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Research Article

Fabrication of Porous Microspheres of Azithromycin, Incorporating Different Porogens

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ABSTRACT
Several antibiotics loaded nebulized formulations allow high local concentrations and a decreased risk of systemic toxicity but this formulation require time-consuming hygienic procedures for administration several times a day. An innovative way to increase drug product stability, patient comfort, and therapeutic effectiveness is to formulate an easily administered sustained-release, biodegradable, and biocompatible porous microspheres. Here the Ionotropic Gelation method has been opted for the preparation of a porous microsphere loaded with Azithromycin by inclusion of different porogens. The structural morphology of porous microspheres was investigated by Scanning Electron Microscopy. Microspheres were in the range of 600 -900 µm. with the pores in the size of 2-15µm.
Among the three porogens used, $NaHCO_3$ showed the maximum porosity of almost 10%, whereas it was 4.9 and 6 for Sucrose and NaCl respectively. Micromeritics properties of porous microspheres were found to be satisfactory in accordance with their flow properties. In Drug release profile it was observed that after 8 hours 65%,57% and 53% drug was released from porous microspheres using NaHCO3, NaCl and sucrose as porogens respectively. To ascertain the drug release mechanism and release rate, data of all the formulations were fitted to Zero order, Higuchi Matrix, Korsmeyer Peppas model to explain the kinetics. The dug was released in a controlled way and the pattern indicated that they obeyed Higuchi kinetics. The value of release exponent 'n' calculated for all the formulations indicates that the formulations released drug in non Fickian (anomalous) release mechanism (n> 0.5) i.e., erosion followed by diffusion.

Keywords: Porous microspheres, Porosity, Micromeritics, Porogens, Higuchi kinetics.

INTRODUCTION

Microspheres are free-floating multiarticulate drug delivery vehicles with particle sizes ranging from 1 to 1000 micrometers that are designed to achieve delayed or regulated drug administration in order to boost bioavailability, stability, and safety at a predetermined pace to a specific location (1). They have several pluses over other particle geometries for biomedical applications. For example, they can be designed and built to have a uniform size and shape, which can improve sphere delivery to the specific target site, a larger surface area allowing for sufficient therapeutic coatings and an increase in degradation rate and ion release, and they can be engineered to be porous or hollow, which allows for encapsulation of other biomedicals (2). Porous microspheres are consisted of interconnective external and internal pores which leads to very low mass density and wide surface area. This feature enables them to have excellent adsorption criteria (3). Due to this uniqueness over the traditional microsphere, they find extensive pharmaceutical applications. Porous microspheres are quite efficient in gastro retentive drug delivery, Pulmonary drug delivery and also as carrier of biopharmaceuticals (4). Pore structure and porosity are the main factors that control their use. In fact, Porosity plays the important role to determine the capacity efficiency and release kinetics from the formulations. The diameter, the number and the structure of the pores are the main factors to affect the properties of porous microspheres. Porous materials are of four types according to their pore sizes: microporous materials (less than 2 nm), mesoporous materials (2–50 nm), macroporous materials (50–200 nm), and gigaporous materials (more than 200 nm). The differences in pore sizes dominate the practical applications of the microspheres (5). The pores are generated by

employing porogens. They are also called pore-forming agents. The mechanism of porogens is dependent on the solvent casting/particulate leaching. The porogen is usually an inert agent added into the materials for porous microsphere preparation. Once the porogen particulates are removed, leaving the pores both in external and internal features. The commonly used porogens are effervescent salts like sodium bicarbonate, inorganic salts like sodium chloride, hydrocarbon waxes, linear polymers, carbohydrates and sugar (6).

There are different well-established methods for the synthesis of microspheres like seed swelling (7), solvent evaporation (8), polymerization (9), spray drying (10), Ionotropic gelation (11) and phase separation (11). The size, shape and pore structure of the particles depend on many experimental variables like temperature, pH, stirring speed, type and concentration of porogen, polymer and its concentration (12). In the Ionotropic gelation method, the process involves the interaction of an ionic polymer with an oppositely charged ion to initiate cross linking. The three-dimensional structure and presence of other groups influence the ability of cations (or anions) to conjugate with anionic (or cationic) functionalities and some kind of selectivity is found (13). The objective of this work was to investigate the effect of porogens on porous structure and release behaviour of porous microspheres prepared by the Ionotropic gelation method.

