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Synthesis and Characterization of Nanoparticulated Rifampicin

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ABSTRACT

Rifampicin is a naturally made, non-peptide antibiotic. It is bactericidal, killing by disabling the protein expression system universally conserved by all bacteria. [1] Specifically, it inhibits the RNA polymerase protein, which is responsible for binding to a strand of DNA as a template and using it to construct a strand of mRNA. The reason rifampicin works so well is that it is a rigid molecule, and sits tightly in the pocket where it binds, allowing the bonds to be very strong. However, this also means if an amino acid with the edge of the channel with a small side chain is replaced by an amino acid with a large side chain, rifampicin may not be able to bind, simply because it cannot fit in the space. Rifampicin is mainly useful in the treatment of tuberculosis and meningococcal infections.[2, 3,4,5,6]

Coming to the pharmacokinetics of the drug, rifampicin is easily absorbed from the gastro intestinal tract. After about six hours almost the entire drug is deacetylated by esterases even in this deacytylated form rifampicin is a potent antibiotic. However, it can no longer be absorbed by the intestines and it is subsequently eliminated from the body. About 7% of the administered drug will be excreted unchanged through the urine, and urinary elimination accounts for about 30% of the dose of the drug that is excreted.[4, 5,6]

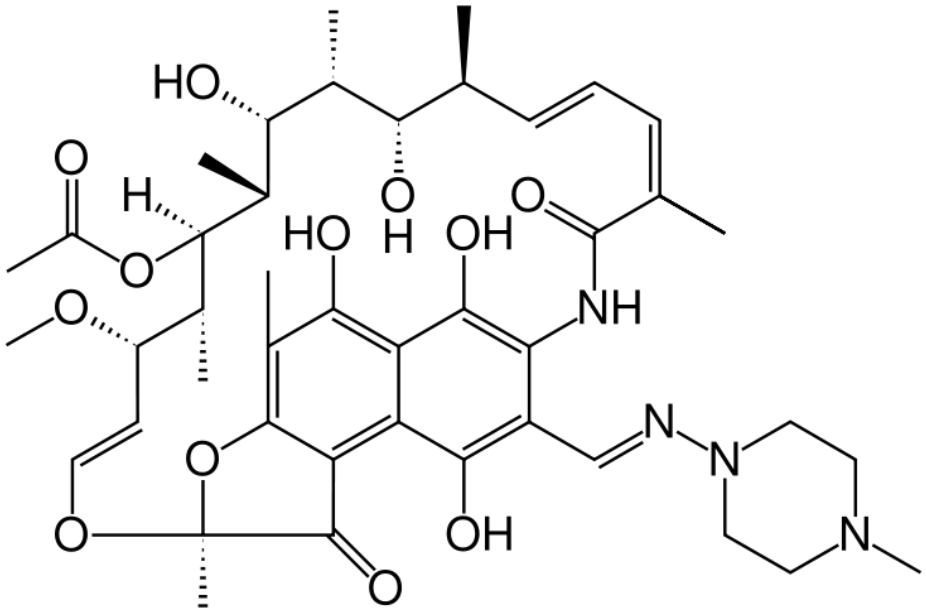
About 60% - 65% is excreted through feces. As a result of this, the drug dose has to be increased or the drug must be given at regular intervals to maintain the minimum effective concentration.

Rifampicin shows polymorphism and therefore, it is necessary to select a suitable crystal form to ensure optimum solubility and dissolution rates[4,5,7]. An increase in amorphous content significantly reduces the dissolution rate of the powders in water [8,9]. Because of the disadvantages both in physical properties and biologically rifampicin has to be synthesized in the form of nano particles which have low surface area and show good solubility and

dissolution properties. Nano particles can also be designed to allow sustained drug release from the matrix. This property of nano particles enables improvement of drug bio-availability and reduction of the dosing frequency and may resolve the problem of drug resistance[10], which is one of the major obstacles in the control of tuberculosis epidemics.

In this method, equal amounts of PEG200 and tween 60(polysorbate 60) and calculated amount of rifampicin was dissolved in that mixture. After that, the solution was stirred with the help of high speed magnetic stirrer[11]. After dissolving the compound completely, this solution was added to the water drop wise while the water was stirred with the magnetic stirrer. Then the nano particles are formed. If the nano particles are not formed after this procedure, homogenizer can be used. After the formation of nanoparticles, characterization using scanning electron microscope is done to confirm the surface properties of the nanoparticle.

