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Effects of iron-alginate ratios and pHs of gelation on characteristics of iron bead encapsulation

Astuti, W.I.S.T., *Wardhani, D.H., Ratnawati, Sagala, E.A.P.P. and Siregar, B.E.I.

Department of Chemical Engineering, Faculty of Engineering, Universitas Diponegoro, Jl. Prof. Sudarto, SH, Tembalang, Semarang 50239, Indonesia

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Abstract

Encapsulation is one of the methods to protect iron from oxidation. Among the encapsulation methods, gelation could be the simplest one. This method requires the matrix which could form a gel. Alginate fulfills the requirements as an encapsulant by creating beads. However, performances of the encapsulation are determined by the conditions of the gelation process. The aim of this study was to study the effect of the iron -alginate ratios (0.05-0.5, w/w) and pHs of gelation (5-10) on the properties of alginate beads for iron encapsulation. $CaCl_2$ solution was used as a cross-linked agent to form beads. Solution of iron-alginate was dropped into $CaCl_2$ solution (150 mL, 1 M) using 5-mL unneedled syringe from about 5 cm above the cross-linked solution surfaces. The results showed that the ratio of iron-alginate and pH have a negative correlation with moisture content, diameter beads, and encapsulation efficiency but iron loading. Neutral pH-gelation produced softer bead texture. The highest efficiency encapsulation was found in pH 5 of gelation. Release of iron was higher in pH 6.8 than that in pH 1.2. Increase in iron-alginate ratio led to have more syneresis effect. However, higher pH-gelation tended to have lower syneresis.

1. Introduction

Iron is considered as an essential mineral to make hemoglobin, a part of blood cells (Abbaspour *et al*., 2014). When body iron level is low, it decreases red blood cell production and results in health problem (Camaschella, 2019). This deficiency which known as iron deficiency anemia has affected 50% of all anemia worldwide. The recommended daily intake of iron is depended on gender and age. Iron fortification has shown to be efficacious to alleviate the burden of the iron deficiencies (Goh *et al*., 2012). Among the challenges in the fortification are to ensure the bioavailability of iron and maintain the sensory quality, stability of the fortified food (Goh *et al*., 2012). Encapsulation has a potency to prevent sensory changes and decreases interactions of iron with other food components that could lower iron bioavailability (Bryszewska, 2019). Various methods can be applied for the encapsulation of iron, including ionic gelation (Liyanage and Zlotkin, 2002)

The ionic gelation is a method of forming hydrogel beads by dropping encapsulant polymer solution into aqueous solution of polyvalent using tools such as pipettes, syringes and vibrating nozzle (Nedovic *et al*., 2011). These polyvalent diffuses into the loaded polymeric drops to form a three-dimensional bead, due to ionic crosslinking. This method involves a crosslinking process between the polyelectrolytes in the presence of their multivalent ion pairs. Low processing costs, mild operating conditions, avoidance of harmful organic solvents, ability to protect encapsulated active compound, and maintain their activity during encapsulation are among the advantages of the ionic gelation method (Chan *et al*., 2006). Conditions of gelation such as solution composition, type of cross-linker, temperature, and pH, affect the characteristics of bead product (Khazaeli *et al*., 2008).

Alginate has been reported to be a polymer that has good potential as an encapsulation material. This polysaccharide consists of mannuronic and guluronic acid groups (Lee and David, 2012). The guluronic group will be ionically cross-linked with the divalent cation. Ca^{2+} ions are commonly used as the divalent cation source (Ramos *et al*., 2018). The availability of divalent ions influences the gelation process to cross-link with the alginate polymer groups. In addition, gelation conditions such as pH also affect the ability of Ca^{2+} ions to bind to alginate polymer groups. Study on the properties of iron

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*Corresponding author.
Email: *dhwardhani@che.undip.ac.id*

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encapsulation using alginate through the gelation method is still limited. Wardhani *et al*. (2021) reported the iron encapsulation using alginate but use the spray drying method. Different in encapsulation method requires different characteristic of the encapsulant. The ionic gelation process was selected because of the mild operating conditions. This study aimed to examine the effect of the iron-alginate ratio and the pH of $CaCl_2$ on the characteristics of iron-alginate beads using the gelation method, including water content, bead size and syneresis. Meanwhile, the ability of alginate beads to encapsulate iron was studied through the encapsulation efficiency and the release profiles.

