

Original Research Article

Characterization of azithromycin tablets formulated as inclusion complex and stability studies

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Abstract

Purpose: To characterize azithromycin inclusion complex for enhanced solubility and improved release from the dosage form.

Methods: Azithromycin was formulated as an inclusion complex with hydroxypropyl- β -cyclodextrin (HP- β -CD) and compressed to produce a tablet by direct compression to enhance the dissolution. The nature of azithromycin molecule in its inclusion complex was investigated using the Infra-Red (IR) and Differential Scanning Calorimetry (DSC) analysis. High-performance liquid chromatography (HPLC) analysis was used to monitor the dissolution rate and stability studies of azithromycin tablets. Two USP protocols of storage were applied at 40 °C with 75 % Relative Humidity (RH), and at 30 °C with 65 % RH.

Results: Pure azithromycin scan showed a prominent endothermic fusion peak between 115 and 122 °C, indicating its melting point. Both pure azithromycin and the inclusion complex with HP- β -CD had the same melting point values, according to the thermogram of the DSC. The molecular structure of azithromycin did not change upon inclusion complexation with HP- β -CD, according to IR spectroscopy. According to the HPLC results, retention time and ionic separation efficiency indicate the ion-pair reversed-phase mode of separation of the applied method.

Conclusion: The inclusion complex of azithromycin with hydroxypropyl- β -cyclodextrin creates tablets with a high dissolution rate, thereby increasing drug absorption and bioavailability. Azithromycin insertion into the structural cavity of HP- β -CD without a chemical reaction protects the drug molecules from environmental factors and enhances their chemical stability.

Keywords: Hydroxypropyl- β - cyclodextrin, Azithromycin, Inclusion complex, Direct compression tablet, Dissolution rate

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INTRODUCTION

Azithromycin is a nitrogen-containing macrolide with an antibacterial action similar to erythromycin. This drug is noted for its activity against some gram-negative organisms associated with respiratory tract infections, particularly *Haemophilus influenza* [1]. It also

acts against *Streptococcus pneumoniae* and *Moraxella catarrhalis*, as well as atypical pathogens like *Legionella pneumophila*, *Chlamydia pneumonia*, and *Mycoplasma pneumonia*. Azithromycin is administered as an injection or orally as capsules and tablets; however, due to its poor solubility, the dissolution rate of the tablets is low, resulting

in low bioavailability [2]. Various techniques have been used to enhance the dissolution and stability of poorly water-soluble drugs, including chemical modification or a novel formulation approach known as the solid dispersion technique, in which natural polymers are used [3,4] or synthetic polymers [5] to increase the solubility of drugs and increase their bioavailability. Other scientists have used polyethylene glycol in the solid dispersion formulation of azithromycin tablets to enhance the dissolution [6]. However, the oily property of this polymer yields additional problems in the manufacturing of tablets and their performance [6]. Other scientists had prepared azithromycin as nanoparticles and revealed that this technique led to an increase in the solubility of azithromycin [7]. A new report describes drug-drug crystal formation using an analgesic drug (especially Paracetamol) in an attempt to enhance the dissolution, resulting in the further enhancement of the pharmacodynamics of azithromycin [8]. This procedure needs a pharmacological study and should be proven for any combination. A novel super disintegrant, starch glutamate-crosscarmellose sodium, was synthesized by some workers and experimented on a carbamazepine tablet. Disintegration time was within seconds and exhibited a high amount of drug release, which therefore influenced the dissolution of the tablet [9], which may be useful in the formulation of an azithromycin tablet.

Recently, the natural, safe, and non-toxic substance β -cyclodextrin and its derivatives have been developed and extensively used in the food industry [10]. The cyclodextrins are natural polymers prepared by hydrolysis of starch, yielding a cyclic oligosaccharide with a ring cavity structure having a lipophilic character inside and a hydrophilic head on the outer surface. Therefore, it can physically carry the poorly soluble drug by forming an inclusion complex with drugs, hence increasing the solubility and stability of the included drug [11].

