



Original Research Article

In Vitro and In Vivo evaluation of hydroxypropylmethyl cellulose phthalate capsules

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Abstract

The aim of this study was to develop a novel HPMCP capsule. The HPMCP capsule containing ^{99m}Tc-DTPA and lactose was evaluated in in vitro and in vivo studies. First of all, the HPMCP capsules were prepared and characterized with length and diameter size, brittleness facility, moisture content and microbiological test. In vitro resistance and solubility studies of prepared capsules were tested at pH 1.2 and pH 7.4 buffers. The radiolabeled HPMCP capsules were administered to fasted volunteers. The disintegration times and positions of capsules were recorded by using gamma scintigraphy. In vitro studies showed that HPMCP capsules were gastro resistant for 2 h at pH 1.2 and dissolved at pH 7.4 in 20-25 minutes. The radiolabeled capsules did not disintegrate in stomach whereas disintegrated in intestines. In conclusion, it was found that, the prepared HPMCP capsules can be an alternative to the hard gelatine capsules and used for intestinal targeting.

Keywords: Capsule, hydroxypropylmethylcellulose phthalate (HPMCP), gamma scintigraphy, radiolabelling, technetium-99m.

Introduction

Controlled drug delivery systems have important advantages in treatment of several diseases. In delayed-release systems, the enteric coating of tablets or capsules modifies the release pattern of active ingredient. These types of formulations can be designed to release the drug in intestines. Two general categories may be distinguished, namely enteric coating and controlled release coatings. Enteric coating process may be required for some active substances irritating the stomach or degrading due to acidic pH of the stomach and it is insoluble in the gastric juices, but dissolves readily on passage into the small intestine. The polymers commonly used to achieve enteric properties are anionic polymethacrylates (copolymerisate of methacrylic acid and either methyl-methacrylate or ethyl acrylate cellulose

based polymers, e.g. cellulose acetate phthalate or polyvinyl derivatives, e.g. polyvinyl acetate phthalate [1,2]. Polymers with ionizable phthalic acid groups dissolve much faster at a lower pH than those with acrylic or methacrylic acid groups [3].

The most common mode of action of enteric coatings is pH related solubility; i.e., insoluble at gastric pH but soluble at some pH above 4.5. Cellulose acetate phthalate (CAP) and hydroxypropylmethylcellulose phthalate (HPMCP) are widely used as enteric film coating materials. HPMCP is completely insoluble in gastric fluid but dissolves above pH 4.5 in proximal end of duodenum. [4-7]. In one study, enteric coated HPMC capsule has been designed to achieve intestinal targeting by manufacturing of two different Eudragit® HPMC capsules [1].

Methods based on pH-sensitive delivery systems such as enteric coated dosage forms could be a simple and practical means for colon-specific drug delivery. However such methods do not have sufficient site specificity because, with this type of dosage form, most of the drug is released in the upper small intestine after gastric emptying, even though drug release is effectively prevented in the stomach. Failure of pH-dependent system may be expected due to inter and intra subject variation of GI pH, pH variation due to pathological conditions and diet composition [8,9].

The most commonly used material for manufacturing capsules is gelatin. Although it is possible to coat hard gelatin capsules. The process is at best very sensitive, especially if an aqueous coating system is used, and can lead to shell embrittlement and poor adhesion of the coat to the smooth gelatin surface. A pre-coating can reduce interactions between the gelatin and the enteric polymer but is time consuming and complicated. On the other hand, HPMC capsules have been available commercially, mainly to the dietary supplement industry as a vegetarian alternative to gelatin, for approximately 10 years. As HPMC is often used as a pre-coating material for enteric coated tablets, it may be expected that the application of enteric type polymers to a capsule made from HPMC would result in 'good polymer to polymer' adhesion and compatibility [10].

Scintigraphy is currently one of the most powerful techniques for the interpretation of in vivo behaviour of pharmaceutical formulations [4]. Gamma-scintigraphy is a non-invasive method for imaging the in vivo behaviour of dosage forms. Gamma scintigraphy is an elegant imaging technique which allows the intestinal performance of pharmaceutical formulations to be visualized [16]. A complete picture of drug delivery following oral administration can be

obtained from sequential images. Determination of gastric residence times, times of transit through the small intestine and colon, times and locations of in vivo release and correlation of findings with kinetic data can be evaluated [11,12].

