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Research Article

A novel formulation of ketoconazole entrapped in alginate with anionic polymer beads for solubility enhancement: Preparation and characterization

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Abstract

Ketoconazole has low solubility in intestinal pH, whereas drug absorption is largest in the small intestine, which can reduce the bioavailability of the drug. Alginate can be combined with a suitable polymer and cross-linked with divalent ions and another polymer to enhance the solubility of the drug. Ketoconazole could be loaded into a matrix polymer consisting of alginate and anionic polymer through hydrogen bonds formed with the N atom of the ketoconazole. The method employed to produce ketoconazole beads involved ionic gelation with $CaCl_2$ as a cross- linking agent, and various polymer combinations were used: alginate 100:0 (AL100), alginate:pectin 75:25 (AP75) and 50:50 (AP50), alginate:gum acacia 75:25 (AG75) and 50:50 (AG50), and alginate:carrageenan 75:25 (AK75) and 50:50 (AK50). The beads were characterized by using differential scanning calorimetry (DSC), scanning electron microscopy (SEM), Fourier transform infrared (FT-IR), X-ray diffraction (XRD), swelling study, in vitro drug release study, and solubility determination. The incorporation of ketoconazole into combination matrices of AL100, AG75, AP75, AP50, and AK75 resulted in significantly higher solubility in FaSSIF-2X (Fasted State Simulated Intestinal Fluid) at pH 6.5 compared to pure ketoconazole.

Keywords

anionic polymer, solubility, encapsulated, beads, ketoconazole

Introduction

Ketoconazole is classified as BCS Class IIb due to its low solubility. Its solubility increases in low pH or stomach pH (pH 1-3) (Ullrich and Schiffter 2018) but decreases in basic or intestinal pH at neutral levels (Spadari et al. 2017). While drug absorption occurs in the small intestine, the poor solubility of ketoconazole in base pH environments leads to reduced oral bioavailability. Several techniques have been developed to enhance the solubility of ketoconazole, including the formation of solid dispersion and inclusion complexes (Balata et al. 2010; Mahore et al. 2019), co-amorphous formation (Hatanaka et al. 2021), co-crystal formation (Hiendrawan et al. 2015; Liu et al.

2016; Indra et al. 2019; Vasoya et al. 2019; Martin et al. 2020), multicomponent complexation (Zoppi et al. 2020), binary mixture formation (Soltanpour and Nazemi 2018; Hatefi et al. 2019; Barzegar-Jalali et al. 2020; Zadaliasghar et al. 2020), and the use of polymeric formation nanostructure (Hosmani and Thorat 2011; Jose and Narayana Charyulu 2016). However, these techniques require complex methods and may involve chemical materials that can be toxic to the body.

Alginate is widely used to produce cross-linked hydrogels for drug delivery. Polymer-based delivery systems can address certain limitations by improving the solubility of the drug. However, biopolymer materials are often characterized by poor mechanical properties. Nevertheless, cross-linked alginate is usually fragile (Nayak et al. 2013). Therefore, alginate can be mixed with a suitable polymer and cross-linked with divalent ions to overcome this limitation through ionotropic gelation (Nayak and Pal 2011). Several studies have demonstrated that adding alginate to other polymers can enhance structural and functional properties. This method involves surrounding and entrapping the active molecule within a semi-permeable biopolymeric matrix (Lozano-Vazquez et al. 2015). Ionotropic gelation initiates cross-linking by interacting the ionic polymer with the oppositely charged ions. The beads rapidly form when an alginate

solution is dropwise added to a divalent cation solution. Ionotropic gelation is widely used for producing beads and can be achieved by injecting the formulation solution through a syringe into a dilute $CaCl_2$ solution (Velings and Mestdagh 1995).

Valence cations, such as calcium from $CaCl_2$ salts, can interact with the negative groups of polymers, including hydroxyl, carboxyl, amino, and sulfate groups, through hydrogen bonds. The addition of calcium ions can enhance the mechanical strength of the polymer combination (Annisa et al. 2021). Following our previous preliminary research, the addition of $CaCl_2$ produces a synergistic effect using viscosity and gel strength data in combination with the polymer alginate-gum acacia, alginate-pectin, and alginate-carrageenan in a specific ratio (Annisa et al. 2022b). Including gum acacia in the alginate solution helps reduce the side-by-side aggregation of the alginate egg-box structure caused by the presence of Ca^{2+}. This aggregation can lead to a loss of the swelling capacity of calcium alginate. Gum acacia is an ampholytic polymer, allowing it to attract alginate molecules through electrostatic forces (Tsai et al. 2017). The combination of alginate and pectin can form a hydrogel. In the presence of Ca^{2+}, pectin can form an egg-box structure similar to alginate. The formation of the egg box structure occurs through the interaction of -COOH groups with Ca^{2+}, resulting in cross-linking between separate polymer chains and reducing electrostatic repulsion between the polymers. The main driving force for forming egg-boxes is a combination of alginate and pectin electrostatic interaction (Cao et al. 2020). The combination of alginate and carrageenan can form hydrogels with Ca^{2+} through the interaction of carboxyl groups from alginate and sulfate and carboxyl groups from carrageenan (Baek et al. 2019). The interaction of carrageenan with Ca^{2+} occurs through electrostatic attraction, forming intramolecular bridges between the oxygen from OSO^{3-} of carrageenan. Subsequently, a cross-linking network is formed between carrageenan macromolecules. Ketoconazole has two weak basic groups with pKa values of 6.5 (imidazole) and 2.9 (piperazine). The most potent ketoconazole interactions occur at the N atom in the imidazole ring and the amide carbonyl group (Basheer et al. 2015). The negatively charged groups of the polymer can interact with ketoconazole through hydrogen bonds from the N atom of the ketoconazole imidazole ring or the ketoconazole acetyl group (Martin et al. 2013; Chen et al. 2020).

In this study, the development of ketoconazole entrapped in alginate-pectin, alginate-gum acacia, and alginate-carrageenan-based hydrogel beads was prepared by ionic gelation to increase the solubility of ketoconazole. The method employed to produce ketoconazole beads involved ionic gelation with $CaCl_2$ as a cross-linking agent, and polymers combination were used: alginate 100:0 (AL100), alginate:pectin 75:25 (AP75) and 50:50 (AP50), alginate:gum acacia 75:25 (AG75) and 50:50 (AG50), and alginate:carrageenan 75:25 (AK75) and 50:50 (AK50). The beads were characterized using Differential Scanning Calorimetry (DSC), Scanning Electron Microscopy (SEM), Fourier Transform Infrared (FT-IR), X-ray diffraction (XRD), swelling study, and in vitro drug release study.

Material and methods

Ketoconazole was obtained from PT. Kimia Farma, Indonesia. Sodium alginate (origin from brown seaweed, viscosity of 1% is 150 mPa, pH 7.3) was manufactured by Shandong Jiejing Group Corporation, gum acacia (origin from the stems and branches of Acacia Senegal, viscosity of 1% is 12.4 mPa, pH 5.0) by Spectrum Chemical MFG Corporation, pectin (origin from citrus peel, viscosity of 1% is 8.7 mPa, pH 3.4) by Danisco USA Inc, kappa-carrageenan (origin from red algae, viscosity of 0.5% is 35.7 mPa, pH 7.9) by Top P&P Co, $CaCl_2$ by PT. Smart-Lab Indonesia and deionized water was supplied from CV. Alfa Kimia, SGF (Simulated Gastric Fluid) medium consisting of NaCl, HCl, and water, FaSSIF-2X (Fasted State Simulated Intestinal Fluid) consisting of NaH_2PO_4, NaCl, Lecithin, Sodium taurocholate, and water. This study used FaSSIF-2X as a double concentration of FaSSIF composition. All chemical materials were supplied from Merck.

Preparation of SGF medium

SGF (Simulated Gastric Fluid) medium was prepared by dissolving 1.6 g of pepsin and 1.0 g NaCl in 500 mL water, then stirring for 30 minutes until homogen. Add 3.5 mL of HCl to the mixture, stirring for 30 minutes. Then, measure the pH of the mixture and adjust with 2M NaOH to get a pH of 2.0. The mixture was put into a 500 mL mea-

suring flask and added with distilled water until the mark, then sonicated for 10 minutes.

Preparation of FaSSIF-2X medium

FaSSIF-2X (Fasted State Simulated Intestinal Fluid) medium was prepared by dissolving 4.43 g NaH_2PO_4 and 2.265 g NaCl into 500 mL water, stirring for 10 minutes until homogen. Added 0.24 g of lecithin to the mixed solution, then stirred for 20 minutes until homogeneous. Added 0.8 g Sodium Taurocholate to the mixture, then stirred for 20 minutes until homogeneous. Then, measure the pH of the mixture and adjust it with 2M NaOH to get a pH of 6.5. The mixture was put into a 500 mL volumetric flask, distilled water was added to the mark, and then sonicated for 10 minutes.