MATERIALS AND METHODS

Materials

Azithromycin was a kind gift from Windlas Biotech Pvt Ltd. Chitosan and Sodium Alginate, Calcium chloride and other chemicals used were of analytical grade.

Methods

Formulation Design: Formulations of Azithromycin loaded porous microspheres containing different concentrations of polymer: porogens were prepared using Factorial design and grouped as Batch F1, F2 and F3 shown in Table 1.

Table 1: Formation of Porous microspheres with different percentage of porogens and their flow properties

Porogen used	Formulations	% of porogen (w/w)	Bulk Volume (ml)	Tapped Volume (ml)	Bulk Density (g/ml)	Tapped Density (g/ml)
NaCl	F1a	12.5	3.48	2.61	0.043	0.057
	F1b	9.3	2.23	1.74	0.047	0.063
	F1c	6.25	2.9	2.23	0.058	0.073
$NaHCO_3$	F2a	12.5	0.754	0.638	0.358	0.423
	F2b	9.3	0.464	0.435	0.475	0.505
	F2c	6.25	0.348	0.29	0.517	0.62
Sucrose	F3a	12.5	4.06	2.9	20.3	14.5
	F3b	9.3	4.35	3.48	13.59	10.87
	F3c	6.25	6.09	5.22	12.42	10.65

Preparation of Porous Microspheres

The microspheres were prepared by the Ionotropic Gelation Method using the formulations as shown in Table 1. Chitosan is dissolved in 5% acetic acid solution by homogenizer. The alginate solutions comprising 2.5% w/v sodium alginate were prepared by initially dissolving the polymer in deionized water using gentle heat, being stirred magnetically (14) . Then the porogens were added to the mix according to the ratio specified (Table 1). On complete solution, an accurately weight quantity of Azithromycin was added to each solution to afford homogeneous dispersions. The dispersions were sonicated for 30 min for the removal of any air bubbles that might be generated during the stirring process. The sodium alginate-drug dispersions (25 ml) were added drop wise via a 20-gauge hypodermic needle fitted with a 10 ml syringe into 50 ml of 5% w/v Acetic acid solution containing Chitosan and cross-linking agent (CaCl2) (15). It was being stirred at 200 rpm for 10 min. The droplets from the dispersions instantaneously gelled into discrete microspheres.

Morphology and Size distribution:

Microspheres were exposed for SEM studies. The morphology and porous structures of the microspheres were examined using ZEISS

EVO 18, CARL ZEISS MICROSCOPY (PENTA FET X 3) OXFORD INSTRUMENTS with an operating voltage of 30 kV. Images were captured using a digital capture card. Prior to examination samples were gold coated under vacuum to render them electrically conductive (16). The samples include various microspheres prepared using different porogens before release study. The microspheres were not subjected to SEM studies after release study because they converted to gel type of matrix when dissolution was performed.

Size and size distribution of the microspheres were measured by sieve analysis. They were separated into different size fractions (% weight fraction) by sieving for 10 min using standard sieves having nominal mesh apertures of 1.4 mm, 1.2 mm, 1.0 mm, 0.85 mm and 0.71 mm, 0.59 mm, 0.35 mm and 0.25 mm (sieve no. 14, 16, 18, 22, 25, 30, 45 and 60 respectively) (14) . The particle size distributions of the microspheres were reported and the mean particle size of microspheres were calculated.