The formulation to be investigated is labelled with an appropriate short-lived gamma-emitting isotope (^{99m}Tc , ^{153}Sm). A gamma-camera can then be used to monitor transit past to the intended site of delivery. In in vivo studies, Technetium 99m -diethylenediaminepentaacetic acid (^{99m}Tc -DTPA) was used as radiopharmaceuticals for gamma-scintigraphy. ^{99m}Tc -DTPA of oral administration does not pass through the digestive barrier. After oral administration of the ^{99m}Tc -DTPA labelled substance is used for gastro-oesophageal reflux and gastric emptying studies [13,14]. In this paper, we describe the manufacture and characterization of HPMCP capsules and their in vitro/in vivo performance.

Materials and methods

Materials

HPMCP (type HP 55, Shin-Etsu Co. Ltd., Japan) was a kind gift from Syntapharm (Germany). Eosin is supplied from Scarlet Fluka. Technetium 99m (^{99m}Tc)-diethylenediaminepentaacetic acid (DTPA) was obtained from the Department of Nuclear Medicine of Dokuz Eylül University.

Preparation of the capsules

The HPMCP capsules were prepared at desired sizes using empty gelatine capsules. 12% solution of HPMCP (type HP 55) with colouring agent (0,1% eosin) in acetone were poured into the moulds and allowed to evaporate approximately 4-8 hours depending on the size of moulds at room temperature. After evaporating, the film layer was formed inside the capsule.

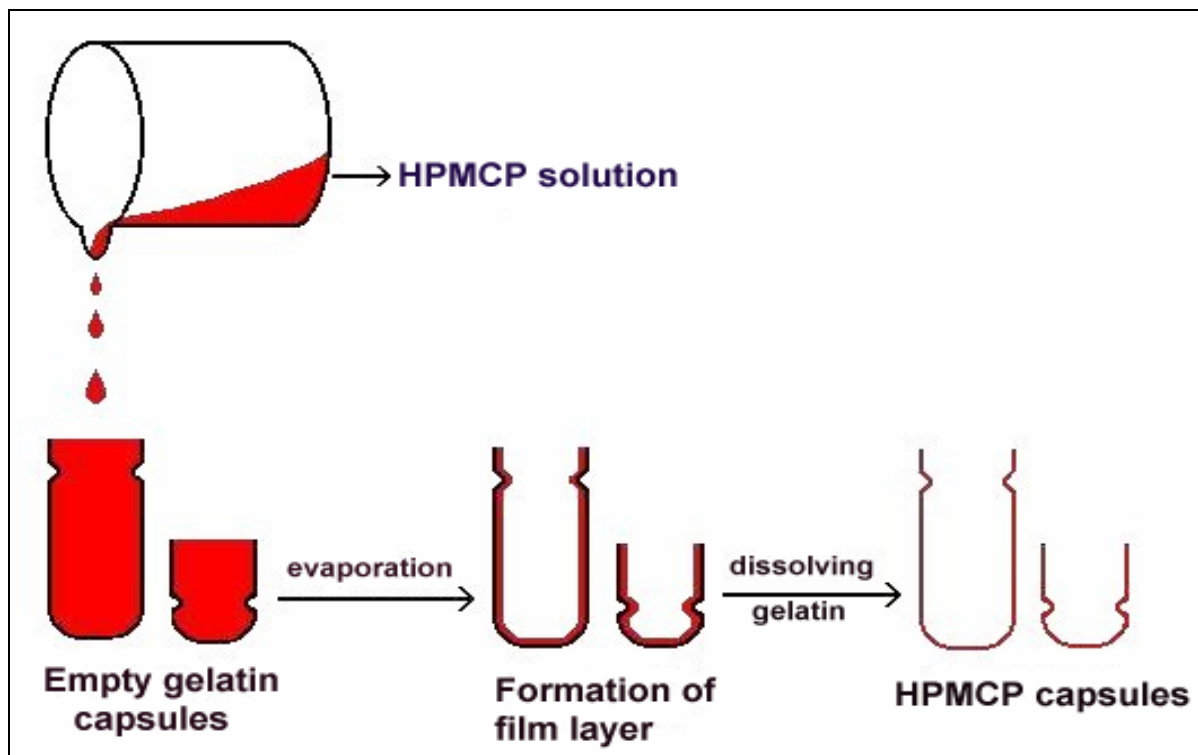


Figure 1: Schematic representation of the preparation of HPMCP capsules.