2. Materials and methods

2.1 Materials

Food-grade sodium alginate was purchased from PT. Multi Kimia Raya (Semarang, Central Java, Indonesia). $FeSO_4.7H_2O$ as iron source was purchased from Merck Chemical Co. (Germany). $CaCl_2$ as a crosslinking agent, HCl, and all other chemicals used were analytical grade.

2.2 Iron bead gelation

Ferrous sulphate and alginate (2 g) were dissolved in 100 ml distilled water under constant stirring at 350 rpm for 15 min. Ratio of iron-alginate was varied to give 0.05, 0.0625, 0.075, 0.0875 and 0.1 (w/w). This solution was pipet-dropped wisely to pH 5 of $CaCl_2$ solution (150 mL, 1 M) using 5 mL unneedled syringe from about 5 cm above the solution surface to prepare the beads. After 1 hr gelation process, fresh beads were collected and rinsed using distilled water and dry with cloth to remove excess water.

2.3 Moisture content and bead size

Moisture content of fresh beads was determined by drying 5 g of the beads in an oven at 105°C until constant weight. Bead diameter was measured as the average diameter of 5-fresh beads. The beads were examined using a vernier calliper (Type 530-104, Mitutoyo, Kawasaki, Japan) with a minimum reading scale of 0.05 mm.

2.4 Encapsulation efficiency and loading capacity

A hundred milligram of beads was dissolved in 50 ml distilled water for 30 min under stirring. This solution (10 mL) was placed in 100 mL-volumetric flash with 1,10-phenanthroline (10 mL, 1 g/L), sodium acetate buffer (8 mL, 1.2 M), and hydroxylamine hydrochloride solution (1 mL, 100g/L) then diluted to 100 ml. After 10 min shaken for color development, the absorbance of the solution was read using spectrophotometer at 508 nm against iron standard curve. The encapsulation efficiency (EE) was further calculated using Equation (1).

$$\text{Encapsulation efficiency } (\%) = \frac{\text{Fe}_{\text{sample}}}{\text{Fe}_{\text{initially added}}} \times 100\% \qquad (1)$$

$$\text{Loading capacity } (\%) = \frac{\text{Fe sample}}{\text{w dried sample}} \times 100\% \qquad (2)$$

2.5 Iron release

The iron bead (0.5 g) was dissolved in 150 mL HCl (0.1 M, pH 1.2) or phosphate buffer solution (0.1 M, pH 7.4) under constant stirring. After 90 min, the beads were collected and the filtrate was determined for the iron release.

2.6 Syneresis

The percentage of syneresis was determined by measuring the amount of weight loss during storage for 24, 48 and 72 hrs at room temperature after the bead gel was formed, using the equation (Sartika *et al*., 2021).

$$\text{Syneresis } (\%) = \frac{w_o - w_t}{w_o} \times 100\% \qquad (3)$$

where w_0 was the initial weight of hydrogel before syneresis and w_t was the weight of hydrogel after syneresis at certain time.

3. Results and discussion

Alginate was used to encapsulate iron through gelation method using $CaCl_2$ solution as a cross link agent to form the iron beads. Hence, ability of alginate to form a gel is an important property. The beads were formed when the Na^+ ions of the alginate were displaced by divalent, in this case Ca^{2+} . A high availability of Ca^{2+} in the cross-link solution led to increase the ability to coordinate with the guluronate blocks of alginate and formed beads (Zhang *et al*., 2021) which encapsulated iron.

3.1 Ratio of iron-alginate

Ability of alginate beads in trapping iron was determined in terms of iron loading and encapsulation efficiency. Higher iron led to encapsulate more iron as shown in the loading result (Figure 1A). In this work, the iron (II) was dissolved in alginate solution prior to be dropped to Ca^{2+} solution as a crosslinking agent to create beads. Since, both cations are divalent hence it seemed that the presence of iron contributed on creating more crosslink with alginate. This resulted to more iron trapped in the beads. Similar result was reported by Goh *et al*. (2012) who found either Cu^{2+} and Zn^{2+} contributes to the crosslink of alginate-Ca^{2+} in different affinity. This higher crosslink decreased free volume space within the beads, hence reduced the efficiency (Figure 1A). This result was supported by Swamy *et al.* (2015).