Determination of drug incorporation efficiency:

Twenty-five milligrams of drug-loaded porous microspheres from each batch was placed in a 100 ml conical flask containing 50 ml of phosphate buffer of pH 7.4. The microspheres were magnetically stirred to promote swelling and break of the cross-linked structure. This afforded liberation and subsequent dissolution of Azithromycin. The solution was filtered through a 0.45 µm membrane filter (17). Then the drug was quantified at 231 nm spectrophotometrically after appropriate dilution with phosphate buffer of pH 7.4. The incorporation efficiency was determined by the following empirical relationship:

Drug incorporation efficiency (%) = (AQ/TQ) × 100, where AQ is the actual quantity of drug present in the microspheres and TQ is the 100% theoretical quantity of drug present in the microspheres (i.e., actual initial loading dose) (12).

Infrared spectroscopy

The drug-polymer interactions were studied by infrared spectroscopy. The IR spectra were recorded between 400 to 4000 cm−1 for pure Azithromycin, blank porous microspheres and drug-loaded porous microspheres from KBr pellet using Perkin Elmer IR spectrophotometer.

In vitro dissolution testing

The dissolution studies were performed in a fully calibrated six station dissolution test apparatus (37 ± 0.5° C, 50 rpm) using the USP rotating basket method in phosphate buffer media (pH 7.4, 900 ml) (18). A quantity of porous microspheres equivalent to 50 mg Azithromycin for each formulation was employed in all dissolution studies. The samples of 5 ml each were withdrawn at predetermined time intervals and were replenished immediately with the same volume of fresh prewarmed phosphate buffer maintaining sink condition throughout the experiment. The aliquots, following suitable dilution, were analyzed spectrophotometrically at 231 nm. The concentrations of Azithromycin in the test samples were calculated using a regression equation (Absorbance = 0.020 X concentration, R2 = 0.999) of the calibration curve in phosphate buffer of pH 7.4.

Kinetic modelling

For the investigation of the release mechanism, the release data (≤60%) from the porous microspheres were fitted to the following power law expression, i.e. $Mt/M\infty = Ktn$ … (1), where Mt and M∞ are the amounts of drug released at time t and the overall amount released, respectively, K stands for release rate constant and n is the release exponent to indicate release mechanism. The release data were further subjected to the modified form of the power law expression to accommodate the lag time (tL) in the beginning of the drug release from the microspheres: $Mt/M\infty = K(t - tL)n$ …(2). The data were fitted into various kinetic models to determine the correlation coefficient. The value of n was calculated from the slope of the plot of log (Mt/M∞) vs. log (t) and log (Mt/M∞) vs. log (t−tL) to interpret the release mechanism (14).

Statistics

Significant differences were calculated using a paired student's t-test. Values of $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Several antibiotics loaded nebulized formulations are already on the market that allows high local concentrations and a decreased risk of systemic toxicity but this formulation require time-consuming hygienic procedures for the administration devices and long administration times, several times a day.

An innovative way to increase drug product stability, patient comfort, and therapeutic effectiveness is to formulate an easily

administered dry powder for inhalation. In addition, using sustained-release, biodegradable, and biocompatible microspheres (MS) in dry powder inhalers may boost bioavailability, stability, and safety at a predetermined pace to a specific location and by enhancing patient comfort by lowering the frequency of delivery. In case of porous microspheres additional features like interconnected external and internal pores, resulting in a low mass density and a large specific surface area, allows them for efficient adsorption, significant impact on capacity efficiency and release kinetics.

The objective of the current study was to develop Antibiotic loaded porous microspheres using different porogens and optimize the formulation and to evaluate the prepared formulations in the aspect of different parameters and find out the suitable porogens for the same.

Ionotropic Gelation method has been opted for the preparation of microsphere loaded with Azithromycin where Chitosan has been used as a polymer as Chitosan is a cationic natural biomaterial

with distinct advantages which can improve drug absorption, CaCl2 and sodium alginate as fabricating agent and biopolymer respectively (19). In the same combination NaCl, NaHCO3 and sucrose was incorporated as porogen to impart porosity.