INTRODUCTION



[12]

Rifampicin is a very popular anti- tubercular drug, effective against Mycobacterium tuberculosis [9]. Because of its high bactericidal action, Rifampicin forms a backbone in short-course chemotherapy for various forms of TB along with other antibiotics like Isoniazid [9]. As an adjuvant, it is used in the treatment of leprosy. On the molecular level, Rifampicin interferes with the metabolism of the susceptible bacteria by blocking RNA synthesis [9]. This effect is the consequence of the tight binding of the drug to a single and highly specific binding site on the DNA-dependent RNA polymerase. The drug-enzyme complex strongly inhibits the initiation of RNA chain synthesis not affecting chain elongation [9].

The treatment of Tuberculosis involves chemotherapy which lasts from 12-18months [9]. Proper prescriptions and patient compliance almost always cure. The emergence of drug resistant bacteria is the serious concern about this disease [10]. The emergence of strains resistant to any of these drugs causes major concern. Multidrug-resistant TB (MDRTB), associated with high death rates of 50% to 80%, spans a relatively short time (4 to 16 weeks)

from diagnosis to death [10]. Next to the MDRTB, the resistance is attributed primarily to improper prescriptions or patient noncompliance [10]. For several months rifampicin must be administered daily, if not the drug resistance increases for tuberculosis and is quick too. So rifampicin must be used along with other antibiotics. The drug cannot readily inhibit the rifampicin resistant bacteria as they produce RNA – polymerase with different beta subunits. Rifampicin inhibits DNA – dependent RNA polymerase synthesis by binding to its beta subunit in bacteria and therefore preventing transcription and translation to proteins. The lipophilic nature of rifampicin makes it to treat meningitis from tuberculosis. Messenger RNA synthesis is obstructed by rifampicin. Prokaryotic DNA – primed RNA polymerase mainly gram positive stains gets inhibited. The drug moves easily into the cell as the membrane of bacterial cell is mycolic acid made up of peptidoglycan.

Hepatotoxicity is the serious adverse effect when patients are treated with rifampicin, so they must undergo liver function tests. Breast feeding must be avoided as it may be excreted through milk. Gastro intestinal tract easily absorbs rifampicin and the bile hydrolyzes its ester functional group and is catalyzed by high pH enzymes called esterases. It is deacetylated after six hours and even in this form rifampicin is potent. Approximately 85% of Rifampicin is metabolized by the liver [9], so in order to maintain uniform levels of drug in the body and reduce the number of doses attempts encapsulation of this drug into nanoparticles promises a good dosage form.

EXPERIMENTS

Preparation of nanoparticles:

Oil/Water:

Attempt 1: Dissolve Rifampicin (0.03g) in the mixture of Polyethylene Glycol 200 (PEG, 150mL) and Tween 60 (0.1459g) using magnetic stirrer. After this take the UV readings using water as

blank and solution (0.5mL) with water (1.5mL) as sample. Then the solution was added to water (450mL) drop wise using magnetic stirrer. Then take the UV readings using water as blank and the solution as sample. There is no color change in the solution since there is unencapsulated rifampicin in the final solution. The concentration of rifampicin was too high.

Attempt 2: Prepare the solution of Tween 60 (0.1529g) and PEG 200 (81mL) using magnetic stirrer. This solution was added to the previously prepared solution (150 mL, attempt 1) drop wise using magnetic stirrer. There is no color change in the solution.

Attempt 3: Prepare the solution of Tween 60 (0.1528g) and PEG 200 (81 mL) using magnetic stirrer. This solution was added to previously prepared solution (100 mL, attempt 2) drop wise using magnetic stirrer. Now the solution is colorless. This solution is sonicated using ultrasonicator for 45 minutes. Carbopol ultrez 10NF polymer is added in order to stabilize the preparation. This solution is tested for the size of the nanoparticles on Transition Electron Microscope (TEM) Phosphotungstic acid (PTA; $H_3PW_{12}O_{40}$) staining method on a copper grid.

Blank is prepared in the same way without Rifampicin.

Water/Oil:

Dissolve Rifampicin (0.0056g) in the mixture of PEG 200 (25 mL) and Tween 60 (0.023g) using magnetic stirrer. Water (75mL) was added to the solution drop wise using the magnetic stirrer. Then the solution was sonicated using ultrasonicator for 45 minutes. Carbomer interpolymer type A is added to stabilize the preparation.

Blank is prepared in the same way without Rifampicin.

Bacterial Study:

S *aureus* (gram positive) and E *coli* (gram negative) are cultured using nutrient broth and incubated for 1 day. Then these cultures was transferred to 20 labeled (+ and -) test tubes (8 mL) and incubated for another day. Then the test tubes are labeled as Dark (D) and light (L) and these are differentiated into Oil/Water (O/W), Oil/Water Blank (O/W B), Water/Oil (W/O), Water/Oil Blank (W/O B), Control. Add 2 mL of the formulations to respective tubes and incubated for 1 hr. The tubes that are marked as D are kept in dark and the other ones are irradiated for 90 min. Transfer 10 mL of Trypsin soy broth into the tubes that are labeled as before and add 1mL of bacterial culture into the respective tubes. Take 20 petri dishes and label them as said above and transfer 3mL of these cultures into the petri dishes (trypsin soy) and incubate them at 37°C for 3 days.

