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Research Article

Evaluation of Biocompatibility of Capsaicin-Loaded Dendrimers on Zebrafish Embryos

Carlin Geor Malar, Bavanilathamuthiah*

Department of Biotechnology, Sathyabama University.

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ABSTRACT

Dendrimers are the nano-sized structures which consist of tree-like branches with many functional terminal ends and are the monodisperse macromolecules which contain symmetric branching units built around a small core molecule. The properties and the applications of these structures depend on the functional terminals. One of the important properties of Dendrimers is its solubility which improves the bioavailability of the insoluble drugs and used as a carrier for drug delivery. Capsaicin can be used against many kinds of cancers. In the present study, the dendrimers were synthesized and capsaicin was bound to it. The successful binding of capsaicin onto the dendrimers was confirmed by the fourier transform infrared spectroscopy (FT-IR) and UV-Vis spectroscopy. The solubility was checked with various solvents and their biocompatibility was evaluated in zebra fish embryos. The results revealed that the prepared dendrimers were biologically compatible in the invivo conditions.

Keywords: Dendrimer, Capsaicin, Zebrafish, FT-IR, Biocompatible.

INTRODUCTION

Dendrimers are the nano-sized structures which consist of tree-like branches with many functional terminal ends. Dendrimers are also considered as the monodisperse macromolecules which contain symmetric branching units built around a small core molecule[1-3]. These are only architectural structures and are not any compound[4]. The Dendrimers can be prepared by either convergent method or divergent method[5] and the growth involves the cascade reactions. Depending on the functional groups attached at the terminals, dendrimers has significant properties and vary in applications. Because of its polyvalent nature, it can exhibit multiple interactions with the target. These Dendrimers have different applications within the medical field. The dendritic polymers are similar to proteins which can be easily functionalized[4]. The drugs if bound to the polymeric carriers' increases the half-life, stability, water solubility and decrease the immunogenicity and antigenicity[6].

One of the naturally occurring phytochemicals, Capsaicin which is the major pungent component of hot chili peppers of the genus Capsicum (family Solanaceae) are used as a food additive. Capsaicin was found to have several physiological and pharmacological effects[7,8]. Capsaicin can be used as an anti-cancer drug because of its anti-proliferating effects expressed on many kinds of cancer cell lines derived from including breast cancer[9,10], colon carcinoma[11,12], nasopharyngeal carcinoma[13], oesophageal carcinoma[14], gastric cancer[15] and pancreatic cancer[16,17]. The poor solubility of capsaicin reduces the bioavailability of it to be used as an anti-cancer drug and hence dendrimer can be used as an efficient carrier.

The *Danio rerio* (Zebrafish) is a tropical freshwater fish belonging to the minnow family (Cyprinidae) of the order Cypriniformes[18]. Since it has the physiological and anatomical characteristics similar to that of the higher vertebrates, it is widely used as an important model organism. They breed rapidly and yield more embryos which are transparent to be used for the experimental studies.

MATERIALS AND METHODS

Chemicals

Oleoyl chloride, capsaicin and coomassie brilliant blue G 250 was purchased from Sigma-Aldrich (Bangalore, India). Polyethylene glycol 400 (PEG 400), chloroform, acetone, methanol and dimethyl sulphoxide and triethylamine were the products from Merck (Mumbai, India). Bovine serum albumin was purchased from CDH Laboratory Chemicals (New Delhi, India). All the chemicals used were of analytical grade and Millipore water was used for all the experimental studies.

Maintenance of zebrafish

The aquarium for culturing and breeding the zebrafishes were set up and all the quality parameters were maintained. The zebrafishes were transferred to the aquarium and acclimatized to the cement tanks for 15 days. The tap water was changed in alternate days in the tanks. The temperature was maintained to be 25 °C to 28 °C and the lighting was maintained on a cycle of 14 hrs of light and 10 hrs of dark for the fish to breed. The next day morning, the embryos were collected and used for the study.

