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Research paper

Effect of drug loading on the transformation of vesicular into cubic nanoparticles during heat treatment of aqueous monoolein/poloxamer dispersions

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Abstract

Colloidal dispersions of the pre-equilibrated cubic phase in the monoolein/poloxamer 407/water system, which are under investigation as potential drug carriers, often contain a considerable fraction of undesired non-cubic particles, particularly when prepared with high concentrations of poloxamer. Recent investigations revealed that the non-cubic particles can be transformed into particles of cubic internal structure by heat treatment. The present study investigates the effect of drug loading on the non-cubic to cubic transformation process during autoclaving of the dispersions. The results indicate that the process can also proceed in dispersions loaded with different concentrations of ubidecarenone, tocopheryl acetate, betamethasone-17-valerate, chloramphenicol or miconazole. At low concentration, none of the drugs had pronounced influence on the autoclaved dispersions whereas with increasing drug concentration different effects were observed. Depending on the type of drug no effects (betamethasone-17-valerate), increasing particle size of the dispersions (chloramphenicol, miconazole) or phase separation upon autoclaving (high load of miconazole) was observed. Except for loading with high amounts of chloramphenicol, which led to the formation of cubic phase particles already without additional heat treatment, the properties of the thermally untreated dispersions were virtually unaffected by drug incorporation.

Keywords: Colloidal drug carriers; Dispersions of cubic phase; Autoclaving; Monoolein; Drug loading; Ultrastructural transformation

1. Introduction

Colloidal dispersions of bicontinuous cubic phases formed in the monoolein/poloxamer/water system have been proposed as potential drug carriers, e.g., for intravenous administration [1]. Such dispersions can be obtained by high pressure homogenization of a pre-equilibrated cubic phase in an aqueous medium [2–5]. The ultrastructure of the resulting dispersions depends highly on the composition and preparation parameters, in particular concerning the particle size distribution and the presence of an additional vesicular fraction [3,5,6]. The attempt to prepare dispersions with a particle size distribution in the lower colloidal range frequently leads to dispersions with a large fraction of non-cubic, vesicular particles. Recently, we observed that the non-cubic particles in aqueous colloidal monoolein/poloxamer 407 dispersions can be transformed into colloidal particles of cubic structure by heat treatment, e.g., by autoclaving [7,8]. Heat treatment can thus be used to optimize the content of cubic particles in poloxamer stabilized monoolein dispersions. Further investigations have shown that the properties of dispersions

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resulting from heat treatment can be modified by variation of the dispersion composition [9].

Detailed reports on the ultrastructural transformation by heat treatment are hitherto only available for drug-free dispersions. In order to further assess the pharmaceutical applicability of this novel procedure, we were interested in whether the heat-induced transformation would also take place in drug-loaded dispersions and which effect drug loading might have on the properties of the resulting dispersions. The latter question is also of more general interest, since the influence of drug loading on the ultrastructure of colloidal particles in the monoolein/poloxamer/water system has not yet been studied in much detail. As lipophilic and amphiphilic substances are expected to interact most prominently with the membrane structure of the cubic phase, the compatibility of the autoclaving process was tested with a selection of lipophilic/amphiphilic model drugs (ubidecarenone, tocopheryl acetate, miconazole, betamethasone-17-valerate, and chloramphenicol (Fig. 1)).

2. Materials and methods

2.1. Materials

Monoolein (GMOrphic-80®, Eastman Chemical Company, Kingsport, TN, USA), containing >94% monoglycerides with >75% of the fatty acid residues being oleic acid, <10% saturated, and <15% more highly unsaturated fatty acids (manufacturer's specification), poloxamer 407 (Pluronic® F127, BASF, Ludwigshafen, Germany), ubidecarenone (Kyowa Hakko Kogyo, Tokyo, Japan), tocopheryl acetate (Synopharm, Barsbüttel, Germany), miconazole (Erregiere, San Paolo d'Argon, Italy), betamethasone-17-valerate (Synopharm, Barsbüttel, Germany), chloramphenicol (Synopharm, Barsbüttel, Germany), and thiomersal (Synopharm, Barsbüttel, Germany) were used for preparation as received from the manufacturer. Purified water was prepared by subsequent filtration and deionization/reverse osmosis (Milli RX 20, Millipore, Schwalbach, Germany) from drinking water.