Table 2: Particle size and Drug incorporation efficiency of the porous microspheres (± SD, N=3)

Formulations	Batch Yield (%)	Mean Particle Size (µm)	drug incorporation efficiency (%) (± SD. N=6)
F1a	76.29 ± 3.85	671 ± 5	56.91 ± 2.87
F1b	70.56 ± 2.90	623 ± 4	60.63 ± 1.08
F1c	72.87 ± 3.28	603 ± 5	45.34 ± 2.18
F2a	75.08 ± 2.08	651 ± 5	65.09 ± 2.12
F2b	70.56 ± 3.87	660 ± 4	66.12 ± 1.11
F2c	72.87 ± 2.24	620 ± 5	48.87 ± 2.04
F3a	78.24 ± 2.91	887 ± 8	41.54 ± 1.08
F3b	82.36 ± 3.23	710 ± 10	39.74 ± 1.23
F3c	80.37 ± 2.87	728 ± 7	43.29 ± 2.15

The drug Azithromycin was scanned under the UV Spectrophotometer at a range of 200 - 400 nm in phosphate buffer pH –7.4. Before the development of any formulations, it is crucial to examine drug excipient interaction. FTIR Spectroscopy demonstrates the interaction of molecules at the level of functional groups. Here, drug excipient interaction was done using FTIR Spectroscopy. The identification of the drug was done by (FT-IR) spectroscopic method using the PerkinElmer FTIR spectrophotometer.

FTIR Spectroscopy (Figure 1) shows the interaction between the molecules at the level of functional groups. Here, drug excipients interaction was done using FTIR Spectroscopy

It was observed from the FTIR Spectra that there may be some physical interactions due to generation of weak bonds as no such shifting of the peaks were marked.

It is suggested by the FTIR spectra that there may be some physical interactions due to generation of weak to medium intensity bonds as no major shifting of peaks was marked (20). Mixtures of polymers can change the rate of diffusion of drug molecules by changing entanglement in the polymeric network. Blend of polymers is known to change the rate of diffusion of drug molecules by varying the entanglement in the polymeric network, leading to the change of tortuosity of diffusion pathways (18). Thus, the interaction might be helpful in sustaining the release of drug molecules from the experimental formulations.

For each of the porogens, three concentrations were selected: low (6.25 %), medium (9.3 %) and high (12.5%). Hence nine formulations were made.

