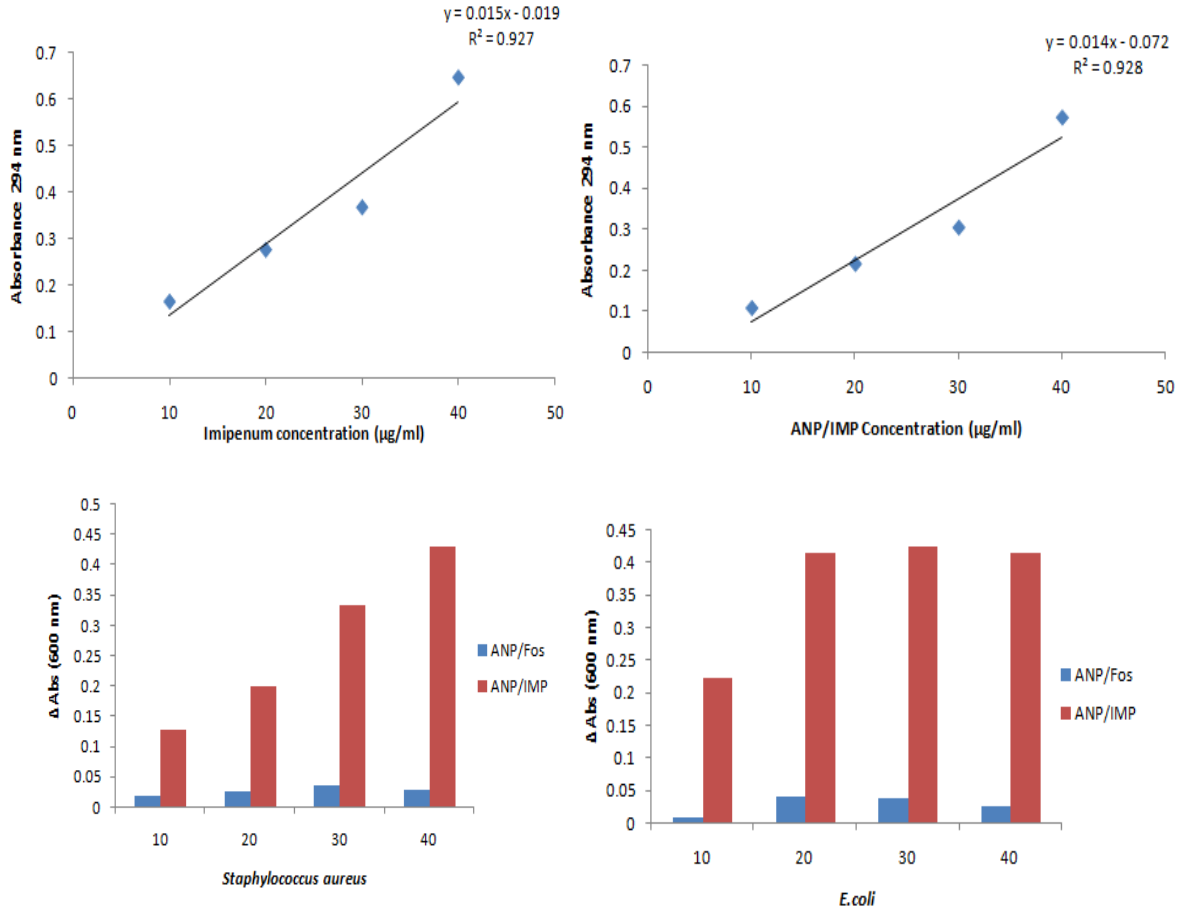
Drugs that are once entrapped take different amount of time to get release with respect to different concentrations. Dialysis technique was used for releasing the drug. Consideration of the interactions of drugs and dialysis must include an understanding of the mechanism of transport during dialysis, i.e., diffusion. Different dilutions of ANP/imipenem were checked for their antibiotic activity on Staphylococcus and E.coli.



