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Stabilization and Target Delivery of Nattokinase Using Compression Coating

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ABSTRACT The aim of the work is to develop a new formulation in order to stabilize a nutraceutical enzyme Nattokinase (NKCP) in powders and to control its release rate when it passes through the gastrointestinal tract of human. NKCP powders were first compacted into a tablet, which was then coated with a mixture of an enteric material Eudragit® L100-55 (EL100-55) and Hydroxypropylcellulose (HPC) by direct compression. The activity of the enzyme was determined using amidolytic assay and its release rates in artificial gastric juice and an intestinal fluid were quantified using bicinchoninic acid assay. Results have shown that the activity of NKCP was pressure independent and the coated tablets protected NKCP from being denatured in the gastric juice, and realized its controlled release to the intestine based on in vitro experiments.

KEYWORDS Coating, Compaction, Controlled release, Eudragit L100-55, Nattokinase, Nutraceutical

INTRODUCTION

Natto is a traditional Japanese food made from fermented soybeans. For centuries, it has been used both as a staple food and a folk remedy for heart and vascular diseases, fatigue and beriberi. However, it was not until the early 1980s that one of the key properties of natto was discovered by Sumi et al. (1987). They found that natto contains a fibrinolytic enzyme, which has a unique potency. It is later named 'nattokinase' which means 'enzyme in natto'. Nattokinase (NKCP) is produced when a kind of microorganism, *Bacillus natto*, acts on boiled soybeans. It has similar properties to a subtilisin-like serine protease. Its molecular weight and an isoelectric point were measured to be approximately 27.7kDa and 8.6, respectively (Fujita et al., 1993; Peng et al., 2003).

Thrombosis is a serious medical condition that arises as a result of fibrins accumulating in a blood vessel, causing the blockage of blood flow to the muscle tissues. It is one of the main causes of health problems such as angina, heart attacks and in more severe cases, strokes and senility. The human body's solution to these problems is the natural production of a tissue-type plasminogen activator (t-PA) and plasminogens. In its natural form, the t-PA is used to convert the plasminogens into a fibrinolytic enzyme called plasmin, which in turn dissolves the blood clot. In many

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thrombosis sufferers, the level of production of this enzyme is impeded and thrombolytic agents such as artificial tissue-type plasminogen activator and urokinase should be used. One of the disadvantages of such agents is their high dependency on the existing level of plasminogens present in the human body. They also have a relatively short half-life and large quantities are needed for them to work effectively.

Owing to its natural ability to fight blood clots, NKCP has been considered to be an excellent alternative treatment option. It closely resembles plasmin and is able to dissolve fibrin directly. In addition, it has the ability to convert plasminogens to plasmins as well as to enhance the natural production of tissue-type plasminogen activator (t-PA) (Peng et al., 2003). In other words, NKCP can prevent the coagulation of blood and dissolve existing thrombus (Sumi et al., 1990; Omura et al., 2005). However, the activity of enzyme is sensitive to temperature and pH of its environment. It has been reported that the activity of enzyme was unstable below pH 5.0 (Sumi et al., 1987). Current commercial products of NKCP are in the form of water-soluble powders. It is likely that most of the enzyme activity is lost via oral administration to the human gastric intestinal tract. In order to stabilize it, direct exposure of the enzyme to the gastric fluid should be avoided.

The objective of this work is to develop a new product formulation in order to stabilize a nutraceutical enzyme Nattokinase (NKCP) in powders and to control its release rate when it passes through the gastrointestinal tract of human. In pharmaceutical industry, fluidized-bed coating of particulate active ingredients with enteric materials is a common practice. However, the NKCP powders with a typical diameter of 20 μm are difficult to handle by this process. There is an alternative process, called compression coating, which is based on powder compaction and possesses many attractive manufacturing features since it is relatively, easy to operate and does not use any liquid (Chan and Zhang, 2002). However, the choice of a coating material is crucial since it should not only be enteric but also form rigid tablets at low compression pressure since some active ingredients, particularly biological materials may be sensitive to compression pressure. Recent work has demonstrated that pure Eudragit® L100-55, which is a methylacylic acid-methylmethacrylate copolymer, is highly compatible (Yap et al., 2006). In this work, the compression coating was used to encapsulate NKCP powders with a mixture in different ratios of the enteric coating material Eudragit® L100-55 (EL100-55), a release modifying agent Klucel® hydroxypropylcellulose (HPC) (Leopold and Eikeler, 2000; Kumar and Kumar, 2001), and a binder magnesium stearate (MgSt). It is hoped that the mixture of the coating materials can form a rigid tablet, which can prevent the direct contact of the acid with NKCP when the tablet is exposed to gastric juice, and allow the enzyme to be released in intestines, based on in-vitro studies.