Synthesis of Dendrimer

*Author for Correspondence

Table 1: Estimation of encapsulation efficiency

S.No	Amount of Capsaicin added (mg)	Amount of Capsaicin bound (mg)	Amount of dendrimer added (mg)	Percentage of encapsulation
1	7	4	100	10
2	21	4	100	10
3	35	5	100	13
4	50	17	100	44
5	64	38	100	100

The esterification process was used for the synthesis of the dendrimer[19]. The oleoyl chloride (0.01 mol), polyethylene glycol 400 (0.01 mol) and triethylamine (0.012 mol) was esterified in the presence of chloroform for 4 hours at 25 °C. The organic phase was removed and the dendrimers were collected, dried under vacuum until completely dried.

Synthesis of Capsaicin bound Dendrimer

Various concentrations of capsaicin (7 to 64 mg) taken in acetone was added to the same amount of dendrimer (100 mg). The acetone was evaporated and filtered with 22 µm filter. The amount of unbound capsaicin was analysed by bradford's method of protein estimation. The successfully capsaicin bound dendrimers were recovered from the filter using acetone and dried. The encapsulation efficiency (Table 1) was calculated and the highly efficient ratio was found to be 64mg of capsaicin for 100 mg of Dendrimers.

Characterization

The formation of dendrimer was confirmed by Fourier transform infra red (FT-IR) spectroscopy (Perkin Elmer Spectrum RX 1) using the potassium bromide (KBr) pellet method in the range of 4000-400 cm^{-1}.

Properties of dendrimers and DNCps

Solubility

The prepared dendrimers and DNCps were checked for the solubility in polar and non-polar solvents. Equal amount of dendrimers (5 mg) were added to the equal amounts (200 µl) of different solvents like acetone, ethanol, dimethyl sulphoxide, chloroform and water. The mixture was mixed thoroughly for few minutes and the solubility of dendrimers and DNCps were checked.

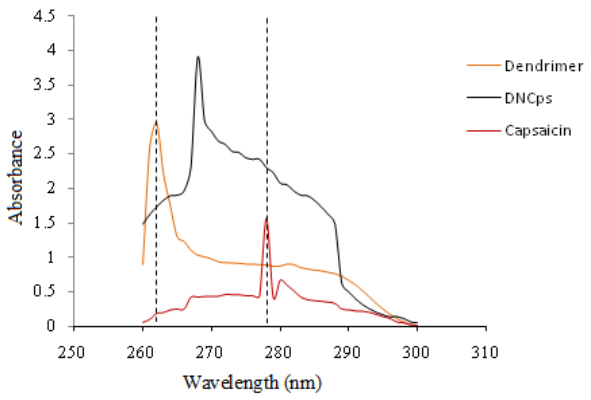


Figure 1: Comparison of the UV-Vis spectrum

Biocompatibility

The synthesized dendrimers were checked for the biocompatibility invivo on the zebra fishes from its embryo stage. The embryos of zebra fishes were subjected to various concentrations of dendrimers and DNCps and observed for its development. The embryos subjected only in tap water were considered as control. The stages of development of zebra fishes were observed from 0^{th} hour to 96^{th} hour of treatment with Dendrimers and dendrimer-capsaicin conjugates.

RESULTS AND DISCUSSIONS

UV-Vis Spectroscopy

Figure 2 shows the UV-Vis spectrum of capsaicin, dendrimer and dendrimer-capsaicin. The absorbance peaks were recorded using the UV-Visible Spectroscopy. The absorbance peak was found to be at 262 nm for the naked dendrimers. Capsaicin showed the characteristic peak at 278 nm. There was a shift in the peak from 262 nm to 268 nm for the capsaicin bound dendrimers confirming the successful binding of capsaicin onto the dendrimer.

