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DESIGN AND DEVELOPMENT OF INVITRO RELEASE MUCOADHESIVE MICROSPHERES EMBEDDED *CLERODENDRUM PHLOMIDIS* (CP) EXTRACT FOR PROLONGED ANTIDIABETIC ACTIVITY

K. Jesindha Beyatricks*, S. Kavimani[1], N. Habeela Jainab[2], Manvitha K.[2] and Madhuri Sarode[2]

*Research Scholar, Prist University, Thanjavur.
[1]Professor, Mother Therasa Post Graduate Institute of Health Sciences, Pondicherry.
[2]Hillside College of Pharmacy & Research Centre, Bangalore.

***Corresponding Author: Dr. K. Jesindha Beyatricks**
Research Scholar, Prist University, Thanjavur.

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ABSTRACT

In this study an attempt was made to prepare mucoadhesive microcapsules of clerodendrum phlomidis extract using alginate polymers for prolonged release. Encapsulation of extract into sodium alginate polymer was done by ionic-gelation technique. The microcapsules were evaluated for surface morphology and particle shape by scanning electron microscope. Microcapsules were also evaluated for their microencapsulation efficiency, in vitro wash-off mucoadhesion test and in vivo study. The microcapsules were discrete, spherical and free flowing. The microencapsulation efficiency was in the range of 60–70% and microcapsules exhibited good mucoadhesive property in the in vitro wash off test. Extract loading and entrapment efficiency was increased with increasing the concentration of polymer. The percentage of microcapsules adhering to tissue at pH 7.4 after 6 h varied from 20–30%, whereas the percentage of microcapsules adhering to tissue at pH 1.2 after 6 h varied from 40–60%. In vivo testing of the mucoadhesive microcapsules in diabetic albino rats demonstrated significant antidiabetic effect of extract. The hypoglycemic effect obtained by mucoadhesive microcapsules was for more than 16 h whereas plain CP extract produced an antidiabetic effect for only 4 h suggesting that mucoadhesive microcapsules are a valuable system for the long term delivery of CP extract.

KEYWORDS: Microspheres, Mucoadhesive, Ionic gelation technique, Microencapsulation.

INTRODUCTION

Novel technologies have been developed recently for drug delivery systems.[1] Use of herbal medicines has been increased all over the world due to their miraculous therapeutic effects and fewer adverse effects as compared to the modern medicines. However, delivery of herbal drugs also requires modifications with the purpose to achieve sustained release, to increase patient compliance etc. In ancient time before the arrival of high throughput screening concerned to drug discovery; 90-95% drug materials were natural products.[2] Information on source of new drugs nearby 1981-2007 specify that approximately half of the drugs are based on the NPs.[3,4] It has been proved that NPs are more voluntarily absorbed than synthetic drugs. NPs have extensively predictable for their wide-ranging structural diversity as well as their spacious series of pharmacological and pharmacognostical activities in the pharmaceutical organizations.

phytosterols (plant sterols) are triterpenes that are important structural components of plant membranes, and free phytosterols serve to stabilize phospholipid bilayers in plant cell membranes just as cholesterol does in animal cell membranes. Early phytosterol-enriched products contained free phytosterols and relatively large dosages were required to significantly lower serum cholesterol.[5] In the last several years two spreads, one containing phytostanyl fatty-acid esters and the other phytosteryl fatty-acid esters, have been commercialized and were shown to significantly lower serum cholesterol at dosages of 1–3 g per day. The popularity of these products has caused the medical and biochemical community to focus much attention on phytosterols and consequently research activity on phytosterols has increased dramatically. b-Sitosterol has promising antidiabetic as well as antioxidant effects and may be considered in clinical studies for drug development.[6]

β-sitosterol is well-known natural sterol in composition of known herbal drugs for treatment of benign prostatic hyperplasia and prostate cancer. Besides, the compound elevated enzymatic and nonenzymatic antioxidant in cells making it effective anti-diabetic, neuroprotective and chemoprotective agent as well. High potential of this compound and its analogues in treatment of various

illnesses, classifies this compound as the noteworthy drug of the future, although its role in treatment of BPH is now approved via clinical trial confirmations.[7]

