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# INFLUENCE OF ELECTRON IRRADIATION FACTOR ON HARUAN TRADITIONAL EXTRACT (HTE) FOR ORAL DRUG DELIVERY

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## *ABSTRACT*

*Haruan or Channa striatus is source of protein that is widely consumed in the region and its extract is well known for having medical values. It is of great advantage if this product could be taken orally rather than by injection because the oral route of drug delivery is still preferred by the vast majority of patients. However protein and peptides can be denatured or degraded by the acidic pH of the stomach and the presence of endogenous enzymes. In order to protect or prevent digestion and degradation of the protein in the stomach and to ensure the protein reaches the gastro intestinal (GI) tract, Carboxymethyl Starch (CMS) nanogel system was developed using electron irradiation method. However stability of HTE during the irradiation process needed to be studied before being developed further. In this study, the HTE was irradiated using electron beams. Its stability was analysed in terms of physical and chemical changes by looking at colour difference, melting point by using Differential Scanning Calorimetry (DSC) and molecular bonds by using Fourier Transform Infrared (FTIR) respectively. The results of this study were that no apparent colour difference was observed with HTE before and after irradiation. These observations were supported by the FTIR and DSC results that showed that there were no changes in molecular bonds and melting point, compared between no irradiation and irradiation HTE during electron irradiation up to 10 kGy. Statistically the test showed no significant difference at $p < 0.005$ between melting temperatures.*

**Keywords**: Electron irradiation, gastro intestinal (GI) tract, melting point, nanogels, protein

## INTRODUCTION

The oral route of drug delivery is still preferred by the vast majority of patients because oral administration is convenient, simple to administer, compact, cheap and painless. However, *per oral* administration of drugs has disadvantages such as hepatic first pass metabolism and enzymatic degradation within the GI tract, that prohibit oral administration of certain classes of drugs especially peptides an proteins (Shojaei, 1998 ; Swarbrick and Boylan, 1998). The success of proteins and peptides as a therapeutic agent depends on the development of a suitable formulation, whereby native protein or peptides retains its structure and activity during preparation and delivery as well as during shipping and long-term storage.

Haruan or *Channa striatus* is widely consumed in the region is well known for having medicinal properties. In Malaysia, haruan is popularly used as a remedy for promoting wound healing process especially after surgical intervention or among caesarean mother after giving birth. Its anti-nociceptive properties alleviate post-operative pain and discomfort (Zainal A. et al., 2005; Zakaria et al., 2007). It is also good for trauma, induction platelets, has anti-inflammatory and anti-microbial properties (Gam et al., 2005; Mat Jais, 2007; Mat Jais et al., 1994; Mat Jais et al., 1997;

Mat Jais et al., 1998; Wach et al., 2000). The *Channa striatus* extract can also be used as an alternative treatment in osteoarthritis (Zainal et al., 2005).

Polypeptide and protein drugs such as insulin, growth hormone, interferon, G-CSF and calcitonin have an important place in therapy. It is of great advantage if these products of biotechnology and synthetic peptide synthesis could be administered orally rather by injection or via transmucosal routes such as through the nose and the lung. However polypeptides can be denatured or degraded by the acidic pH conditions in the stomach and presence of endogenous enzymes when given orally.

The aim of this study was to investigate the physico- chemical stability of Haruan Traditional Extract (HTE) upon electron irradiation. The studies are based on physical appearance (colour changes) before and after irradiation, differential scanning calorimetry (DSC), to study changes of melting point and fourier transform infrared analysis (FTIR), to study stability of functional groups. Based on this study, the results will be used for development of natural material *carboxymethyl* starch (CMS) as nanogels forms using electron irradiation for targeted drug delivery to gastrointestinal (GI) tract.

## MATERIALS AND METHOD

### Extraction of Haruan Traditional Extracts (HTE)

Haruan fish was caught in the wild in Rawang, Selangor, Malaysia. Samples were kept in a tank and transferred to Physiology Laboratory, Faculty of Medicine and Health Sciences, University Putra Malaysia. The extract was prepared by cooking under pressure (1 amp) at 120$^{o}$C for 3 hours with an additional hour for the seasoning process. The preparation followed a standard procedure previously establish (Mat Jais et al., 1997). Briefly, fresh boneless fillet were weighed and place on a stainless steel wire mesh mounted in a stainless steel tripod cooker. Distilled water was added to the fish water in ratio of 1:1. At the end of the extraction process, the fillets were discarded and the liquid extract was collected, filtered using a serial filtration process with 0.45 µm and 0.22 µm microfilter (Sartorius, Germany), centrifuged (1500 rpm, 25$^{o}$C) and stored at 4$^{o}$C.