Preparation of beads ketoconazole

The polymer solution was prepared by accurately weighing1.0 g for alginate (AL) / gum acacia (GA) / pectin (PC) powder and 0.5 g carrageenan (CR) powder into 100 mL deionized water with continuous stirring for 30 min, 50 °C to obtained alginate 1%w/v, gum acacia 1%w/v, pectin 1%w/v, and carrageenan 0.5%w/v. The alginate solution was mixed with another polymer solution in the following ratios:25:75, 50:50, 75:25, and 100:0 v/v to obtain a final mass of 15.0 g (Table 1). The mixture was stirred for 1 hour at 50 °C. The 5% w/v ketoconazole in methanol was added to the mixed polymer solution of 10 mL under continuous stirring for 1 hour. Subsequently, the mixed solution was dropwise added using a syringe into 1 M calcium chloride ($CaCl_2$) under continuous magnetic stirring. The droplets were retained overnight to complete the curing reaction and produce rigid beads. The wet beads were collected and washed with deionized water three times. The method for collecting the beads used filter paper Whatman No.42. Finally, the wet beads were dried in the oven overnight at 37 °C. The preparation of ketoconazole beads is presented in Fig. 1.

Table 1. Description of the name beads.

Name of beads	Composition	Ratio
AL100	Alginate	100:0
AG75	Alginate:Gum Acacia	75:25
AG50		50:50
AG25		25:75
AP75	Alginate:Pectin	75:25
AP50		50:50
AP25		25:75
AK75	Alginate:Carrageenan	75:25
AK50		50:50
AK25		25:75

Size measurements of beads

The physical size of beads was determined by directly looking at 5 pieces of beads at the scale bar of rules.

Scanning electron microscopy (SEM)

The scanning electron microscopy (SEM) of bead imaging was performed using a JSM- 6510LA instrument, JEOL Ltd Japan. The SEM instrument has a resolution of 1–10 nm, allowing for detailed observation of the surface morphology of the samples. The samples were placed on metal stubs, and the photomicrographs were taken at different magnifications.

Fourier transform infrared (FT-IR)

The beads were analyzed by Thermo Scientific Nicolet i50 (Madison, USA) with ATR accessories. Spectral scanning was measured between the wavelength region 4000 to 600 cm^{-1}.

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) measurements were performed using the DSC-60 Plus Shimadzu, Japan. The samples were heated in a closed aluminum pan start-

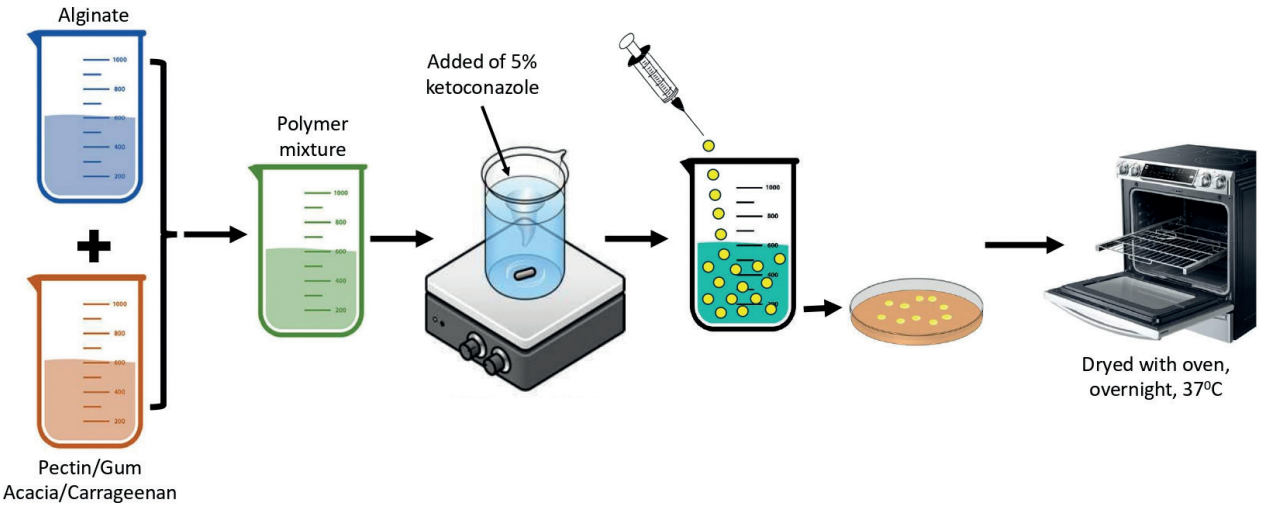

Figure 1. Preparation of ketoconazole beads.

ing from 30 °C with a flow rate of 10 °C/min. The measurements were carried out to investigate the thermal properties of the samples.

X-ray diffraction (XRD)

X-ray diffractograms of the samples were recorded using an X-ray diffractometer (Rigaku Miniplex600, Tokyo, Japan). The instrument was operated with a continuous scanning range of 4 to 80 thetas.

Drug loading

Samples of ketoconazole beads weighed approximately 50 mg and were then crushed with a mortar and stamper. Methanol was added as much as 10 mL, then stirred for 1 hour, 300 rpm. Determination of ketoconazole concentration used the HPLC analysis method. The drug loading of ketoconazole in alginate/pectin, alginate/gum acacia, or alginate/carrageenan beads was calculated by the formula shown in Eq. 1.

$$\%DM\frac{L_A}{L_T}\times 100 \quad (1)$$

where L_A is the actual quantity of drug in beads and L_T is the initial weight of beads.

Swelling study

Swelling tests were conducted using SGF (Simulated Gastric Fluid) at pH 2.0 and FaSSIF- 2X (Fasted State Simulated Intestinal Fluid) at pH 6.5. Three dried ketoconazole beads (W_d) were placed in the wells of a 24-well microplate containing 2 mL of media. The microplate was placed in the incubator shaker at 37 °C for 5 hours. The beads in the hole were weighed at 1, 2, 3, 4, and 5 hours. The beads were drained using filter paper before being weighed (W_t). The swelling ratio (%SR) was determined using Eq. 2.

$$\%SR\frac{Wt-Wd}{Wd}\times 100\% \quad (2)$$

In vitro drug release study

Ketoconazole sample beads were weighted, equivalent to approximately 10.0 mg of ketoconazole. We selected 10 mg ketoconazole because the dissolution medium we used was 1/20 of the normal volume medium, so we scaled down the dose 1/20 too, which was 200 mg to 10 mg. The method for dissolution testing used magnetic stirring because we used a small-scale approach. The sample was dissolved in 50 mL of SGF at pH 2.0, which served as the dissolution medium. Stirring was carried out using a stirrer at 100 rpm. At 15, 30, 60, 90, 120, 150, and 180 minutes, 500 µL samples were withdrawn and transferred to 1.5 mL microtubes. The volume was refilled with 500 µL of SGF pH 2.0 as much after each sampling. Acetonitrile was added to the samples in a 1:1 ratio. Determination of ketoconazole concentration used the HPLC analysis method.

According to the dissolution profile data, a kinetic model analysis was conducted to determine the most suitable kinetic model. Dissolution program performed by DDSolver modeling using a non-linear regression approach, including first-order, Korsmeyer-Peppas, Higuchi, and Hixson-Crowell models. The input for dissolution data consisted of corrected ketoconazole dissolution levels (%) and dissolution time (minutes). Determination of the dissolution model of ketoconazole beads uses the parameter R^2, where the highest R^2 value indicates the best fit of the model (Zhang et al. 2010). Analysis of dissolution efficiency (DE) was defined as the area under the dissolution curve between time points (x-axis) expressed as a percentage of the curve at maximum dissolution (y_{100} axis) over the same period. The DE is calculated by the DDSolver program (Kassaye and Genete 2013).

Solubility determination

The solubility test was carried out using the standard shake flask method by adding an excess of the active drug substance (10 mg for ketoconazole powder and the equivalent of 10 mg for ketoconazole beads) into a test tube containing 2 mL of FaSSIF-2X medium pH 6.5, then vortexed for 30 seconds. The test tube was placed in the incubator shaker at 37 °C for 5 hours. When finished, let it sit for a while until the precipitate drops. Determination of ketoconazole concentration used the HPLC analysis method.

Analytical method

The chromatography system comprised an Elite LaChrom HPLC, a Hitachi UV-Vis detector L- 2420, and a Hitachi pump L-2130. A Phenomenex Luna column (250 x 4.6 mm, 5 µm) was utilized. The mobile phase consisted of an acetonitrile:water mixture with 0.15% TEA (50:50). The injection volume was 20 µL. Detection occurred at a wavelength of 232 nm, while the flow rate was set at 1 mL/min. Before analysis, all samples were filtered through a 0.45 µm nylon filter.