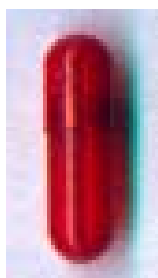


Figure 2: The photograph of HPMCP capsule.

The gelatine capsules containing HPMCP film were dissolved in acidic medium (pH = 1-2) at $\approx 40^\circ\text{C}$. The insoluble HPMCP film layer was remained in the form of capsule in water. Then the capsules were dried at room temperature. The schematic representation of preparation technique and the photograph of HPMCP capsule are shown in Figure 1 and 2.

Characterization of HPMCP capsule

Size of Capsule

The length and diameter of HPMCP capsules were measured by digital micrometer (ID-C112, Mitutoyo Corp., Kawasaki, Kanagawa, Japan).

Their brittleness was measure by using Monsanto GPIT 9544.

Moisture content

The HPMCP capsules were weighted at the beginning of the study, after that they were dried at 105°C during four hours. The weights of capsules were measured at one hour intervals. The loss in weight of capsules was calculated from below equation.

$$\text{Percent loss in weight of capsules} = \frac{b}{a} \times 100$$

a is initial weight of capsule, b is differences between initial and dried weight of capsules.

Microbiological test

The HPMCP capsule was dissolved at pH 7.4 phosphate buffer in sterile condition. The solution was cultivated on EMB Agar, Blood Agar and in Mueller-Hinton Broth (MHB). These were incubated at 37°C for 48 hours. At the end of this period growth results were evaluated.

In vitro resistance and solubility studies

The HPMCP capsules were tested in acidic and alkaline media for their resistance and solubility. In vitro disintegration and dissolution behaviour of capsules were investigated using the gamma counter (SESA Uniscaler I /S). For this purpose, the capsules were filled with 0.1 g lactose and as a radiopharmaceutical agent ^{99m}Tc -DTPA was added as a tracer. The HPMCP capsules containing 0.5 mCi ^{99m}Tc -DTPA were placed into 10 mL 0.1 N HCl solution (pH 1.2) at 50 rpm, 37 °C. At 30 minutes intervals, the radioactivity in test medium was measured for 2 hours (SESA Uniscaler-I/S). After two hours, the undissolved capsules were washed with distilled water and then placed into 10 ml of pH 7.4 phosphate buffer at 37 °C. ^{99m}Tc -DTPA release from the capsules was investigated for 25 minutes (n=6).

In vivo studies

Four healthy volunteers (1 male, 3 female) of 25-30 years of age and 50-60 kg body weight participated in the study after passing a clinical screening procedure that included medical history, physical examination. They were non-alcoholic, non-smokers and were not taken any drugs. All participants signed a written informed consent. After they had been informed of the nature and details of the study in accordance with the Turkish Guidelines for ethics. The study procedures were in accordance with the Helsinki Declaration. ^{99m}Tc -DTPA (0.5 mCi) was added into the capsules containing lactose. Each fasted volunteer ingested ^{99m}Tc -DTPA containing capsule orally with \approx 150 mL of water. The location of the radiolabelled capsule in the gastrointestinal tract of each subject was monitored with a gamma-camera (G.E 600 XR/T). Each volunteer was positioned in front of the gamma camera and anterior images were taken for 180 seconds at frequent intervals over a period of 16–17 h. The lower part of the sternum and umbilicus of volunteers were marked with 0.003 mCi ^{99m}Tc as a marker.

Results and discussion

Characterization of capsule

Size of Capsule

The results of the length and diameter size of capsule are shown in Table 1. The brittleness of HPMCP capsule is about $2\pm 0,045$ kg (mean \pm SD, n=6).

Table 1: The length and diameter of capsules.

Measurement number	Diameter (mm)		Length (mm)	
	Body	Cap	Body	Cap
1	6,930	6,460	18,630	10,990
2	6,850	6,390	18,650	10,940
3	6,920	6,400	18,640	10,960
4	6,900	6,430	18,610	10,930
5	6,870	6,470	18,670	10,980
6	6,910	6,440	18,630	10,950
Average	6,897	6,432	18,638	10,958
SD	0,031	0,032	0,020	0,023

SD: standard deviation

Moisture content

The moisture content of capsule was determined. HPMCP capsule moisture content was about $4.8\%\pm 0,019$ (mean \pm SD) w/w at the end of 4h. The results of the moisture content of capsule (% loss in weight of capsule) are shown in Table 2.