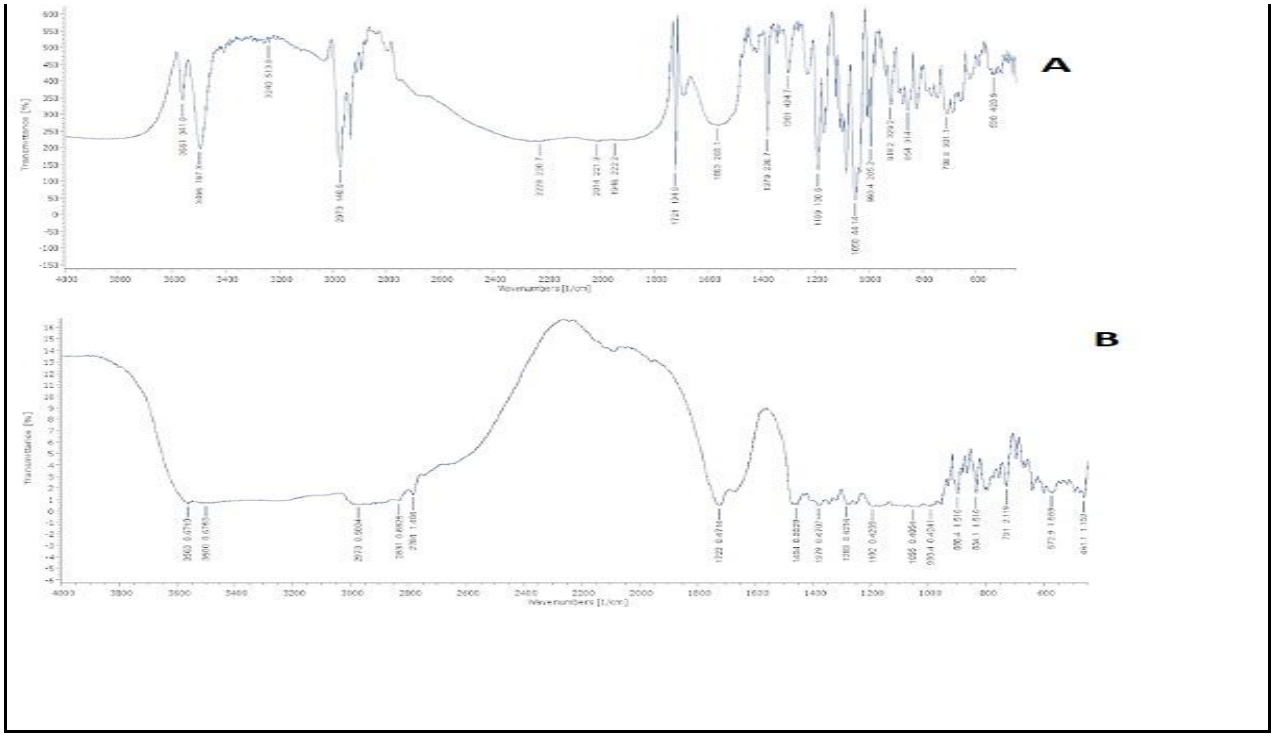

Fig 1: FTIR spectra of A. Drug (Azithromycin) B. Drug with all excipients for porous microspheres

The structural morphology microspheres were investigated by SEM, and some representative SEM micrographs are given in Figure 2,3. It was found that plenty of pores were formed. The pores are in size ranges of 2-15 µm proving the formation of porous microspheres.

In particle size distribution study, it was observed that the particles were on gathered on sieve no 22 (850 µm), 25 (710 µm) and 30 (595 µm). Finding the average size of the microspheres, it can be reported that it was within 600 - 900 µm (Table 2).

Since the microspheres have lots of pores, the density of them is lower compared with the conventional microspheres.