Clerodendron phlomidis is well known drug in ayurveda and siddha medicine for treatment of diabetics. *Clerodendron phlomoidis* L. (Family: Verbenaceae) is commonly known as *Thazhu thaazhai* in Tamil and *Arni* in Hindi. Recently, dosage forms that can precisely control the release rates and target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems.[8]

Microspheres, in general have the potential to be used for targeted and controlled release drug delivery but coupling of mucoadhesive properties to Microspheres has additional advantages. Mucoadhesive and biodegradable polymers undergo selective uptake by the M cells of payer patches in gastrointestinal mucosa.[9,10]

Microspheres constitute an important part of novel drug delivery system by virtue of their small size and efficient carrier capacity. Microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers having a particle size ranging from 1-1000 μm. The range of Techniques for the preparation of microspheres offers a Variety of opportunities to control aspects of drug administration and enhance the therapeutic efficacy of a given drug.[11]

Mucoadhesive formulations orally would be to achieve a substantial increase in length of stay of the drug in the GI tract. Stability problem in the intestinal fluid can be overcome. Therapeutic effect of drugs insoluble in the intestinal fluids can be improved.[12] Mucoadhesive drug delivery systems utilize the property of bioadhesion of certain water-soluble polymers that become adhesive to mucous membranes on hydration[13] and hence can be used for targeting a drug to a particular mucus tissue (e.g. gastrointestinal. buccal, nasal, etc.) extended period of time.[14,15]

MATERIALS AND METHODS

Leaves of Clerodendrum phlomidis were collected from out-skirts of Andhra, India. The plant materials were authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupathi, India. Beta sitosterol from sigma chemicals, Bangalore, India and all other reagents and solvents used were of pharmaceutical or analytical grade.

Preparation of Mucoadhesive Microcapsules containing CP extract[16]

Mucoadhesive microcapsules containing CP extract were prepared employing sodium alginate in combination with three mucoadhesive polymers—sodium CMC, carbopol 934Pand HPMC as coat materials. Orifice-ionic gelation method was employed to prepare the microcapsules. Sodium alginate and the mucoadhesive polymer were dissolved in 50 ml of purified water to form a homogenous polymer solution. The active substance CP extract was added to the polymer solution (in a ratio of CP extract: polymer solution 1:1) and mixed thoroughly to form a viscous dispersion. The resulting dispersion was then added manually dropwise into calcium chloride (10% w/v) solution (100 ml) through a syringe with a 26 gauge needle. The addition of dispersion in the $CaCl_2$ solution was completed within 3 h of the preparation of the dispersion. The added droplets were retained in the calcium chloride solution for 15 min to complete the curing reaction and to produce spherical rigid microcapsules.

Microencapsulation Efficiency

An appropriate amount of microcapsules were first crushed and then weighed and suspended in methanol to take out the CP extract from microcapsules while assuring that there was no loss of material in the process. After 24 h, the filtrate was assayed spectrophotometrically.

Particle Size Analysis

Particle size distribution of the microcapsules was done by sieve analysis procedure. The microcapsules were shaken on a mechanical shaker, using a nest of British standard sieves, for 15 min.

Scanning Electron Microscopy (SEM)

SEM was performed for morphological characterization of microcapsules using scanning electron microscope (SEM— LEICA, 5430, London, U.K). They were mounted directly onto the SEM sample stub using double - sided sticking tape and coated with gold film (thickness, 200 nm) under reduced pressure (0.001 mmHg).

Mucoadhesion Testing by In Vitro wash-off Test

The mucoadhesive properties of the microcapsules were evaluated by in vitro wash-off test. A 2 cm wide and 2 cm long (2×2) piece of rat intestinal mucosa was tied onto a glass slide (3 in. long and 1 in. wide) using thread. About fifty microcapsules were spread onto the wet, rinsed, tissue specimen, and allowed to hydrate for 30 s. The prepared slide was hung onto one of the grooves of a USP 24 disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in the test fluid at 37°C contained in one litre vessel of the machine. At the end of 1 h and at hourly intervals up to 6 h, the machine was stopped and the number of microcapsules still adhering to the tissue was counted. The test was performed at both gastric pH (0.1 N HCl, pH 1.2) and intestinal pH (phosphate buffer, pH 7.4).