Result

Physical characterization of beads

In this study, ketoconazole beads were formulated using a combination of alginate-gum (AG), alginate-pectin (AP), and alginate-carrageenan (AK) polymers with respective ratios of 75:25, 50:50, and 25:75. The successful bead formulations include AL100, AP75, AP50, AG75, AG50, AK75, and AK50. The resulting wet beads are depicted as white balls. The wet beads (Fig. 2) size is bigger than the drying beads by looking directly at 5 pieces of beads on the scale bar of rules (Fig. 3), causing the wet beads to shrink to around 1 mm using rules. Beads AG25, AP25, and AK25 (25% alginate composition) were not subjected to the drying stage because no bead formation was observed. These beads with 25% alginate composition appeared to stick together in all combinations.

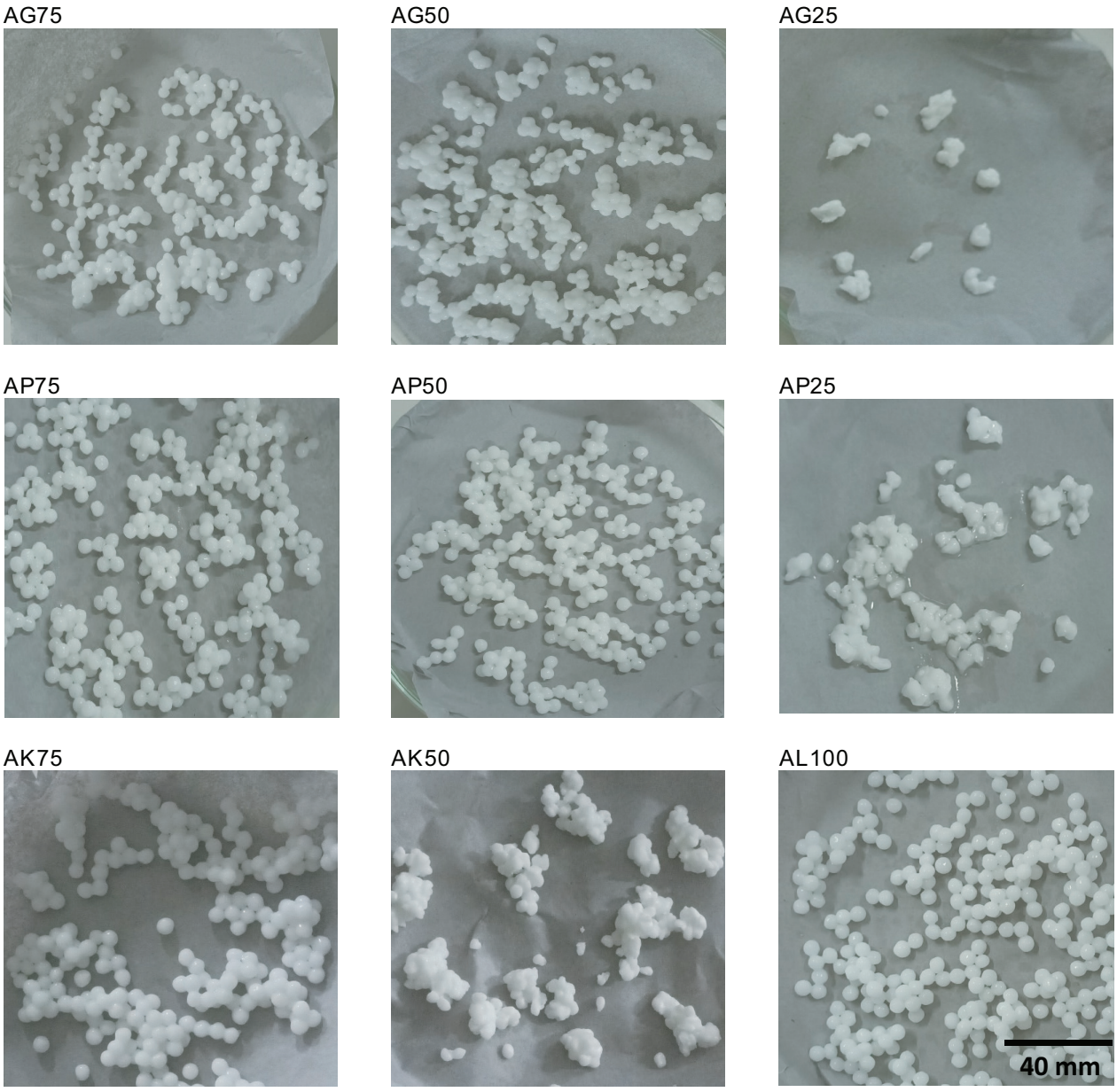

Figure 2. Visualization of wet ketoconazole beads before drying by direct photo. AG75 (alginate:gum acacia 75:25), AG50 (alginate:gum acacia 50:50), AG25 (alginate:gum acacia 25:75), AP75 (alginate:pectin 75:25), AP50 (alginate:pectin 50:50), AP25 (alginate:pectin 25:75), AK75 (alginate:carrageenan 75:25), AK50 (alginate:carrageenan 50:50), AL100 (alginate 100).

Scanning electron microscopy (SEM)

SEM testing was carried out to determine the morphological characteristics of the surface of ketoconazole beads. As shown in Fig. 4, the ketoconazole beads exhibit a generally round shape with a rough and wrinkled surface and small pores. These wrinkles are a result of water removal during the drying process of the beads in the oven to shrink. The results of SEM testing of AP75 beads show that their surface has a rigid structure compared to AP50. Similarly, the AG75 beads showed a surface with a rigid structure compared to AG50. The AK75 and AK50 beads showed a porous surface and a network-like appearance. Notably, the SEM examination of all ketoconazole beads showed the absence of drug crystals on the surface of the beads, indicating successful dispersion of the drug molecules within the polymer matrix. The absence of crystals suggests no leakage of the beads, ensuring that the drug remains encapsulated within the beads.

Fourier transform infrared (FT-IR)

FT-IR testing was conducted to analyze the specific interactions formed and their impact on precipitation. The samples used in the analysis included single polymer powders, pure ketoconazole powders, polymer combination beads, and ketoconazole beads. Ketoconazole possesses a donor group (NH-) and a receptor group (-O-) that can engage in hydrogen bonding interactions with polymers. In the spectra of ketoconazole beads, there was a

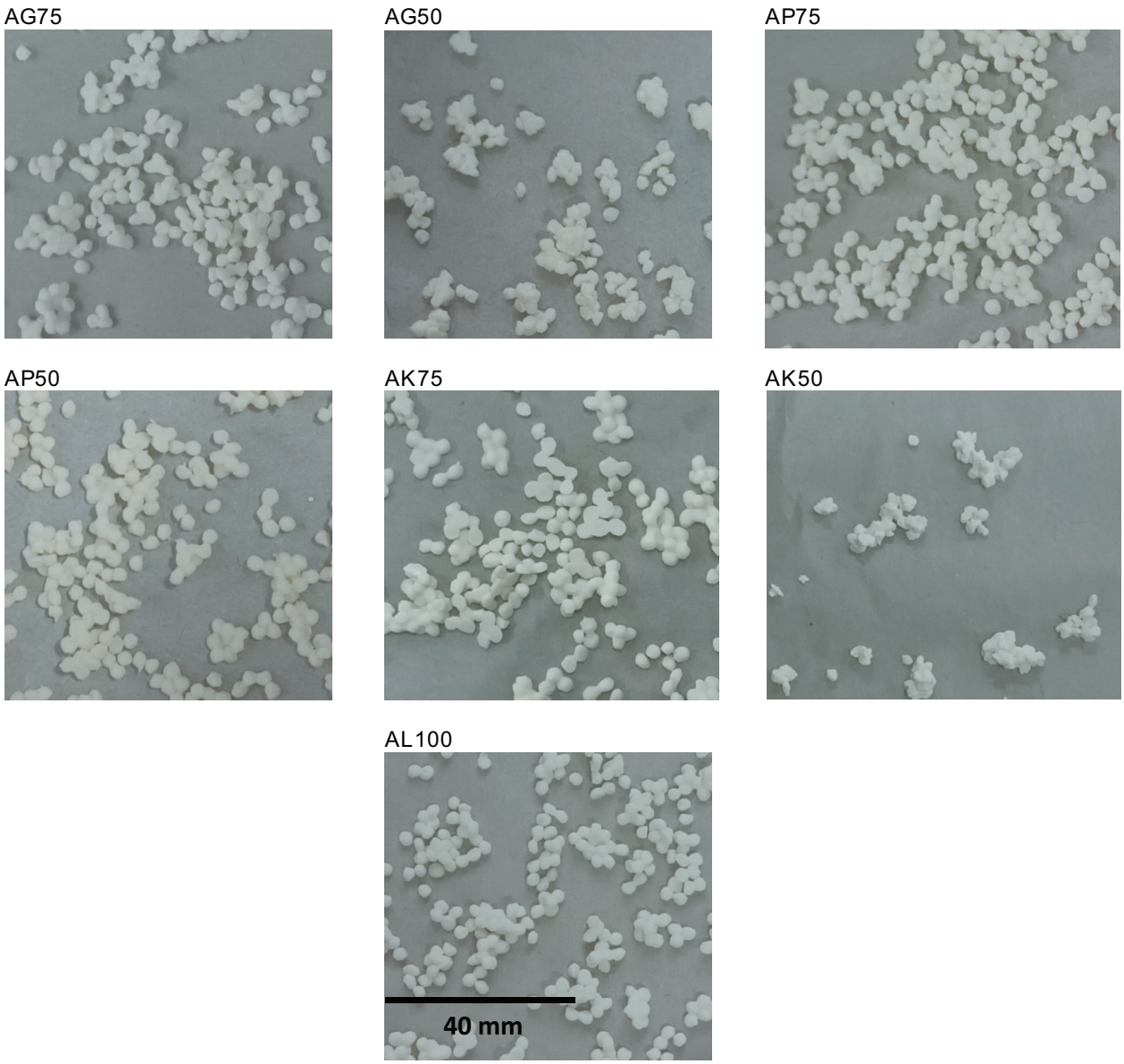

Figure 3. Visualization of dried ketoconazole beads after drying by direct photo. AG75 (alginate:gum acacia 75:25), AG50 (alginate:gum acacia 50:50), AP75 (alginate:pectin 75:25), AP50 (alginate:pectin 50:50), AK75 (alginate:carrageenan 75:25), AK50 (alginate:carrageenan 50:50), and AL100 (alginate 100).