Briefly 100 µl of bacterial culture were spread over the LB agar different dilution of imipenem were checked for MIC against Staphylococcus and E.coli.

Standard of Imipenem (µg/ml)	Absorbance 294 nm	ANP/IMP
10	0.165	0.107
20	0.276	0.215
30	0.367	0.304
40	0.645	0.573

ANP/IMP (600nm) Staphylococcus aureus	Absorbance	ANP/IMP (600nm) E.coli	Absorbance
0.945	0	0.841	0
0.817	0.128	0.619	0.222
0.745	0.2	0.428	0.413
0.612	0.333	0.417	0.424
0.514	0.431	0.428	0.413

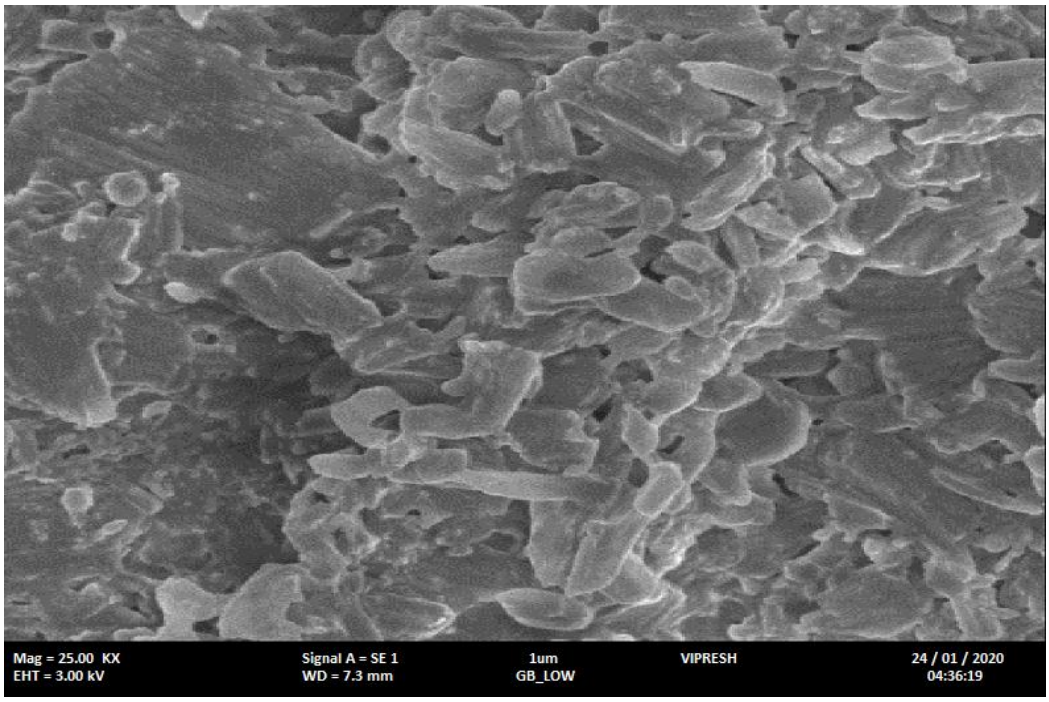


Loading Imipenem on ANP

The loading of Imipenem on ANP was performed according to the method of Chakraborty et al. with slight modifications. Four formulations of IMNP with different drug to nanoparticles (Imipenem:ANP) ratios were prepared. Accordingly 10, 40, 80 and 120 mg ANP was mixed in 1 mL phosphate-buffered saline (PBS) (pH 7.4) and sonicated for 5 minutes at 100 W with a resting interval of 15 seconds. A total of 10 mg Imipenem was dissolved into each ANP suspension to achieve Imipenem: ANP ratios of 1:1 (IMNP1), 1:4 (IMNP2), 1:8 (IMNP3), and 1:12 (IMNP4). The ANP: Imipenem ratios of 1:1 (IMNP5), 1:4 (IMNP6), 1:8 (IMNP7) and 1:12 were prepared using the same procedure by dissolving 10, 40, and 80 and 120 mg of Imipenem into 10 mg ANP suspension, respectively. All the suspensions were agitated at

200±1 rpm for 48 hours at room temperature using an orbital shaker. In total, 10–120 mg of naked ANP was prepared concurrently using a similar procedure and treated as negative control.