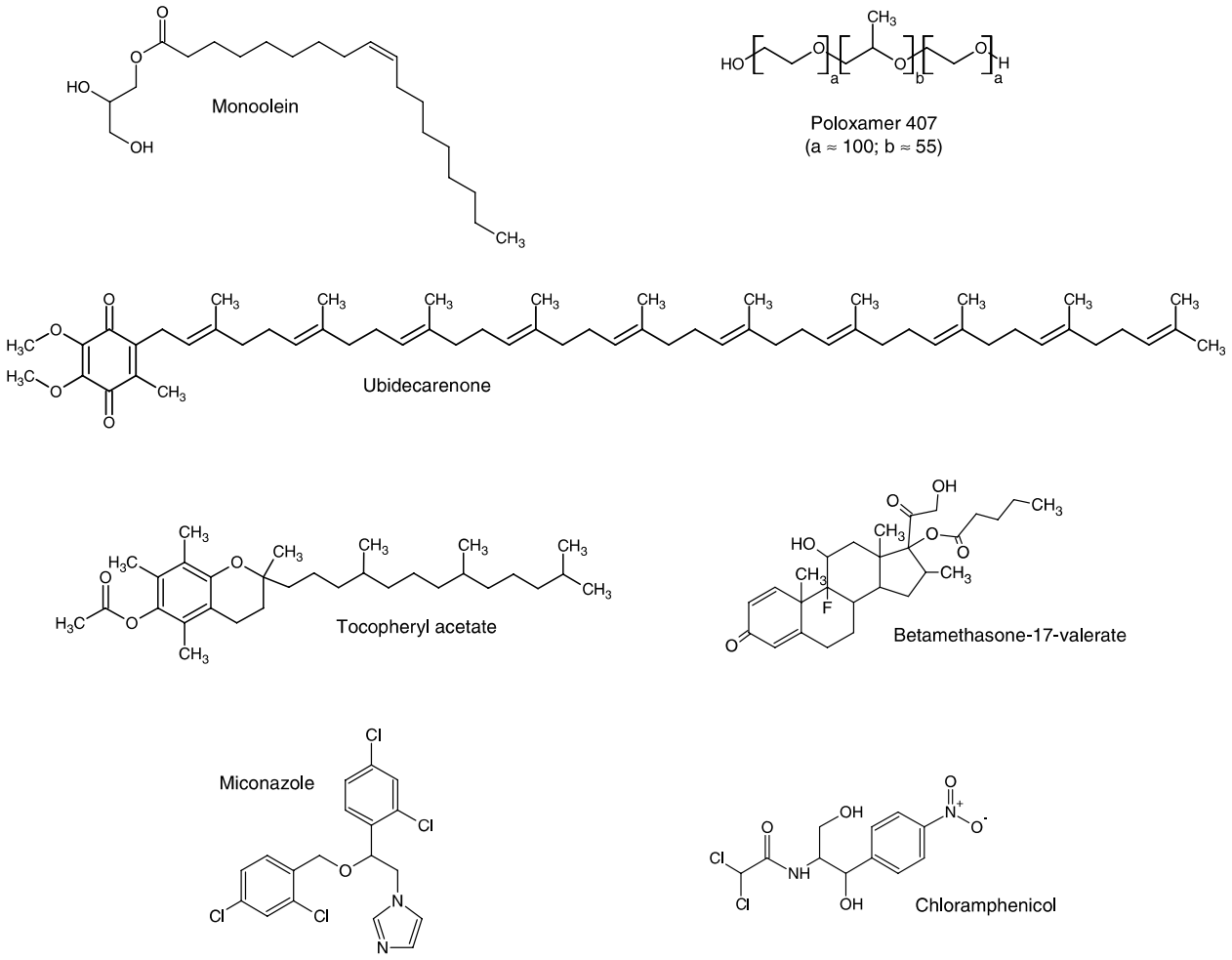

Fig. 1. Molecular structure of the drugs under investigation.

2.2. Methods

2.2.1. Dispersion preparation

The drug-free dispersion contained 4.4% monoolein and 0.6% poloxamer 407 in 95% water (with 0.01% thiomersal for preservation). For dispersion preparation, monoolein and poloxamer were mixed in the melt and the molten mixture was added dropwise to water under stirring at room temperature. The resulting coarse dispersion was equilibrated for at least about 1 day at room temperature before homogenization in a microfluidizer M110S (Microfluidics, Newton, MA, USA) at 350 bar for 15 min at 40 °C (using a water bath to thermostatize interaction chamber and heat exchanger). For the preparation of drug-loaded dispersions, different amounts (Table 1) of ubidecarenone, tocopheryl acetate, betamethasone-17-valerate, chloramphenicol or miconazole were dissolved in the monoolein/poloxamer 407 melt at 60 °C (unless otherwise specified) and processed with this melt in the same manner as the drug-free dispersions.

Within 1 day after homogenization, fractions of the dispersions were autoclaved at 121 °C in a laboratory autoclave heated with an external heater plate (Sanoclav KL-12, Wolf, Geislingen, Germany) for 15 min plus an equilibration time of 5 min. Before pressurizing, the autoclave was kept at 100 °C for 5 min to remove air from the chamber.

Non-autoclaved and autoclaved dispersions were stored in glass vials at 23 °C protected from light.

2.2.2. Characterization

2.2.2.1. Visual inspection. About 1 week after preparation, the dispersions were visually assessed for optical appearance (e.g., color, turbidity, homogeneity and presence of macroscopic particles).

2.2.2.2. Particle size analysis by laser diffraction. The particle size of the dispersions was investigated with a Coulter LS 230 (Beckman Coulter, Krefeld, Germany) combining information from simple light scattering (LS) and polarization intensity differential scattering (PIDS) to obtain information on the particle size in the nanometer as well as in the micrometer range. For data evaluation based on the Mie theory, an optical model was created using the instrumental software assuming 1.45 as the real and 0 as the imaginary part of the refractive index of the particles. Results given are means of five successive measurements of 120 s each.

2.2.2.3. Light microscopy. A Leica DMRXP microscope (Leica, Wetzlar, Germany) calibrated with a micrometer slide was used with polarized light or differential interference contrast at magnifications between 100 and 1000×.

2.2.2.4. Small angle X-ray diffraction. Samples were investigated with a Kratky camera (Hecus Braun X-ray Systems, