Research Article

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Therapeutic Delivery

Simultaneous improvement of ketoconazole solubility, antifungal and antibiofilm activity by multicomponent complexation

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Background: A novel multicomponent complex (MC) of ketoconazole (KET) with β-cyclodextrin (β-CD) and *N*-acetylcysteine (NAC) was developed with the purpose of improving the solubility as well as the antifungal and antibiofilm activity of KET against *Candida albicans*. **Results & methodology:** The interactions among the components were studied using nuclear magnetic resonance, thermal analysis, powder x-ray diffraction, infrared spectroscopy and scanning electron microscopy. Phase-solubility studies demonstrated a considerable increase in the solubility of the MC. An enhancement in antibiofilm and antifungal activity of MC was determined against *C. albicans* by XTT assay and microbiological studies. **Conclusion:** This MC, with improvements in the drug pharmaceutical performance, might have an important potential in the development of new pharmaceutical formulations of KET.

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Keywords: antibiofilm activity • antifungal activity • ketoconazole • multicomponent complex • solubility

Ketoconazole (KET, Figure 1A) is an imidazole antifungal, its mechanism of action is to inhibit the production of fungal cell membranes and is utilized to treat a diversity of deep and superficial tissue fungal infections [1]. Chemically, it is a dibasic compound with a solubility profile dependent of pH. KET is very soluble at pH below 3, but its solubility decreases to <1 mg/ml at pH 4 and drops to only 0.002 mg/ml with higher pH. KET is completely soluble at an acidic pH in a typical stomach. Nevertheless, in the small intestine (pH 6–7), precipitation of KET is a concern [2]. The low solubility of KET causes difficulties in the manufacture of pharmaceutical products and restricts its bioavailability and its therapeutic use. One of the prime objectives of the pharmaceutical industry is to increase the solubility of poorly water-soluble drugs, since inadequate water solubility is usually associated with inadequate dissolution profiles and, therefore, limited oral bioavailability [2–4]. The obtainment of a multicomponent complex (MC): drug, cyclodextrins (CD) and a third component, is an approach that has been utilized many times to improve the desired properties of a drug, including those we are looking for in an antibiotic, such as antimicrobial activity and antibiofilm activity [5–11].

Other authors previously reported the formation of binary KET: CD complexes, demonstrating that complexation was an effective strategy to increase the solubility of these drugs [12–15]. In our previous work, we demonstrated that the multicomponent complex formed by KET, β-cyclodextrin (β-CD) and proline was a good strategy to enhance the solubility and antifungal activity of KET [16].

In this work we evaluated the effects of the complexation of KET with β-CD (Figure 1B) and *N*-acetylcysteine (NAC, Figure 1C) on the drug's aqueous solubility, antifungal and antibiofilm activity. NAC was selected because it is known for decreasing biofilm development by a diversity of microorganisms and reducing the secretion of an extracellular polymeric substance, which disrupts the mature biofilm [17,18]. NAC is widely used in medicine by different routes of administration, as it has a very good safety profile [19]. Moreover, it is synergistic with tigecycline [20], colistin [21]. A new strategy at the moment is the search for drug combinations with synergistic action. This combination could reduce the dose of the drug and its toxicity, increasing its effectiveness. Since a disease is caused by multiple effects, the goal of using two drugs at a time is to attack more than one target at a time. Additionally,

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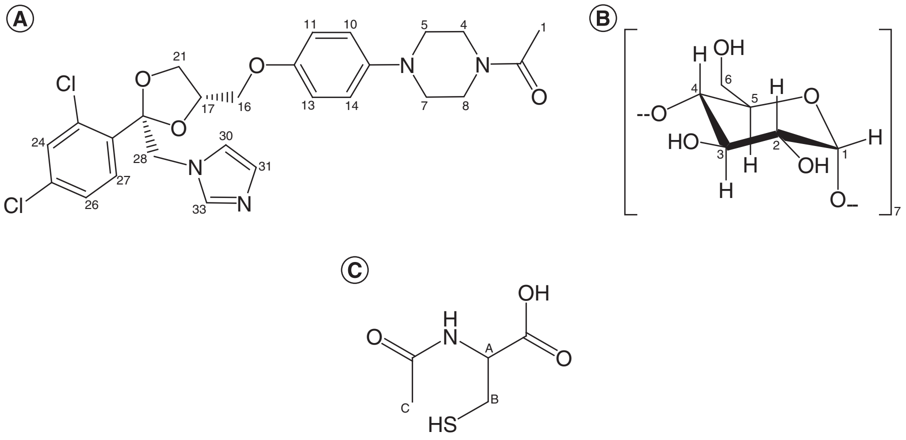

Figure 1. Chemical structure and proton atom assignment scheme of **(A)** KET, **(B)** β-CD, **(C)** NAC.
β-CD: β-cyclodextrin; KET: Ketoconazole; NAC: *N*-acetylcysteine.

the development of multidrug resistance can be diminished by the multitarget strategy [22]. Additionally, NAC has therapeutic effects over an extensive variety of illnesses such as cystic fibrosis, chronic bronchitis, heavy metal toxicity, AIDS, etc. [23]. Since NAC possesses the capacity for modulation of proinflammatory cytokine synthesis and anti-inflammatory activity, it can be used to modulate oxidative stress and diseases related with inflammatory disorders [6,24,25]. These previous scientific contributions make NAC a suitable third component to be used in a formulation. In addition, in our country it is a drug approved by our regulatory agency (ANMAT).