ANP (mg)	Imipenem (mg)	Imipenem:ANP	%Imipenem	ANP (mg)	Imipenem (mg)	ANP:Imipenem	%Imipenem
10	10	1:1	1	10	10	1:1	1
40	10	1:4	1	10	40	1:4	4
80	10	1:8	1	10	80	1:8	8
120	10	1:12	1	10	120	1:12	12



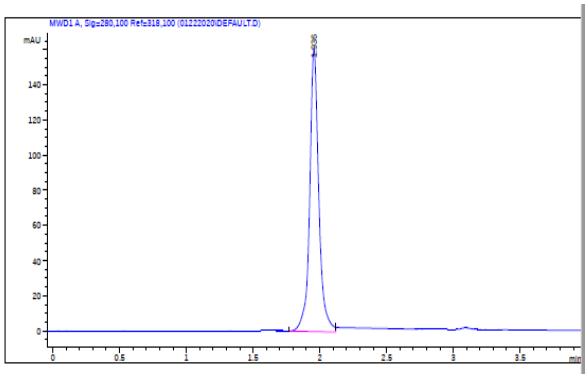
SEM Image of Imipenem Loaded Calcium Carbonate Nanocrystals.

CHARACTERIZATION AND QUANTIFICATION

Drug release was estimated by HPLC. Agilent 1100 series with Agilent G1322A degasser, Agilent G1311A Quat pump, Agilent G1329A ALS, Agilent g1330b alstherm Agilent G1316A Colcom and Agilent G1315B DAD (Santa Clara, CA, USA) using empower 2 (database version 6.10.00.00) acquisition software were used in analytical method validation. Analytical balances mettler XP205 (Columbus, OH, USA) and Sartorius genius (Goettingen, Germany), Microbalance Sartorius ME5 (goettingen, Germany) were used throughout the study for weighing. Millipore milli-Q gradient A10 (Billerica, MA, USA), was used for production of HPLC grade water. Millipore millexnylon filters of 0.45 micrometre porosity (Billerica, MA, USA) were used to filter blank, standard and sample preparations. Low temperature incubator (Model 815) used for storage of reconstituted Imipenem. A mixture of

methanol, propan-2-ol and water in a ratio of 250:100:50 respectively was used as mobile phase as well as diluent. The analysis was performed using a chromatograph equipped with zorbax extend C18, 250 mm x 4.6 mm, 5 micrometre. HPLC column was thermostat at 30 °C temperature. Ultraviolet detection was done at 225 nm. An injection volume of 100 µL. Sample tray temperature was kept ambient. Diluent was used for blank analysis. To prepare mobile phase and diluent, HPLC grade methanol and propan-2-ol were procured from Merck (Mumbai, India) and HPLC grade water was produced from milli -Q gradient A10 water purification system. Preparation of standard solution About 10 mg of Imipenem standard was accurately weighed into a 10 mL dried volumetric flask.