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Figure 1B shows that increase of iron-alginate ratio tended to decrease the water content. Alginate polymers have carboxyl and hydroxyl groups, which could bind water molecules. Addition of iron content led to decrease those functional groups to bind the water. Moreover, increase the iron created more electrostatic interaction with the present functional groups. As a result, less sites were available to absorb water in the alginate. Wichchukit *et al*. (2013) found a decrease water absorption on a mix alginate matrix.

Figure 1B also shows an increase of iron-alginate ratio led to reduce the bead size. Lower alginate decreased viscosity of the solution which made the solution dropped easier and resulted smaller size of beads. Similar result was reported by Ramos *et al.* (2018). Moreover, Swamy *et al*. (2015) reported higher crosslink resulted a more compact and rigid network structure of the beads.

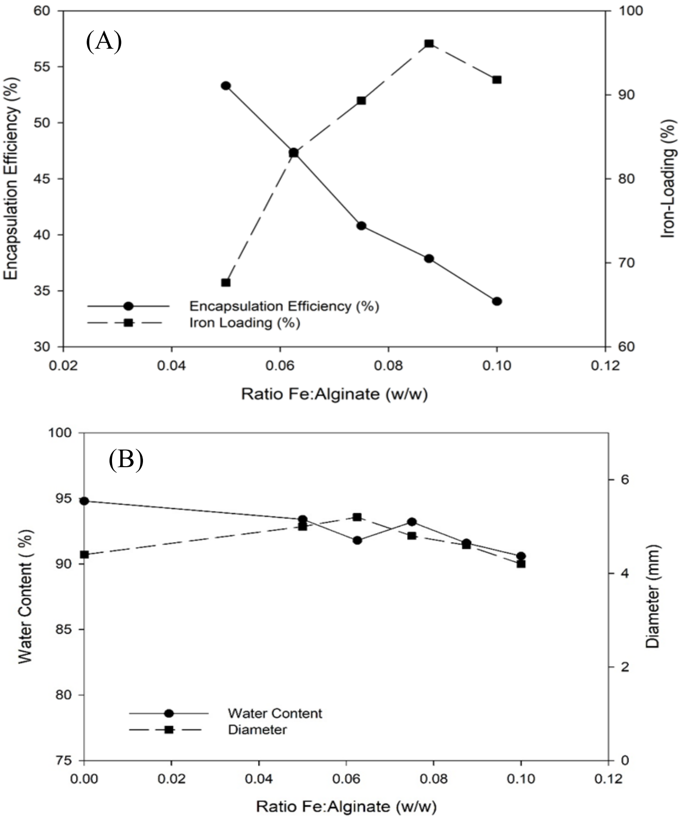

Figure 1. Effect of iron-alginate ratio on encapsulation efficiency and iron loading (A) and moisture content and bead diameter (B).

Increasing iron-alginate ratio changed the appearance of the bead from transparence into cloudy (Figure 2A). A low iron-alginate ratio had a brittle texture, while an increase in the iron-alginate ratio resulted in the chewier texture of the beads. Ramdhan *et al*. (2018) reported higher crosslinked alginate resulted stronger beads. Moreover, the ratio related with shape of the bead. The bead of low ratio of iron-alginate (0, 0.05 and 0.0625, w/w) has a spherical bead shape, while a further increase in the iron-alginate ratio (0.075, 0.0875 and 0.1w/w) produces a tailed bead type. The tail was shaped because the bead was quickly formed, probably due to the large number of cross-links that were created in short time of gelation. This condition supports the loading results.