wide peak -OH at ~3400 cm^{-1} (Fig. 5C), whereas, in single ketoconazole, this peak was not observed (Fig. 5A). This observation indicates the formation of strong hydrogen bonds between ketoconazole and the polymer combination. Furthermore, the interaction of hydrogen bonds can be identified by the vibrational shift of the functional groups involved. A shift towards lower wave numbers in the hydroxyl peak suggests the presence of stronger hydrogen bonds, whereas a shift to a higher wave number suggests weaker bond formation.

The spectrum of ketoconazole beads exhibits a broader peak at wave number 1654 cm^{-1} (COO-) (Fig. 5C) compared to pure ketoconazole (Fig. 5A). The spectra of the polymer combination with and without ketoconazole showed similar patterns, suggesting that the gel matrix structure formed by combining the polymer with Ca^{2+} is maintained even after the addition of ketoconazole. Molecular interactions between polymers can form complex matrix structures in beads, with or without ketoconazole. In the spectra of ketoconazole beads (Fig. 5C), peaks appeared at wave numbers ~ 1200 cm^{-1} (C-N) and 814 cm^{-1} (C-Cl), indicating axial deformation of the C-Cl group and axial stretching of the C-N group in ketoconazole. These peaks did not appear in the spectra of beads without ketoconazole (Figure 5B), indicating the successful encapsulation of ketoconazole in the polymer combination. The FT-IR spectrum of ketoconazole beads shows the same pattern as pure ketoconazole, indicating no interaction between ketoconazole and the polymer combination.

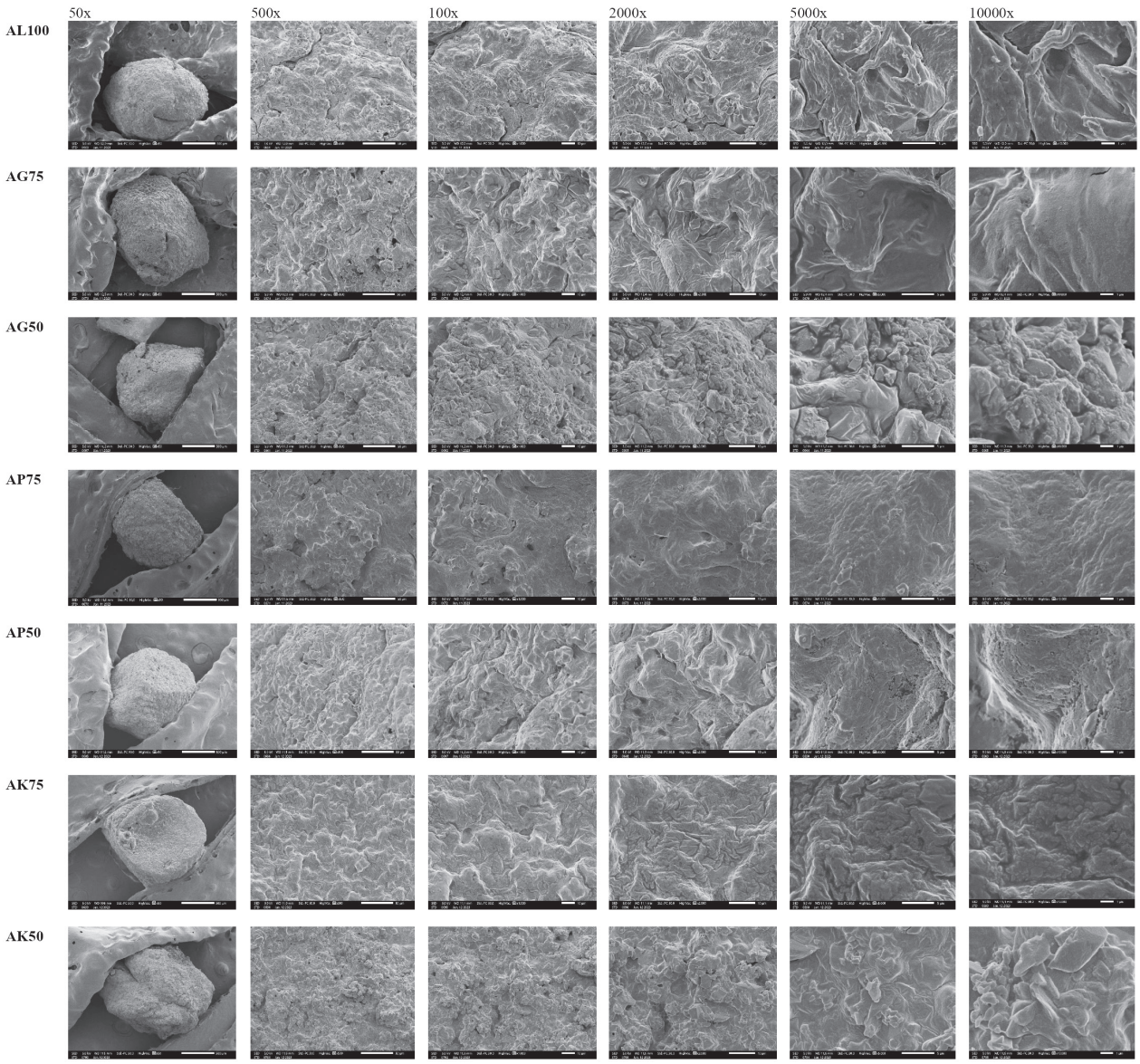

Figure 4. SEM micrographs of AL100 (alginate 100), AG75 (alginate:gum acacia 75:25), AG50 (alginate:gum acacia 50:50), AP75 (alginate:pectin 75:25), AP50 (alginate:pectin 50:50), AK75 (alginate:carrageenan 75:25), AK50 (alginate:carrageenan 50:50) with magnified at 50×, 500, 100×, 2000×, 5000×, dan 10000×.

Differential scanning calorimetry (DSC)

DSC testing was conducted to analyze the thermal profile of single polymer powder samples, pure ketoconazole powder, polymer combination beads, and ketoconazole beads. The results of the DSC test revealed the presence of an endothermic peak in all samples (Fig. 6). The endothermic peak is attributed to the evaporation of water during crystallization. Ketoconazole beads exhibited two endothermic peaks, namely a sharp peak at 120 °C and a broad peak at 163- 181 °C (AL100=170.24 °C, AG75=181.38 °C, AG50=178.25 °C, AP75=173.95 °C, AP50=172.55 °C, AK75=163.79 °C, AK50=169.19 °C). The first peak was not observed in the polymer combination without ketoconazole. The appearance of this new peak may be attributed to the influence of ketoconazole on the molecular structure of the polymer-Ca^{2+} complex. The first peak shows the eutectic melting point, while the second peak shows the melting point. The formation of the first peak may also represent the stages of loss of water content from the beads.

