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# Konjac gum and maltodextrin compound tablets as carriers of IgY for sustained release in stomach

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**Abstract**

Egg yolk immunoglobulin (IgY) is a biologically active ingredient with high immunogenicity; however, its instability in the acidic environment of the upper gastrointestinal tract limits its application in oral formulations. In the present work, an encapsulation system based on maltodextrin (MD) and konjac gum (KGM) was developed as a protective carrier for IgY for targeted release to retain stability. A simulated gastric model was used to compare the release characteristics of the different formulations, and to explore the optimal release mode. To better understand the controlled release mechanism of MD and KGM composite tablets, the release curve, macrostructure, microstructure, and water mobility were analysed. Results indicated that the sustained release of IgY from MD and KGM composite tablets was mainly driven by Fick diffusion and dissolution. As the concentration of KGM increased, the release rate of IgY from the tablets decreased, and the release mechanism gradually changed from diffusion to erosion. The dense cross-linkage between MD and KGM helped prevent tablet disintegration, and slowed down the release of IgY. In addition, the dissolved KGM formed a film on the tablet surface to control the erosion rate. It can be concluded that the optimal formulation of an IgY-loaded tablet was a mass fraction of 50% MD, 30% KGM, and 20% IgY. The present work provided a practical method to protect the biologically active ingredients from acidic destruction in the stomach during oral treatment.

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## Introduction

Immunoglobulins (Ig), also known as antibodies, are glycoproteins secreted by plasma cells in response to external antigenic stimuli, and play a vital role in humoral immunity (Amro *et al*., 2018). Immunoglobulins are widely present in the bodily fluids of living organisms, and their main role is to bind to antigens to eliminate pathogenicity, and protect the body from foreign invaders. Immunoglobulins can be classified into five different types: IgA, IgM, IgG, IgE, and IgD (Leslie and Clem, 1970). Eggs contain three types of Ig, namely IgY, IgM, and IgA. The IgM and IgA are mainly found in egg whites, while IgY is only found in the yolk, hence it is called egg yolk immunoglobulin or egg yolk antibody (Rose *et al*., 1974). The advantages of IgY as compared to other animal-derived Ig can be summarised as high precision of immunoassay, strong immune response, good stability, low production cost, high yield, high purity, and no drug resistance.

As the crystallisable fragment (Fc) of IgY is less cross-reactive with mammalian IgG, false positives and negatives occur less frequently, thus leading to unusually high detection accuracy (Larsson *et al*., 1991). The antigen is poorly immunogenic in mammals, and it is difficult to obtain the corresponding antibodies. Immunised poultry can be used to produce IgY, and the corresponding specific antibodies can be produced with a small amount of antigen. The IgY is stable over a wide temperature and pH range (-20 ~ 70°C, pH 3.5 ~ 11.0), and can withstand high pressure (Thu *et al*., 2017). Eggs are readily available, and can be stored under refrigerated conditions for up to six months. The preparation process is convenient, fast, and does not require blood collection from animals (Spillner *et al*., 2012; Xu *et*

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*al*., 2012). The IgY, which contains about 100 ~ 200 mg/egg, is the main Ig in egg yolk, and can be easily purified. Eggs have a much higher yield of Ig than similarly sized mammals (Akita and Nakai, 1992; Hau and Hendriksen, 2005).

The IgY has a strong safety profile, and promising application prospects in the fields of nutrition, animal husbandry, biotechnology, and pharmaceutical chemistry. As an ideal food additive, IgY can be used for food preservation and disease prevention. Often, IgY can also be added to infant food, and middle-aged and elderly healthcare products, to enhance the immunity of immune-vulnerable groups. Heo *et al*. (2002) added specific anti-*Helicobacter pylori* IgY to dairy products such as cheese, ice cream, and yogurt drinks, and found that it inhibited the growth of the bacterial pathogen in the stomach after consumption. Horie *et al*. (2004) prepared specific anti-*Helicobacter pylori* IgY after immunising layers with *H. pylori* urease, added it to yogurt, and found that it inhibited *H. pylori* infection after consumption. Sarker *et al*. (2001) found that anti-HRV IgY alleviated diarrhoea symptoms in children.