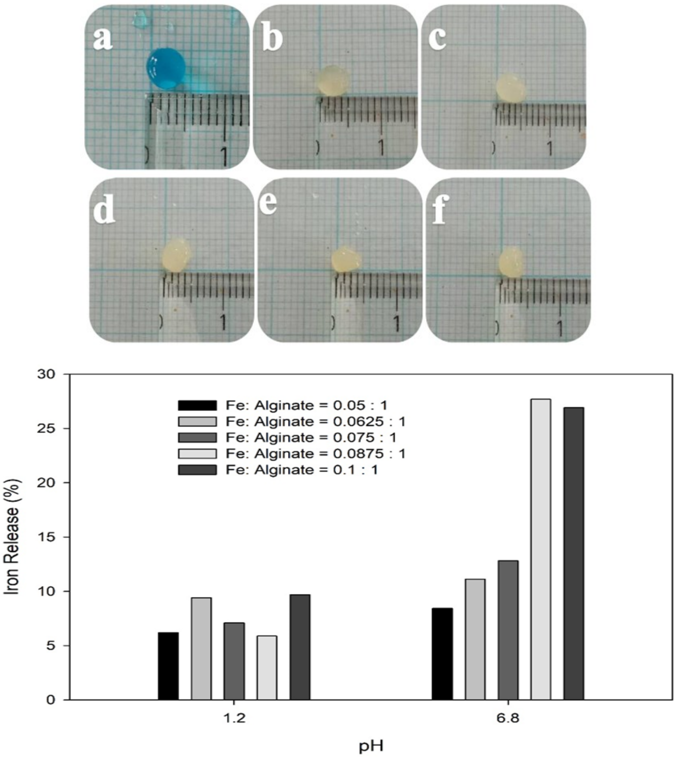

Figure 2. The appearance of the bead at iron-alginate ratio (a) 0, (b) 0.005, (c) 0.0625, (d) 0.075 (e) 0.0875, (f) 0.1, subsequently (top) and iron release at pH 1.2 and 6.8.

The release profile of iron from alginate powder was determined using two solutions, which represent, the nonenzymatic fluid of gastric solution (pH 1.2) and the intestinal condition (pH 6.8) (Ching *et al*., 2017). In general, the effect of the iron-alginate ratio was directly proportional to iron release at both pHs (Figure 2B). The iron releases at pH 1.2 was lower than at pH 6.8. Lower release in acidic than in neutral condition has also been reported by other authors (Hurrell and Egli, 2010; Ching *et al*., 2017). Chan *et al.* (2011) stated, this trend is due to suppressing disassociation of the carboxyl group in the alginate molecule at low pH conditions. The protonated carboxyl groups formed a more compact gel network due to the reduced electrostatic repulsion between the alginate polymers (You *et al*., 2001). At neutral pH, the gel network was more tenuous because the carboxyl group did not protonate, resulting in larger gel pores and greater iron release (Jeffre *et al*., 2022). The result showed that the range of iron release was 5.88-9.68% under acid conditions and 8.42-26.9% under neutral conditions. These results were lower than Pratap *et al.* (2018) who reported 90% release of iron from chitosan at pH 2 within 30 mins and 15% at pH 7 within 2 hrs. The results showed that alginate has better potential as an iron encapsulant when using ionic gelation compared to chitosan in protecting iron release.

Figure 3 shows syneresis was in line with increasing iron-alginate ratio. The addition of iron created competition between iron and Ca^{2+} in binding the polymer. Polymer chains that were not cross-linked, created hydrogen bonds either with other polymer or water. Since the hydrogen bonds were weak, they allowed the water to release and resulted in syneresis (Rajmohan and Bellmer, 2019). Hence, syneresis was higher at a higher crosslink alginate. Similar trend was reported by Ramdhan *et al.* (2018).

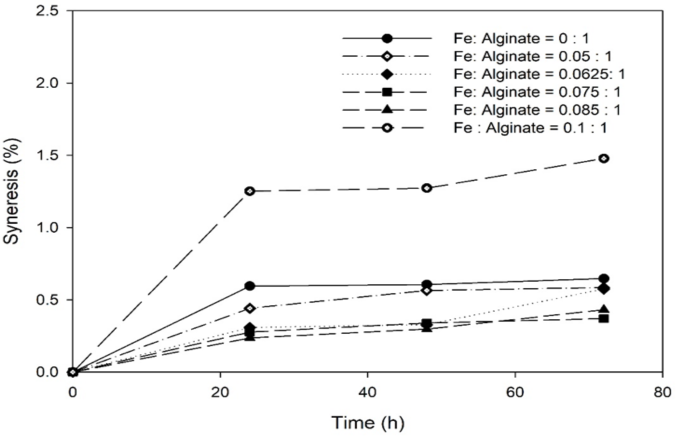

Figure 3. Effect iron-alginate ratio on syneresis.