On the other hand, the stability study requires a specific analytical method to determine the percent of azithromycin in the dissolution process and to follow the degradation products of azithromycin. Several analytical methods have been reported for the quantitative analysis and dissolution rate determination of azithromycin tablets, including the spectrophotometric method by oxidation of azithromycin in solution with the formation of a coloring solution measured at an absorbance of 410 nm. This means that different factors

will affect the precision of the determination, such as the coloring agent and the time of the procedure [12]. However, the high-pressure liquid chromatographic (HPLC) methods made the analysis simple, rapid, and sensitive to detection. Therefore, several chromatographic methods of stability-indicating HPLC have been applied for drugs and their degradation products [13,14].

In the present work, a novel ion-pair reversed-phase liquid chromatographic method was developed for the determination of azithromycin, and the new generation derivative of cyclodextrin, Hydroxypropyl- β - cyclodextrin (HP- β -CD), was used to form an inclusion complex of azithromycin to enhance the dissolution rate and stabilize the formulated tablets of azithromycin. In addition, the surfactant sodium lauryl sulfate was incorporated in the innovative formula of azithromycin complex as it is beneficial for poorly water-soluble drugs [15].

EXPERIMENTAL

Materials

Azithromycin dihydrate, Hydroxypropyl- β -cyclodextrin (HP- β -CD), sodium lauryl sulfate (SLS), acetonitrile, and methanol solvent (HPLC grade), PEG 6000, Croscarmellose, and magnesium stearate were supplied by Safa Co. for Pharmaceutical Industries, Baghdad. For apparatus, see Differential Scanning Calorimeter (DSC, Shimadzu Co.), Infra-Red spectrometry, and HPLC instrument (Bruckner Co.).

Methods

Inclusion complex preparation

An amount of azithromycin dihydrate equivalent to 250 mg of azithromycin was mixed with Hydroxypropyl- β - cyclodextrin (HP- β -CD) in a ratio of 1:1; the powder mixture was moistened with ethanol. The mixed powder was passed through a sieve mesh No. 60, then dried in a hot air cabinet at about 60 °C [16]. The dried powder mixture of azithromycin with HP- β -CD was tested by Differential Scanning Calorimetry (DSC) and by an IR spectrometer to investigate any change that might have occurred in azithromycin molecules after complexation with HP- β -CD.

Pre-formulation study

An amount of azithromycin dihydrate equivalent to 250 mg of azithromycin was mixed with 100, 200, and 250 mg of HP- β -CD, respectively. The inclusion complex of these different concentrations of HP- β -CD was formed as tablets by direct compression and evaluated for their solubilizing potency [11]. The proper concentration of HP- β -CD as a potent solubilizing agent in complexation was 250 mg, which was selected for complexation to produce a tablet of azithromycin in the newly developed formulation (F4 and F5).

Evaluation of different formulas

Five different formulas of azithromycin complex tablets were manufactured by weighing an equivalent amount of azithromycin (250 mg) for each tablet, and mixing it with other ingredients. The mixed powder was compressed by direct compression using the prepared direct compression excipients (DC-excipients), which were self-prepared in the laboratory according to the previously reported procedure [16]. The list of ingredients per tablet for the studied formulations of azithromycin tablets is presented in Table 1.

HPLC assay

This was done using a chromatography column of 5- μ m particle size, and 150 x 4.6 mm diameter, in an oven at 50 °C. The mobile phase consisted of acetonitrile and phosphate buffer, pH 8.0, in portions (55:45 mL) of each.

The buffer solution was composed of 0.44 % dibasic potassium phosphate and 0.02 % sodium 1-octane-sulfonate, and the detection was by UV at 210 nm with a flow rate of 1 mL per minute. The sample for injection was prepared at a concentration of 0.025 % using a diluent of phosphate buffer pH 8.0, with acetonitrile (80:20).

Dissolution test

All the formulations in this study were subjected to a dissolution test following the USP method by using pH 6.0 phosphate buffer, 900 mL, and apparatus 2:75 rpm. The dissolution time was carried out for 45 minutes, and the percentage of dissolved azithromycin in the dissolution medium was measured at intervals by the HPLC method to establish the dissolution profile of each formulation.

Stability study

Samples of azithromycin tablets in their final packaging were stored in stability chambers using two stability protocols: 6 months at 40 °C and 75 % relative humidity. Test samples were stored at 30 °C (65% RH) for two years.

In the above protocols of stability study, the physicochemical properties of the tablet samples were tested, including appearance, friability, hardness, disintegration time, dissolution rate, and the presence of degradation products in storage.

Table 1: List of Ingredients in different formulations of azithromycin tablets under stability study

Name of substance	F1	F2	F3	F4	F5
Azithromycin dihydrate (mg)	262	262	262	262 mg in HPB-CD complex	262 mg in HPB-CD complex
Sodium lauryl sulfate (mg)	0	0	6	0	6
PEG 6000 (mg)	0	100	100	0	0
DC-Excipients (mg)	300	300	300	300	300
Croscarmellose (mg)	30	30	30	30	30
Magnesium stearate (mg)	10	10	10	10	10

RESULTS

The DSC interpretations

DSC scan of pure azithromycin showed a sharp endothermic fusion peak between 115 and 122°C, which corresponds to the melting point of azithromycin. The thermogram of the azithromycin-(HP- β -CD) complex indicated no change in the physical properties of azithromycin; the melting points of both pure

azithromycin and that of the inclusion complex with HP- β -CD are the same, as shown in Figure 1.

The IR-spectrum

Azithromycin showed peaks of (C = O) stretching at 1728 cm⁻¹ and that of (C – O – C) symmetrical stretching at 1051 cm⁻¹. These

characteristic peaks have also been identified in the IR spectrum of the azithromycin complex with a very slight difference due to the influence of HPB-CD complexation. This IR

spectroscopy indicated no chemical change like azithromycin upon inclusion complexation with HP- β -CD (Figure 2).

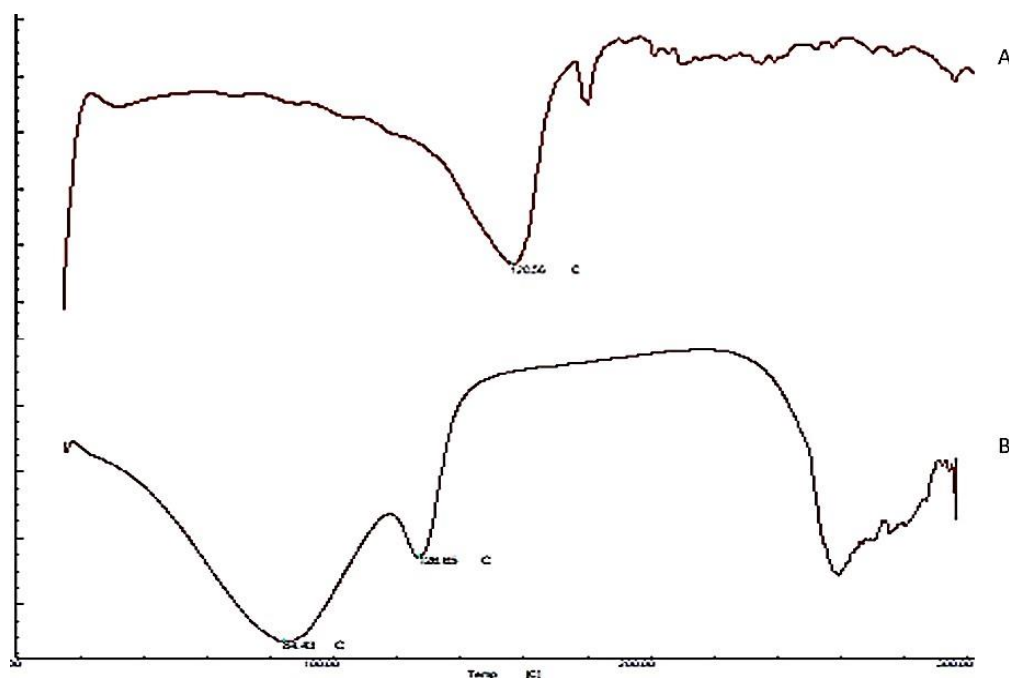


Figure 1: Differential Scanning Calorimeter thermogram of (A) Azithromycin pure and (B) Azithromycin complex with HP- β -CD

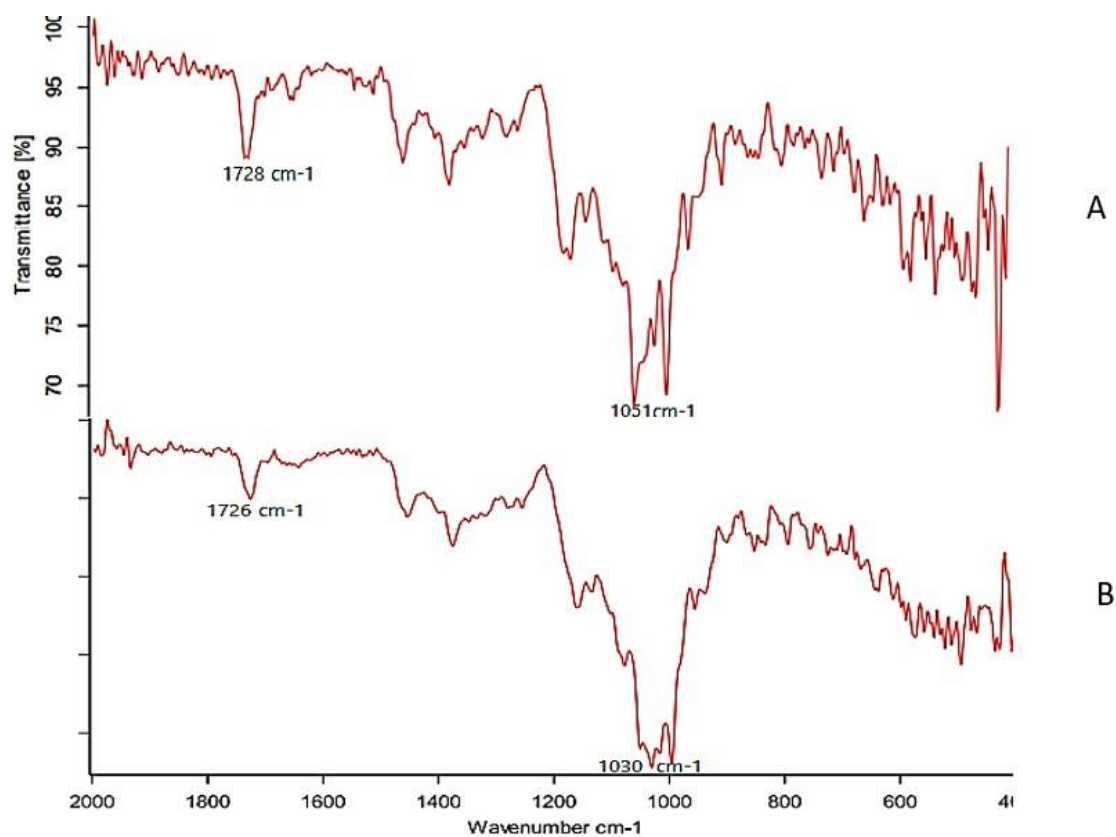


Figure 2: Infra-Red spectrum of (A) pure Azithromycin, (B) Azithromycin-inclusion complex

HPLC analysis

The HPLC results show the separation of azithromycin at about 6.5 minutes due to the use of the counter ion 1-octane sulfonate at a concentration of 0.02% in the mobile phase. This retention time and the efficiency of ionic separation are reasonable and can be improved upon with the increase of ionic substance concentration (Figure 3). This increase in retention time and efficiency of separation with an increment of the ionic substance indicated the ion-pair reversed-phase mode of separation.

Degradation product formation

A solution of azithromycin was stored in an oven at 60 °C for 24 hours and then

analyzed by HPLC. The result indicated the formation of a degradation product, which was detected at a retention time of about 4 minutes. This method of HPLC is therefore considered suitable for stability study (Figure 3).

Dissolution

The results of the dissolved amount of azithromycin at intervals of up to 45 minutes are reported as dissolution profiles in Figure 4, for all the studied formulations. These dissolution profiles show a higher rate in the azithromycin tablet formulated by inclusion complex with HP- β -CD, which is enhanced further when combined with SLS in the formulation (F5), Figure 4.

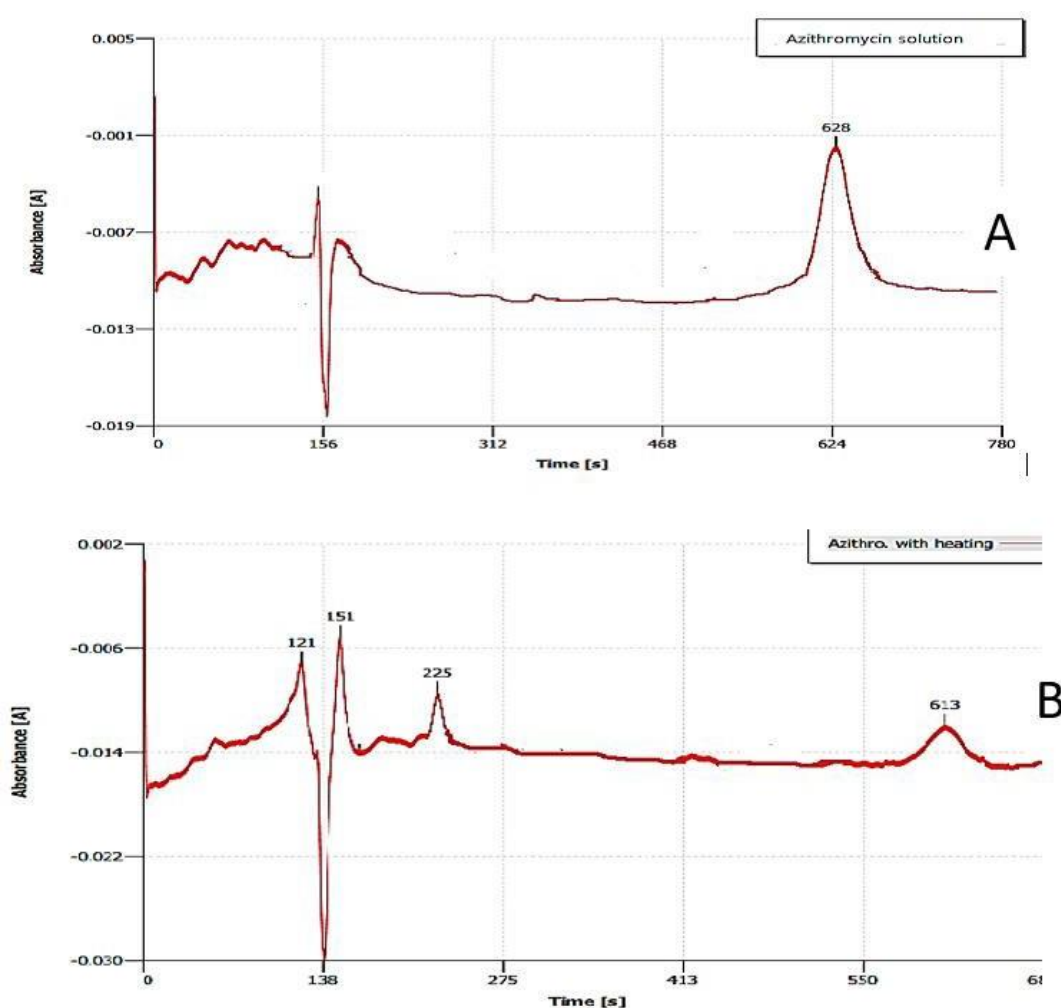


Figure 3: The chromatogram of (A) Azithromycin solution freshly prepared, (B) Azithromycin heated solution showing a peak of the parent compound at about 6.5 minutes with a peak of degradation product at about 4 minutes