However, the application of IgY in oral formulations is limited by the instability of stomach juice under extreme acid conditions. Studies have shown that the secondary structure of IgY is destroyed under the acidic conditions prevailing in the upper gastrointestinal tract, such as low pH, thereby rapidly inactivating and reducing the stability of IgY to pepsin (Shimizu *et al*., 1992). Based on the above background, in the present work, a sustained-release chewable tablet of IgY in a simulated gastric environment was designed to control the rate of IgY release from gastric juice, thus increasing its active action time. Maltodextrin was used as the main raw material for the preparation of chewable tablets. The effect of konjac gum addition on the microscopic, macroscopic, and mesoscopic structural changes of the tablets during the swelling process and the slow-release mechanism of the composite system were also analysed. The present work demonstrated IgY as an oral nutritional chewable tablet and a nutritional additive in food systems.

## Materials and methods

*Sample preparation*

Egg yolk immunoglobulins were prepared with different ratios of konjac gum and maltodextrin. The percentage of IgY in the tablets was fixed at 20%. The percentages of konjac gum were set to 0, 10, 20, 30, and 40%. The percentages of maltodextrin were set to 80, 70, 60, 50, and 40%. Each tablet weighed 250 mg with a 10 mm diameter. The maltodextrin-konjac gum composite tablet samples containing 0, 10, 20, 30, and 40% konjac gum were labelled KGM 0, KGM 10%, KGM 20%, KGM 30%, and KGM 40%, respectively.

*Determination of release curve of IgY*

The prepared IgY tablets were placed in simulated gastric juice (1% NaCl solution with pH 2.0), shaken at 120 rpm in a constant temperature water bath at 37°C, and digested for 15 min, 30 min, 45 min, 1 h, 2 h, 3 h, 4 h, and 5 h, respectively. The tablets were then removed from the simulated medium at 6 h to determine the protein concentration. The egg yolk Ig release curve was then plotted using time as the abscissa, and protein concentration as the ordinate.

*Observation of microstructure of maltodextrin-konjac gum tablets*

The maltodextrin-konjac gum tablets with konjac gum concentrations of 10 and 30%, and the control group tablets, were placed in simulated gastric juice for digestion. After digestion for 10 min, the microstructure of the tablets was observed under an optical microscope.

The swelled tablets were dried using a freeze dryer, and the surface structure of the dried tablets was observed by scanning electron microscopy. The dried samples were fixed on the sample table, and sprayed with gold under vacuum conditions for final observation.

*Determination of water mobility of maltodextrin-konjac gum tablets after swelling*

IgY-konjac gum (10 and 30%) composite tablets and the control tablets were mixed with 200 μL of deionised water, and the spin-spin relaxation time ($T_2$) of the tablets was measured using a low-field nuclear magnetic resonance analyser, according to the method of Xu *et al*. (2020). The samples were tested in a tube with a diameter of 25 mm. $T_2$ was measured according to the Carr-Purcell-Meiboom-Gill (CPMG) sequence (Xu *et al*., 2020). The number of echoes was set to 3,000, the echo time was 0.25 ms, and data were obtained for eight scan repetitions.

*Statistical analysis*

All experiments were performed at least three times in parallel. SPSS version 17.0 (SPSS Inc., Chicago, USA) for Windows was used for the analysis of variance (ANOVA), and Duncan's multiple range test was used to evaluate the level of significance ($p < 0.05$). Origin 2018 was used for chart drawing and formula simulation.

## Results

*Effect of konjac gum percentage on gastric release characteristics of IgY-loaded tablets*

The percentage of IgY release from tablets prepared with different proportions of konjac gum is shown in Figure 1.

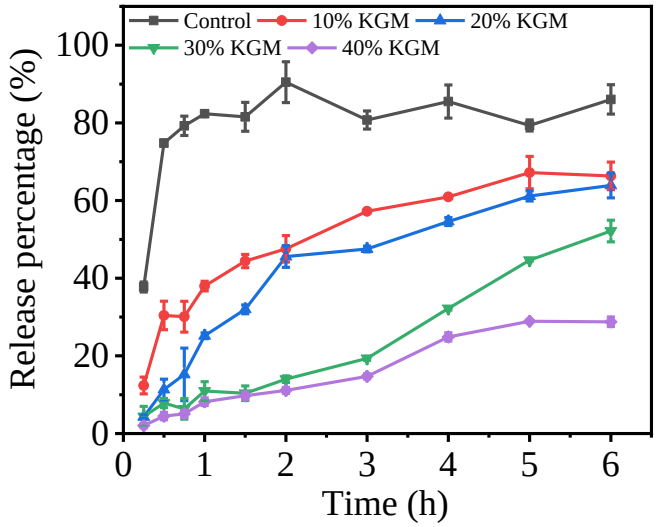


**Figure 1.** Release percentages of IgY in simulated gastric fluid from maltodextrin-based tablets in combination with various percentages of konjac gum.