As for the CD and the pharmaceutical industry, these are used to increase the solubility, bioavailability and stability of drugs. On the world market there are currently about 30 products with a complex drug/CD. Therefore, NAC and CD were not subjected to toxicity studies. *C. albicans* is often associated with immunocompromised hosts and nosocomial infections [26]. A problem related to therapeutic failure in antifungal treatments against *C. albicans* infections is the resistance phenomenon, which is very often associated with biofilm formation [27].

C. albicans-related infections frequently involve biofilm formation, a process in which free yeast cells adhere to a living or inert surface, finally resulting in the establishment of a complex 3D structure of fungal cells, filaments and extracellular polymeric matrix. There are notable differences between free and sessile cells, including an augmented tolerance of the latter toward antifungal drugs [28–30].

The experiments included in this work allowed an exhaustive characterization of the multicomponent complex KET:β-CD:NAC in solution (phase-solubility studies and nuclear magnetic resonance [NMR]), in a solid state (thermal analysis, powder x-ray diffraction [PXRD], infrared spectroscopy [FTIR] and scanning electron microscopy [SEM]). In addition to this and, unlike other works on the subject, we included tests of the biological performance (antimicrobial and antibiofilm activity against *C. albicans*) of the developed MC, with which it was possible to confirm that the system has advantages over the pure KET.

Materials & methods

Chemicals & reagents

KET was obtained from Parafarm (Argentina), β-CD (MW: 1135) was kindly supplied by Ashland Argentina SRL, NAC and the D_2O 99.9 atom % D used in spectroscopic studies were purchased from Sigma®. Sabouraud glucose agar was obtained from Britania (Buenos Aires, Argentina). The other solvents and reagents were of analytical chemical grade. The water acquired from a Millipore Milli-Q purification complex was used in all experiments.

Phase solubility analysis

An excess of KET (15 mg), was added to solution containing 5 ml of increasing concentrations of β-CD (0–15 mM) in absence (binary complex) or presence (MC) of NAC (15 mM) by triplicate. The suspensions were placed at 37.0 (±0.1)°C in a shaker (Ferca) at 130 r.p.m. for 72 h, to ensure dissolution equilibrium. At the end of the experiment, the suspensions were filtered (0.45 μm pore diameter, Millipore, USA) and quantified by UV spectrophotometry at 295 nm (Agilent Cary 50 spectrophotometer) [31].

The apparent stability constants (Kc) for the KET complex were determined as a function of the CD concentration added from the intrinsic solubility (S_0) and the value of the slope of the phase-solubility plots using the following equation:

$$K_C = \text{slope}/S_0(1 - \text{slope}) \quad \text{(Eq. 1)}$$

^{1}H NMR assay

A Bruker Avance II 400 spectrometer was used to record the NMR spectra at 298 K using D_2O and tetramethylsilane as an internal standard. The following Equation 1 was used to calculate the changes induced in the ^{1}H chemical shifts for β-CD, originated due to their complexations [32]:

$$\Delta\delta = \delta_{complex} - \delta_{free} \quad \text{(Eq.2)}$$

Preparation of KET:β-CD:NAC solid samples

Freeze dried (FD) KET:β-CD:NAC (1:1:2 M ratio) was obtained by the freeze-drying technique using a Freeze Dryer 4.5, Labconco Corp., MI, USA, with 50 mbar vacuum for 96 h. KET:β-CD:NAC complex (1:1:2) and pure drugs aqueous solutions were prepared, sonicated (2 h), filtered, frozen at -48° until complete solidification and then carry out the freeze-drying process. On the contrary, a physical mixture (PM) was prepared by simple mixing of KET, β-CD and NAC in an agate mortar [32].

Characterization of KET:β-CD:NAC solid samples

FTIR spectroscopy

To investigate the interaction between KET, β-CD and NAC in the complex prepared by PM and FD, the FTIR spectra of the complex and pure compounds (KBr disks) and MC were registered on a Nicolet Avatar 360 FTIR spectrometer in the wavelength range of 400 to 4000 cm^{-1}. The software EZ OMNIC E.S.P v.5.1 software was used to obtain and process all spectra [33].

SEM studies

Morphological structures of the solid samples were examined and photographed at the Laboratorio de Microscopía y Análisis por Rayos X (LAMARX) of the National University of Córdoba, using a SEM (Carl Zeiss Σigma). To increase the conductivity, samples were gold/palladium covered under vacuum using a sputter coater Quorum 150 [33].

PXRD

A PAN analytical X'Pert PRO diffractometer was used to obtain the PXRD diffractograms using Ni-filtered Cu-K α-radiation. The diffractograms were obtained between a 2θ of 4 and 35°, operating at 40 kW and 40 mA, at a scanning rate of 23.0 s/step with a step size of 0.026° [34].

Differential scanning calorimetry

A differential scanning calorimetry (DSC) Discovery series instrument (TA, USA) was utilized to obtain the DSC profiles. The thermal behavior was analyzed by heating *approximately 1 mg of the sample using hermetic aluminum pans with a pin hole by applying a heating stage between 60 and 160°C, at a rate of 10°C/min and under a nitrogen flow (50 ml/min) [34]. The cooling rate is not included because the effect of cooling on the sample was not studied in this work.