### Stability Study of HTE upon Irradiation

HTE was added to 100 ml of water and mixed homogenously with a disperser (Ultra Turrax T25 IKA, Switzerland). These Samples were transferred into a plastic mould and sealed. Irradiation was carried out using electron beam machine-EPS 3000 that generates electron from an accelerator with the following irradiation parameter: Current = 1 mA, Voltage = 2.0 MeV and Dose 2, 4, 6, 8 and 10 kGy. The effects on HTE upon irradiation were studied.

### Colour Changes during Irradiation

The appearance of the irradiated samples was compared with non-irradiated samples.

### Fourier Transform Infrared Analysis (FTIR)

FTIR spectra were recorded on Perkin Elmer FTIR V2000 Spectrometer in the range of 4000-400 cm$^{-1}$. The attenuated total reflection (ATR) technique was used for analysis purpose.

### Differential Scanning Calorimetry (DSC)

DSC was performed using Mettler Toledo DSC 1* E System. Samples were placed in an aluminum pan, sealed and weighed. An empty aluminum pan was used as a reference. The sealed pan was heated from $50^{o}$C to $150^{o}$C at the rate of 10 $^{o}$C/min.

### Statistical Analysis

The results were analysed using one-way analysis of variance (ANOVA). The data were statistically analyzed using SPSS program version 12.0. A statistically significant difference was considered at $p < 0.05$.

## RESULTS AND DISCUSSION

### Colour Changes upon Electron Irradiation

Figure 1 shows the HTE samples before and after electron irradiation at dose 0 kGy to 10 kGy. The appearance of the irradiated samples was compared with the non-irradiated samples; there were no observable colour changes for irradiation samples. The brown residues found at the bottom of the bottles are sedimented HTE samples. In this study, it is found that the exposure of HTE to electron beam irradiation doses up to 10 kGy does not affect the colour of HTE.

### Fourier Transform Infrared Analysis (FTIR)

An infrared spectrometer determines the positions and relative size of all the absorption or peak in the infrared region and plots them. This plot of absorption intensity versus wavenumber (or wavelength) is referred to as the infrared spectrum of a compound. In Figure 2 a spectrum exhibits two strong peaks at 3274 $cm^{-1}$ and 1637 $cm^{-1}$ representing the O-H stretch and C=O stretching vibration respectively.

The O-H stretching vibration in carboxylic acids occur in this region 3400 $cm^{-1}$ to 2400 $cm^{-1}$ (Figure 3). It is easily distinguishable from alcohol and phenols by the presence of a very broad band extending from 3400 $cm^{-1}$ to 2400 $cm^{-1}$ and the presence of carbonyl absorption. If this very broad O-H stretch band is seen, along with a C=O peak, it almost certainly indicates that the compound is a carboxylic acid. The resulting C(O)NH bond (Figure 3) is called a peptide bond, and the resulting molecule is a polypeptide.