It was apparent that the IgY release rate gradually increased as the amount of konjac gum decreased. The release curves of 10 and 20% konjac gum showed similar trends, and the IgY release from 10% konjac gum was slightly higher than that of 30 and 40% konjac gum. The release curves of the samples containing 30 and 40% konjac gum had similar trends over 4 h, and the release rate of 30% konjac gum increased after 4 h. To better explain the release properties of the maltodextrin-konjac gum composite IgY-loaded tablets, the curves in Figure 1 were fitted to the release model. The commonly used release model formula is shown in Table 1, where $M_t$ denotes the release amount at time t, M denotes the total loading capacity, k denotes the rate constant, and n denotes the diffusion index in the Ritger-Peppas release model formula, and Table 2 displays the specific release mechanism represented by n (Ritger and Peppas, 1987). The release mechanism can be classified into three types: swelling system, where the drug release carrier swells to dissolve the drug; diffusion system, where the drug is diffused and released from the drug release carrier; erosion system, where the drug release carrier will be degraded and eroded to a degree that controls the release of the drug.

**Table 1.** Expression formula of release models.

| Number | Release model | Expression |
|---|---|---|
| 1 | Zero-level release | $M_t/M_\infty$=kt |
| 2 | First level release | $M_t/M_\infty$=1-$e^{-kt}$ |
| 3 | Higuchi | $M_t/M_\infty$=$kt^{1/2}$ |
| 4 | Ritger-Peppas | $M_t/M_\infty$=$kt^n$ |

**Table 2.** Relationship between diffusion index n and release mechanism in Ritger-Peppas release model.

| Drug release mechanism | Diffusion index n | | |
|---|---|---|---|
| | Sheet model | Cylindrical model | Spherical model |
| Fick diffusion | < 0.5 | < 0.45 | < 0.43 |
| Irregular diffusion (the synergistic effect of dissolution and diffusion) | 0.5 ~ 1.0 | 0.45 ~ 0.89 | 0.43 ~ 0.85 |
| Dissolution mass transfer | > 1.0 | > 0.89 | > 0.85 |

Table 3 shows the fitted parameters for each set of release curves and models. As shown in Table 3, the fitting $r^2$ of the release curve for the tablets without konjac gum was low, thus indicating a poor fit, and that the formulation did not have sustained release effects. In the zero-order model fitting, the fitting $r^2$ of the release curve of the tablets containing 30 and 40% konjac gum was above 0.96, which was an ideal release curve. In the Higuchi release model fitting (characterising the sustained release behaviour), the fitting $r^2$ of the release curve of the konjac gum-IgY tablet reached above 0.88, thus

indicating that konjac gum-IgY tablets had better-sustained release properties. In the fitting of the Ritger-Peppas release model, the fitting $r^2$ of the release curve of the konjac gum-IgY composite tablet was higher than 0.95, thus indicating a better fitting. The fitting n value of the tablets containing 10% konjac gum was less than 0.5, thus indicating that the tablet release was mainly driven by Fick diffusion. The fitting n value for tablets containing 20 and 40% konjac gum was between 0.5 and 1, thus indicating that the tablet release was mainly due to the synergistic effect of erosion and diffusion. The fitting n value of the tablet containing 30% konjac gum was slightly greater than 1, thus indicating that the tablet release was mainly driven by erosion. It can be seen that the release mechanism of konjac gum-IgY tablets gradually changed from diffusion to erosion as the addition of konjac gum increased, thus indicating that konjac gum as a drug release carrier could control the release of IgY by enhancing the erosion effect. A study by Gu *et al*. (2021) also showed that the release mechanism of IgY from alginate/carrageenan bed was mainly due to the synergistic effect of corrosion and diffusion.