MATERIALS AND METHODS

Eudragit® L100-55 (methacrylic acid) was provided by Röhm Pharma Polymers (Darmstadt, Germany). KLUCEL® Hydroxypropylcellulose (HPC) was provided by Aqualon (Düsseldorf, Germany). NKCP was given by Daiwa Pharmaceutical Co Ltd (Japan) as compliments. Magnesium Stearate (MgSt) was provided by Merck (UK). Chromogenic substrate S-2251 was purchased from Quadratech Diagnostics Ltd., Surrey, UK, Bicinchoninic Acid (BCA) Protein Assay, reagent was purchased from Pierce, Northumberland, UK, Sodium chloride, calcium chloride, 2-amino-2-hydroxymethyl-1,3-propanediol, sodium phosphate, hydrochloric acid, and citric acid were purchased from Sigma, Dorset, UK. All chemicals were of analytical grade and used as received without further purification.

Preparation of Core Pellets

Compaction of NKCP powders into a tablet was achieved using a Lloyd Material Testing Machine (Lloyd Instruments, 6000R, Fareham, UK). The NKCP powders of a given amount (100 mg) were placed into a die with 6 mm diameter and compressed with a flat-face punch to form a tablet. The compression speed was controlled by setting the punch displacement to 2 mm per min. The initial height of the powder bed was measured from the height of the die deducting the traveling distance of the punch before it touched the powder bed (indicated by the increase in force imposing on the punch).

Compression Coating of Core Pellets

The coating materials were mixed thoroughly according to four different formulations shown in Table 1. The powder bed was made by filling a die of 10 mm with 50% of the coating materials. One core tablet produced at a compression pressure of 120 MPa was then placed at the centre of the powder bed using a core locator (Fig. 1), before the remaining half of the coating materials was added to the die. Tablet coating was performed under a compression pressure of 90 MPa and compression speed of 2 mm per minute using the flat-face punch.

Mechanical Characterization of the Coated Tablets

The thickness of the ejected tablets was measured using a micrometer (Mitutoyo, Japan). This was performed soon after they were formed and 24 hr later to allow for any elastic recovery (if any). Elastic recovery of each tablet was determined using the following equation (Armstrong and Hainesnu, 1972)

$$\text{Elastic recovery (\%)} = \frac{H - H_c}{H_c} \times 100 \qquad (1)$$

where H_c is the height of the tablet newly formed (mm) and H is height of the tablet after 24 hr.

A diametral compression test was performed 24 hr after the formation of the tablets to determine their tensile strength, which was calculated using the following equation (Fell and Newton, 1970)

$$\sigma = 10^{-6} \times \frac{2F}{\pi D t} \qquad (2)$$

where σ represents the tensile strength (MPa), F is the force applied to the tablet corresponding to fracture (N), D is the tablet diameter (m) and t is its thickness (m).

TABLE 1 **Formulations of Coating Materials used for Coating NKCP Tablets**

Formulation	Total amount	Eudragit® L100-55 (w/w)	HPC (w/w)	MgSt (w/w)
Formulation 1 (F1)	300 mg	99%	0%	1%
Formulation 2 (F2)	200 mg	89%	10%	1%
Formulation 3 (F3)	200 mg	83%	17%	0%
Formulation 4 (F4)	300 mg	100%	0%	0%

