Research Article **Novel drug delivery system**

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FORMULATION, OPTIMIZATION AND EVALUATION OF SPRAY DRIED MICROSPHERES OF AZITHROMYCIN DIHYDRATE

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ABSTRACT

In the present study formulation and characterization of spray drying microspheres of azithromycin dihydrate, (prescribed extensively in solid dosage forms) in a sustained release form to overcome drug resistance, and dosing non-compliance in pediatric patients. So, the purpose of this research was to formulate sustained release microspheres of azithromycin dihydrate using chitosan as a carrier polymer, which is also a suitable polymer to mask the bitter taste of drug. Drug entrapment efficiency for azithromycin dihydrate reached to highest level of 84.8% and percentage yield to 68%. Formulated spray dried microspheres gave drug release for the initial dosing and maintenance dosing in a sustained manner for 72 hours. This gave a hope to the possibility of single dose treatment for pediatric patients. The formulated microspheres show pharmacotechnical properties in the acceptable range.

KEYWORDS; **Spray Dried Microspheres, Azithromycin Dihydrate, Pediatric patients.**

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INTRODUCTION

For decades an acute or chronic illness is being clinically treated through delivery of drugs to the patients in form of some pharmaceutical dosage forms like tablets, capsules, liquids, creams, pills, aerosols, injectables, and suppositories[1]. However, these conventional dosage forms have some drawbacks. Multiple daily dosing is inconvenient to the patient and can result in missed doses, made up doses and patient incompliance with the therapeutic regimen. When conventional immediate release dosage forms are taken on schedule and more than once daily, there are sequential therapeutically blood peaks and valley associated with taking each dose[2-4]. It should be emphasized that the plasma level of a drug should be maintained with in the safe margin and effective range. For this proper and calculated doses of the drug need to be given at different time interval by conventional dosage form. To achieve and maintain the concentration of administered drug within therapeutically effective range, it is often necessary to take drug dosage several times and these results in a fluctuating drug level in plasma. Greater attention has been focused on development of sustained or controlled release drug delivery systems with concomitant recognition of the therapeutic advantages of controlled drug delivery.[5] Controlled drug delivery systems have been introduced to overwhelm the drawback of fluctuating drug levels associated with conventional dosage forms. A variety of materials and approaches have been proposed which could be effectively used in designing and construction of systems with potential to provide predictable, precise and reproducible pattern of controlled release or even site-specific drug delivery. A sustained release system delivers the active agent at slower rate than the conventional dosage form but the release is substantially affected by external environment. Various terms like 'smart', intelligent', 'novel', therapeutic have been assigned to controlled release systems.[6]An ideal controlled drug delivery system is the one which delivers the drug at a predetermined rate, locally or systemically, for a specified period of time. Microencapsulation is a rapidly expanding technology. It is the process of applying relatively thin coatings to small particles of solids or droplets of liquids and dispersions. Microencapsulation provides the means of converting liquids to solids, of altering colloidal and surface properties, of providing environmental protection and of controlling the release characteristics or availability of coated materials.[7] Many antibiotics, including macrolides and quinolones, are used incorrectly in the treatment of presumed respiratory tract infections. The use of broad-spectrum antibiotics increased considerably in the 1990s, but often this use is inappropriate. Guidelines, such as those for community-acquired pneumonia, encourage rational therapy and more prudent prescribing. There are strong links between appropriate use, compliance and resistance as well as between regimen complexity and compliance. These works provide a platform for thinking about a low dose, high-compliance drug therapy with good efficacy. Such therapy will need to be combined with programs to promote rational antibiotic use, particularly targeting inappropriate prescribing for viral infections and use of agents with a broader antimicrobial spectrum than is necessary. Azithromycin dihydrate, a macrolide antibiotic of the azalide subclass, exerts its antibacterial action by binding to the 50s ribosomal subunits of susceptible bacteria and suppressing protein synthesis. Azithromycin is an azalide antibiotic that contains a nitrogen atom in the macrolide aglycone ring. It is active in vitro against Streptococcus pneumoniae group A streptococci, Streptococcus agalactiae, Staphylococcus aureus, Haemophilus influenzae, Moraxella catarrhalis, and intracellular organisms such as Chlamydia, Mycoplasma, and Legionella species. Azithromycin retains the gram-positive activity of erythromycin as the result of a common mechanism of action but provides enhanced activity against gram-negative organisms. To a greater extent than other macrolides, azithromycin is concentrated in phagocytic cells

and has shown invitro, to reduce the viability of intracellular bacteria. The pharmacokinetics of azithromycin is characterized by rapid and extensive concentration within the intracellular and interstitial compartments of tissues. High and sustained tissue antibiotic concentrations are accompanied by relatively low concentrations in serum. Given the efficacy of a one-time-dose for these common infections and the assured compliance, the hope would be that this type of regimen can help minimize the emergence of antibiotic resistance. A one-dose-only treatment is a significant advancement, giving physicians and patients an option that can effectively treat the most common respiratory tract infections, while also providing an additional benefit regarding noncompliance with therapy. The present research work was carried out with the aim to try to reduce azithromycin dosing frequency, as it is a broad spectrum antibiotic producing a resistance if given in high frequency from the childhood. So, if we can reduce the dosage frequency it will be more beneficial to all small aged respiratory tract infection patients and treat then up to older age. At the same time looking to the general child nature, such a single dosing for a treatment would lead to patient compliance, and complete treatment with appropriate dosing.[8]