In vitro Release of CP extract (β-sitosterol as a Marker) from microspheres

Dissolution studies of the microspheres were performed in triplicate employing USP XIII dissolution rate test apparatus-1 (Electrolab, TDL-08L, India) simulating the

GIT conditions. Weighed quantities of the microspheres were loaded into the basket of the dissolution apparatus, 500 ml of the phosphate buffer of pH 7.4 with 0.5% Sodium lauryl sulphate was used a dissolution medium. The temperature of the dissolution fluid was maintained at 37±1°C with a stirring speed of 100 rpm. The samples were withdrawn at intervals of 1, 2, 3, 4, 6, 8, 10, 12hr and filtered with 0.22 µm filter (Millipore). The amount of β-sitosterol was estimated by HPLC.

RESULTS AND DISCUSSION

The average percentage yield of methanolic extract of leaves of *C. phlomoidis* was found to be 3.92% w/w. The qualitative phytochemical tests carried out for the identification of the nature of phytoconstituents present in Methanol extract of leaves of *C. phlomoidis* showed mainly the presence of alkaloids, phytosterols, glycosides, saponins, phenolic compounds, proteins and flavonoids.

The microcapsules were discrete, spherical and free flowing. The microencapsulation efficiency was in the range of 60–70% and microcapsules exhibited good mucoadhesive property in the in vitro wash off test. Extract loading and entrapment efficiency was increased with increasing the concentration of polymer. The percentage of microcapsules adhering to tissue at pH 7.4 after 6 h varied from 20–30%, whereas the percentage of microcapsules adhering to tissue at pH 1.2 after 6 h varied from 40–60%. In vivo testing of the mucoadhesive microcapsules in diabetic albino rats demonstrated significant antidiabetic effect of extract (Fig.01). The drug entrapment efficiency of formulated CP extract entrapped alginate microspheres ranged from 51.04 ± 1.02 to 57.20 ± 1.46%, according to the composition of the polymer concentration. The results indicated that the percentage of extract entrapment increased with increase in polymer concentration. This was attributed to physical interaction and/or entanglement of the greater amount of extract inside the intricate cross-linked calcium alginate gel network. The divalent metal ions Ca^{++} fit into electronegative cavities of the sodium alginate like eggs in an "Egg Box" model to form ionically gelled alginate due to electrostatic ionic interaction between positively charged metalcations and negatively charged COO−groups of SA. At the cross-linking sites, metal cations cause inter-polysaccharide binding, which are called as junction zones. These divalent metal cations compete with Na^{+}ions of SA and thus, bringing 2 polymer chains together. Divalent metal cations are accommodated in the interstites of 2 polyuronate chains having a close ion-pair inter-action with $COO^{-}$of the SA and sufficient coordination by other electronegative oxygen atoms. The CP extract-loaded alginate microspheres prepared using lower concentration might have larger pores due to insufficient cross-linking and/or drug leaching through the pores. The insufficient cross-linking and/or drug leaching through the pores could result in lower drug encapsulation.

SEM of chitosan microspheres is shown in the above Figure. The microspheres are spherical in shape with smooth surfaces. It can be observed that polymer crosslinking with calcium chloride was successful, which led to uniform formation of spheres.