Antimicrobial activity assay

Since KET is practically insoluble in water we decided to determine the antimicrobial activity by the method of diffusion from agar plates instead of the dilution method. The antimicrobial activity of free or complexed (obtained by FD) KET (7 µg) was determined according to the method reported by Marzouk *et al.* with some modifications [15]. Furthermore, β-CD and NAC at the concentration found in the complex were used as a control. An inoculum of *C. albicans* clinical strains (256 or 216) diluted to approximately 10^7 colony forming units per milliliters (CFU/ml^{-1}) were spread on the surface of agar Saboureaud plates. It is important to use clinical strains to test multicomponent complexes instead of using ATCC strains, considering the future therapeutic application of the developed system. A disk of 6 mm of sterile Whatman N° 1 filter embedded in 10 µl of a complexed or free

KET solution (7 µg) were added on an agar culture medium. Pure β-CD and NAC were used as a negative control. After 18 h of incubation at 37°C, the plates were observed and the inhibition zone diameters measured. The assay was made by triplicate.

Antibiofilm activity, XTT assay

Biofilm formation & treatment

Biofilms were prepared in 96-well culture plates containing 200 µl of 1 × 10^6 *C. albicans* ml^{-1} in Saboreaud glucose broth complemented with 0.5% glucose to promote biofilm development. Then, plates were incubated overnight at 37°C on an orbital shaker at 130 r.p.m. Finally, free cells were removed with care and each well was washed twice with 200 µl of PBS. The biofilms were incubated in fresh nutrient medium containing KET (250 µg ml^{-1} = 10 × MIC) and KET:β-CD:NAC (1:1:2 molar ratio relation 250 µg ml^{-1} of KET). XTT assay was performed after 24 h of exposure to antimicrobial agents (control and tested samples) [35].

XTT assay

The XTT reduction assay was utilized by the quantification of biofilm metabolic activity. After exposure to KET, its complexed form and PBS (control), the biofilms were washed with PBS. Before, each well was added with 200 µl of a solution with XTT (200 mg l^{-1}) and PMS (20 mg l^{-1}). The plates were incubated in the light absence at 37 ± 0.5° for 3 h. Finally, the absorbance was determined at 490 nm in a Biotek Synergy 2 multimode microplate reader [36].

Statistical analysis

The results were statistically analyzed by Student's *t*-test. The studies were repeated at least three-times and the means and standard deviations were calculated. Differences were considered to be significant at $p < 0.05$.

Results & discussion

Characterization of KET:β-CD:NAC complex in solution

Phase solubility analysis

The phase solubility analysis of KET:β-CD and KET:β-CD:NAC showed a linear increase in the solubility of KET with respect to the concentration of β-CD (Figure 2). Consequently, they can be considered as AL-type diagrams [31] suggesting the formation of 1:1 complexes in the absence and in the presence of NAC. The apparent stability constant value calculated from the phase solubility analysis was 51 ± 4 M^{-1} (pH ~3.5) with an efficiency of solubility (ES, S_{max}/S_0) of 459. This K_C value was less than that corresponding to the binary KET:β-CD complex (4350 ± 54 M^{-1}, pH ~6.8, ES = 54) which is similar to that obtained by Demirel *et al.* (3706 M^{-1}) [12]. According to the KET ionization, a decrease with pH and consequently, the interaction with β-CD, is better at pH 6.8 in the binary complex. On the other hand, organic acid has been used as a pH modifier to improve the KET solubility [2]. In this work, NAC acts as a pH modifier, synergizing the increase in KET solubility with β-CD, causing a marked rise in the ES. An increase in the solubility of KET it is sought with the formation of the MC.

^{1}H NMR spectroscopic studies

NMR is an effective technique to elucidate the interactions between drugs and CD and to confirm the formation of an inclusion complex. The establishment of an inclusion complex induces changes in the chemical shifts of the drug and CD [12].

To study the interactions between the three compounds, the chemical shifts of β-CD protons in a free and complex form were evaluated (Figure 1 SI). The chemical shifts of KET could not be analyzed due to the poor solubility of the drug in D_2O. As seen in Table 1, the β-CD protons H3 located inside the cavity, suffer major changes in the chemical shifts in the presence of KET and NAC. The H3 proton shielding suggests the insertion of a portion of the KET molecule rich in π electrons (such as an aromatic ring) into the β-CD cavity. On the other hand, protons located outside the cavity (H1, H2 and H4) only showed minor variations.

Characterization of KET:β-CD:NAC complex in solid state

FTIR

To confirm whether KET interacts with NAC and β-CD in solid state, the FTIR spectra of the pure compounds and the solid complex prepared by FD and PM were performed.