FT-IR analysis

Figure 1 shows the fourier transform infrared spectrum of the dendrimer, DNC and capsaicin. The various peaks in the fourier transform infra red spectroscopy analysis confirmed the presence of the characteristic functional groups present in the dendrimer as well the binding of capsaicin on the dendrimer. The characteristic peak at 2929.8 cm^{-1} shows the presence of aliphatic C—H (stretching band). The stretching band of carbonyl group of ester (C=O and C—O) can be seen at 1720.3 cm^{-1} and 1104.2 cm^{-1} respectively. The C—H bending vibration of CH_2 can be seen at 1474 cm^{-1}. The stretching vibration of C=C was seen at 1652.8 cm^{-1}.

The characteristic absorption bands of capsaicin were found at 3447.3, 2927.4, 2369.5, 1637.7, 1403.0, 1067.2 and 806 cm^{-1}. The shifting of peaks were seen in the DNCps at 2939.3, 2358.3, 1638.4, 1474.7, 1067.9 cm^{-1}. The disappearance of the peaks at 1720.3 and the appearance of peak at 804.3 cm^{-1} confirmed that the capsaicin was bound to the dendrimer.

Solubility

Figure 3 shows the solubility of the Dendrimers in various solvents. The solubility test was performed to know the nature of the terminal ends in the branches of Dendrimers as well as the binding of capsaicin onto the dendrimers. The Dendrimers were found to be soluble in