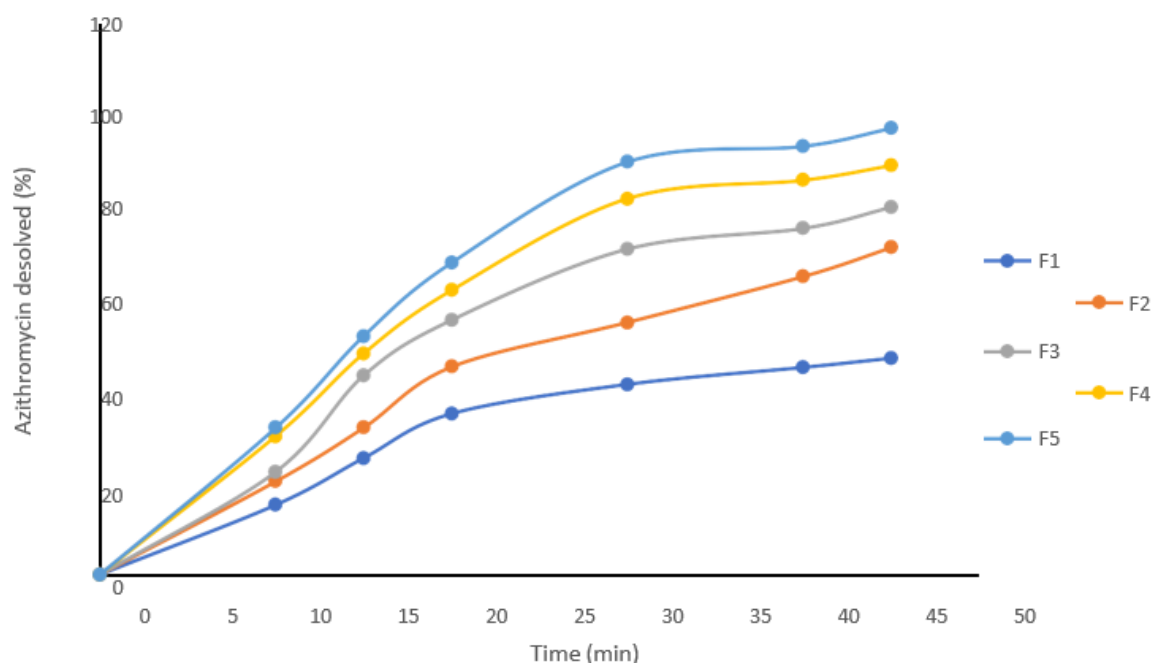


Figure 4: Dissolution profiles of the different formulations of azithromycin tablets

Table 2: Results of storage temperature and relative humidity on azithromycin tablets for 6 and 24 months

Test	Initial results	6 months	24 months
Appearance	White, smooth surface	White, smooth surface	White, smooth surface
Hardness (kg)	4.5	4.4	4.6
Friability (%)	0.2	0.21	0.23
Disintegration time (min)	3.0	3.5	4.0
Dissolution After 30 min (%)	90.0	90.5	89.5
Content assay (%)	100.04	99.2	99.5
Degradation detection by HPLC	Not detected	Not detected	Not detected

Stability

The resulting data of the accelerated and long-term stability studies are presented in Table 2, where the physical-chemical properties tests are carried out on azithromycin tablets prepared as HP- β -CD inclusion complex (F5) and stored under two types of storage conditions.