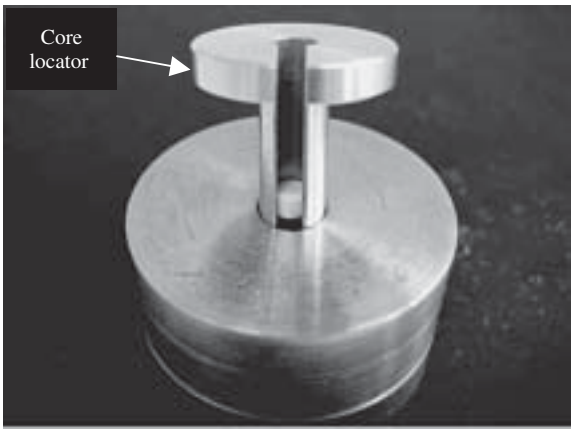

FIGURE 1 **Photo of the Core Locator.**

Determination of Enzyme Activity by Amidolytic Assay

NKCP activity was determined by an amidolytic method (Fujita et al., 1993). The assay started with the incubation of the mixture of 0.1 mL Tris buffer (0.5M), pH 7.4, containing 17.5 mM NaCl with 0.1 mL enzyme solution at 37°C for 1 min. Soon after, 0.1 mL substrate S-2251 (4 mM, CHROMOGENIX) was added to the mixture, which was then incubated in a water bath at 37°C for 10 min. The reaction between NKCP and substrate S-2251 was stopped by the addition of 1 mL citric acid solution (2% w/v) acting as a stopping reagent. Absorbance of the sample was then measured at wavelength of 405 nm, using a spectrophotometer (Cecil CE1020, UK) to measure the liberation of *p*-nitroaniline. Blank solution for zero adjustment was made using the same procedure as stated above, but distilled water was used instead of the substrate solution.

The effect of compression pressure on the NKCP activity was investigated, and its activity after the compression coating was also determined in order to identify whether the enzyme was denatured after it was coated by a mixture of materials including Eudragit® L100-55 which has acid side chains.

In-Vitro Dissolution Tests

The dissolution of coated tablets was performed in a basket apparatus tuned at 37°C with a rotating speed

of 100 rpm according to the British Pharmacopoeia Standard. A tablet was immersed into 500 mL of in-vitro solution in a dissolution vessel with a jacketed round bottom and a basket stirrer. In order to mimic human gastrointestinal (GI) tract, the tablet was first immersed in a simulated gastric fluid (0.1 N HCl, pH 1.2) for 2 hr, and then transferred to a simulated intestinal fluid (phosphate buffer solution, pH 7.4) for approximately 5.5 hr. 100 μL of the solution were withdrawn from the vessel regularly and placed into a microplate with 96 wells and the same amount of fresh solution was added to the dissolution vessel, which maintained its constant liquid volume during the experiment. The relative concentration of the enzyme released from the tablet was detected using BCA assay at the wavelength of 540 nm (SLT Spectra, Austria) using NKCP as a reference protein. Since the NKCP concentration in the dissolution fluids (500 mL) was very low, and its activity could not be detected accurately using the method described in Section 2.4, the enzyme, which still remained within the tablet during the dissolution, was recovered and its activity was measured. For a control purpose, 5 tablets of NKCP were dissolved in 25 mL simulated gastric and intestinal fluids respectively, which were maintained at 37°C, and its activity, as a function of exposure time was determined.

RESULTS AND DISCUSSION

Effect of Compression Pressure on Enzyme Activity

NKCP powders were compressed into a tablet at different pressures ranging from 60 to 400 MPa. The lowest pressure of 60 MPa was chosen because below this value the tablets were not rigid enough to handle. Fig. 2 show the relative activity of enzyme versus compression pressure. As can be seen, there was no significant loss of the enzyme activity for the pressures investigated. Based on these results, NKCP powders were compressed into a tablet at 120 MPa for further experiments. It was also found that the activity of NKCP before and after being coated by the mixture of materials including Eudragit® L100-55 powders via compression at 90 MPa did not change significantly (data not shown), which indicates the enzyme was not denatured after it contacted the solid Eudragit® L100-55 powders.