As can be seen in Figure 3A, the FTIR spectrum of pure KET was characterized by its absorption peaks at

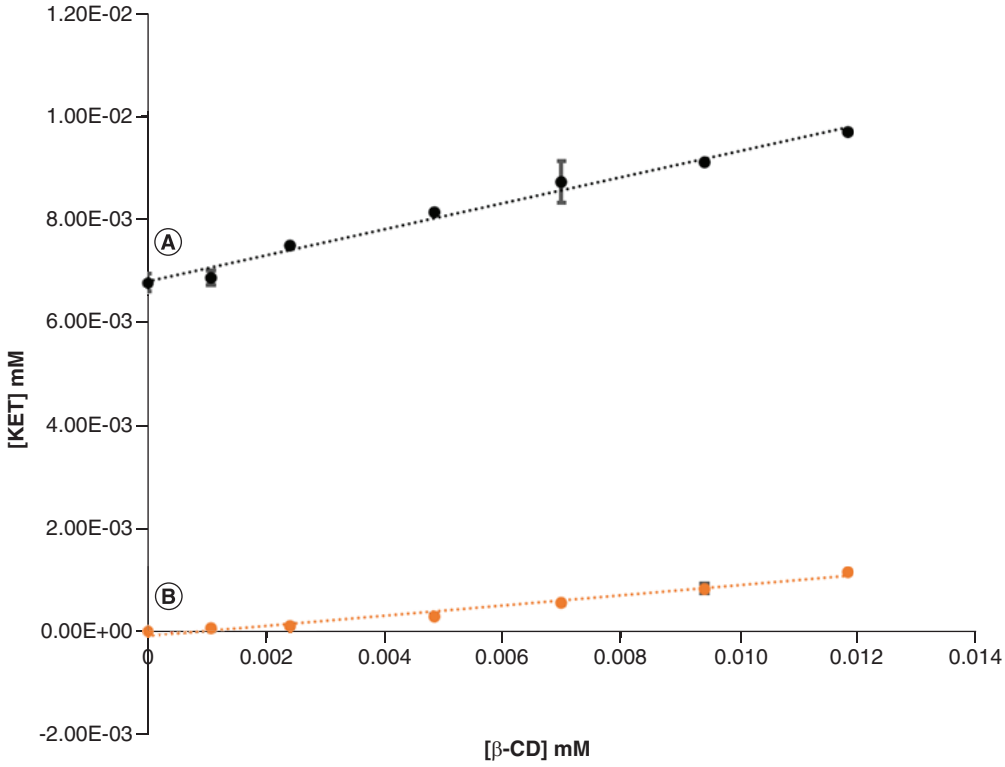

Figure 2. Phase solubility analysis of the BC **(A)** KET:β-CD and MC **(B)** KET:β-CD:NAC.
β-CD: β-cyclodextrin; BC: Binary complex; KET: Ketoconazole; MC: Multicomponent complex.

Table 1. Chemical shifts displacements for the protons β-cyclodextrin in complex forms.

β-CD protons	δ (p.p.m.)	δ (p.p.m.) KET:β-CD:NAC	Δ δ (p.p.m.)
H1	5.07	5.07	0.00
H2	3.65	3.68	0.03
H3	**3.96**	**3.80**	**-0.16**
H4	3.58	3.62	0.04
H5	**3.87**	**Overlapped**	**-**
H6	3.88	3.90	0.02

β-CD: β-cyclodextrin.

3114 cm^{-1} (aromatic ring), 2884 and 1455 cm^{-1} (alkanes), 1645 cm^{-1} (carbonyl), 1242 and 1104 cm^{-1} (C=O bond), 1292 cm^{-1} (cyclic amine), 1416 and 1201 cm^{-1} (aliphatic amine).

The IR spectrum of β-CD (Figure 3B) is characterized by having an intense band of absorption between 3500 and 3300 cm^{-1}, which is caused by the hydroxyl groups of the macromolecule. Then, we can see bands located at 2927 cm^{-1}, which are due to -CH and -CH2 groups, as well as between 1158 and 1030 cm^{-1}, due to the stretching vibrations of the primary and secondary hydroxyl groups. While in the case of NAC (Figure 3c), representative bands were observed at 1716 cm^{-1} (carboxyl), 2546 cm^{-1} (sulfhydryl) and 3375 cm^{-1} (secondary amine).

The spectrum of the KET:β-CD:NAC PM (Figure 3D) demonstrated a superposition spectrum of the three pure compounds indicating the absence of an intermolecular interaction in this complex.

In the complex prepared by FD (Figure 3E), the absorption peak at 3114 cm^{-1} (aromatic ring) of KET disappears, the peak of 2884 cm^{-1} decreases in intensity, signals between 2800 and 3000 cm^{-1} undergo shifts and enlargements and the peak of 1645 cm^{-1} (carbonyl) undergoes a widening. As for the peaks of the NAC, the absorption peak at 3373 cm^{-1} disappears and the absorption peak at 2546 cm^{-1} decreases markedly in intensity. These findings suggest that some interactions among the components occurred during the complex formation [12].