CHROMATOGRAPHS OF STANDARD



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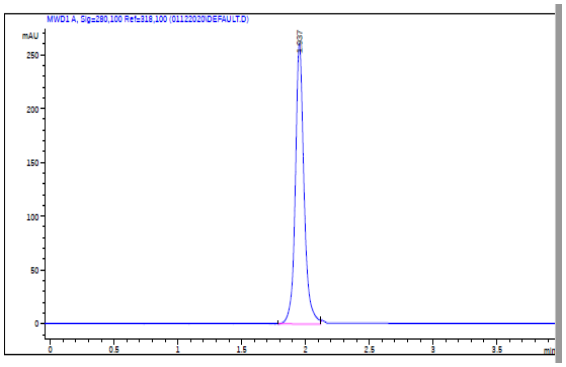

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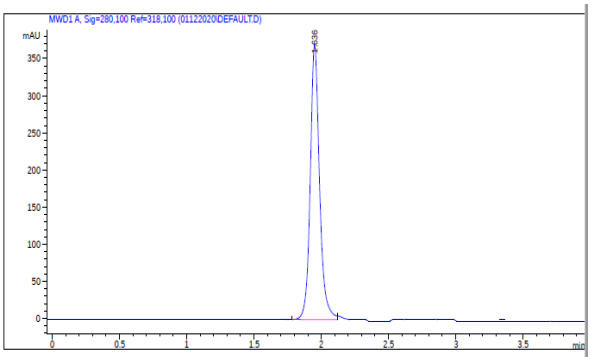

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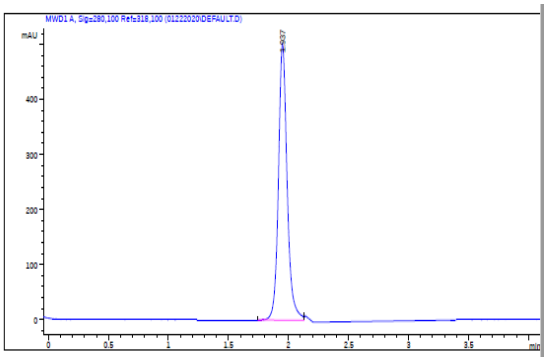
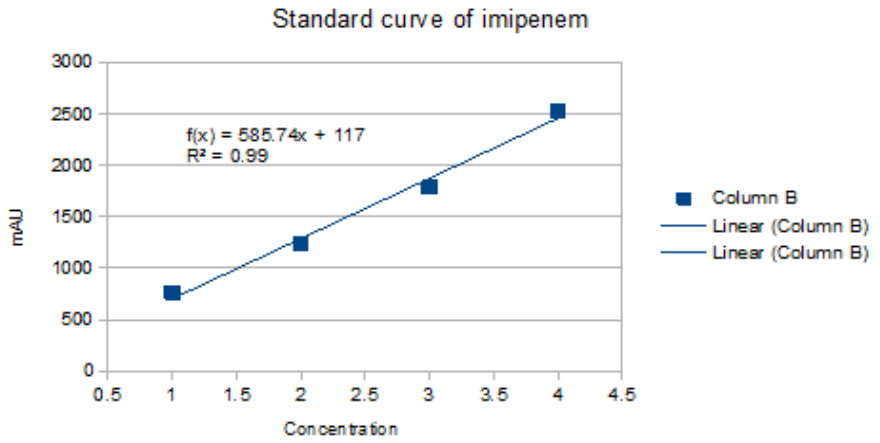
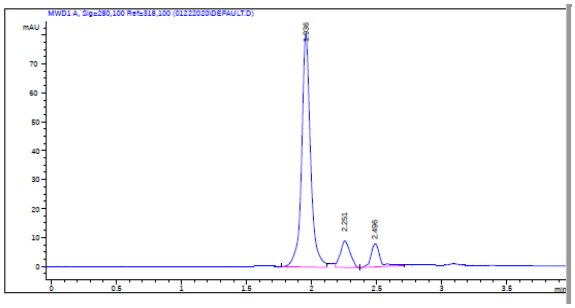