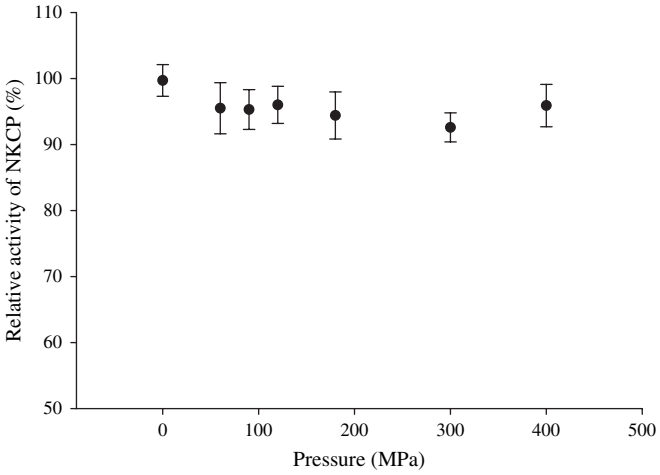

FIGURE 2 **Effect of Compression Pressure on the Remaining Enzymatic Activity of NKCP in Tablets. The Error Bars in all Figures Represent 95% Confidence Limits.**

Analysis of Compaction Pressure and Punch Displacement Data

Fig. 3 shows the punch displacement versus pressure for compacting NKCP powders into a tablet at a pressure of 120MPa. As expected, the displacement increases with the pressure. Their relationship was fitted by Kawakita equation (Kawakita and Ludde, 1971) with two constants *a* and *b*.

$$\frac{P}{C} = \frac{1}{ab} + \frac{P}{a} \tag{3}$$

where P is the applied pressure, C is the relative volume reduction which can be determined from the punch displacement ΔH and the initial height of the powder bed H_p,

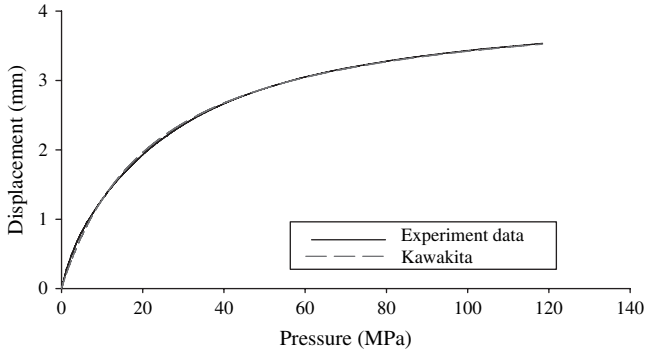

FIGURE 3 **Punch Displacement Versus Pressure Data and Fitting using Kawakita Equation for Compacting NKCP Powders into a Tablet at a Speed of 2mm min^{-1}.**

$$C = \frac{(V_0 - V)}{V_0} = \frac{\Delta H}{H_p} \quad (4)$$

where V_0 is the initial volume of NKCP powders and V is the volume under a given applied pressure. 'a' represents the initial porosity of the powder bed and 'b' is considered to be related to the resistance towards volume reduction. 'b' should reflect the inter-particle friction, particle-die wall friction, cohesion, and densification of particles that resist repacking during compaction. In general, 'b' is inversely proportional to the compression resistance (Adams et al., 1994; Adams and McKeown, 1996; Guo et al., 2002). The constants 'a' and 'b' were calculated by firstly plotting a graph of P/C versus P, which is a straight line. According to Eq. (3), the slope of the straight line equals '$1/a$' and the intercept represents '$1/ab$'. The least square method was used to determine the values of 'a' and 'b' from the data of compaction of NKCP powers into a tablet, which were found to be 0.690 ± 0.014 and 0.044 ± 0.001, respectively, with the correlation coefficients all greater than 0.98, see Table 2.

Punch displacement versus pressure data for compacting the coating materials (F1 to F4 in Table 1) with a NKCP tablet entrapped were obtained, and their relationship was also fitted by Kawakita equation in order to gain insights into the porosity 'a' and the compaction resistance 'b' of the coated materials used. All the fittings show similar trend and hence a typical figure is presented (Fig. 4). As can be seen, the fitting based on Kawakita equation deviates slightly from the experimental data at the initial stage of compaction. This deviation could result from a slight uneven top surface of powders in the die after die filling since it was not sensible to tap the die hard enough to flatten the surface layer as it might misplace the position of the core tablet. Overall, the fitting by Kawakita equation shows good agreement with the experimental data as the relative mean error between the fitting and experimental data is 9% and the maximum error 27%. The values of constants 'a' and 'b' were obtained and are presented in Table 2. Interestingly, the constant value 'a' did not vary dramatically among different formulations, which implies that the initial porosity of the powder bed was about the same for individual formulations. This is logical as majority of the powders used in each of the formulations is Eudragit® L100-55.