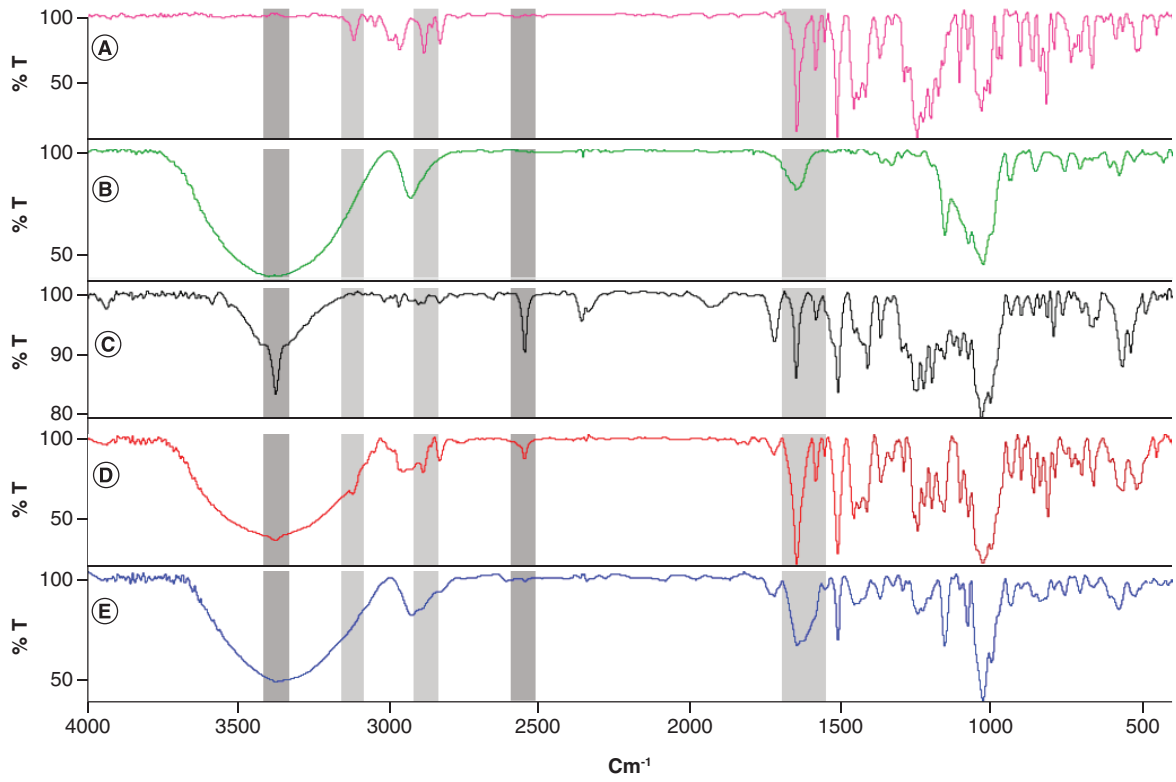

Figure 3. FTIR spectra of: **(A)** KET, **(B)** β-CD, **(C)** NAC, **(D)** KET:β-CD:NAC PM system, **(E)** KET:β-CD:NAC FD system. β-CD: β-cyclodextrin; FD: Freeze dried; FTIR: Infrared spectroscopy; KET: Ketoconazole; NAC: *N*-acetylcysteine; PM: Physical mixture.

DSC & PXRD

Figure 4 shows the DSC curves of the pure components and ternary complex (PM and FD). It is shown that NAC displays an endothermic peak at 113°C and KET presents a sharp endothermic peak at 150°C corresponding to the melting point of the drug. For β-CD, an endothermic peak between 50 and 120°C can be observed, attributable to dehydration [37]. In the analysis of DSC of KET:β-CD:NAC PM, an endothermic peak was observed between 34 and 103°C, attributable to dehydration and a little endothermic peak at 112.6°C, attributable to the melting process of NAC (indicated with the arrow). On the other hand, the FD complex did not show any endothermic peak (at 113°C or 150°C), this last phenomenon can be attributable both to drug amorphization and/or inclusion complex formation [12].

Thermal analysis findings are reinforced by results obtained by the PXRD measurements.

The PXRD (Figure 5) showed crystalline solid patterns of the pure components and of the complex obtained by PM, while the KET:β-CD:NAC FD complex presented an amorphous pattern. Both results (thermal analysis and PXRD) showed that the new solid complex prepared by FD is a phase of amorphous nature, different from the KET:β-CD:NAC complex obtained from the PM where the pure components do not interact. Similar results were obtained by Balata *et al.* in their binary complex KET:β-CD [37].

SEM

SEM microphotographs was utilized to analyze the morphological characteristics of the pure compounds, the PM and the FD complexes (Figure 6). PM, prepared with pure powders, presented the crystals of KET mixed with NAC and β-CD, confirming that the drug continues to crystalline and reveals that there is not an existing interaction in the solid complex.

Considering the severe change in the morphology of the solid particles obtained by the FD method in which the original shape of the three components disappeared, together with their differences with the PM, reveals a solid state interaction [38].