**Table 3.** Fitting parameters of releasing curves of KGM-IgY tablets.

| Konjac gum | Zero | | Higuchi | | Ritger-Peppas | | |
|---|---|---|---|---|---|---|---|
| proportion (%) | K | $r^2$ | k | $r^2$ | k | $r^2$ | n |
| 0 | 0.0014 | 0.2101 | 0.0391 | 0.1850 | 1.1293 | 0.4555 | 0.1180 |
| 10 | 0.0034 | 0.8434 | 0.0807 | 0.9358 | 0.1852 | 0.9550 | 0.3839 |
| 20 | 0.0042 | 0.8852 | 0.1086 | 0.9896 | 0.0535 | 0.9500 | 0.5887 |
| 30 | 0.0034 | 0.9677 | 0.1033 | 0.8830 | 0.0020 | 0.9708 | 1.0993 |
| 40 | 0.0021 | 0.9665 | 0.0512 | 0.9557 | 0.0058 | 0.9692 | 0.8310 |

*Morphological analysis of maltodextrin-konjac gum composite tablet*

The macroscopic view of maltodextrin-konjac gum composite tablets in simulated gastric juice is shown in Figure 2.

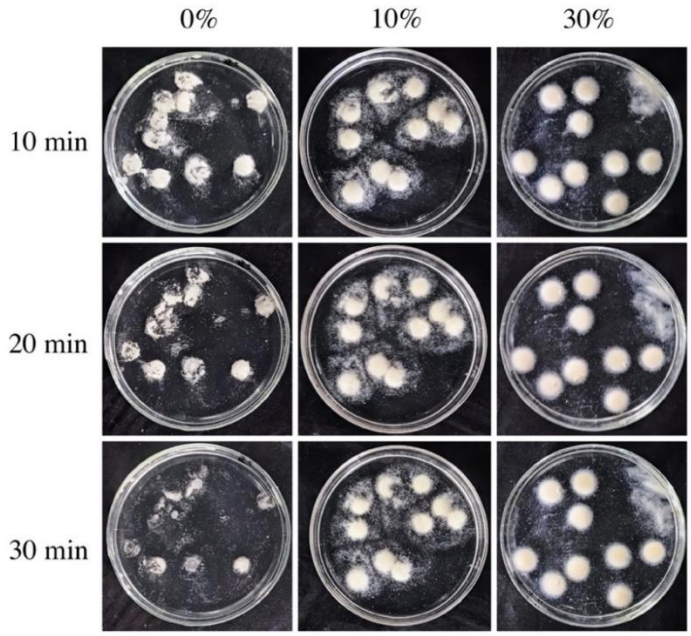


**Figure 2.** Macrographs of KGM-IgY compound tablets (at KGM ratios of 0, 10, and 30%) soaked in simulated gastric fluid for 10, 20, and 30 min.

It was observed that the tablet without konjac gum gradually dissolved in the simulated gastric juice, and lost its original shape after 30 min of immersion in the simulated gastric juice. The tablet with 10% konjac gum swelled and dissolved slightly in the simulated gastric juice. As the digestion time increased, more small particles were released around the tablets. The tablets with 30% konjac gum swelled more obviously in the simulated gastric juice, with a translucent gelatinous substance appearing around the tablets and a change in tablet volume. The tablets containing 40% konjac gum were more stable during disintegration, with fewer small particles dissolving around them. The macroscopic phenomenon was associated with the release curve as shown in Figure 1 which visualises the tablet swelling phenomenon.

After 10 min of immersion in simulated gastric juice, IgY tablets containing 0, 10, and 30% konjac gum were placed under an optical microscope to observe the edges of the tablets. As shown in Figure 3, the edges of the tablets without konjac gum were not significantly swollen, and the surface displayed was smooth. The tablet with 10% konjac gum showed a three-dimensional gel-like film with many tiny bubbles wrapped around the film. The edge of the tablets with 30% konjac gum had a more pronounced three-dimensional structure with a lower density of

wrapped bubbles. Other studies have shown that tablets containing konjac gum form a pseudo-gel barrier upon hydration (Alvarez-Manceñido *et al*., 2008). The formed gel-like surface layer could prevent water from entering the interior of the tablet, thus protecting the drug inside the tablet, and achieving a sustained release effect.