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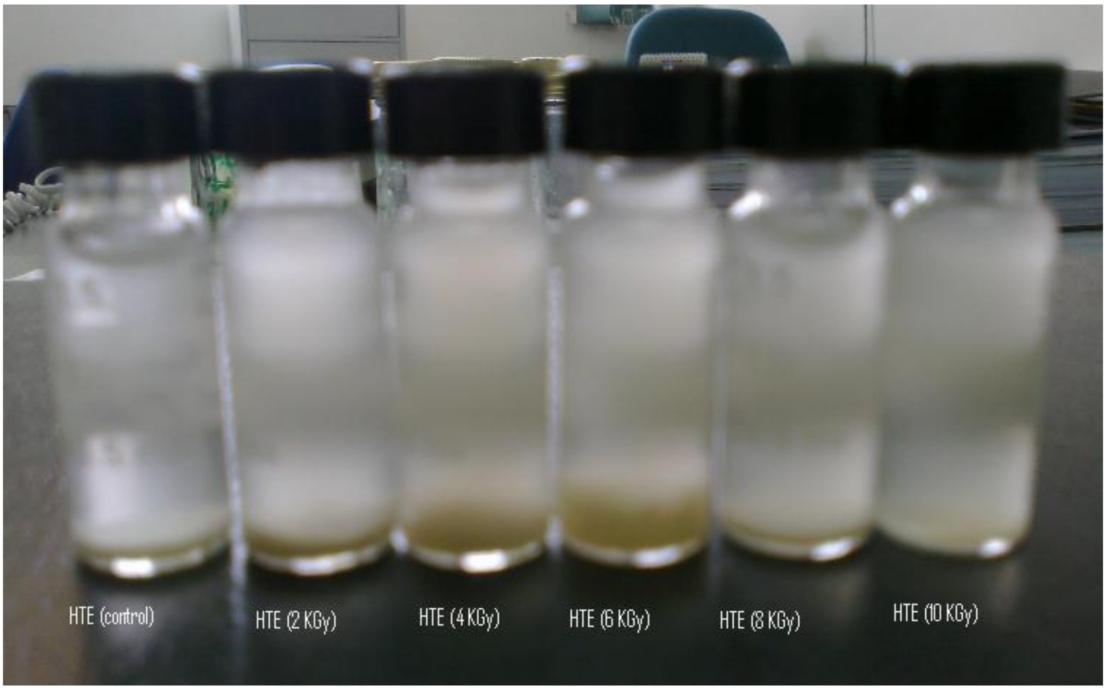


Figure 1: HTE samples before and after irradiation at different dose (2 kGy to 10 kGy) using electron beam machine

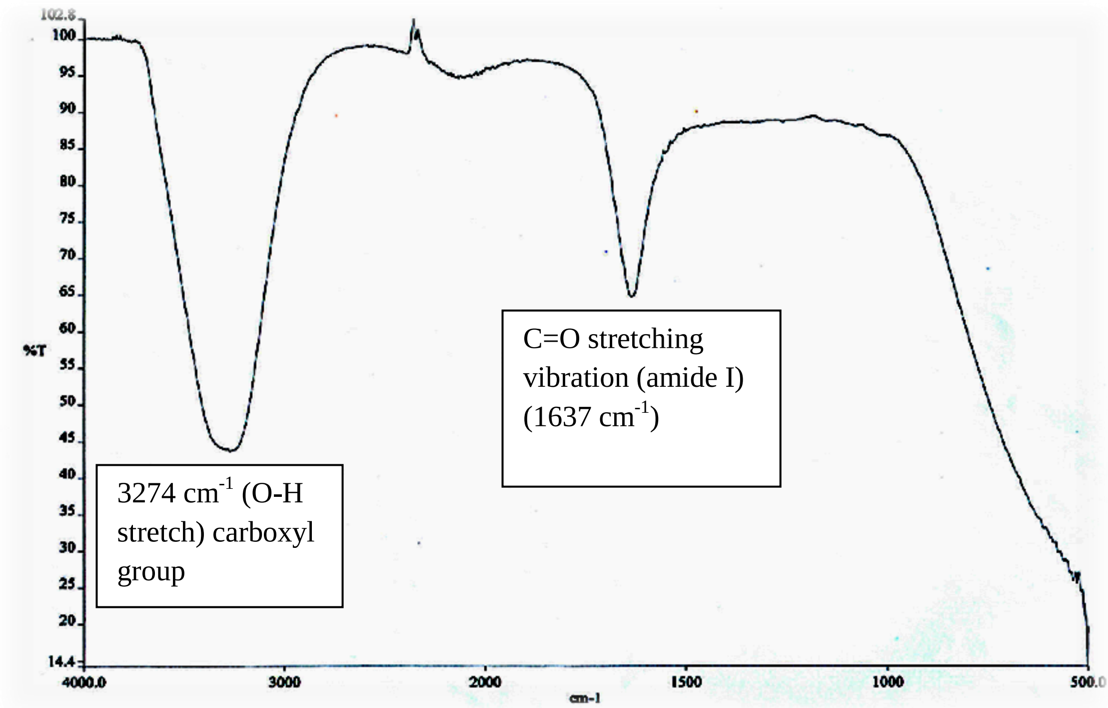


Figure 2: The infrared spectrum of HTE before electron irradiation

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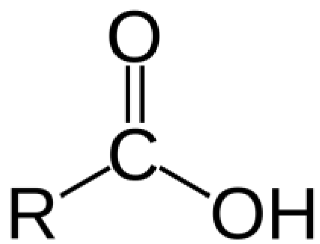