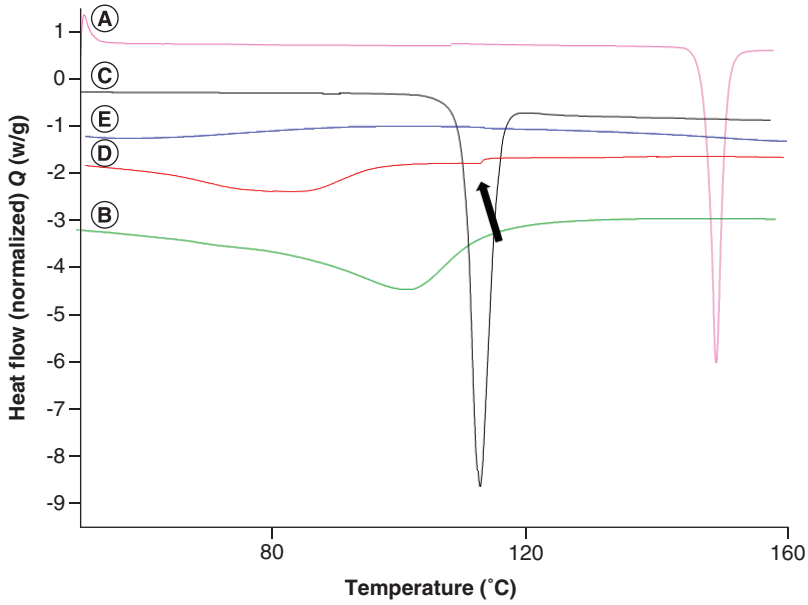

Figure 4. DSC curves of **(A)** KET, **(B)** β-CD, **(C)** NAC, **(D)** KET:β-CD:NAC PM system and **(E)** KET:β-CD:NAC FD system.
β-CD: β-cyclodextrin; DSC: Differential scanning calorimetry; FD: Freeze dried; KET: Ketoconazole; NAC: *N*-acetylcysteine; PM: Physical mixture.

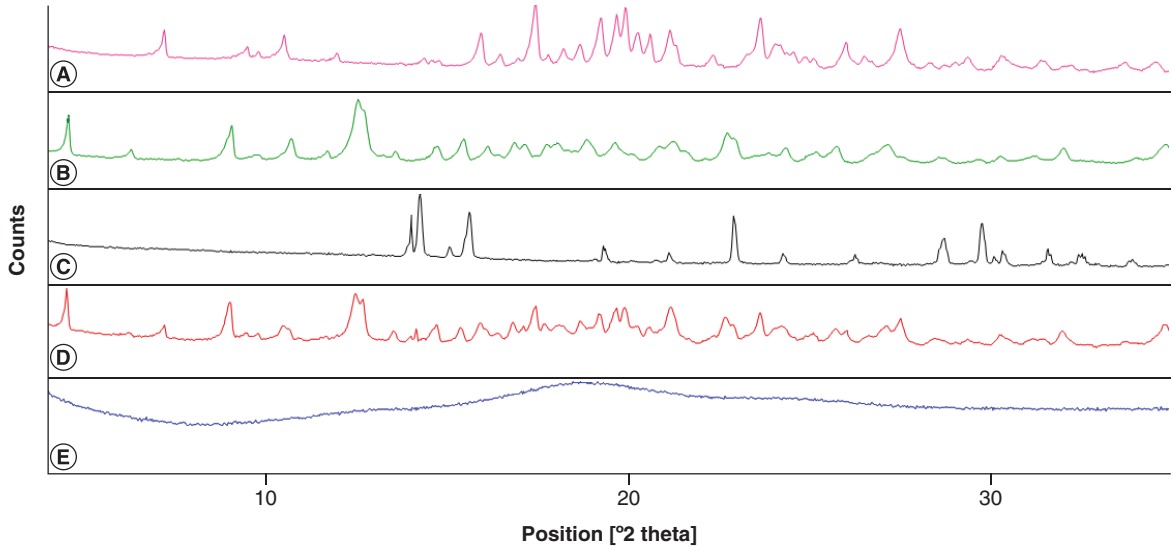

Figure 5. PXRD of **(A)** KET, **(B)** β-CD, **(C)** NAC, **(D)** KET:β-CD:NAC PM system and **(E)** KET:β-CD:NAC FD system.
β-CD: β-cyclodextrin; FD: Freeze dried; KET: Ketoconazole; NAC: *N*-acetylcysteine; PM: Physical mixture; PXRD: Powder x-ray diffraction.

All these change, observed and discussed in FTIR, DSC, PXRD and change in the morphology in SEM of MC confirmed the complex formation necessary for increasing solubility.

Antimicrobial activity

The antifungal activity of KET, β-CD, NAC, KET:β-CD and KET:β-CD:NAC was evaluated by the diffusion agar method (standardized by CLSI) described by Marzouk *el al.* [15] against clinical strains of *C. albicans* 216 and 256. β-CD and NAC used as a negative control did not display inhibition zones (data not shown). Figure 7 showed the inhibition zones and in Figure 7B we can view the antifungal results from pure KET and KET:β-CD and KET:β-CD:NAC complexes. The results showed that KET:β-CD and KET:β-CD:NAC complexes have a more