MATERIALS AND METHODS

Azithromycin dehydrate was collected as gift sample from Lincoln Pharmaceutical PVT. LTD., Kalol, Gujarat, India. Chitosan was collected also as a gift sample from Troikka pharmaceutical Pvt. Ltd., Ahmedabad. Acetic acid (Glacial 100%GR) was purchased from Merk Speciality PVT LTD. F. C. Reagent and Hydrochloric acid was purchased from Finar Chemicals Limited, Ahmedabad, Gujarat, India.

Preparation of microspheres of chitosan and Azithromycin

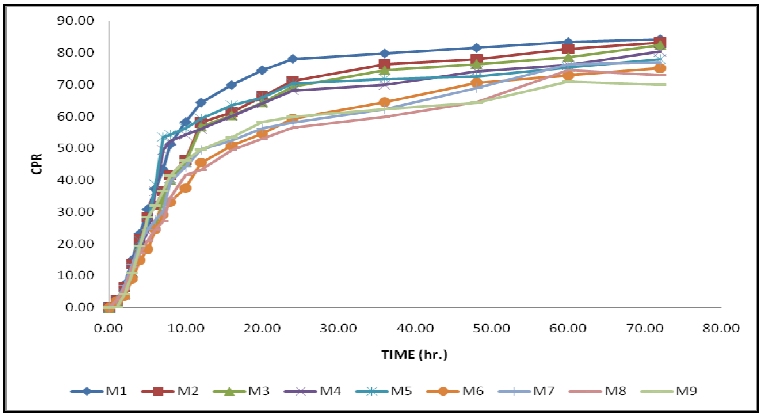
Chitosan microspheres were prepared by a spray drying technique. The chitosan solution to be spray dried was prepared by dissolving chitosan in water containing 0.5 %v/v acetic acid and then adding solution of Azithromycin to above solution until clear solution formed with continuous stirring up to 2 hrs with 800 rpm of top lab stirrer. Drug to Polymer ratio of 1:1 was selected for preliminary trials. The resultant solution was spray dried by using "LU-222 advanced lab spray drier (Labultima, Mumbai, India) for preparing microspheres at inlet temperature 150^{o}C, feed rate 10 ml/min and aspiration rate: 50 mBar. In the present study a 3^2 full factorial design was employed to study the effect of independent variables, i.e. drug: polymer ratio(X_1) (1:1, 1:2, 1:3) and the concentration of Glacial acetic acid used to dissolve chitosan (X_2) (0.5, 1, 1.5) on dependent variables % drug release at Q_{72}, drug loading efficiency, mucoadhesion property, in vitro wash off test, percentage compressibility, angle of repose, percentage yield and particle size. A statistical model (equation below) incorporating interactive and polynomial terms was utilized to evaluate the responses.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}X_1^2 + b_{22}X_2^2$$

Where, Y is the dependent variables, b_0 is the arithmetic mean response of the nine runs, and b_1 is the estimated coefficient for the factor X_1. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. The interaction terms (X_1X_2) show how the response changes when two factors are simultaneously changed. The polynomial terms (X_1^2 and X_2^2) are included to investigate non-linearity.[9]

RESULT

Drug release study



Graph 1
In vitro Drug Release

The drug release study was carried out using USP XXIV basket apparatus (Electro lab, TDT-06T, India) at 37°C ± 0.5°C and at 100 rpm using 900 ml of 0.1 N HCL as a dissolution medium (n=5) as per USP XXVI. Micro particles equivalent to 50 mg of azithromycin were used. 10 ml of sample solution was withdrawn at predetermined time intervals, filtered through a 0.45 µm membrane filter, diluted suitably and analyzed spectrophotometrically at 760 nm using U.V Spectrophotometer, Shimadzu 1700. Percentage drug dissolved at different time intervals was calculated. The percentage drug release of batches M1 to M9 is shown in Figure 1.[10]Error! Not a valid link.