Figure 3: Structure of a carboxylic acid

The amide vibration seen at 1637cm$^{-1}$ (Figure 3) further indicates the presence of a peptide bond. The amide groups gives up to 9 characteristic bands namely amide A, B, I, II, III, IV, V, VI and VII. Amide I and amide II are two major bands of the protein infrared spectrum. The amide I band (between 1600 cm$^{-1}$ and 1700 cm$^{-1}$) is mainly associated with the C=O stretching vibration (70 – 85%) and is directly related to the backbone conformation. It is the most intense absorption band found in protein. It is primary governed by the stretching of the C=O (70 – 85%) and C-N group (10 – 20%). The exact band position is determined by the backbone conformation and the hydrogen bonding pattern.

Figure 4 is the infrared spectrum of HTE before and after electron irradiation at different doses showed no changes of the functional group of the HTE during irradiation.

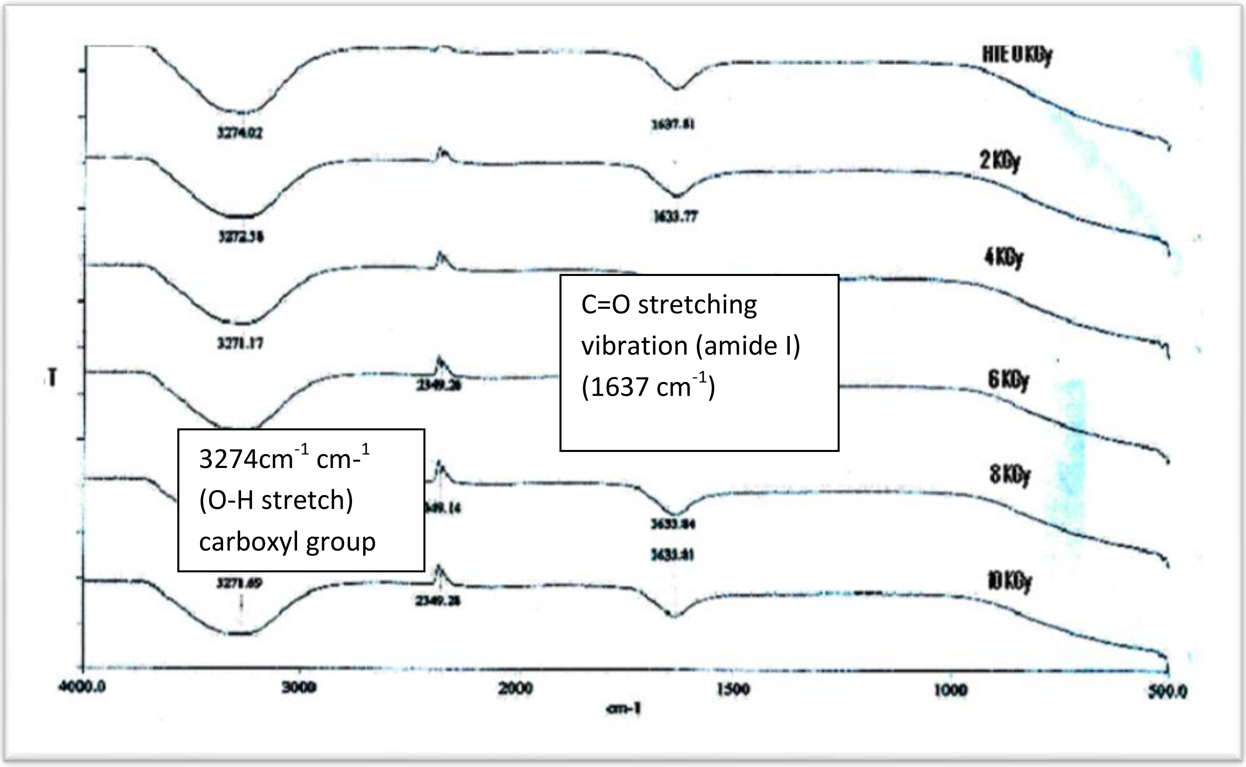


Figure 4: The infrared spectrum of HTE before and after electron irradiation at different doses (0, 2, 4, 6, 8 and 10 kGy)

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**Differential Scanning Calorimetry (DSC)**

DSC is a thermo analytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned.