There is a slight shift in the endothermic peak from the polymer combination of the ketoconazole beads. The peak of the ketoconazole is observed at a lower temperature, indicating the development after adding the drug, which causes the free volume in the matrix to increase. At the peak of ketoconazole, a melting point of 153.23 °C was obtained. These results are consistent with ketoconazole, which has one sharp endothermic peak at a melting point of 154 °C (Demirel et al. 2011). In the results of the ketoconazole thermogram beads, the peak of 153.23 °C is not visible because ketoconazole has been encapsulated

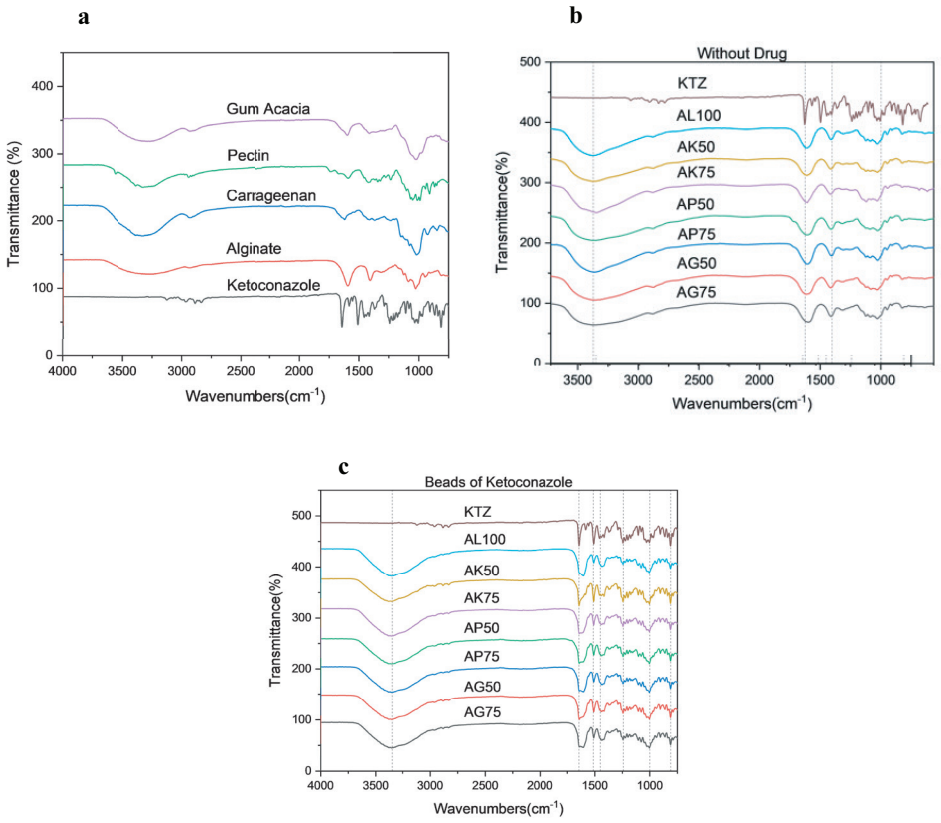

Figure 5. FTIR spectra of **a.** Sole polymer; **b.** Polymer combination without drug, dan; **c.** Drug loaded in a combination of polymer (beads ketoconazole). KTZ (ketoconazole), AL100 (alginate 100), AG75 (alginate:gum acacia 75:25), AG50 (alginate:gum acacia 50:50), AP75 (alginate:pectin 75:25), AP50 (alginate:pectin 50:50), AK75 (alginate:carrageenan 75:25), AK50 (alginate:carrageenan 50:50).

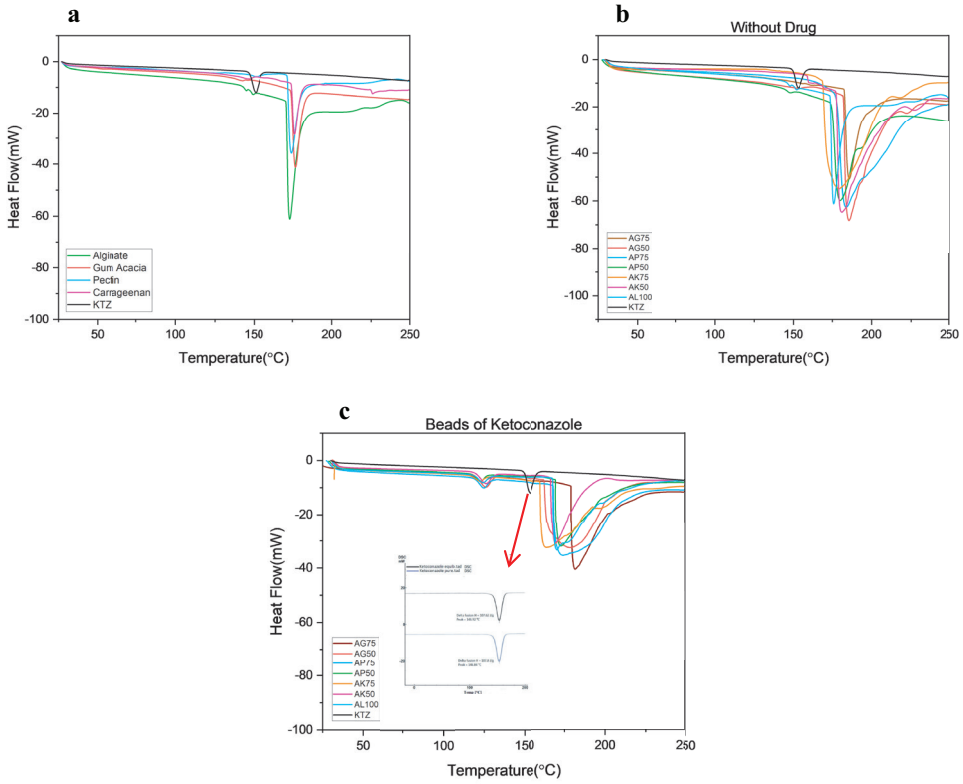

Figure 6. DSC thermograms of **a.** Sole polymer; **b.** Polymer combination without drug, dan; **c.** Drug loaded in a combination of polymer (beads ketoconazole). KTZ (ketoconazole), AL100 (alginate 100), AG75 (alginate:gum acacia 75:25), AG50 (alginate:gum acacia 50:50), AP75 (alginate:pectin 75:25), AP50 (alginate:pectin 50:50), AK75 (alginate:carrageenan 75:25), AK50 (alginate:carrageenan 50:50).

into the polymer combination beads. It also shows that the dispersion of the drug with the polymer has become a uniform amorphous form.

X-ray diffraction (XRD)

XRPD testing is carried out to obtain basic information about a material's crystal structure and properties. In this study, the ketoconazole diffraction pattern (Fig. 6a) showed several sharp peaks with high intensity at the diffraction angles according to the literature, which is 15, 17, 20, 21, 23, and 270 (Demirel et al. 2011). These sharp peaks in the ketoconazole diffraction pattern indicate the high crystallinity of pure ketoconazole. In the diffraction pattern of the ketoconazole beads (Fig. 7b), many of the sharp peaks observed in the pure ketoconazole diffraction either disappeared or decreased in intensity, indicating a transformation of the crystalline phase from pure ketoconazole to an amorphous form of ketoconazole beads. This change in the diffraction pattern suggests that ketoconazole was successfully encapsulated into beads.

The electrostatic interaction between carboxyl groups in the polymer-ketoconazole combination can disrupt the original structure of the polymer and the drug, weakening the previous crystal structure arrangement (Kiaei Pour et al. 2020). Polymers also offer additional benefits, as they can act as excipients for stabilizing the amorphous form of ketoconazole in the formulation and preventing its recrystallization within a certain period.

Drug loading

The drug loading percentage data of ketoconazole beads is shown in Table 2. A higher drug loading percentage was found in AK50 beads than other beads, whereas the AK50 beads form weak beads after cross-linking with Ca^{2+}. This could be attributed to the lower strength of beads and the easier drug comes out of the beads. Hence, ketoconazole concentration detected in medium increase, following the amount of drug loading will be higher too.

Table 2. Drug loading of ketoconazole in combination with polymers beads.

Sample	Drug Loading (%)
AL100	20.56±0.99
AG75	24.96±1.79
AG50	31.73±2.62
AP75	20.49±1.05
AP50	18.34±1.52
AK75	19.55±0.16
AK50	37.59±5.59

AL100 (alginate 100), AG75 (alginate:gum acacia 75:25), AG50 (alginate:gum acacia 50:50), AP75 (alginate:pectin 75:25), AP50 (alginate:pectin 50:50), AK75 (alginate:carrageenan 75:25), AK50 (alginate:-carrageenan 50:50). Data are expressed as mean ±SD (n=3).