3.2 pH-gelation

Various pHs of gelation were conducted for 0.05 (w/w) iron-alginate ratio. Iron loading was in line with pH gelation, while the efficiency showed reverse trend (Figure 4A). The highest efficiency (56.23%) was observed at pH 5 of gelation, followed with decreasing efficiency on higher pH gelation (Figure 4A). Similar phenomenon was reported by Zhang *et al.* (2016). They suggested that this trend is related to the strength of the electrostatic interactions between the active compound and alginate molecules. At higher pH, a strong electrostatic repulsion occurs between anionic active compound and anionic alginate that results on difficulties in trapping the active one. Moreover, at higher pH the beads tend to be swollen due to unprotonated of the carboxyl group that make the pores within the alginate are bigger and results on losing the iron from the trapping (Ramdhan *et al.*, 2019).

Effect of pH-gelation on water content and diameter of the beads was represented in Figure 4B. Alginate is a copolymer that consists of mannuronate and guluronate blocks with pKa values for each carboxylate are 3.38 and 3.65, respectively. When pH was below the pKA, the carboxylic group was mainly in protonated state with the present of H^+, the condition that disrupts the coordination between the Ca^{2+} and alginate. This reduced the available sites for crosslinking, thus weakens the crosslinking network (Buenaflor *et al.*, 2022). This weak condition allowed more water to penetrate the hydrogel structure and resulted in higher water content and bigger bead size in lower pH (Figure 4B).

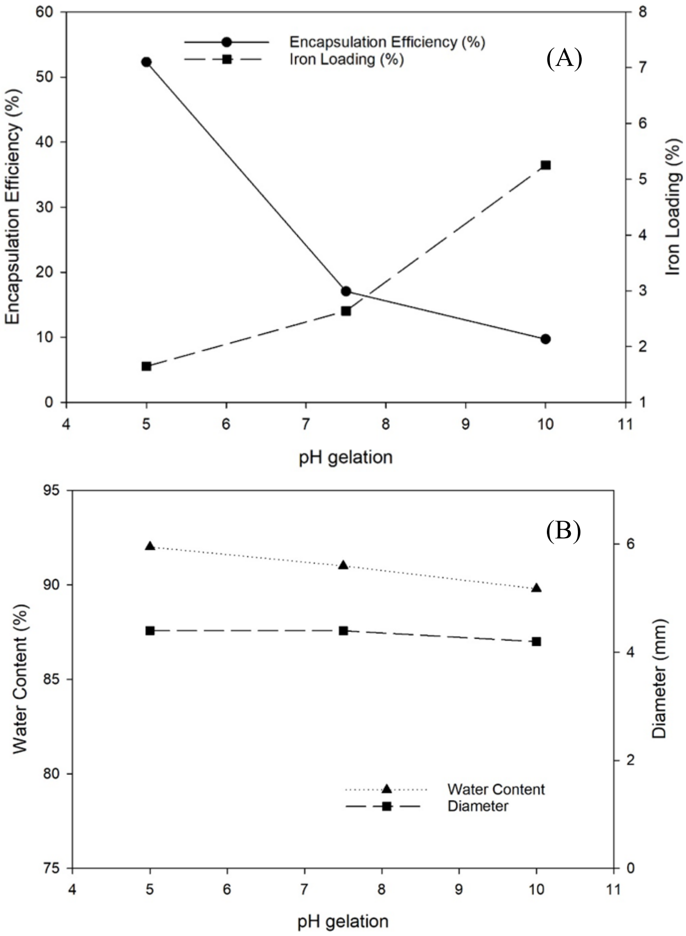

Figure 4. Effect the pH gelation on (A) iron loading and efficiency encapsulation and (B) water content and beads diameter.

Figure 5A shows all the beads in round shape regardless the pH. However, low pH-gelation tended to form unfirm and more fragile beads. Buenaflor *et al.* (2022) reported on acid gelation solution, the bead does not retain shape and squishy. However, the result obtained showed that the beads maintain their round shape even in lower pH. This suggested that alginate and the crosslink agent availability in this work were in balance to create the crosslink. Chuang *et al.* (2017) reported a low pH value demonstrates more significant effect on the particle shape at a low alginate concentration.