On the other hand, F2 and F3 have slightly greater 'b' values than F1 and F4, which mean they have relatively less compaction resistance. It is believed that HPC, which is often used as a lubricant, reduced the friction between the die wall and the powder bed during the compaction process and hence facilitated tablet compaction, which might also create tablets with lower porosity.

Elastic Recovery

The elastic recovery of the coated tablets made of the 4 formulations is also presented in Table 2. The tablets made from F1, F2, and F3 had similar elastic recovery, which is significantly less than what was produced by F4. When the data of F1 and F4 are compared, the effect of MgSt on reducing elastic recovery in F1 is evident. It is worth mentioning that all the tablets formed from F4 were capped whereas tablets formed from F1 were fine. Capping is a common problem during tablet formation, which attributes to either the elasticity of the materials, presence of entrapped air within the interstices of the tablet during compaction or friction from the die wall and internal shear stress that causes initiation and propagation of cracks (Long and Alderton, 1960; Long, 1960; Ritter and Sucker, 1980; Mann et al., 1981; Kuppuswamy et al., 2001). However, it is not surprising that MgSt reduced the capping phenomenon and elastic recovery (F1 and F2) as it acted as a lubricant to prevent

TABLE 2 **Kawakita Equation Constants and Elastic Recovery for NKCP Tablets with a Coating Made of Different Formulations. The Relative Maximum Error (*M*), Relative Mean Error (*A*) and the Correlation Coefficient of Each Fitting (*R*) using KAWAKITA EQUATION are also Given. The Values after the Sign ± Represent Two Times of the Standard Error of the Mean Based on Five Repeated Measurements**

Formulation	Constant 'a'	Constant 'b' $(Mpa)^{-1}$	*M*	*A*	*R*	Elastic recovery (%)
F1	0.68 ± 0.01	0.032 ± 0.001	16%	4%	0.998	9.3 ± 0.4
F2	0.62 ± 0.01	0.041 ± 0.001	22%	7%	0.993	7.8 ± 1.1
F3	0.61 ± 0.01	0.044 ± 0.001	27%	9%	0.989	9.1 ± 0.6
F4	0.69 ± 0.03	0.027 ± 0.001	17%	5%	0.998	15.1 ± 1.6

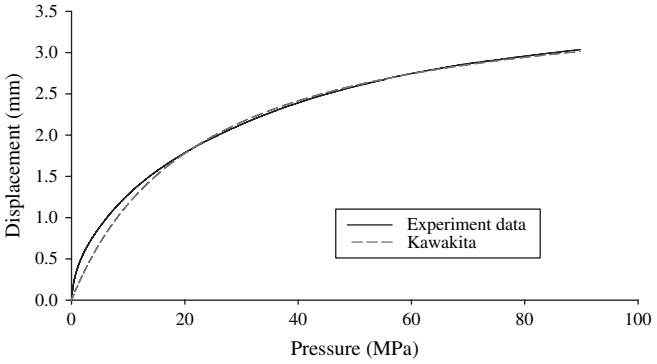

FIGURE 4 **Kawakita Equation Fitting to Punch Displacement Versus Pressure Data for Compression Coating of a NKCP Tablet with Coating Materials of F3.**

tablets from sticking to the die and punch as well as decreased the yield strength of powder mixtures (De Boer et al., 1978; Ertel and Carstensen, 1988; Zuurman et al., 1999).

However, tablets formed from F3 without MgSt showed no tendency of capping, and relatively low elastic recovery. This might be due to the effect of HPC included in F3. There are reports claiming that HPC is widely used as a binder in direct compaction and exhibits good plasticity (Yoshiharu and Tsuneji, 1974; Guo et al., 1998; Alvarez-Lorenzo et al., 2000), which enhanced particle-particle interactions during tablet coating by compaction. Nevertheless, tablet capping was observed when 90% (w/w) Eudragit® L100-55 and 10% (w/w) HPC were used (data not shown), which implies that the content of HPC in the mixture must reach a certain ratio in order to increase its plasticity and to produce desirable tablets. Due to the observed capping phenomenon, F4 was excluded from further experiments.