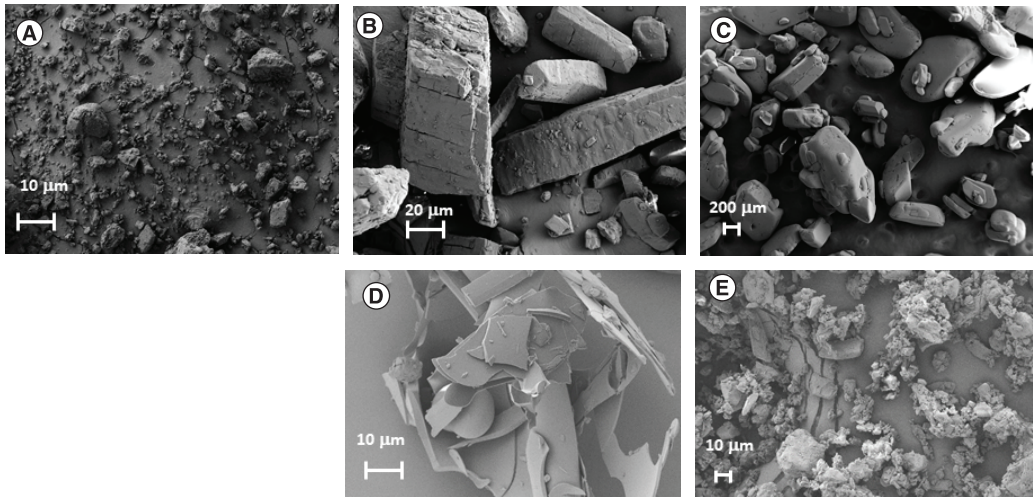

Figure 6. SEM of **(A)** KET, **(B)** β-CD, **(C)** NAC, **(D)** KET:β-CD:NAC FD system and **(E)** KET:β-CD:NAC PM system.
β-CD: β-cyclodextrin; FD: Freeze dried; KET: Ketoconazole; NAC: *N*-acetylcysteine; PM: Physical mixture; SEM: Scanning electron microscopy.

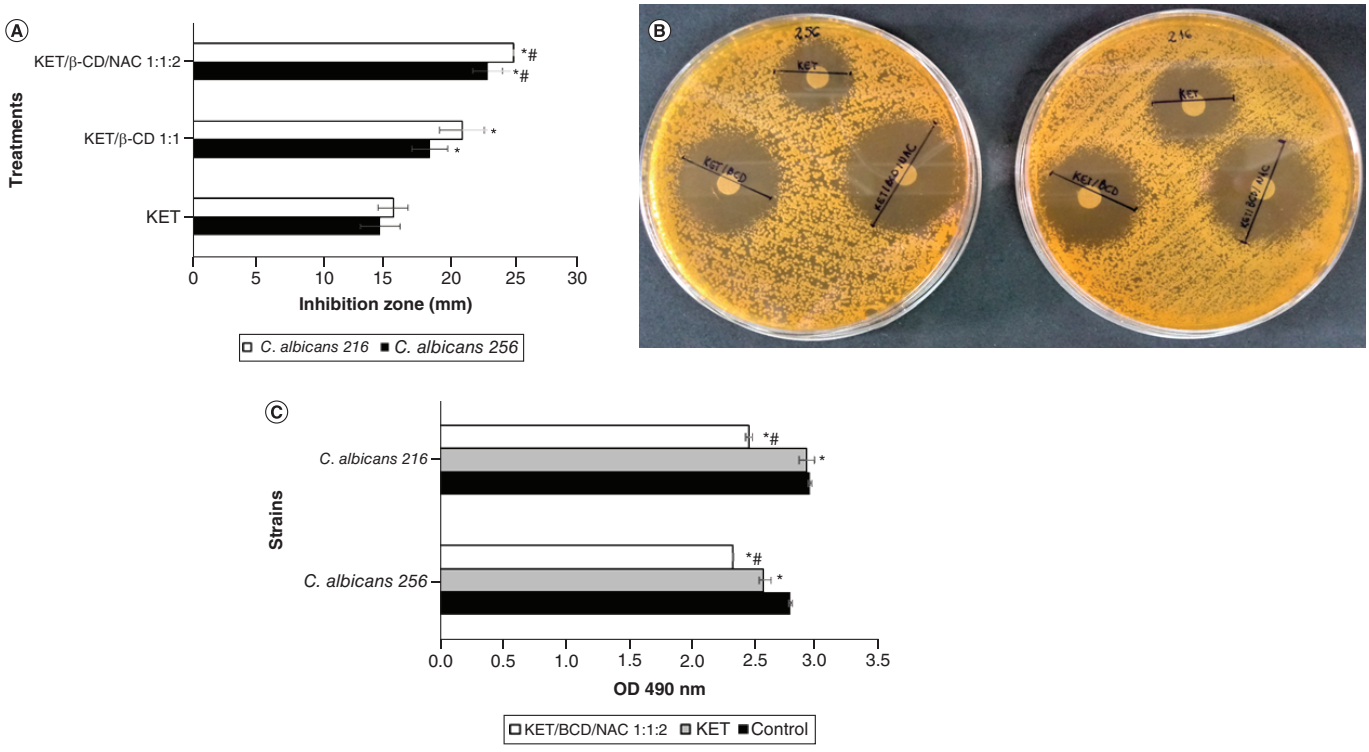

Figure 7. **(A)** Histogram of the mean values of the inhibition zones diameters of KET, KET:β-CD and KET:β-CD:NAC. *$p < 0.05$ with respect to KET, #$p < 0.05$ compared with KET:β-CD, **(B)** Images of the inhibition zone from *C. albicans* 256 and *C. albicans* 216, **(C)** Effect of KET and KET:β-CD:NAC on metabolic activity determined by XTT assay of *C. albicans*. Error bars represent standard deviation. *$p < 0.05$ with respect to the control, #$p < 0.05$ compared with KET.
β-CD: β-cyclodextrin; KET: Ketoconazole; NAC: *N*-acetylcysteine.