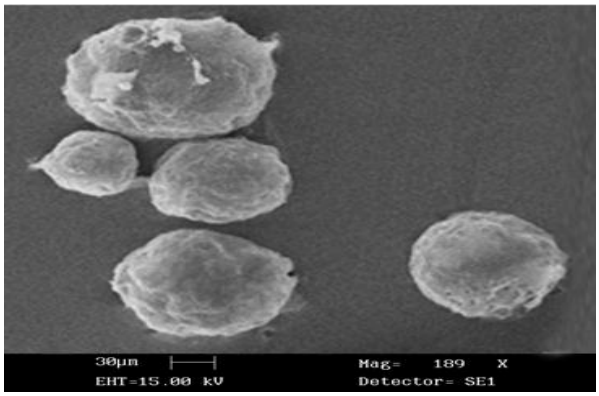

Figure 1: SEM of CP extract loaded alginate microspheres

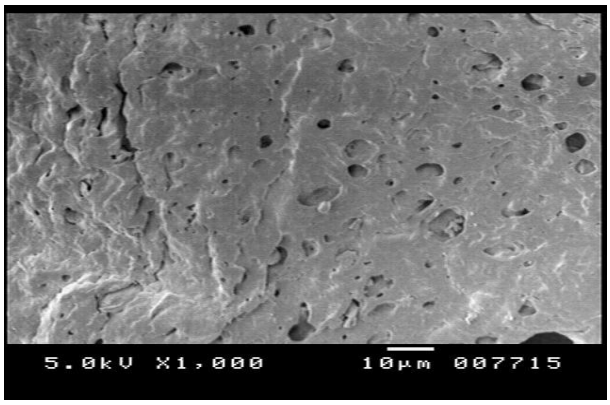

Figure 2: SEM of CP extractloaded alginate Microspheres after dissolution

The mucoadhesive properties of the microcapsules were evaluated by in vitro wash-off test. A 2 cm wide and 2 cm long (2×2) piece of rat intestinal mucosa was tied onto a glass slide (3 in. long and 1 in. wide) using thread. About fifty microcapsules were spread onto the wet, rinsed, tissue specimen, and allowed to hydrate for 30 s. The prepared slide was hung onto one of the grooves of a USP 24 disintegrating test apparatus. The disintegrating test apparatus was operated such that the tissue specimen was given regular up and down movements in the test fluid at 37°C contained in one litre vessel of the machine. At the end of 1 h, and at hourly intervals up to 6 h, the machine was stopped and the number of microcapsules still adhering to the tissue was counted. The test was performed atphosphate buffer, pH 7.4.

CP extract loaded microspheres was observed good mucoadhesive properties in the in-vitro wash-off test. The mucoadhesion test for microspheres was performed at pH 7.4 medium continuously for 6 h. The wash-off was faster for plain CP extract loaded alginate microspheres. The percentage of mucoadhesive polymer coated CP extract loaded alginate microspheres adhering

to tissue at pH 7.4 after 6 h varied from 50 to 70, whereas the percentage of plain CP extract loaded alginate microspheres adhering to tissue after 6 hours varied from 20 to 30. The results of the wash-off test indicated that the mucoadhesive polymer (carbopol) coated CP extract loaded alginate microspheres had fairly good mucoadhesive property.

Particle size can be determined by optical microscopy method. The mean diameter of CP extract loaded alginate microspheres was found 130± 3.4µm.

In vitro **CP extract (β-sitosterol as a Marker)** released amount of mucoadhesive microspheres as a function of time were shown in Fig. x. The release of the β-sitosterol was fast at the initial hours and became relatively slow later period. The in vitro release profile specifies that the percentage mean cumulative release was found 99% in the formulations after 12 h.

The amount of β-sitosterol released from alginate microspheres increased with decreasing alginate concentrations. For this reason, the result of in vitro release study showed that formulations prepared with low concentration of alginate released the drug faster than formulations prepared with higher concentration alginate. The alginate microspheres swell and then disintegrate due to the release of the calcium ions by sodium or phosphate. The results of in vitro release studies also exhibited that 1.75% alginate formulation released the drug slower than the 0.75% polymer concentration. Polymer and extract could bind better with water to form viscous gel structure, which may block the pores on microspheres surfaces and sustain the drug release. The swelling and disintegration of alginate microspheres is an important factor in the release of drug. To prevent these factors, alginate microspheres were bind with extract, which could strengthen the alginate matrix and reduce membrane permeability.

One of the ways of changing drug release from the microspheres is to change the crosslinking density of the matrix by employing various time of exposure to crosslinking agent. The effect of the exposure time to calcium chloride on the release rate of β-sitosterol has been investigated by varying the time of exposure to calcium chloride as 15 - 45 min. The results were given in Fig 3, 4**.**, which clearly indicated that increasing exposure time to $CaCl_2$ decreased the cumulative release of β-sitosterol. The β-sitosterol release was found more slowly with the percentage increase of cross-linker concentrations ($CaCl_2$) in cross-linking solutions, which can be attributed by high degree of cross-linking by higher $CaCl_2$ concentration might slower the drug release from highly cross-linked microspheres. The higher concentration of cross-linker used for the preparation of ionically gelled alginate microspheres might produce a rigid polymeric structure due to contraction of microvoids, which could facilitate poor entry of dissolution medium into the calcium ion induced ionically gelled extract-loaded alginate polymer and slow the drug release.