STANDARD 4

Concentration ug/ml	Area mAU
1	764.69684
2	1240.23999
3	1784.85461
4	2535.62793



CHROMATOGRAPHS OF SAMPLE



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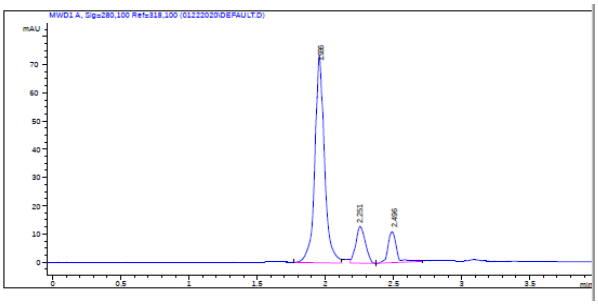

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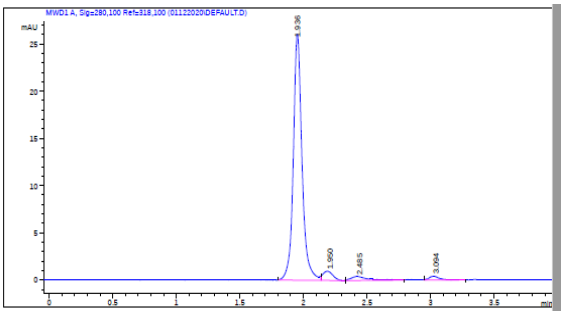

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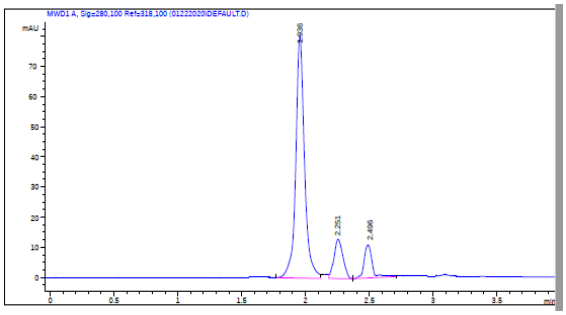

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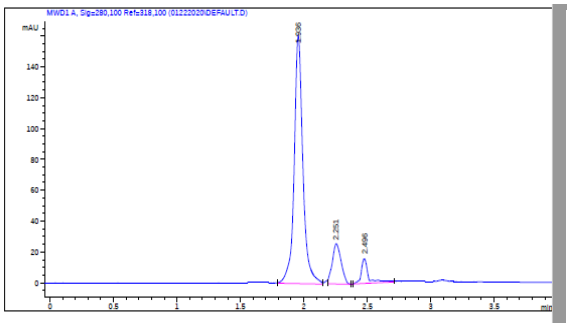

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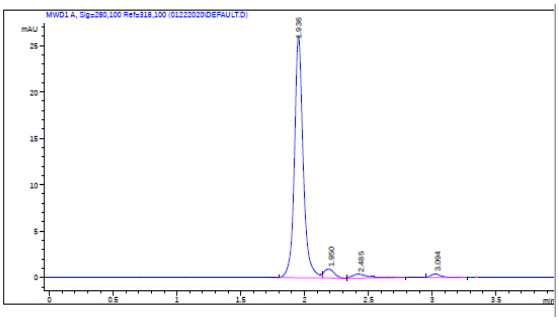

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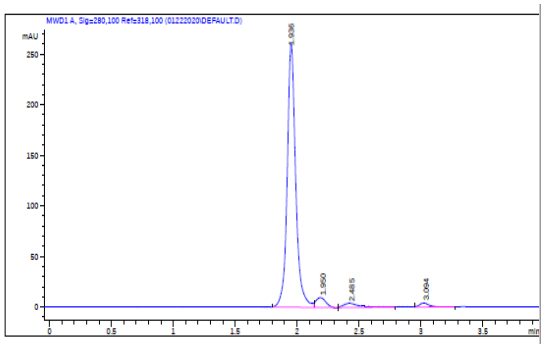