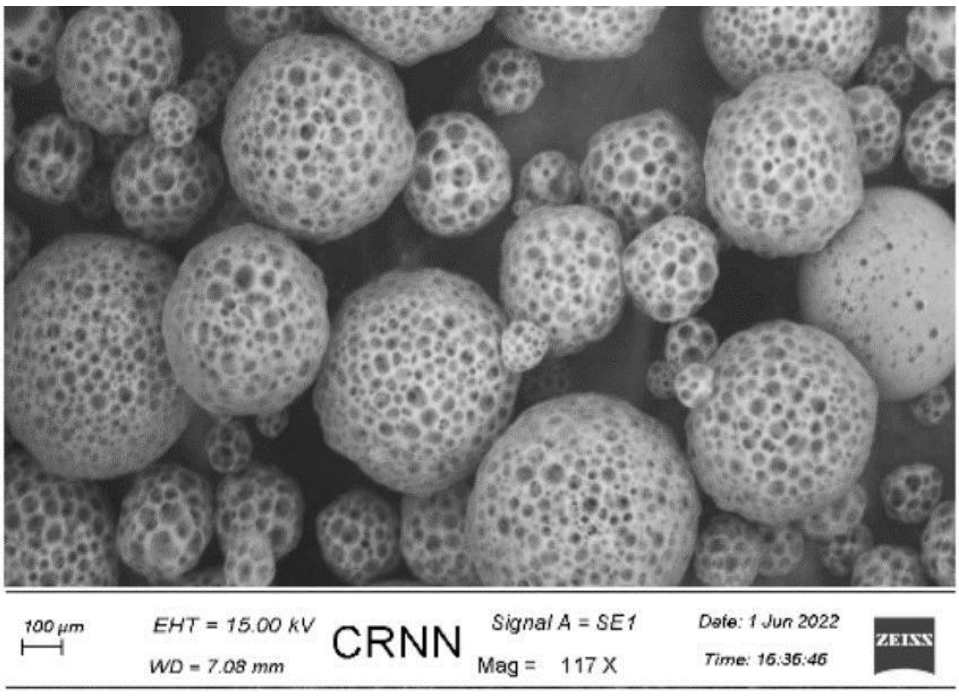

Fig 2: Scanning Electron Microscopy of Porous microsphere

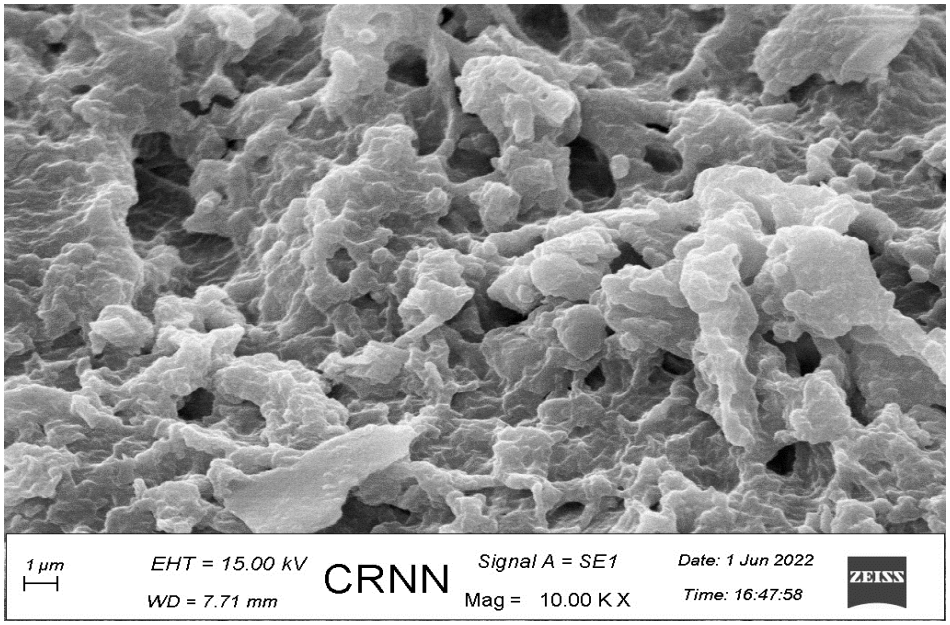

Fig 3: Scanning Electron Microscopy of Porous microsphere in higher magnification

After the preparation, the formulations were subjected to various evaluation parameters.

The physicochemical tests included their Micromeritics properties, drug loading.

Among the three porogens used, $NaHCO_3$ showed the maximum porosity of almost 10 %, whereas it was 4.9 and 6 for Sucrose and NaCl respectively. Micromeritics properties of porous microspheres were evaluated and found to be satisfactory in accordance with their flow properties. In the determination of drug incorporation efficiency, it was found to be in the range of 40-66 % (Table 2). Porous Microspheres made of $NaHCO_3$ as porogen showed around 48-66 % of drug loading. whereas NaCl and Sucrose reflected maximum 64 and 43 % of the drug loading respectively.

Fig. shows the release of Azithromycin versus time. The concentrations in the graph are the average of three readings. The error bars shown in this Figure 4 are the highest and lowest values of the measured concentrations for each time. Some amount of the active agent was released from the microspheres initially at zero time due to surface absorption (21). After the initial burst, release was curvilinear with time.

In the release profile it has been found that after 8 hours 65%,57% and 53% drug was released from porous microspheres using $NaHCO_3$, NaCl and sucrose as porogen respectively.

To ascertain the drug release mechanism and release rate, data of all the formulations were fitted to Zero order, Higuchi Matrix, Korsmeyer peppas model to explain the kinetics of the drug release microspheres. The cumulative percent of drug released versus time plot exhibits curvilinear nature. This suggests that drug release is not governed by zero-order kinetics. This observation is confirmed by fitting the dissolution data to a zero-order model where comparatively low values of correlation coefficients (R2) are obtained. The results of the above-mentioned studies show that drug releases from the porous microspheres are much more acquainted with Higuchi Kinetics (Table 3). Drug release followed was matrix. The value of release exponent 'n' calculated for all the formulations indicates that the formulations released drug in non Fickian (anomalous) release mechanism (n> 0.5) i.e., erosion followed by diffusion.