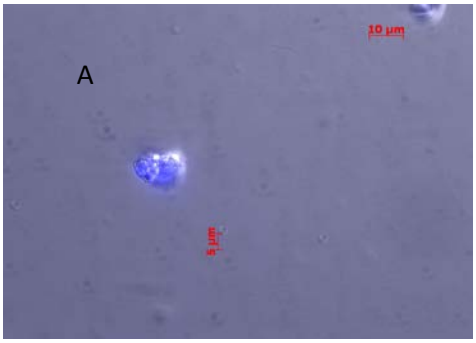
RESULTS

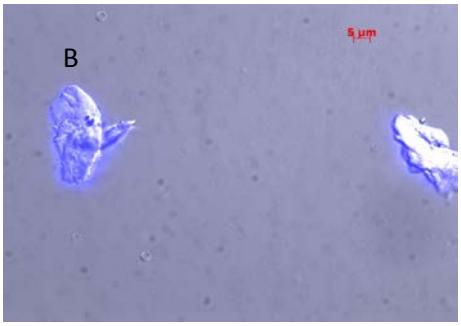
Nanoparticles:

The UV reading of the solution without adding water was wavelength, λ-392.47 nm and Absorbance, A-1.589

The UV readings of the final solution was wavelength, λ-475 and Absorbance, A-0.8

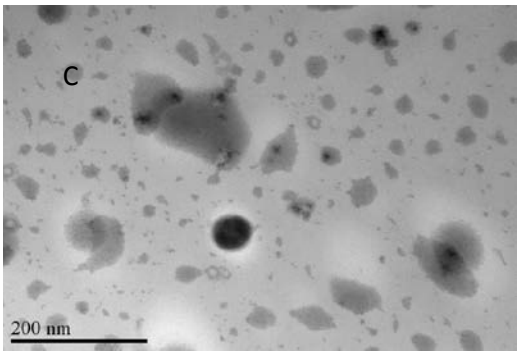
The size of the nanoparticles is in the theoretical range. The following are the images from Fluorescence microscopic and TEM images

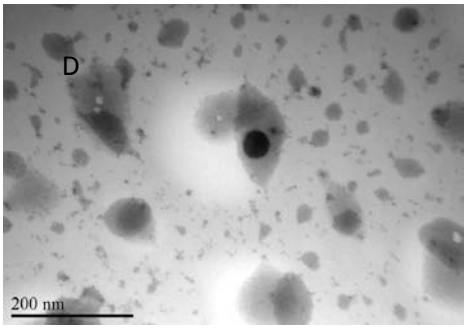




A and B Fluorescent microscopic image of aggregated nanoparticles

C and D TEM images after sonication





Bacterial Study:

There is no growth of gram negative (E *coli*) bacteria for the Light and Dark.

There is visible growth in gram positive bacteria on O/W and W/O in both L and D. But when compared to both W/O has no effect as it does with O/W.

In order to confirm this effect, this procedure was repeated for 2 times.

+D W/O has 8470 colonies and +L W/O has 102 colonies.

The original count was calculated by multiplying the colonies count with dilution factor.

The first dilution factor was 20 since we took 0.5 mL out of 10 mL.

The second dilution factor was 400 since we took 20 µL from 8000 µL.

Culture	Plate	Tube	Original
+D W/O	8470	28233	$1.13*10^7$
+ L W/O	102	340	$1.36*10^5$

The percentage was 12%

Attempt 1: +D O/W has 207 colonies and +L O/W has 180 colonies

Culture	Plate	Tube	Original
+D O/W	207	4140	$1.66*10^6$
+ L O/W	180	3600	$1.44*10^6$

The percentage was 86%

Attempt 2: 1mL of culture on plate

Culture	Plate	Tube	Original
+D O/W	71	710	284000
+ L O/W	98	980	392000

The percentage was 72%

Attempt 3: 1mL of culture on plate

Culture	Plate	Tube	Original
+D O/W	191	1910	764000
+ L O/W	66	660	264000

The percentage was 34.5%.

By taking the average of the above results (1, 2 and 3), the result was 64.17%.

DISCUSSION

We prepared both the types of nanoparticles (O/W and W/O). These solutions were tested for the particle size and on bacteria to know which formulation would be best for the maximum effect. As seen from the results, there is no effect on the negative bacteria. Even on positive bacteria, O/W has maximum effect.

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