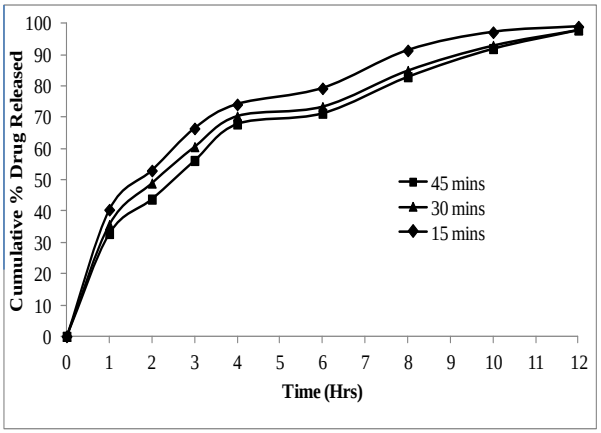

Fig No 3: Effect of polymer concentration on β-sitosterol release

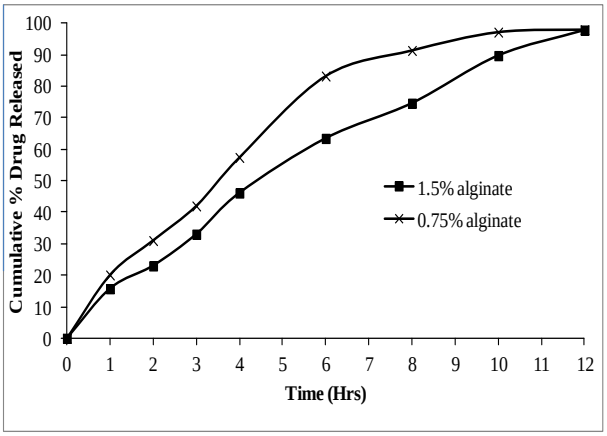

Fig No. 4: Effect of cross linking time on β-sitosterol release

Kinetic evaluation of *in vitro* release data

Data obtained from *in vitro* release studies of mucoadhesive microspheres was explored various kinetic models used are zero-order, first-order and Higuchi equations. The data obtained from the in vitro release were fitted to various kinetic equations to determine the mechanism of drug release and release rate. As indicated by the higher correlation coefficient (r^2 = 0.99), the drug release from mucoadhesive microspheres followed the Higuchi model rather than the first-order and zero-order equations. These findings indicated that the drug release from the mucoadhesive microspheres was diffusion controlled. In sustained release formulations, diffusion, swelling and erosion are the three most important rate controlling mechanisms followed. The drug release from the polymeric system is mostly by diffusion and is best described by Fickian diffusion. But in case of formulations containing swelling polymers, other processes in addition to diffusion play an important role in exploring the drug release mechanisms. These processes include relaxation of polymer chains, imbibitions of water causing polymers to swell and changing them from initial glassy to rubbery state. Due

to swelling, considerable volume expansion take place leading to moving diffusion boundaries complicating the solution of Fick's second law of diffusion. So the release data were further treated by equation given by Ritger and Peppas or also called as the Power law.