Table 2: The moisture content of capsule (% loss in weight of capsule) with SD (n=6).

Time(minute)	% loss in weight of capsule
60	4,298 \pm 0,023
120	4,432 \pm 0,044
180	4,835 \pm 0,058
240	4,835 \pm 0,019

Microbiological test

The results of microbiological test are shown that no growth was observed on the EMB, Blood Agar and MHB in which HPMCP capsules were tested for microbiological control.

Results of In Vitro Studies

The release of ^{99m}Tc -DTPA from HPMCP capsules, in simulated gastric and intestinal media was investigated. The dissolution profiles from the HPMCP capsule is shown in Figure 3. It was seen that ^{99m}Tc -DTPA was not released from HPMCP capsules during 2 h in pH 1.2. All amount of the ^{99m}Tc -DTPA was released rapidly in pH 7.4 at the end of 25 min. At this pH, the capsules completely dissolved. It was considered that HPMCP capsule to achieve 2 hour in vitro lag time would be suitable to achieve in vivo capsule opening either in the intestinal zone.

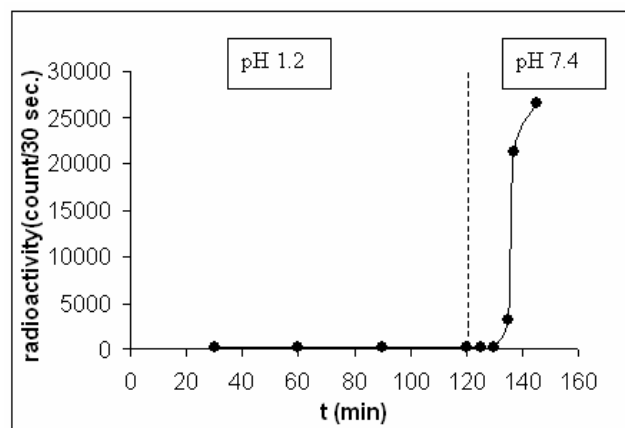


Figure 3: The in vitro resistance and solubility control of HPMCP capsule in acidic and alkaline media using ^{99m}Tc -DTPA.

