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Investigation of Controlled Release Microparticles for Fish Feeds

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## 一、中文摘要

水產養殖類似人類其他活動對環境有不良影響，因此應有更大的省思盡全力來保護水中生態系統。由於在水中與在魚腸道中酸鹼值的劇烈差異，本研究中我們嘗試發展酸鹼敏感性微粒來控制魚飼料釋放。魚飼料購自市場並研磨成粉，不同比例的藻酸鈉與 Eudragit EP 100 溶液是作為凝集液，將魚飼料粉導入凝集液中進行微包而成為微粒，體外釋出研究是以改良式 Franz 擴散槽與 pH1.2 鹽酸溶液以及 pH=7.0 磷酸鹽溶液進行 24 小時。結果顯示，魚飼料微粒在中性環境下，可延緩釋離達 12 小時；微粒在酸性的環境下，最初的一小時即經酸催化水解成小粒子並釋出包覆物質。

關鍵詞：控制釋放，酸鹼敏感性，微粒，魚飼料

### Abstract

Aquaculture, like any other human activity can have adverse effects on the environment. Hence, greater consideration is needed towards a general strategy of protecting the aquatic ecosystem at all levels. Owing to drastic variation of pH value between in water and in fish gut, we attempt to develop a pH-sensitive microparticles for controlled release of fish feed in this work. Original fish feed was bought from market and then grinded to powder. Homogenous aqueous solution of

sodium alginate and Eudragit EP 100 in various ratios were used as coagulation fluid. The powder was encapsulated to form microparticle by transferring microparticles into coagulation fluid. The in vitro release study was determined over 24 hours, using modified Franz diffusion cells at HCl solution (pH=1.2) and PBS solution (pH=7.0) as a dissolution medium. The results show that the release of encapsulated microparticle can be reduced at least for 12 hours in neutral medium. Moreover, the resulting microparticles undergo acid-catalyzed hydrolysis into small parts and should therefore release encapsulated material (i.e. Vitamin B2) at an accelerated rate in acidic environments during first one hour.

Keywords: Controlled release, pH sensitive, Microparticles, Fish feeds

## 二、Introduction

Aquaculture appears to have strongest potential to meet the increasing demands for aquatic products in most regions of the world. Global production of farmed fish and shellfish has more than doubled in the past 15 years. Asia contributes more than 90% to the world's aquaculture production. (1-3) Fish farmers actively seek to exploit certain aspects of feeding behaviour to optimize and hopefully therefore to maximize their production. Two aspects are of prime interest, namely increasing appetite and therefore consumption of food and ensuring that dietary energy is maximized for growth and minimized for general daily expenditure (swimming, feeding). (4,5) Thus, fish growers maximize their productivity by using feeds that provide balanced nutrition and result in good feed conversion efficiency. Proper nutrition is an essential component to increased weight gain and overall fish health. An alternative aspect through the production cycle has on occasion come to the fore in recent years in Taiwan is well health management. (6-8) Successful fish health management begins with prevention of disease rather than treatment. Prevention of fish disease is accomplished through good water quality management, nutrition, and sanitation. In regard to water quality management and nutrition, the good feed should not contaminate the water quality. (9)

It is well known that hydrochloric acid is secreted to reduce gut pH and to allow enzymes to work in fish with a stomach. The level of pH value before and after digestion is a key indicator of disintegration of feed. The pH-sensitive controlled release of feed to maximum food consumption is a minimization of waste food, leaching and overall pollution. In the present study, we attempt to investigate controlled release microparticles of fish feed in intensive culture as following: cost-effectiveness of feed (i.e. the cheapest feed that can adequately supply nutrient requirement), attractiveness to the fish, efficiency of the feed conversion ratio, and stability in water to prevent feed loss and minimize water pollution. The results show that the release of

## 三、Results and discussion

The formulations used in the experiments are shown in Table 1. Vitamin B2 was added as a marker for dissolution tests. The mixtures of vitamin B2, CaCl<sub>2</sub> and fish feeds as bulk materials were dry mixed thoroughly. After the water was added as a binding agent, the moistened mass was immediately passed through a 20 mesh sieve. The resulting extrude was dried at temperature 50°C and then crush to form

microparticles. Homogenous aqueous solution of sodium alginate and Eudragit EP 100 in various ratios were used as coagulation fluid. The microparticle was encapsulated by transferring microparticles into coagulation fluid and dried at room temperature. Light scattering measurements of particle size were performed with a Zetasizer particle size analyzer. The microparticle preparations were diluted 1:20 with filtered distilled water. The result shows in Fig 1 and indicates that the microparticles have a mean particle size ranging from 20  $\mu$  m to 60  $\mu$  m. The disintegration studies were carried out in modified Franz diffusion cells and analyzed by turbidimeter. The in vitro release of vitamin B2 from the prepared microparticles was determined over 24 hours, using modified Franz diffusion cells at 700 rpm, 15 ml of HCl solution (pH=1.2) and PBS solution (pH=7.0) at 37°C  $\pm$ 0.5°C were used as a dissolution medium. At predetermined time intervals, an aliquot of the samples was withdrawn and passed through 0.45 $\mu$ m filter. The concentration of Vitamin B2 in each sample was measured spectrophotometrically. It can be seen that the powder of original fish feed bought from market disintegrated significantly at pH 7.0 as well as at pH1.2. The release of encapsulated microparticle can be reduced at least for 12 hours in neutral medium. Moreover, the resulting microparticles undergo acid-catalyzed hydrolysis into small parts and should therefore release encapsulated material (i.e. Vitamin B2) at an accelerated rate in acidic environments during first one hour.

Table 1. Formulae of fish feed pellet product

Composition Amount ratio  
(%,W/W)

Feed dried powder 72.2~92.7

Vit.B2 0~1.0

Eudragit EP100 0~1.3

Sodium alginate 0~2.0

CaCl<sub>2</sub> 0~4.0

Water

0~25

Total

100

Fig. 1. Particle distribution of fish feed microparticles were performed with a Zetasizer particle size analyzer.

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