Figure 5, Figure 6 and Table 1 show the differential scanning calorimetry (DSC) thermal profile and the transition temperatures and enthalpy of HTE before and after electron irradiation at 0 to 10 kGy. The plots show the DSC heat as a function of HTE samples temperature with an exothermic peak at 103.24 to 104.35$^{o}$C response oriented downwards and enthalpy of -1226.51 to 1914.45 J/g. Exothermic reaction occur because change in specific heat is negative; power decrease because heat is released by the reaction and less power is required to maintain equivalent temperatures in the chambers. Table 1, showed melting temperature of sample HTE non irradiation at 104.35$^{o}$C and irradiation samples (2 to 10 kGy) at range 103 to 104.35$^{o}$C, therefore from the comparative studies between for HTE samples before and after electron irradiation, they were not significant different at $p < 0.05$.

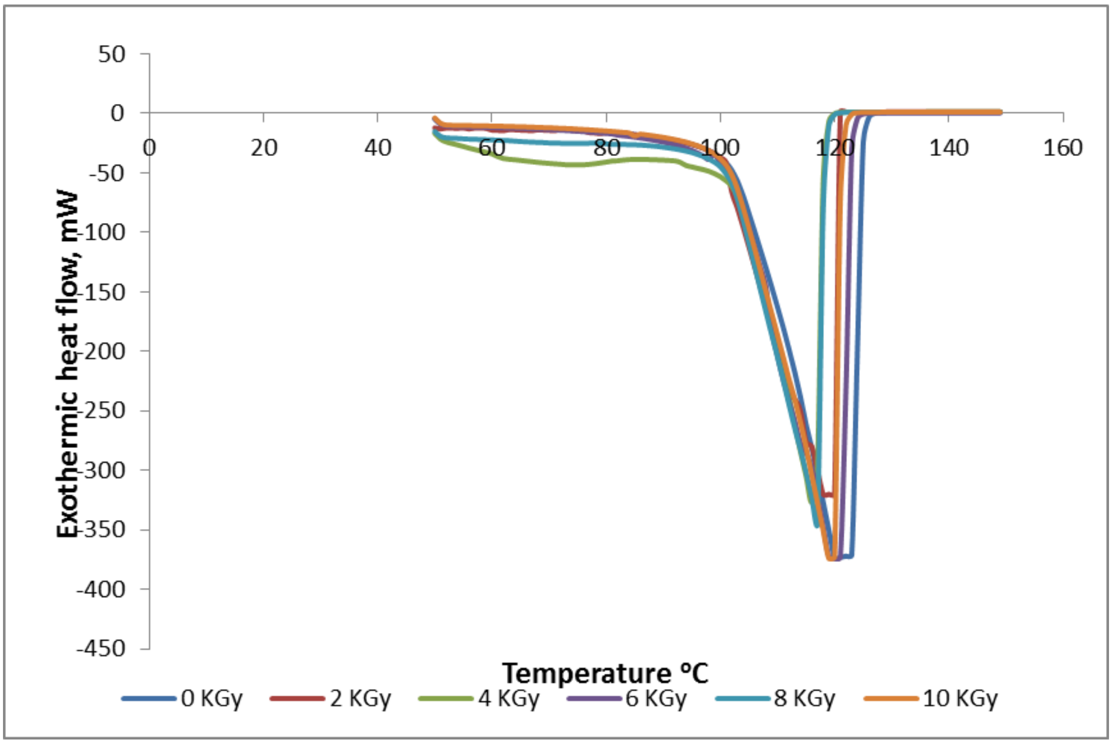


Figure 5: Differential scanning calorimetry (DSC) of HTE before and after electron irradiation at 0 to 10 kGy