potent antifungal activity than KET Figure 7A, which may be due to the complexation of KET that considerably improves its water solubility, this can increase the available KET concentration, which coincides with what was published by Adachi *et al.* [2]. Moreover, the multicomponent complex showed an activity significantly greater than the binary complex, highlighting the relevance that the addition of NAC has for the antifungal activity of

this complex. When we carried out this investigation, we did not consider it necessary to carry out the MIC and minimum factionary concentration because our method showed a better performance for the MC. However, we will conduct these tests in future work. NAC has antimicrobial activity, though it is not an antimicrobial drug. Since 1997, when the *Staphylococcus epidermidis* biofilm was inactivated for the first time with NAC [39], numerous studies have established the effectiveness of NAC in decreasing biofilm formation of a wide range of pathogen microorganisms [20,40,41]. NAC has antimicrobial activity against Gram-positive and Gram-negative microorganisms. Although the mechanism of action of NAC is not clearly understood, the antioxidant action of its -SH residue is known, which removes free radicals and helps the immune system to break disulfide bridges. It also has antibiofilm activity, decreasing microbial adhesion, extracellular polysaccharide production and delaying the structure of mature biofilms [42]. This shows us that the selection of the third component was adequate because it synergizes KET's antifungal activity as well as improving its solubility.

Antibiofilm activity, XTT assay

First, we include a control, to which PBS is added, because we are interested in knowing firstly if the strains are biofilms-forming and secondly if the complex eradicates the biofilm with respect to the control and also to evaluate what happens between KET and the multicomponent complex. Since the KET:β-CD:NAC multicomponent complex showed a greater antimicrobial effect with respect to KET:β-CD binary complex, its activity against biofilms was tested. Figure 7C presents the results determined by the XTT reduction assay expressing the decrease in the biofilm viability, after treatment with KET and the multicomponent complex. These results showed that the KET:β-CD:NAC complex caused a significant reduction in cellular metabolic activity. In general, it is accepted that a weakening may be a possible tool for the human immune system to fight infections related to biofilm and this association is able to decrease *C. albicans* biofilm viability. Dispersal of biofilm, that is, changing the biofilm microbials to free cells is another major focus for antimicrobial drugs. These free cells will be more susceptible to antimicrobials that were before unable to infiltrate the biofilm; NAC disrupts the biofilm and facilitates the arrival of antimicrobials to the fungal cell [42,43]. Our third component also allows MC to have better antibiofilm activity than KET.

Summarizing, in a first stage we analyzed the possibility of forming MC between KET, CD and NAC in aqueous solution and in the same test we determined if the formation of complexes allowed an adequate increase in the solubility of the drug (phase-solubility studies). The characterization in solution was completed with NMR. In a second stage, we studied whether the multicomponent complex could be obtained in the solid state using a technique suitable for industrial use (lyophilization). The solid obtained was characterized using various analytical techniques (thermal analysis, PXRD, FTIR and SEM) that corroborated the obtaining of a new solid with amorphous characteristics. The third stage consisted of determining how the formation of this MC affected or not the microbiological behavior of the drug (antimicrobial and antibiofilm activity). As a limitation of our work, toxicity studies of β-CD and NAC were not performed and we will include this in future research. In other words, we have used the traditional scheme used in the synthesis and characterization of a supramolecular system [7,11,12].

Conclusion

This work presents the preparation and evaluation of a KET:β-CD:NAC MC. The formation of this complex in aqueous solution was established by phase solubility analysis and NMR technique. In a solid state, the MC was prepared by the FD method. The FTIR, studies exhibit a significant molecular interaction among the components. The SEM microphotographs evidence marked changes in the size and morphology of the particles of the MC compared with the pure components, while DSC and PXRD techniques showed that the solid obtained by the FD method was amorphous. Finally, the new complex was able to significantly improve the antifungal activity of KET and reduce the fungal viability in the biofilms of *C. albicans*. The greater antifungal activity and the better activity against the biofilms of *C. albicans* are a relevant aspect of this multicomponent complex, in the face of a current public health problem, such as the resistant microorganisms and their biofilm formation capacity. Based on these results, it can be concluded that the KET:β-CD:NAC will be valuable in optimizing the properties of new KET pharmaceutical formulations.

Future perspective

In the near future, pharmaceutical formulations of KET would be optimized in terms of the drug solubility and antifungal performance by means of complex formation, thus leading to a higher therapeutic effectiveness of the drug.

Summary points

Solubility analysis

- The highest efficiency of solubility (S_{max}/S_0) was obtained with ketoconazole (KET):β-cyclodextrin (β-CD):*N*-acetylcysteine; (NAC) multicomponent complex (MC).
- KET:β-CD:NAC showed a solubility of approximately eight-times as much as the KET:β-CD at the same β-CD concentration (Figure 2).

Nuclear magnetic resonance studies

- The shielding effect observed for the H3 proton can be attributed to the insertion of a portion of the KET molecule rich in π electrons into the β-CD cavity.

Infrared spectroscopy studies

- The modification of some bands in the complex prepared by freeze dried indicate the formation of a MC by the intermolecular interactions observed among its groups.

Thermal analysis, x-ray diffraction & electronic microscopy

- These studies provide evidence for a strong solid-state interaction of KET with β-CD and NAC obtained by freeze drying.

Antimicrobial & antibiofilm activity

- Both studies showed a better antifungal performance of the KET:β-CD:NAC MC.

Financial & competing interests disclosure

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