Iron release in 2 pHs is presented in Figure 5B. Iron release at pH 6.8 was slightly higher than that of pH 1.2. Lin *et al.* (2022) reported that the alginate beads are shrink in low pH leads to increase mechanical strength and denser beads. As a result, lower iron release is observed in pH 1.2. Meanwhile, an ion exchange was suggested occurred in the low pH-gelation that maintain the bead firmness.

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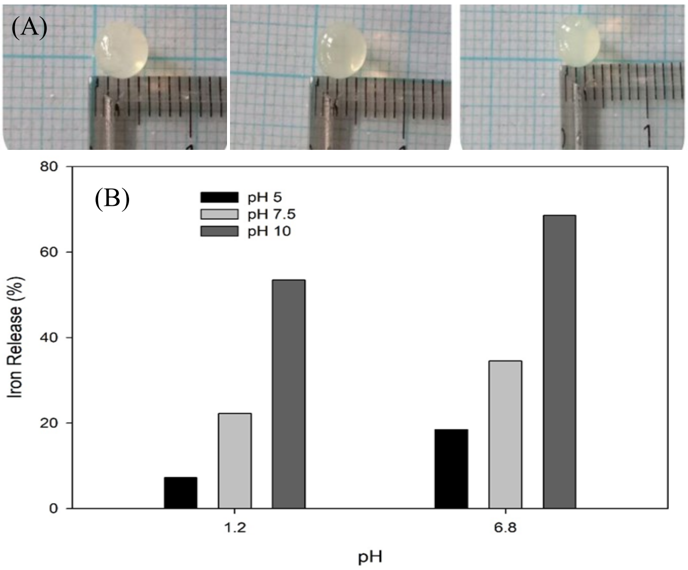

Figure 5. Effect pH gelation on (A) bead appearance and (B) iron released from encapsulation at pH 1.2 and 6.8.

Figure 6 shows higher syneresis was found in the beads prepared in lower pH-gelation, and this syneresis increased with time. The syneresis starts straight after the gel formed (Ramdhan *et al*., 2019). Hence, the result obtained could be a continue phenomenon that starts since the bead formation where in the acidic-gelation medium the beads had decreased in repulsion between protonated carboxyl functions that allowed more water to loss during storage. Higher syneresis in lower gelation pH also was reported by Ramdhan *et al*. (2018).

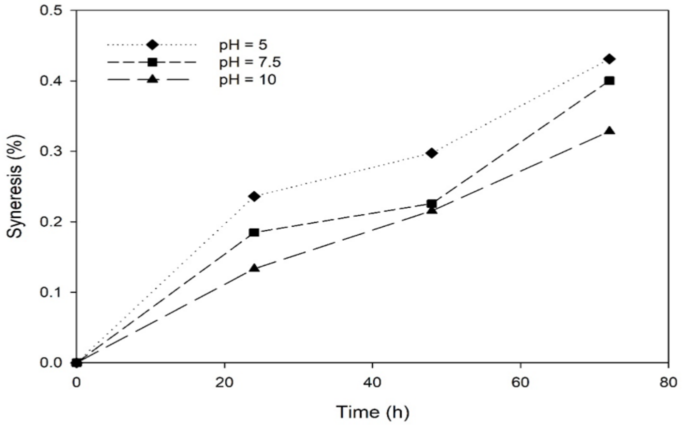

Figure 6. Effect the pH gelation on syneresis.

4. Conclusion

Neutral pH-gelation produced softer bead texture. Higher ratio of iron-alginate and pH-gelation had a negative correlation with moisture content, diameter of the iron bead and encapsulation efficiency. The highest efficiency encapsulation (56.23%) was found in lower iron-alginate ratio and acid pH-gelation. In ratio and pH variation, iron release was higher in pH 6.8 than that in pH 1.2 solution. Increase in iron-alginate ratio led to have more syneresis effect. However, higher pH-gelation tended to have lower syneresis.

Conflict of interest

The authors declare there is no conflict of interest.

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