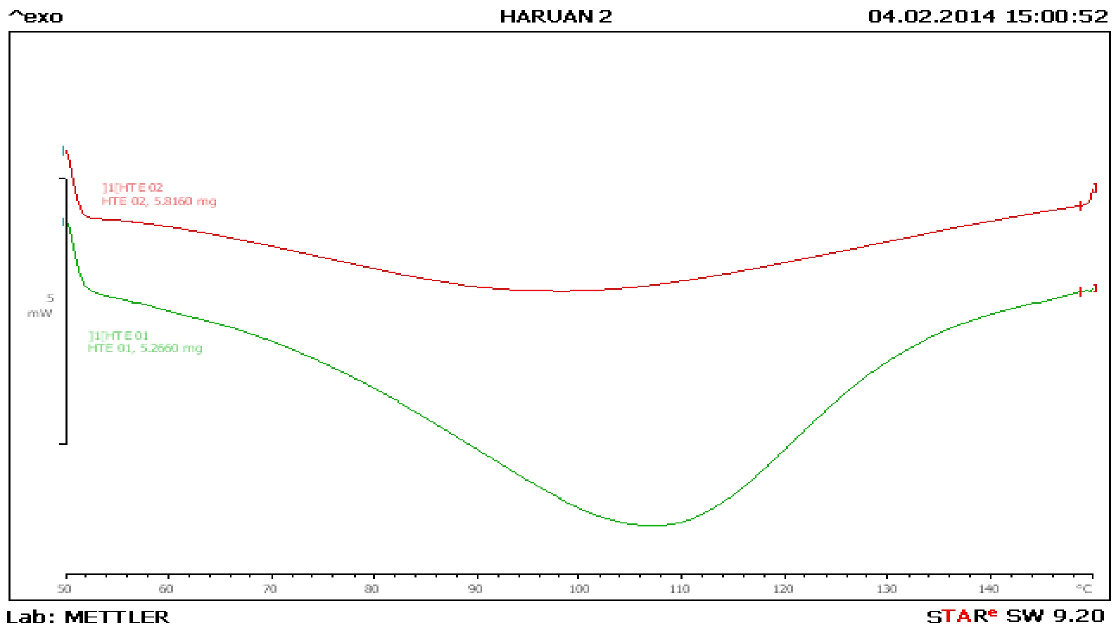


Figure 6: Differential scanning calorimetry (DSC) of HTE sample powder before irradiation

Table 1: Transition temperatures and enthalpy of HTE before and after electron irradiation at 0 kGy to 10 kGy

| **Samples** | **Irradiation Dose (kGy)** | **Transition Temperatures (°C)** | | **Enthalpy, Δ*H* (J/g)** |
|---|---|---|---|---|
| | | **Onset Temp. $T_o$** | **Melting Temp. $T_m$** | |
| HTE 0 | 0 | 101.92 ± 0.2 | 104.35 ± 0.2 | -1914.45 ± 0.5 |
| HTE 1 | 2 | 100.06 ± 0.3 | 103.24 ± 0.1 | -1110.86 ± 0.6 |
| HTE 2 | 4 | 101.10 ± 0.1 | 103.11 ± 0.3 | -1238.63 ± 0.7 |
| HTE 3 | 6 | 101.43 ± 0.2 | 104.18 ± 0.2 | -1808.39 ± 0.1 |
| HTE 4 | 8 | 100.44 ± 0.3 | 103.32 ± 0.3 | -1226.51 ± 0.2 |
| HTE 5 | 10 | 101.04 ± 0.2 | 104.35 ± 0.1 | -1831.06 ± 0.3 |

## CONCLUSIONS

The results presented showed that the physicochemical properties of Haruan Traditional Extract (HTE) remain stable during electron irradiation up to 10 kGy. Based on physical changes (colour change), differential scanning calorimetry (DSC) and fourier transform infrared analysis (FTIR), the changes/ differences between irradiated and non-irradiation samples were not found significantly different at $p < 0.05$. Based on this study, the results can be used as a drug for development of natural material carboxymethyl starch (CMS) as nanogels forms using electron irradiation for targeted drug delivery to gastrointestinal (GI) tract.

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