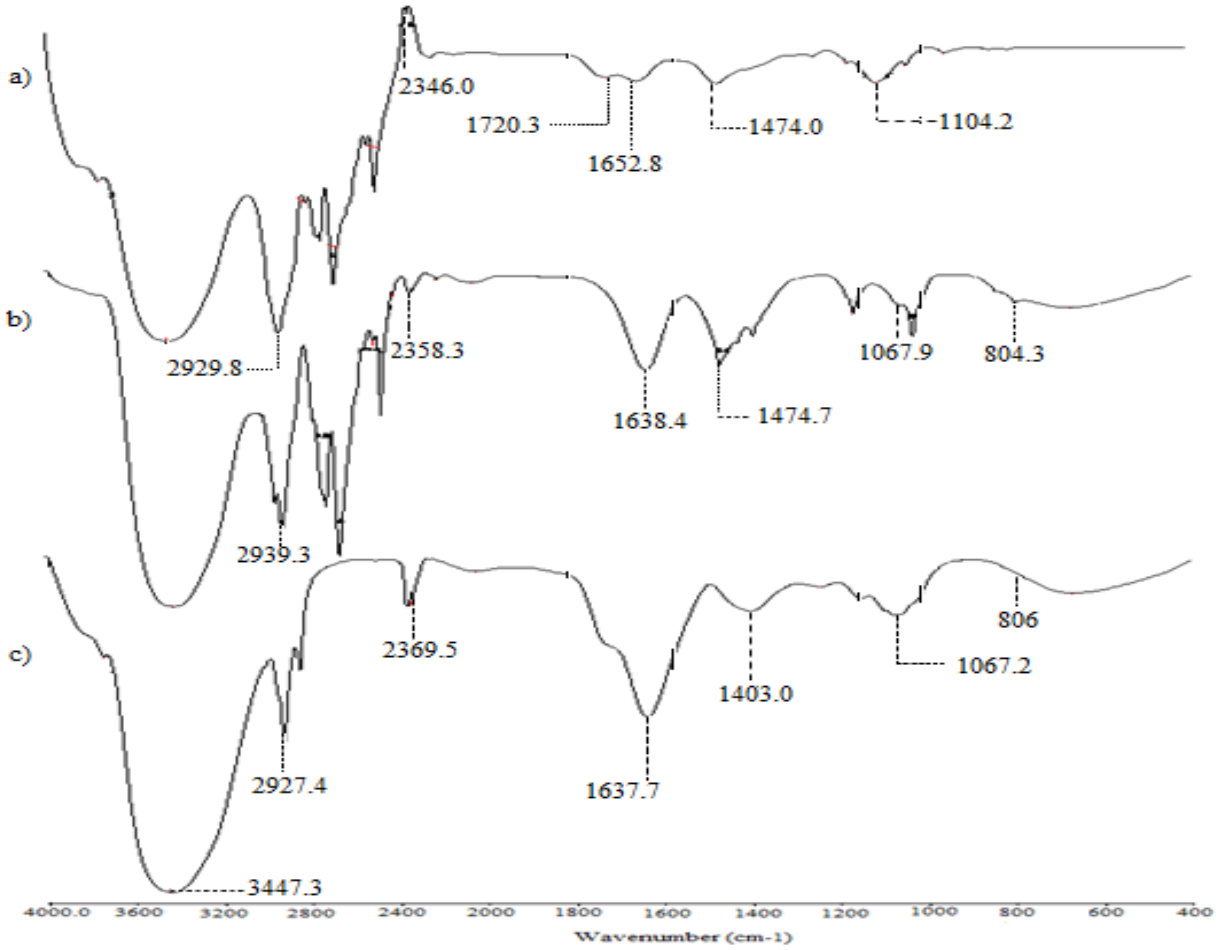


Figure 2 : FT-IR spectra of Dendrimer (a), DNCps (b) and Capsaicin (c).

polar solvents like water, acetone, ethanol and dimethyl sulphoxide confirming the presence of hydrophilic (-OH) groups on the terminals. The capsaicin bound Dendrimers (DNCps) were observed to be partially soluble in water whereas soluble in alcohols indicating the binding of capsaicin onto the Dendrimers.

Biocompatibility

The control was maintained and the development at various stages was observed. At the 0th hour, the chorion was observed to be swelled up and lifting the newly fertilized egg. At the 24th hour of development of the embryos, the segmentation period was observed. The tail bud became more prominent and there was elongation in the embryo length. The pharyngula stage was observed at the 48th hour of development[20]. The development of notochord was observed and the nervous system was hollow and expanded anteriorly. The protruding mouth was observed anteriorly behind the eyes at the 72nd hour Figure 4 shows the different stages of development of embryos after the treatment with Dendrimers and DNCps. The stages observed from 0th hour to 96th hour after treatment with the Dendrimers and DNCps were found to be normal (similar to that of control) even at higher concentration. The Dendrimers were found to be non-toxic in the invivo conditions.