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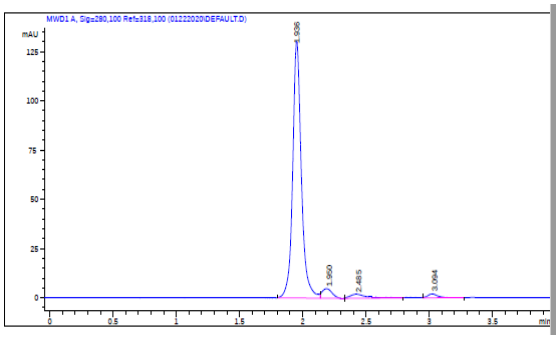

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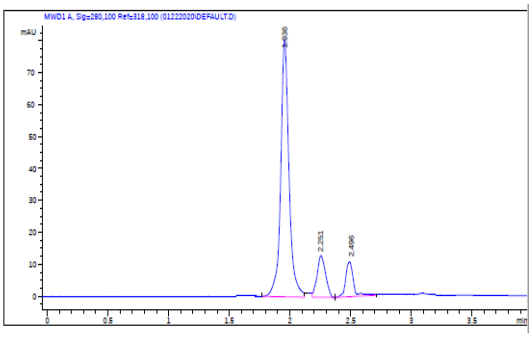

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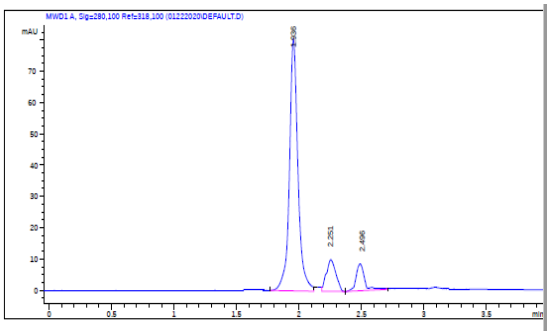

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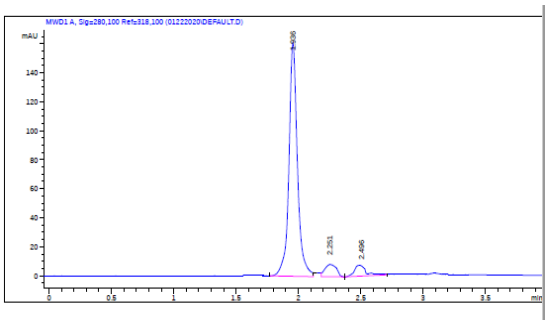

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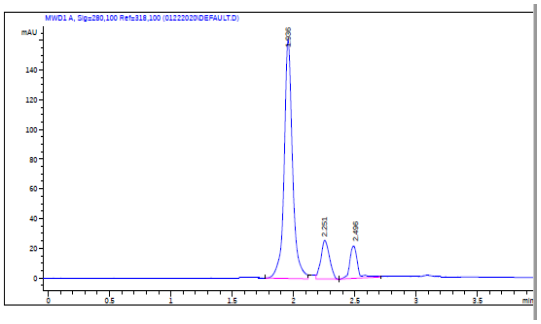

SAMPLE 12

RESULTS

Sample	Area (m A U)	Concentration
Day 1-1	382.34642	0.454
Day 1-2	361.10025	0.417
Day 1-3	124.02399	0.012
Day 1-4	382.34642	0.454
Day 2-1	764.69684	1.108
Day 2-2	124.62399	0.013
Day 2-3	1240.23999	1.921
Day 2-4	620.24	0.861
Day 3-1	382.34642	0.454
Day 3-2	382.34622	0.454
Day 3-3	764.69684	1.108
Day 3-4	764.69684	1.108

So the concentration of the drug increases with the time & shown increased area of antibacterial activity respectively.

CONCLUSION

Through this study we conclude a novel dosage form of the drug Nanocrystal Hydrogel. Hence the solubility as well as permeability of the imipenem nanocrystal enhanced as compared to the pure drug of Imipenem respectively.

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