Tensile Strength

The fracture modes of the tablets made of different formulations are shown in Fig. 5. As can be seen, the tablet of F1 fractured into approximately two equal halves after diametral-compression whilst tablets produced from F2 and F3 fractured into two unequal parts, a chunk in the core area and a piece near the edge. Strictly speaking, Eq. (2), can only be used to calculate the tensile strength of the tablets with a fracture mode described by Fig. 5A (Fell and Newton, 1970). Nevertheless, the equation was still used to estimate the tensile strength of tablets made of different formulations for comparison, it was found tablets that contained HPC had the higher tensile strength (3.27 ± 0.12 MPa and 3.18 ± 0.08 MPa for F2 and F3, respectively) than those with Eudragit alone for F1 (1.69 ± 0.04 MPa), which is in line with the reports mentioning that HPC, when used as a binder, could produce strong tablets (Joneja et al., 1999). This observation might result from the stronger adhesive bonding between different components than cohesive bonding between Eudragit® L100-55 particles. It is also believed that addition of HPC could prevent propagation of cracks that might exist in the tablets when Eudragit® L100-55 powders alone were used (F4). Enhancement of tensile strength with the addition of HPC might also be due to an increase in densification as it has been reported that HPC powders exhibit good compressibility and compatibility (Fukui et al., 2000).

Generally all the tablets had a tensile strength greater than 1 MPa, which means they were strong enough for handling in typical industrial operations (Chung, 2004).

FIGURE 5 **Fractured coated tablets after diametral compression. (A) Normal tensile failure for F1. (B) and (C) Failure for F2 and F3 respectively.**

In-Vitro Enzyme Release Rate

The cumulative release of enzyme to dissolution liquids (in terms of relative percentage) with time is shown in Fig. 6. Basically, there was very little enzyme (< 5%) released into the simulated gastric juice (SGJ) at the end of exposure for F1 and negligible release for F2 and F3, which indicates the coating materials well protected the enzyme diffusing out of the coated tablets. It was also observed that there was no significant change in the shape of the tablets after being exposed to SGJ for 2 hr. When the tablets (F1) were exposed to the simulated intestinal fluid (SIF), there was a gradual release of the enzyme initially (up to 4.2 hr), and then a faster release until whole of the enzyme was released at a time of 5.2 hr, which might correspond to complete dissolution of their outer shell and the core was disintegrated respectively. Optical photos (not shown) taken at different stages of the dissolution experiments

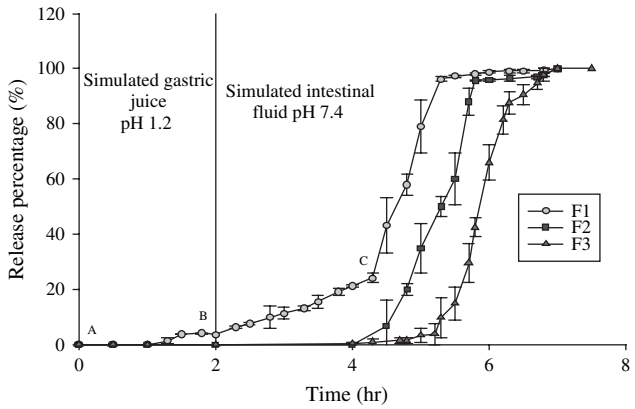

FIGURE 6 **Release Profiles of NKCP from Coated Tablets Made of Different Formulations. The Tablets were Firstly Immersed in SGJ for 2 hr and Then Exposed to SIF for Approximately 5.5 hr.**

proved that a fraction of the NKCP core embedded within the tablet was dissolved by liquid diffused in until point C (Fig. 6) where the coating disintegrated, which allowed the core to be exposed to the dissolution liquid directly and hence a faster release was observed from this point. A relatively shorter complete release time than the other two formulations was observed for F1. The tablets of F1 contained only Eudragit® L100-55 and MgSt. The presence of MgSt might create a more porous structure that enabled a faster liquid uptake, which facilitated wall disintegration and subsequently released their contents (Sungthongjeen et al., 2004).