Scanning electron microscopy (SEM)

Scanning electron photomicrographs of drug-loaded chitosan microspheres were taken. A small amount of microspheres was spread on metal stub. Afterwards, the stub containing the sample was placed in the scanning electron microscope chamber. Scanning electron photomicrograph was taken at the acceleration voltage of 20 KV, chamber pressure of 0.6 mm Hg, at different magnification. The photomicrograph of batch M1 and M5 obtaining spherical in shape and particle size 414nm to 3.97 µm. The photograph of scanning electron microscope, as shown in Figure: 1,2.[11]

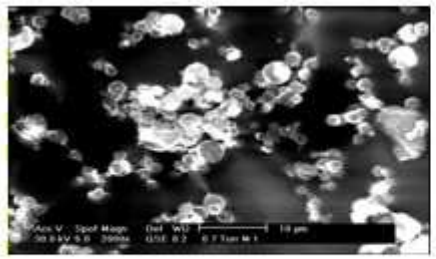


Figure: 1
SEM Batch M1

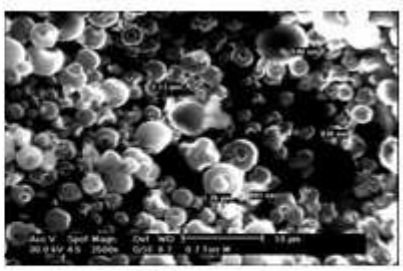


Figure: 2
SEM Batch M5

Percentage Drug entrapment efficiency

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment (PDE) as per the following formula.

PDE = (practical drug loading / theoretical drug loading) × 100

Theoretical drug loading was determined by calculation assuming that the entire drug present in the chitosan solution used gets entrapped in microspheres and no loss occurs at any stage of preparation of microspheres. Practical drug loading was determined by tacking a twenty mg of accurately weighed microspheres were crushed in a glass mortar-pastel and the powdered microspheres were suspended in 10 ml of 0.01 N HCl (pH 6.1). After 24 hours the solution was filtered and the filtrate was analyzed for the drug content. Table 1 shows all results.

Table:1

Result of 3^2 Full Factorial Design Batches

Batch No.	M1	M2	M3	M4	M5	M6	M7	M8	M9
Particle size(μm)	2.4	2.9	3.12	3.12	3.25	3.6	3.5	3.6	3.8
% Yield	68	65	58	54	68	44	45	43	38
% drug entrapment	87.87	86.1	86.0	85.1	84.8	83.2	83.4	81.3	79.6
Q72	84.3	83	82	80	78	75	77	73	70

DISCUSSION

The aim here was to correlate the effect of independent and dependent parameter of the formulation. This might help in the prediction of the desirable and acceptable pharmaceutical formulation in shortest possible time using minimum number of man hours and raw materials. The characterization was carried out for all the formulated batches from M1 to M9. The percentage yield of all batches ranged from 43 % to 68%. The study helped in the ease to know the requirement of raw material and effect of the formulation parameters. The percentage drug entrapment efficiency of all batches varied from 79.6% to 87.87%. The idea of percentage of loading and dosage calculation is obtained from the percentage drug entrapment efficiency data. As the drug entrapment efficiency is nearer to 100% for any batch it shows best drug loading and required less amount of formulation dosage to be administered, compared to the less percentage drug entrapmented batch. Here batch M1 gave highest 87.87% drug entrapment efficiency and batch M9 lowest 79.6%. The scanning electron microscopy measurement was carried out for the measurement of actual particle size of the formulation batches. Here the smallest particle had a size of 540 nm while the largest particle had 3.34 μm particle size. This range of the particle size was would prove to be the desired one with respect to the microspheres production. Here by using the spray dryer instrument one can obtain fine and smooth surfaced microspheres. In vitro drug release profile data was obtained to assume a hypothetical percentage of drug release as in the stomach. In vitro drug release profile is a modified hypothetical model to achieve a percentage of drug release in the body. There are Q24 was highest in batch M1 (78.15), intermediate (71.83) in batch M5 and lowest in batch M8 (59.81). Considering the set of all batches that as batches M1 to M3, M4 to M6 and M7 to M9, batches only batch M6 did not reach this value. The highest value was seen in batch M1 (15.03) and the lowest in batch M3and M9 (10.88) without considering M6 (9.0), at three hours. The Q4 release was highest in batch M1 (23.24) and lowest in batch M8 (17.15). The batch M5 (75.41) and batch M7 (73.00) gave nearly same release profile after

60 hrs. The Q3 of batch M7 (13.54) was higher compared to M5 (12.65), so it can be a good candidate in comparison to M5. But after 3 hrs., the release percentage reduced in batch M7 compared to M5. So, this might be because of the higher percent of chitosan that formed hydrogel from which the drug did not diffuse at the desired rate. Since, batch M5 gave continuous release as compare to M7, it was selected for M7 batch. Now to further drop off the batches amongst these t50 was considered. Here t50 for batch all the batches was 8, 11, 11, 8, 7, 16, 14, 18, 14 respectively of batches M1 to M9. The Q24 value for the all the batches was 84.30, 83.20, 82.46, 80.46, 78.00, 75.21, 77, 73, and 70 respectively to batches M1 to M9. Considering t10, t50 and Q24 for the selection of the best batch it is very clear that batch M5 would be a good candidate for both loading release and sustain release.

CONCLUSION

Formulated spray dried microspheres gave drug release for the initial dosing and maintenance dosing in a sustained manner for 72 hours. This gave a hope to the possibility of single dose treatment for pediatric patients. The formulated microspheres show pharmacotechnical properties in the acceptable range. This study clearly demonstrated that one could develop a sustained dosage form of a drug having a long biological half-life as a single dose treatment and thus reduce the drug resistance in patients.

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