DISCUSSION

Hydroxypropyl- β - cyclodextrin (HP- β -CD) is a cyclic oligosaccharide having a ring cavity structure with a lipophilic property inside and a hydrophilic head on the outer surface. It can include the azithromycin molecule and form an inclusion complex with a hydrophilic character, making it soluble in water. This character allows HP- β -CD be considered as a solubilizing agent. On the other hand, it protects the guest molecule (such as azithromycin in its solid state) against environmental challenges, increasing its

stability. Inside the body, azithromycin will be released easily from azithromycin (HP- β -CD) inclusion complex, yielding its effect. This inclusion complex does not affect the nature of azithromycin as a drug, since there was no chemical reaction in the inclusion complex. This finding has been approved by the DSC test and the IR spectrum for both pure azithromycin and azithromycin inclusion complex, which showed no change in melting point and the characteristic IR peak of the azithromycin structure.

The applied HPLC method of analysis was developed by using a C18 column for reversed-phase chromatography, and the mobile phase consists of (55: 45) acetonitrile and phosphate buffer solution, pH 8.0, with the addition of ionic substance 0.02 % 1-Octan- sulfonate, which turns the mode of separation to ion-pair reversed-phase chromatography. The concentration of 1-Octane sulfonate can modify the separation; a high concentration shows a longer retention time, but the most

suitable concentration was 0.02%. This analytical method was approved as an HPLC stability-indicating method as it can differentiate between the parent compound of azithromycin (at 6.5 min.), and its degradation product (at 4.0 min). Dissolution test of the different formulations of azithromycin tablets in this work (Table 1) was operated using the USP method, which evaluates the formula of the azithromycin tablet (F4 and F5), prepared with hydroxypropyl- β -cyclodextrin inclusion complex, as higher percentages of drug were released at 45 minutes of operation, and followed by other formulations. This result indicates the potential solubilization effect of hydroxypropyl- β -cyclodextrin. On the other hand, the increment in the percent of release in F5 over that of F4 tablets is attributed to the use of the surface-active agent (SLS) as an ingredient in the preparation of the tablet.

Azithromycin, as an inclusion complex with the hydroxypropyl- β -cyclodextrin, was formulated as a tablet dosage form and subjected to stability test according to the USP program for 2 years at different conditions of storage. The stability of a drug is the resistance of the active constituent against environmental challenges.

Factors such as moisture, light, and heat during storage can significantly affect drug stability. The stability of a drug's raw materials is often different from that of the drug in its pharmaceutical dosage forms, such as tablets or liquids [14]. Drugs in liquid formulations are generally less stable, whereas in tablet form, the drug remains in a solid state, which usually provides better stability. Some manufacturers apply film coating to tablets to offer additional protection against light and moisture.

In this study, the stability tests were performed on azithromycin tablets prepared as plain, uncoated tablets. However, the drug azithromycin is already included in the ring cavity of the hydroxypropyl- β -cyclodextrin, which exhibits good protection against environmental effects and prevents drug decomposition, permitting long-term stability of the drug.

CONCLUSION

The results indicate that azithromycin prepared as an inclusion complex with hydroxypropyl- β -cyclodextrin produced tablets with a high rate of dissolution, which obviously will increase the rate of drug absorption and lead to higher bioavailability. In addition, the inclusion of azithromycin through the structural cavity of

hydroxypropyl- β -cyclodextrin without chemical reaction results in a potent protection of the drug molecules against any environmental challenges, and it exhibits good chemical stability of its active substance. The pharmaceutical properties of azithromycin tablets were also improved due to the application of direct compression in the manufacturing process of tablets using in-home preparation of DC-excipients.

DECLARATIONS

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Ethical approval

None provided.

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Authors contribution

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to the claims relating to the content of this article will be borne by the authors. Kahtan J. Hasson conceived and designed the study, collected and analyzed the data, and Tiba M. Hameed wrote the manuscript. All authors read and approved the manuscript for publication.

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