Release of NKCP from tablets made of F2 and F3 in SIF only occurred at the 2nd and 3rd hr, respectively. The two stage release rate was not very obvious for these two formulations, which might be due to the fact that the tablets started to release the enzyme only after the outer shell was removed completely by dissolution or erosion of the hydrophile gelation layer formed around the core surface (Turner et al., 2004). Visual observations showed that only part of the core was wet before the outer layer was dissolved. It is the characteristic of HPC to be able to form a gel layer when wet. This offers protection to the core by blocking the voids and resisted the penetration of liquid through it. On the other hand, it is speculated that the tablets of F2 and F3 had smaller voids between particles due to the existence of HPC particles which were smaller and more irregular in shape than Eudragit® L100-55 (data not shown). This observed difference in the release rate of NKCP from the coated tablets of different formulations is also in good agreement with the Kawakita fit, which shows that F2 and F3 had lower compaction resistance and thus might have relatively lower permeability.

Overall, the time required to release all NKCP from the tablets increased with the content of HPC in the formulations, and whole of the enzyme was released between 3–5 hr in SIF, which is desirable.

Determination of Release Kinetics

In order to determine the release kinetics of enzyme in the tablets formed from each formulation, a power law model, which is proposed (Siepmann & Peppas, 2001), is used here.

$$\frac{\mu_t - \mu_{p1}}{\mu_\infty} = k(t - t_{p1})^n (t \geq t_{p1}) \qquad (5)$$

where t_{p1} is lag time after which NKCP began to release, μ_t, μ_{p1}, and μ_∞ (100%) are absolute cumulative amount of enzyme released at time *t,* t_{p1} and infinite time respectively, k is a constant incorporating structural and geometric characteristics of the system, and *n* is Power Law constant, an indication of the mechanism of enzyme release. The value of *n* for the case of cylindrical carriers is 0.45 for Case – I Fickian diffusion (diffusion – controlled drug release) and 0.89 for Case – II transport (swelling – controlled release). Any figure in between those two values indicates anomalous transport mechanism or in the other words non-Fickian release. t_{p1} is included in this equation because the lag time for each formulation was different. All the fittings were performed for the enzyme release in SIF region.

Eq. (5) was found to fit all the curves reasonably well (Fig. 7), with all the correlation coefficients greater than 0.98. The values of the constants in the model for each formulation are shown in Table 3. It is not sensible to fit the entire release profile for F1 as a whole since before and after depletion of the outer shell there were two distinct phases of enzyme release.

The fitting results indicate the release of enzyme for the 1st phase of F1 and the whole phase of F2 was dominated by swelling since the n value is 0.89. Corresponding to the 2nd phase of F1 and the whole phase of F3, the release was controlled by a mixed effect of diffusion and swelling. The results are reasonable in view of the fact that majority of the tablets were made

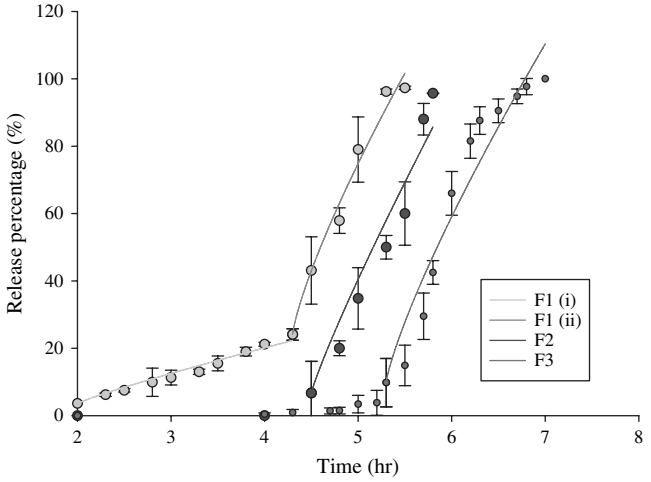

FIGURE 7 **The Experimental Data of the In Vitro Release Profile for F1, F2, and F3 Fitted with a Power Law Model Described by Eq. (5).**