Swelling

Swelling testing in this study was conducted to determine the effect of the external medium pH on the swelling ability of ketoconazole beads. The percentage of swelling ratio (%SR) of the beads was measured in SGF media at pH 2.0 and FaSSIF-2X at pH 6.5. The swelling test on dry beads is related to the hydration of the hydrophilic groups in the alginate combination with other polymers. The liquid from the medium permeates the beads, filling the inert pores between the polymer chains. The %SR data are shown in Fig. 8, and they show that ketoconazole beads exhibit a smaller %SR at acidic pH than at alkaline pH. The osmotic pressure gradient between the alginate beads and the surrounding environment is crucial in swelling. In SGF medium at pH 2.0 (Fig. 8a), the beads experience shrinkage, and drug release occurs through a diffusion mechanism from the insoluble matrix. Among all the polymer combination beads in an acidic pH medium, it can be seen that the AL100 beads have a higher %SR than the other alginate combination beads. The addition of other polymers into alginate can prevent deprotonation between alginate bonds. Another possible reason is that the structure of alginate beads, when combined with other polymers, becomes stronger, resulting in lower shrinkage compared to single alginate. In FaSSIF-2X medium at pH 6.5 (Fig. 8b),

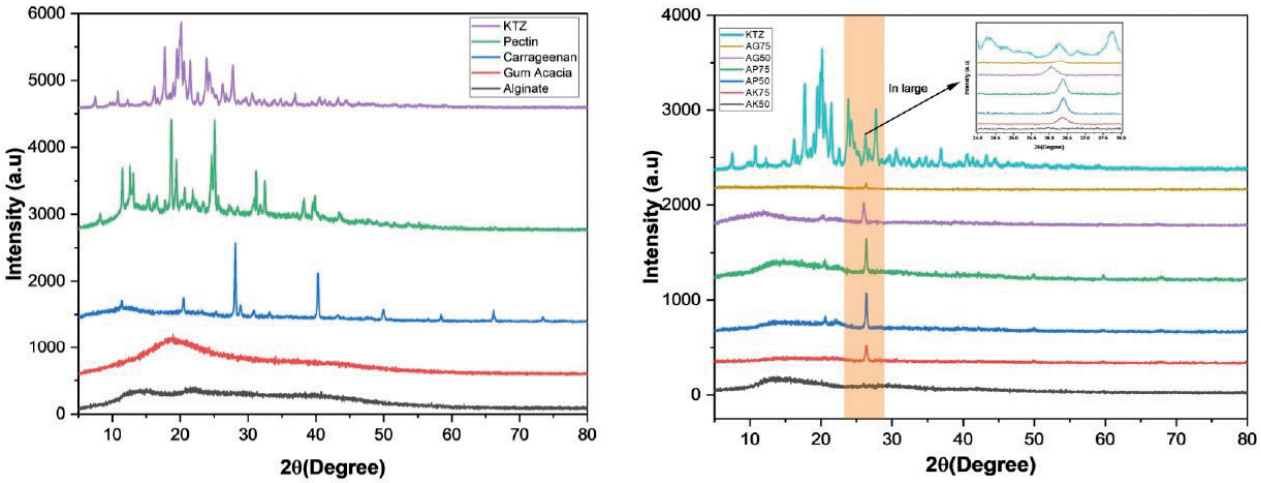

Figure 7. X-ray diffraction spectra of **a.** Sole polymer and **b.** Drug loaded in a combination of polymer (beads ketoconazole). KTZ (ketoconazole), AL100 (alginate 100), AG75 (alginate:gum acacia 75:25), AG50 (alginate:gum acacia 50:50), AP75 (alginate:pectin 75:25), AP50 (alginate:pectin 50:50), AK75 (alginate:carrageenan 75:25), AK50 (alginate:carrageenan 50:50).

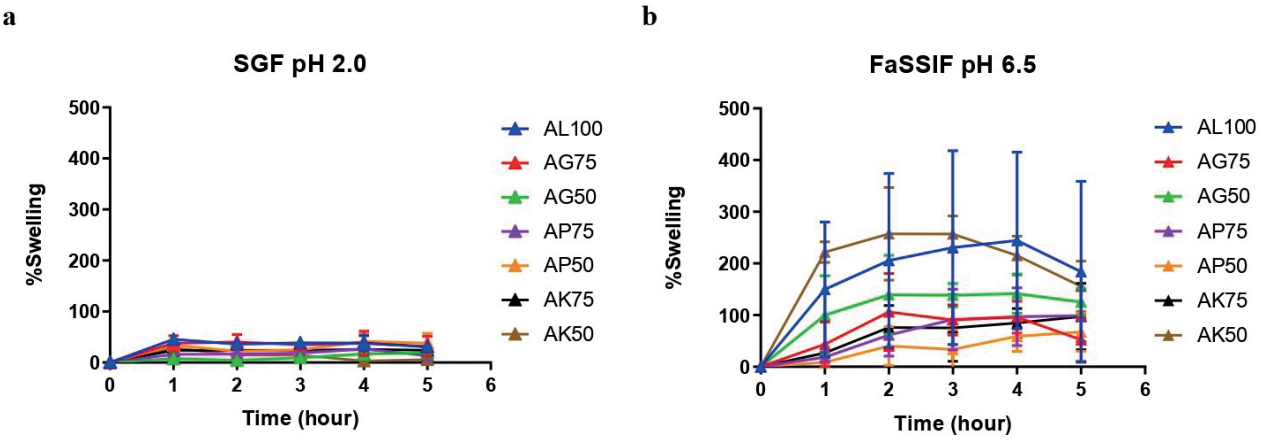

Figure 8. Swelling profile of ketoconazole beads in **a.** SGF pH 2.0 and **b.** FaSSIF-2X pH 6.5. AL100 (alginate 100), AG75 (alginate:gum acacia 75:25), AG50 (alginate:gum acacia 50:50), AP75 (alginate:pectin 75:25), AP50 (alginate:pectin 50:50), AK75 (alginate:carrageenan 75:25), AK50 (alginate:carrageenan 50:50). Data are expressed as mean ±SD (n=3).

the ketoconazole beads swell initially, undergo erosion, disintegration, and degradation, and then dissolve in the medium. Among all the polymer combination beads in an alkaline pH medium, it can be observed that the AL100 beads have a higher swelling ratio than the other alginate combination beads. It is due to the alginate network's high osmotic pressure, which comes from the dissociated acid groups. The resulting SD value is quite large. It may be due to the lack of homogeneity when making the beads so that the swelling ability of the beads produced in acidic and alkaline media has a high variation.

In vitro drug release profile

Dissolution testing in this study was conducted to predict drug performance in vivo. An acidic medium was chosen based on Indonesian Pharmacopoeia V, which utilizes acidic media to assess the dissolution of ketoconazole (BPOM 2012). This study, SGF pH 2.0 was selected as the dissolution medium to represent physiological conditions in the gastrointestinal tract as an alternative to using HCl (Jantratid et al. 2008). By understanding the drug dissolution in an acidic medium, the overall rate of drug absorption can be predicted. The absorption rate of lipophilic drugs is influenced by gastric dissolution and the rate of gastric emptying (Nicolaides et al. 2001).

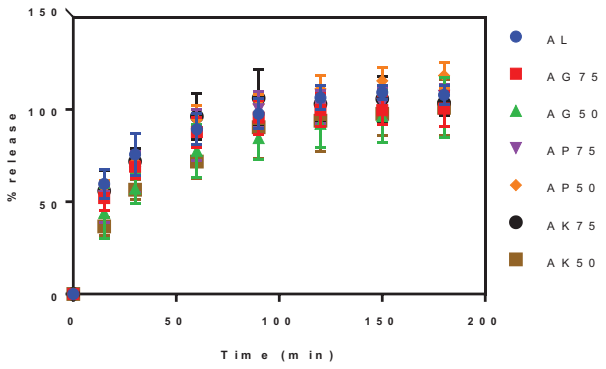

Figure 9. In vitro drug release profile in SGF pH 2.0. AL100 (alginate 100), AG75 (alginate:gum acacia 75:25), AG50 (alginate:gum acacia 50:50), AP75 (alginate:pectin 75:25), AP50 (alginate:pectin 50:50), AK75 (alginate:carrageenan 75:25), AK50 (alginate:carrageenan 50:50). Data are expressed as mean ±SD (n=3).

Fig. 9 displays the dissolution profile of ketoconazole beads in an SGF medium at pH 2.0. In an acidic pH medium, ketoconazole transforms into its HCl salt form, facilitating its dissolution in acidic conditions. Analysis of dissolution efficiency (DE) was performed at 15 (DE_{15}) and 60 (DE_{60}) minutes. There was no significant difference ($p>0.05$) between all ketoconazole beads at DE_{15} and DE_{60}.

The analysis of curve fitting for ketoconazole beads analysis results in SGF medium pH 2.0 (Table 3) revealed that Korsmeyer-Peppas was the most appropriate model for all polymer combinations, with a value of R^2=0.9585–0.9856.

Solubility determination

In this study, the solubility medium used was FaSSIF-2X, representing drug solubility in the intestinal environment (Fig. 10). The solubility of ketoconazole in FaSSIF-2X medium at pH 6.5 was 40.0±4.34 µg/mL. The results of the solubility test (Fig. 10) showed that the beads AL100 (248.1±17.56 µg/mL), AP75 (226.9±20.37 µg/mL), AP50 (221.1±10.81 µg/mL), and AK75 (227.7±3.72 µg/mL)

Table 3. R^2 adjusted from profile model dissolution of ketoconazole beads.

Sample	Dissolution model			
	Korsmeyer-Peppas	First Order	Higuchi	Hixson-Crowell
AL100	0.9839±0.00	0.9599±0.01	0.7990±0.08	0.8663±0.07
AG75	0.9773±0.00	0.9703±0.02	0.8094±0.02	0.9024±0.03
AG50	0.9856±0.01	0.9431±0.04	0.9164±0.02	0.9058±0.07
AP75	0.9718±0.02	0.9641±0.03	0.8383±0.11	0.9083±0.05
AP50	0.9849±0.01	0.9229±0.04	0.8882±0.03	0.9200±0.03
AK75	0.9537±0.01	0.9506±0.02	0.7705±0.05	0.8663±0.04
AK50	0.9585±0.02	0.9255±0.05	0.9217±0.04	0.9026±0.09

AL100 (alginate 100), AG75 (alginate:gum acacia 75:25), AG50 (alginate:gum acacia 50:50), AP75 (alginate:pectin 75:25), AP50 (alginate:pectin 50:50), AK75 (alginate:carrageenan 75:25), AK50 (alginate:-carrageenan 50:50). Data are expressed as mean ±SD (n=3).