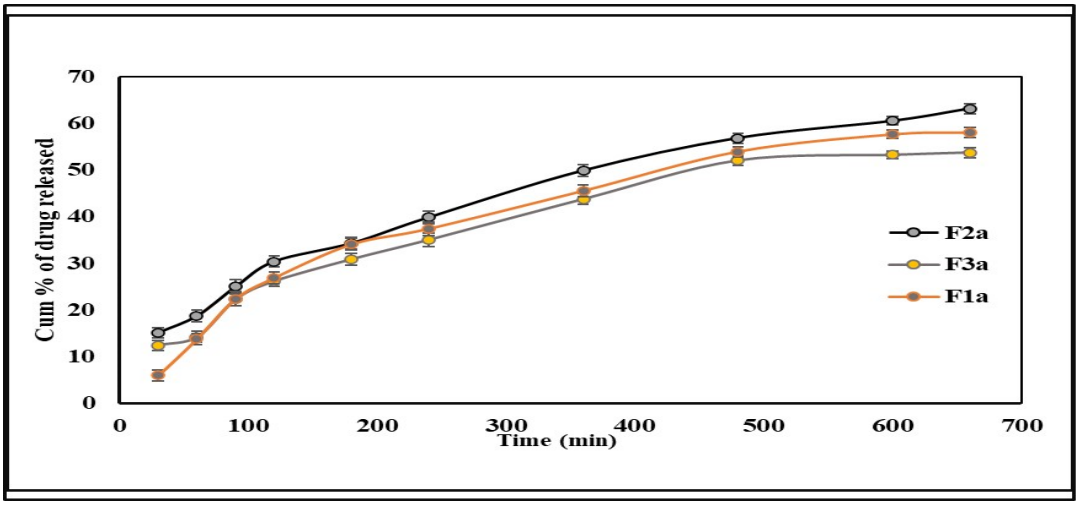

Figure 4: Drug Release profile of Azithromycin from porous microspheres

Table 3: Kinetic modelling of drug release pattern

Kinetic model	Formulation F1a	Formulation F2a	Formulation F3a
Zero order	0.085x + 13.37 = 0.886	y = 0.081x + 17.81 R² = 0.951	y = 0.073x + 14.65 R² = 0.94
Higuchi	2.747x +3.065 = 0.970	y = 2.539x + 1.197 R² = 0.995	y = 2.299x + 0.410 R² = 0.986
Korsmeyer Peppas	y = 0.721x + 0.150 R² = 0.939 N= 0.721	y = 0.822x+ 0.876 R² = 0.992 N= 0.822	y = 0.521x + 0.304 R² = 0.978 N= 0.521

CONCLUSION

Controlled drug delivery via polymer-based systems achieved profound success for prevailing both in the present and in future work. The thought behind developing porous microspheres was to deliver Azithromycin in the pulmonary area in a continual manner for prolonged periods to reduce the frequency In the present study, Porous microsphere loaded with Azithromycin were prepared by Ionotropic Gelation method by inclusion of different porogens. Chitosan, Sodium alginate, Calcium chloride were used for the formulations and NaCl, NaHCO3 and sucrose were introduced as pore formers. SEM photographs of the formulations clearly indicated the formation of a number of pores confirming it as porous microspheres. A significant variation is observed in the in-vitro release pattern of Azithromycin from the porous microspheres in relation to change the porogen.

The prepared microspheres were in the range of 600 -900 µm. with the pores in the size of 2-15µm.

Among the three porogens used, $NaHCO_3$ showed the maximum porosity of almost 10%, whereas it was 4.9 and 6 for Succrose and NaCl respectively. In the present study, it was observed that the n varies from 0.5 to 0.8 represents a change in the drug transport mechanism. The formulations released drug in non Fickian (anomalous) release mechanism (n> 0.5) means erosion followed by diffusion.

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CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors are responsible for the content and writing of this article.

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