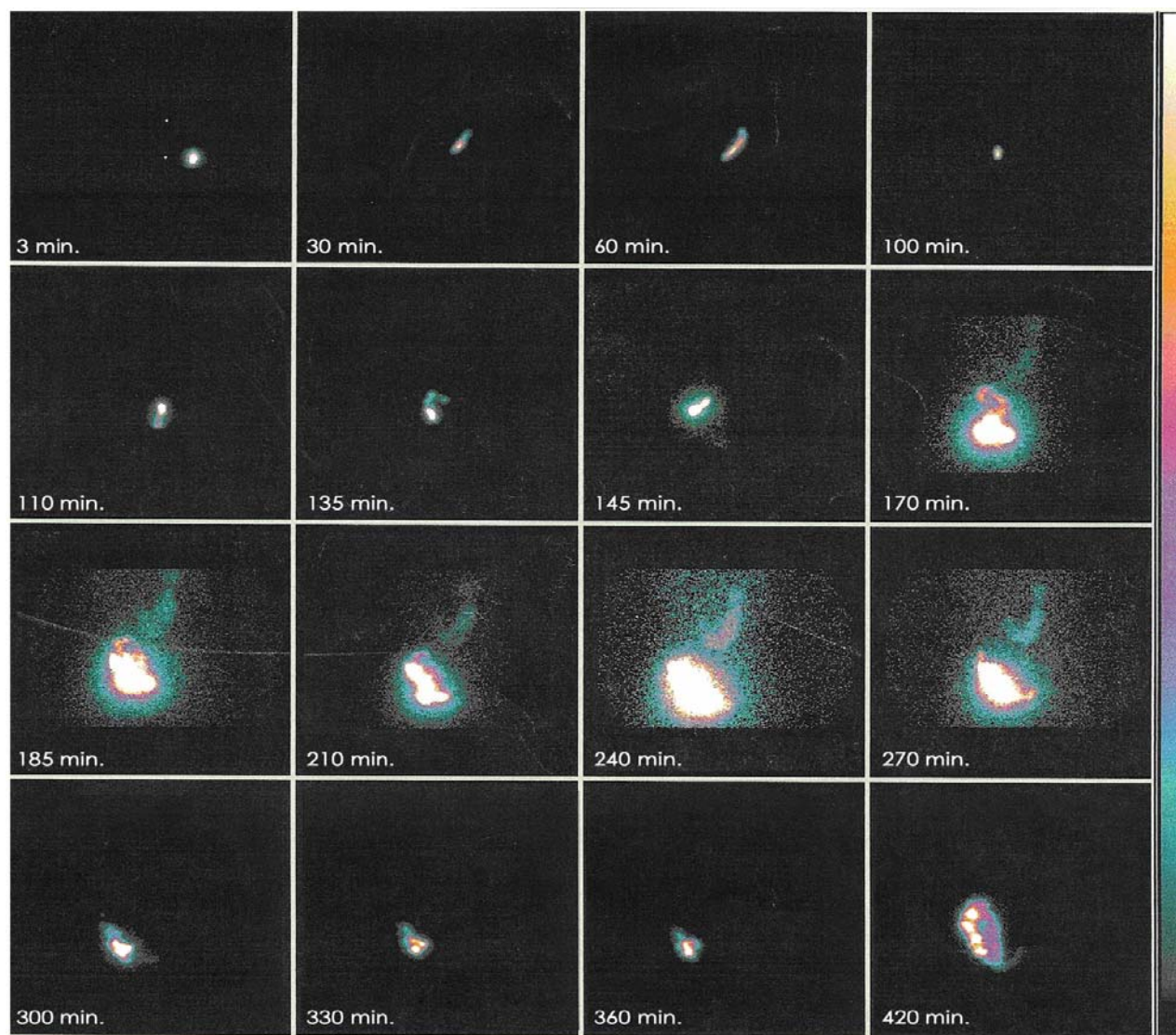


Figure 4: Time lapse drug delivery from the HPMCP capsules.

Results of In Vivo Studies:

In order to find out the in vivo performance of the HPMCP capsules, gamma scintigraphic studies were carried out on the capsules, using ^{99m}Tc -DTPA as tracer, in human volunteers.

The locations of the HPMCP capsule in the human digestive tract at different times after its administration are given in Figure 4. The HPMCP formulation was expected to be intact in stomach and to dissolve in intestine because of alkaline pH.

The difference of capsule transit time can be seen in digestive system of volunteers. Gastric residence time of HPMCP capsule was 120 minute in stomach. At 135 minute the capsule was in duodenum and at 185 minute the capsule was started to dissolve in small intestine. It was dispersed in intestine at 430 minute. The small intestine transit time for HPMCP capsules ranged between 3 and 4 hour. The capsule type remained intact in the stomach which confirmed the gastro-resistant properties of HPMCP polymer. The data from this study demonstrate that volunteers dosed with HPMCP capsule ^{99m}Tc -DTPA as tracer disintegration occurred intestinal zone suggesting that the volunteer's intestinal pH was sufficient to dissolve the polymer on this formulation and thereby provide for intestinal targeting.

Conclusion

The moisture content of capsule shell must be such as to prevent brittleness. It is normally 13.0 to 16.0%, determined by drying at 105°C for hard gelatine capsules. The brittleness can be checked by at the centre of the capsule against a smooth hard surface; the capsule must not shatter [15]. The moisture content and brittleness of prepared HPMCP capsules were found 4.8 ± 0.019 , and 2 ± 0.045 kg, respectively. Also, the brittleness of hard gelatine capsule is about 2.1 kg. According to this result, HPMCP capsules had low moisture content, and their brittleness was resistant to applied pressure.

The HPMCP capsules showed a good correlation in in vitro/in vivo conditions as expected. According to in vitro study, ^{99m}Tc -DTPA released from HPMCP capsules was independent of time and it was mainly controlled by dissolution of the HPMCP polymer. The gamma scintigraphic imaging method was utilised to obtain visual data about the movement and disintegration of the HPMCP capsule in the human. The HPMCP capsules can be good container for intestinal targeting and they are convenient for drugs that have gastrointestinal adverse effect and site specific delivery into the intestine.

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