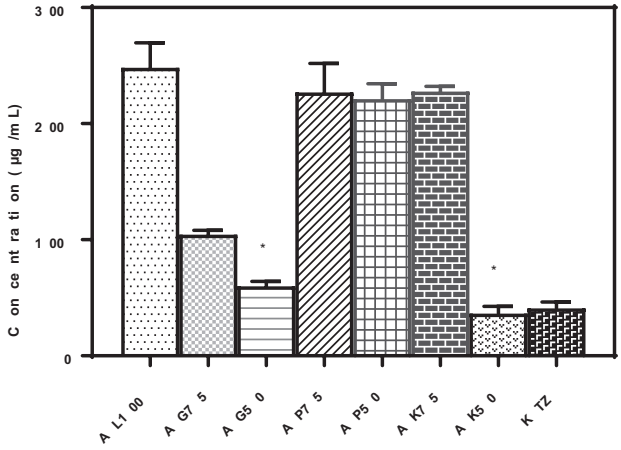

Figure 10. Solubility results of KTZ (ketoconazole), AL100 (alginate 100), AG75 (alginate:gum acacia 75:25), AG50 (alginate:gum acacia 50:50), AP75 (alginate:pectin 75:25), AP50 (alginate:pectin 50:50), AK75 (alginate:carrageenan 75:25), AK50 (alginate:carrageenan 50:50) in FaSSIF-2X pH 6.5. Data are expressed as mean ±SD (n=3). *not significant differences at p>0.05 from KTZ.

had a solubility five times higher than that of pure ketoconazole. In comparison, bead AG75 (104.4±2.99 µg/mL) had a solubility two times higher. Beads AL100, AP75, AP50, AK75, and AG75 showed significantly different ($p<0.05$) solubility compared to ketoconazole. On the other hand, the beads AG50 (59.9±3.43 µg/mL) and AK50 (36.5±5.13 µg/mL) did not show a significant difference ($p>0.05$) in solubility compared to ketoconazole (40.0±4.34 µg/mL).

Discussion

Ketoconazole could be loaded into a matrix polymer consisting of alginate and anionic polymer through hydrogen bonds formed with the N atom of the ketoconazole. The negatively charged groups of the polymer can interact with ketoconazole through hydrogen bonds from the N atom of the ketoconazole imidazole ring or the ketoconazole acetyl group (Martin et al. 2013; Chen et al. 2020).

The strength of the bonds between alginate and the other polysaccharides decreases with a lower alginate ratio. Alginate plays a crucial role in forming a polymer network to create beads. The physical properties of the AK50 beads were not strong, but they were continued for drying and then characterized. Combining alginate and the other polysaccharides with a lower alginate ratio results in weaker and less dense beads. When the drug, in combination with alginate with other polysaccharides, was dropped into $CaCl_2$, ionic cross-linking occurred between the sodium alginate chains. During this process, Ca^{2+} displaces Na^{2+} ions from sodium alginate on the carboxylate group, and the second sodium alginate chain binds to Ca^{2+} to form a bridge. Subsequently, Ca^{2+} co-occupies two other alginate chains, forming an encapsulating matrix with drugs (Boppana et al. 2015). An insufficient concentration of Ca^{2+} as a cross-linker can also cause the resulting matrix to become loose and leaky, allowing the drug to escape from the matrix during the bead-making process (Soppimath et al. 2001).

In this study, AG25, AP25, and AK25 beads were not dried as they did not form firmly, so the SEM test was not carried out.

The SEM results of AP75 beads show that their surface has a rigid structure compared to AP50. In a study by Koo, et al, AP80 produced a smoother surface than AL100, AP60, AP40, and AP80 (Koo et al. 2014). However, an increase in the ratio of pectin or alginate does not always correlate with the surface structure of the resulting beads (Kiaei Pour et al. 2020). Research conducted by Kiaei Pour et al reported that the pectin composition of less than 50% in the alginate-pectin mixture can cause leakage of the beads, leading to the active substance coming out of the beads (Kiaei Pour et al. 2020).

The SEM analysis of AG75 beads showed a surface with a rigid structure compared to AG50. This rigid structure indicates that the beads have a good controlled drug release ability. This finding aligns with research conducted by Tsai, et al who reported that a gum acacia:alginate ratio of 25:75 resulted in the roughest surface and the most rigid structure. Furthermore, an increase in the ratio of gum acacia causes the surface of the beads to become rougher at first, then smooth again (Tsai et al. 2017). The formation of beads using the ionic gelation method with alginate and gum acacia produces a rough and dense surface (Boppana et al. 2015). Tsai et al reported that beads with an alginate: gum acacia ratio of 25:75 produced a very porous surface, resulting in poor ability for controlled release (Tsai et al. 2017).

The SEM analysis of AK75 and AK50 beads revealed a porous surface with a network-like appearance. The higher the composition of carrageenan, the higher the porosity of the beads, making it easier for the drug to enter the beads (Malhotra and Basir 2020). The addition of the cross-linker $CaCl_2$ can reduce the pore size of the alginate-carrageenan compared to without a cross-linker addition (Roh and Shin 2006). This structure of AK beads in the presence of the drug shows a more regular morphology. The result stems from the molecular interactions, such as hydrogen bonding, between the drug and alginate/pectin/gum acacia/carrageenan network during encapsulation into the hydrogel system (Mohamadnia et al. 2007).

No drug crystals were discovered on the surface of any of the ketoconazole beads, according to the SEM examination results. According to Benfattoum et al, this suggests that the drug molecules have been evenly distributed throughout the polymer matrix (Benfattoum et al. 2018). The lack of crystals on the surface of the beads suggests that there is no bead leakage, which would cause the medicine to escape from the beads (Kiaei Pour et al. 2020).

In the swelling study with SGF pH 2.0 medium, the carboxyl group (COO-) of alginate/pectin/gum acacia/carrageenan (Fig. 11) undergoes protonation, resulting in the formation of soluble alginic acid. This protonation process leads to a decrease in the %SR of the beads (Pasparakis and Bouropoulos 2006; Huang et al. 2012). The protonated functional groups come closer to each

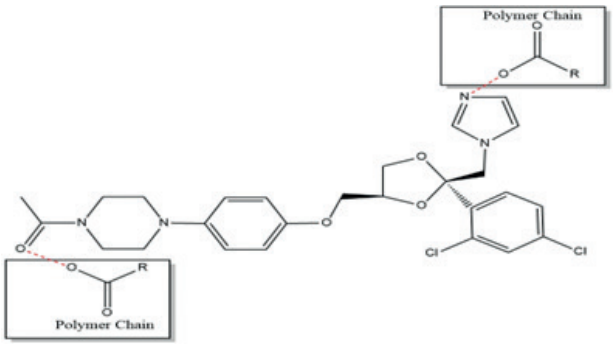

Figure 11. Interaction between ketoconazole with carboxylate groups (COO-) polymer chain of alginate/pectin/gum acacia/carrageenan.

other, thereby reducing electrostatic repulsion, which makes the network structure stronger. This stronger structure inhibits the entry of the medium into the beads (Ma et al. 2023). As a result of the formation of hydrogen bonds between the carboxyl groups in alginate, shrinkage in an acidic solution is also possible as a result of the predominant interaction between the combination of alginate with the other polysaccharides (Kolesnyk et al. 2015). Additionally, polymer concentration can influence the %SR. The higher the alginate/pectin/gum acacia/carrageenan concentration, the greater the viscosity, which strengthens the three- dimensional structure of the beads, causing the swelling ability to decrease (Chopra et al. 2015).

In the swelling study with FaSSIF pH 6.5 medium, carboxyl groups (COO-) of alginate/pectin/gum acacia/carrageenan (Fig. 11) generating molecular electrostatic repulsion forces that cause the %SR increase (Huang et al. 2012). The ion exchange occurs between Ca^{2+} and Na^{+} ions present in the composition of the medium, specifically NaH_2PO_4. Free carboxylate molecules increase during the ion exchange process, leading to electrostatic repulsion between alginate chains, causing the chains to relax the beads to expand (Georgiou et al. 2017). In an alkaline medium, there is a difference in osmotic pressure between the inner polymer network and the medium environment, causing the medium to enter the polymer matrix, and then the beads expand.

Medium in both as only one alkaline (FaSSIF-2X pH 6.5) and only one acidic medium (SGF pH 2.0) was used. In the stomach, the ketoconazole beads remained intact despite acidic pH. Combining alginate and the other polysaccharides in the beads as drug precipitation inhibitors can inhibit drug precipitation when it enters the small intestine.