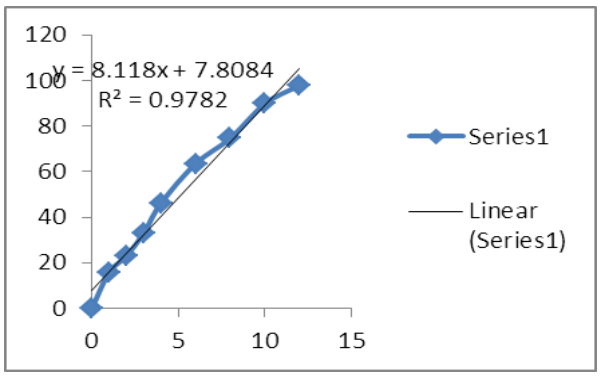

Fig No 5: Zero order release kinetics

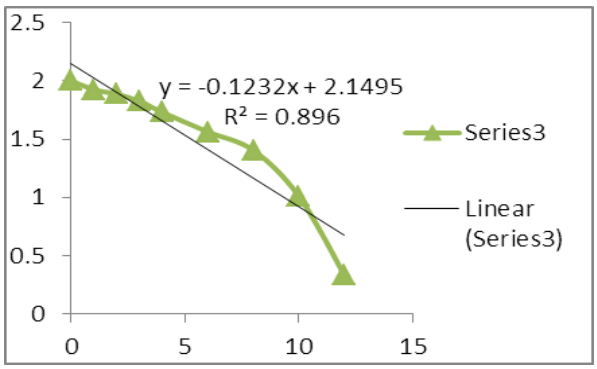

Fig No 6: First order release kinetics

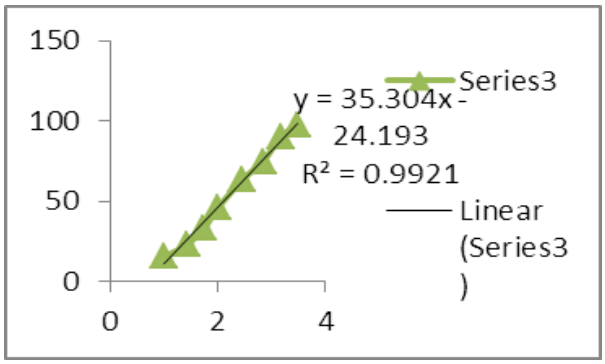

Fig No 7: Higuchi release kinetics

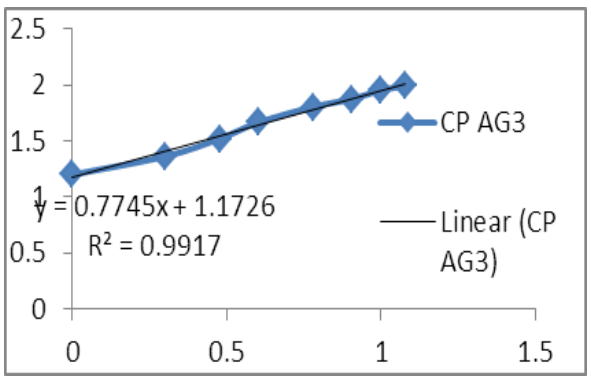

Fig No. 8: Peppas release kinetics

The release data obtained via the above procedure were subjected to the Ritinger and Peppas model to devise its release mechanism. The initial 60% cumulative release data were used to estimate the diffusion exponent 'n' by using equation:

$$M_t / M_\infty = Kt^n$$

Where Mt is the amount of drug released at time t, M∞ the nominal total amount of drug released, K the kinetic constant and n the diffusion exponent that is used to characterize the release mechanism.

This equation is a generalization of the observation that superposes two apparently independent mechanism of drug transport, Fickian diffusion and a case-II transport describes drug release from a swelling polymer. When *n* takes the value 0.5 it indicates diffusion-controlled drug release and for the value 1.0 indicates swelling-controlled drug release. Values of *n* between 0.5 and 1.0 can be regarded as an indicator for the both phenomena (anomalous transport). These extreme values for the exponent *n*, 0.5 and 1.0, are only valid for slab geometry and for spheres and cylinders different values have been derived. For microspheres, a spherical geometry is considered and as per Ritger and Peppas *n* takes values in the range of 0.45–0.89 for anomalous transport. The value of *n* with regression coefficient for optimized mucoadhesive microspheres of CP extract was found to be 0.774 indicating the anomalous transport. The anomalous diffusion mechanism of drug release demonstrated both diffusion-controlled and swelling-controlled drug release from mucoadhesive microspheres containing CP extract.

CONCLUSION

The microcapsules containing CP extract consisting of mucoadhesive polymer alginate could be prepared by an ionic gelation process. The microcapsules exhibited good mucoadhesive properties and antidiabetic acitivity in an in vitro test. From the in vitro study, the developed mucoadhesive microcapsules in vitro study are suitable for prolonged release effect after the oral administration of mucoadhesive microcapsules of CP extract.

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