After drying by freeze dryer, the surface and cross-section of the tablets soaked in simulated gastric juice for 10 min were observed by SEM. As can be seen in Figure 4, the first column shows the surface structure of tablets at low magnification, and the second column shows the cross-section of tablets at low magnification. The third column shows the cross-section of tablets at high magnification. Graphs a, b, and c represent IgY tablets without konjac gum, which had dry and cracked plane surface with many hole-like depressions in the section, and no obvious cross-linked network structure. Graphs d, e, and f represent the tablets containing 10% konjac gum with a smooth surface and slight wrinkles. The cross-section displayed a cross-linked layered structure. Graphs g, h, and i represent the tablets containing 30% konjac gum with a smooth and uneven surface. From the cross-section graph, a cross-linked structure with a cotton-like dense network and small pores was observed. The SEM microscopic images indicated that the incorporation of konjac gum could promote the interaction between components forming the cross-linked network, which then prevented the tablets from breaking in gastric juice. In addition, the dense internal structure could interfere with the diffusion of IgY, thus controlling the release behaviour.

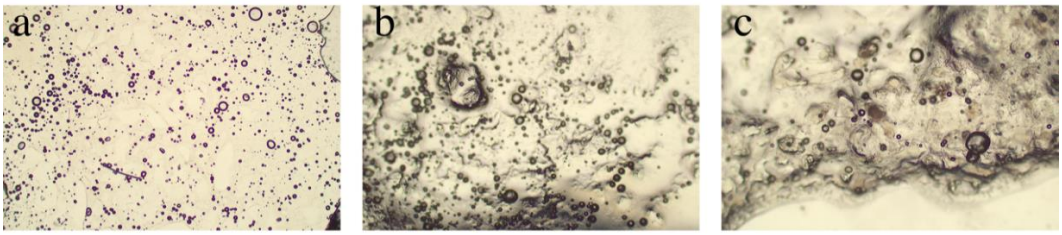


**Figure 3.** Micrographs of KGM-IgY compound tablets (a: 0% KGM; b: 10% KGM; and c: 30% KGM) soaked in simulated gastric fluid for 10 min (40× magnification).

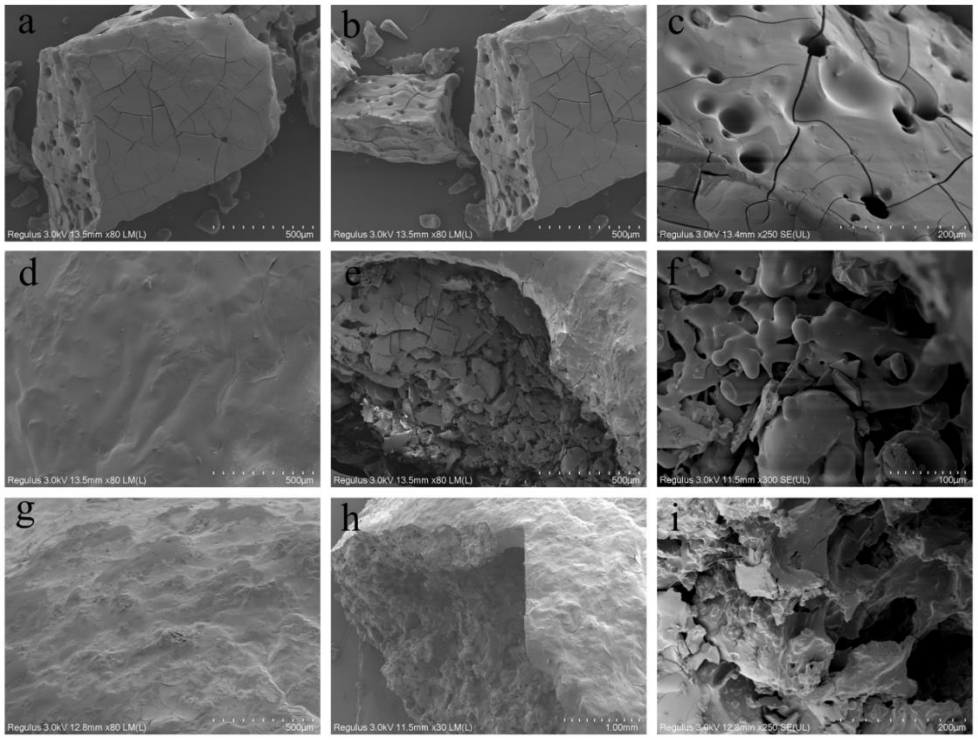


**Figure 4.** SEM micrographs of IgY tablets without KGM (a × 80 surface; b × 80 section; and c × 250 section), 10% KGM-IgY tablets (d × 80 surface; e × 80 section; and f × 250 section), and 30% KGM-IgY tablets (g × 80 surface; h × 80 section; and i × 250 section) which were soaked in simulated gastric fluid for 10 min.