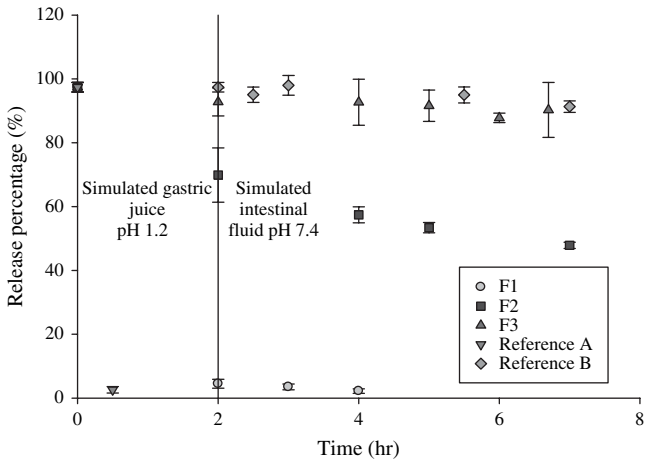

FIGURE 8 **Activity of NKCP in Coated Tablets Made of Different Formulations. Reference *A* and Reference *B* Indicate the Pure NKCP Core Tablets Dissolving in SGJ and SIF Respectively for a Designated Period of Time.**

TABLE 3 **Effect of Coating Formulation on the Constants in the Power Law Model Described by Eq. (5) and the Corresponding Correlation Coefficient (*R*)**

Formulation	μ_{p1}	t_{p1}	*k*	*n*	*R*
Formulation 1 (F1) (i)	3.6	2.0	8.9	0.89	0.997
Formulation (F1) (ii)	24.1	4.3	67.2	0.78	0.998
Formulation 2 (F2)	6.7	4.5	62.6	0.89	0.992
Formulation 3 (F3	9.8	5.3	65.6	0.81	0.993

up of Eudragit® L100-55 and also all the tablets swelled after 2 hr exposure in SGJ (photos not shown).

Enzyme Activity

The activity of the enzyme in tablets of 3 formulations when they were exposed to the two dissolution liquids is shown in Fig. 8. For comparison, pure NKCP core tablets were dissolved in the SGJ and SIF, respectively for a designated period of time and the change of its activity is also presented. Clearly, NKCP lost its activity by 97% after it was exposed to the SGJ for 0.5 hr, which demonstrates again the necessity of using coating for protection. In contrast, there was no significant loss of its activity when the pure NKCP core tablets were exposed to the SIF for 5 hr.

The tablets made of different formulations offered protection of the enzyme to a different extent. It seems that F1 failed to meet the purpose. Even though Fig. 6 shows that less than 5% of enzymes were released after exposure for 2 hr in SGJ, a significant amount of liquid diffused into the core of the tablets (photos not shown) and deactivated NKCP, but the size of pores in the tablets might not be big enough to allow the dissolved NKCP molecules to diffuse out. Similar explanations might be applicable to F2 where some SGJ diffused into the core and denatured the enzyme. However, about 50% of enzyme activity was retained after an exposure time of 5 hr in SIF. It is speculated that the SGJ that diffused into the tablets during the first two hours continued to denature the enzyme until the pH within it was neutralized by the phosphate buffer solution pH 7.4 after the tablets were transferred to SIF. Among the 3 formulations, the most promising one is F3, since the data shows that the coated tablets prevented direct contact between SGJ and enzyme itself as well as allowed the whole entrapped enzyme released in the intestinal fluid within 5 hr. The constant enzyme activity for F3 for the duration of dissolution experiments suggests that the loss of the enzyme activity was negligible.

CONCLUSIONS

The activity of enzyme NKCP was investigated before and after its powders were compacted into a tablet and then coated with materials made of different formulations. Results showed that the punch displacement versus pressure data for compaction of NKCP powders into a tablet fit Kawakita equation well. It has been found that the compression pressure up to 400 MPa did not cause significant denaturation of NKCP. After the NKCP tablet was further coated

by materials, which were made of different formulations, the extent of protection of NKCP from being denatured in artificial gastric juice depended on the formulations. An appropriate mixture of Eudragit® L100-55 and HPC (83% / 17% w / w) seemed to fully protect the enzyme from being denatured after the coated tablets were exposed to the SGJ for 2 hr, and also allowed the enzyme to be fully released after being exposed to SIF for 5 hr. Future work includes structural characterization of the tablets and exploitation of the coating materials for stabilization and controlled delivery of other water soluble active ingredients.

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