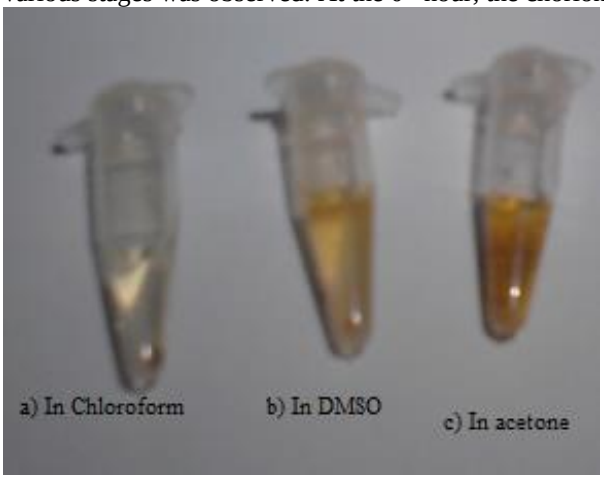
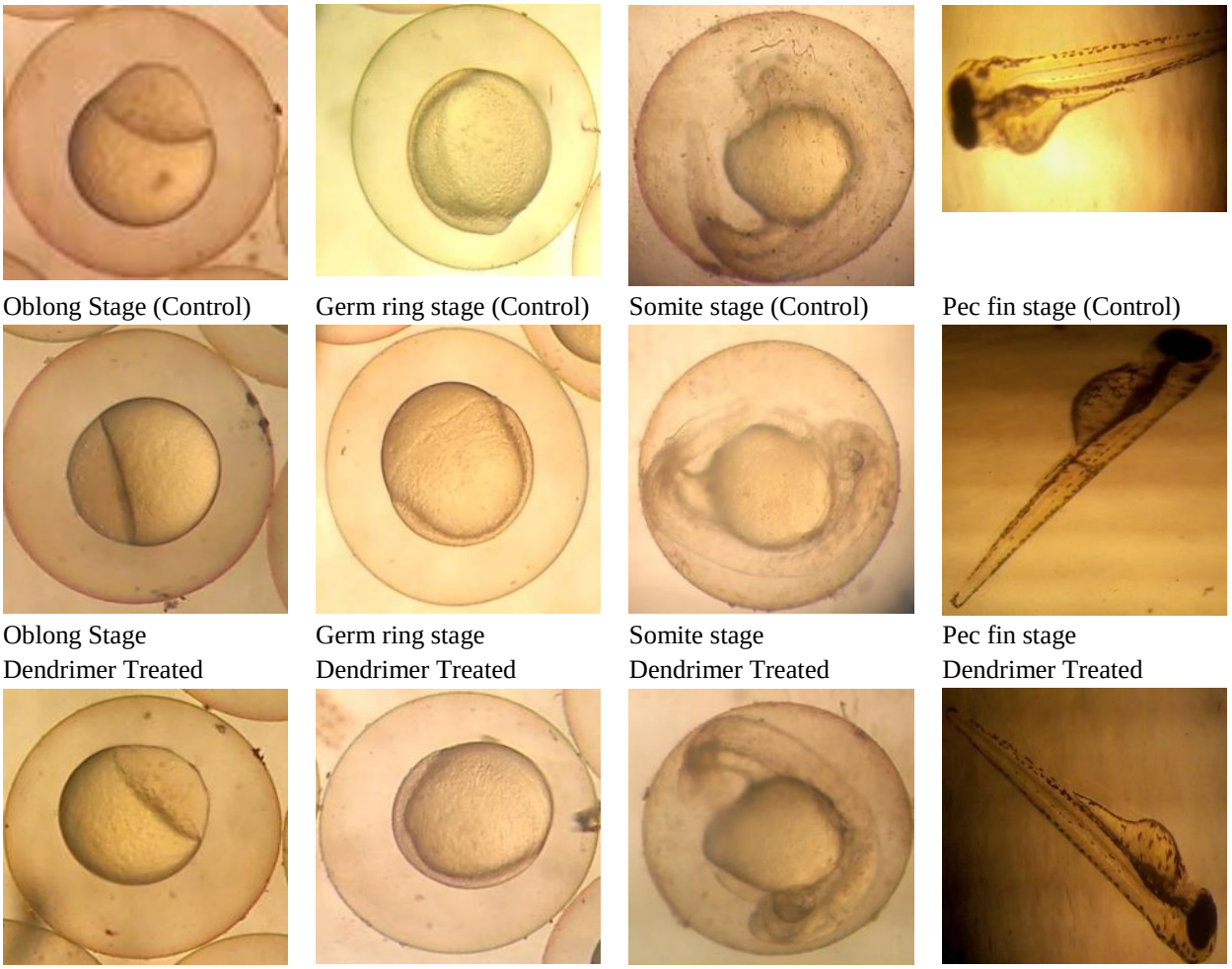


Figure 3: Solubility test of DNCps with various solvents

CONCLUSION

In the present study, the Dendrimers were synthesized and characterized. The solubility of the capsaicin was increased by attaching it on the dendrimers. It was then checked for the biocompatibility in invivo conditions on the embryos of zebrafish. The stages observed from 0th hour to 96th hour after treatment with the Dendrimers and DNCps were found to be normal (similar to that of



Oblong Stage (Control) | Germ ring stage (Control) | Somite stage (Control) | Pec fin stage (Control)

Oblong Stage Dendrimer Treated | Germ ring stage Dendrimer Treated | Somite stage Dendrimer Treated | Pec fin stage Dendrimer Treated

Oblong Stage DNCps Treated | Germ ring stage DNCps Treated | Somite stage DNCps Treated | Pec fin stage DNCps Treated

Figure 4: Different stages of development of zebrafish embryos

control) even at higher concentration confirming the non-toxic nature of the Dendrimers.

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