*Water mobility of composite tablets after soaked in simulated gastric juice*

Figure 5 shows the mobility of water hydrogen protons in konjac gum maltodextrin composite tablets soaked in simulated gastric juice. The protons were determined based on the variation of the spin-spin relaxation time ($T_2$) of the three hydrogen proton pools. The pools were marked as peaks 1, 2, and 3 based on the value of $T_2$ from the minimum to the maximum. The shorter the $T_2$, the lower the degree of freedom of the water hydrogen protons. Peak 1 represented the water hydrogen protons tightly bound to the macromolecule. Peak 2 represented the loosely bounded water hydrogen protons around the macromolecule. Peak 3 represented free water hydrogen protons without boundaries (Au *et al*., 2015). As shown in Figure 5, the peak areas of peak 2 were in the following order: tablets containing 30% konjac gum > tablets containing 10% konjac gum > the tablets without konjac gum. The peak area of peak 3 was in the following order: tablets without konjac gum > tablets with 10% konjac gum > tablets with 30% konjac gum. The results indicated that the incorporation of konjac gum helped to improve the water-binding ability of maltodextrin-based tablets. The water in the tablets combined with macromolecules and lost its fluidity, while the tablets without konjac gum had a weaker ability to restrict the mobility of water. Another phenomenon that we should note was that the tablet samples without konjac gum showed a larger peak 1, thus indicating a relatively higher ability to interact with water. It can be inferred that the decrease in water mobility, when used with konjac gum, was due to the constraint effect of the dense network between macromolecules, which could be verified by the microstructure of the tablet containing 30% konjac gum.

In addition, when soaked in simulated gastric juice, the single maltodextrin-prepared tablet tended to break into particles, thus increasing the rate of dissolution. In contrast, the maltodextrin-konjac gum composite tablets would maintain their intact shape, and gradually erode. In general, the smaller the particle size, the faster the rate of water absorption and swelling, which was not conducive for drug sustained release (Chen *et al*., 2021). Therefore, konjac gum could be used as a drug-release carrier for tablets that controls the erosion rate of tablets by binding to water molecules, thus controlling the release of the drugs.

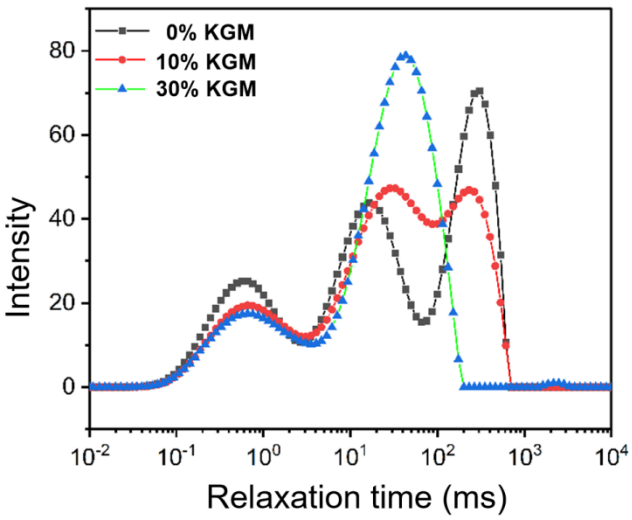


**Figure 5.** Spin-spin relaxation time ($T_2$) spectra of low field NMR of KGM-IgY compound tablets (KGM ratios of 0, 10, and 30%).

## Conclusion

The incorporation of konjac gum facilitated the sustained release of IgY from maltodextrin-based tablets. As shown in the results of the release curve fitting, the release mechanism of IgY from the composite tablets changed from diffusion to erosion with the increase in the percentage of konjac gum. The controlled erosion was related to an increase in the interaction between maltodextrin and konjac gum, thus forming a dense three-dimensional “cotton-like” network. The continuous network on one hand prevented tablet disintegration, and on the other hand, inhibited IgY diffusion, thus controlling the IgY release behaviour. The present work demonstrated a new strategy for the delivery of physically active macromolecules to increase tolerance to the harsh gastrointestinal environment.

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