The dissolution test in this study utilized a reduced scale compared to the standard normal scale dissolution test. The decision to use small-scale testing was chosen to be more efficient in biorelevant media, as it is quite expensive and difficult to obtain since it must be imported. Additionally, using a reduced scale can reduce the number of samples needed for testing (Emmanuel et al. 2010). Previous research has also employed small-scale testing for dissolution studies. For instance, Klein et al reported that small-scale testing could be an alternative method for dissolution testing in multi-particulate powder samples of drug preparations, tablets, or small capsules, as the drug's size and dosage form can impact drug release (Klein 2006). Emmanuel et al stated that small-scale testing is useful for characterizing drug release profiles, especially for immediate-release drugs and tablet preparations (Emmanuel et al. 2010). Small volumes used in dissolution testing can also be applied to drugs with low solubility, even in non-sink conditions (Takano et al. 2006). However, using a magnetic stirrer in this study as a substitute for paddles can lead to different geometric agitator effects originating from hydrodynamic shear forces (Johansson et al. 2018). Using a stirrer can accelerate drug release compared to the paddle method.

The polymer employed in this study is hydrophilic, resulting in similar dissolution profiles among the combinations of alginate and other polymers. The component ratio of alginate/gum acacia, alginate/pectin, or alginate/carrageenan affects the dissolution rate of ketoconazole beads. The hydrophilic nature of the polysaccharide polymer used in the manufacture of ketoconazole beads also contributes to increasing the rate of dissolution because it can enhance the wettability of the drug (Fouad et al. 2021). However, increased alginate/pectin/gum acacia/carrageenan concentration would cause prolonged drug release due to pore obstructions on the bead surface (Pal and Nayak 2012).

Ketoconazole dissolved from the beads by more than 80% at 60 minutes. However, the dissolution requirement for ketoconazole tablets in Indonesian Pharmacopoeia V is that within 30 minutes, 80% should be dissolved (BPOM 2012). Ketoconazole beads did not dissolve as much as 80% at the 30th minute, possibly due to differences in drug dosage form, i.e. ketoconazole is more slowly released from the beads matrix compared to tablets.

The Korsmeyer-Peppas model is used to describe drug release from polymer systems controlled by a diffusion mechanism for sustained-release drugs (Ge et al. 2022). By analyzing the model, we can obtain a diffusional exponent (n) value to distinguish the release mechanism, which can be Fickian (diffusion-controlled release) for n values $\leq$0.43, non-Fickian (anomalous transport) for n values 0.43–0.85, or transport case-II (relaxation-controlled release) for value n$\geq$0.85 (Jana et al. 2013). In this study, the obtained value of the n for ketoconazole beads was between 0.232 and 0.319, indicating the release of the drug from the matrix of polymer combination beads was through a diffusion mechanism (Dimofte et al. 2022). Even though the obtained n value is less than 0.50, this value may indicate that the mechanism for releasing ketoconazole from the matrix is through the diffusion of controlled-release drugs (Permanadewi et al. 2019). The release rate of the drug is decreased due to hydrophobic interactions between the polymer matrix and the drug, while the rate will increase if there are more hydrophilic components than hydrophobic ones (Dimofte et al. 2022).

The drug in the polymer matrix system is a controlled release. When the beads are exposed to the dissolution medium, drug release is modulated by diffusion through the swelling matrix and dissolution/erosion of the matrix. The

process of swelling, dissolution, and erosion is very complex and the rate of drug release decreases with increasing thickness of the matrix (Tonnesen and Karlsen 2002).

The solubility test conducted in this study aimed at determining the solubility of ketoconazole beads in the small intestine environment. Intestinal solubility is a critical biopharmaceutical attribute that describes drug absorption after oral administration (Riethorst et al. 2016). Ketoconazole exhibits poor solubility under alkaline conditions, specifically at pH 6.5, where its solubility is only 0.01 mg/mL (Cristofoletti et al. 2017). This low solubility at a basic pH can be a major factor limiting drug absorption and leading to decreased drug bioavailability. Additionally, ketoconazole may have the potential to precipitate when moving from the stomach to the small intestine. This limitation could be attributed to issues related to either equilibrium solubility problems or kinetics (Dressman et al. 1998).

Research on the solubility of ketoconazole using FaSSIF-2X media has not been found in previous studies. FaSSIF-2X was chosen because it has a higher concentration of phosphate buffer to maintain pH due to changes in pH from gastric juice to small intestinal fluid (Berben et al. 2019; Jede et al. 2019). Therefore, the results of this study were compared to studies conducted using FaSSIF-2X media pH 6.5. In FaSSIF-2X pH 6.5, the solubility results of ketoconazole were obtained as follows: 21.89±0.001 µg/mL (Pathak et al. 2017), 21±0.001 µg/mL (Takano et al. 2006), and 13.2±0.1 µg/mL (Dahlgren et al. 2021). Jamil et al reported the solubility of ketoconazole in FaSSIF-2X-V2 media pH 6.5 in an inter-laboratory manner. That result was 9.46–13.91 µg/mL (Jamil et al. 2021). The solubility of ketoconazole in FaSSIF-2X medium is higher than in the studies using FaSSIF medium. The content of bile salts and phospholipids in FaSSIF-2X media is greater than in the other two media. The equilibrium solubility of ketoconazole in an intestinal medium with pH ≥ pKa is linearly correlated with the total bile salt concentration (Psachoulias et al. 2011). The higher the concentration of total bile salts, the higher the solubility of ketoconazole. The combination of bile salts and phospholipids can form micelles (6.5 nm diameter) and vesicles (50 nm diameter) in the intestinal lumen. The formation of these micelles and vesicles can facilitate the solubility of lipophilic drugs so that the higher the total bile salt concentration, the higher the solubility of ketoconazole (Dahlgren et al. 2021). Bile salts and lecithin are amphiphilic components that greatly affect the solubility of lipophilic drugs. The solubility of ketoconazole can increase 4 to 5 times in the presence of an amphiphilic component (Psachoulias et al. 2011; Ruff et al. 2017). Higher concentrations triggered by supersaturation conditions can directly increase the oral absorption of weak base BCS class II drugs (Tsume et al. 2017).

When comparing the solubility of ketoconazole in phosphate buffer medium pH 6.8, which is 1.84 ± 0.1 µg/mL (Annisa et al. 2022a), it is evident that these results are significantly lower than the solubility of ketoconazole in FaSSIF and FaSSIF-2X medium. The main contributing factor is that phosphate-buffered media lack the composition of bile salts and phospholipids, which enhance the solubility of drugs that are otherwise difficult to dissolve. Apart from the media's composition, other factors that can affect the solubility of active substances also include the physical characteristics of the media solution, such as surface tension, osmolarity, and ionic strength (Fuchs and Dressman 2014).

Dahlgren et al and Kalantzi et al, reported that the solubility of ketoconazole in Human Intestinal Fluid (HIF) was 28 µg/mL (Kalantzi et al. 2006; Dahlgren et al. 2021). The results of in vivo studies have large variations due to the large variation in measured luminal pH values, starting from 3.6 (Ruff et al. 2017).

Using alginate as a bead composition can overcome the problem of poor solubility of ketoconazole in a higher pH environment. Alginate exhibits slow solubility in acidic pH, forming insoluble alginic acid precipitates, while it dissolves more readily in alkaline pH. The contrasting solubility of drugs and polymers can mutually compensate for each other's shortcomings by having opposite solubility (Gutsche et al. 2008). Pectin, on the other hand, is insoluble in both acidic and alkaline pH, so it is more easily dissolved in the pH of intestinal fluids (Das et al. 2011). The characteristics of pectin and alginate have good solubility in an alkaline pH condition, resulting in beads that can increase the solubility of ketoconazole at AP75 and AP50 beads. In contrast to the alginate/gum acacia and alginate/pectin combinations, an increase in the gum acacia/carrageenan ratio results in lower solubility. It suggests that alginate plays a major role in increasing the solubility of ketoconazole in the small intestine, as the ability of gum acacia and carrageenan to increase drug solubility has not been reported in previous studies.

Conclusion

According to the results of this study, the formulation of ketoconazole beads cross-linked with alginate-pectin, alginate-gum acacia, and alginate-carrageenan shows promising characteristics for enhancing solubility of ketoconazole. The physicochemical characterization confirms the formation of ketoconazole beads, an indication of successful loading in the matrix polymer. The results of the solubility test of ketoconazole beads in FaSSIF-2X media at pH 6.5 showed that AL100, AG75, AP75, AP50, and AK75 significantly exhibited higher solubility than pure ketoconazole, whereas AG50 and AK50 did not show significant improvements. Previous studies have already been conducted on using alginate as a polymer to improve the solubility of poorly soluble drugs. Alginate plays an important role in increasing the solubility of ketoconazole in basic pH conditions due to its good solubility in such conditions. However, it is worth noting that alginate is insoluble at acidic pH and forms a precipitate. The differing solubility properties of the drug and polymer can complement each other's limitations by having opposing solubility characteristics.

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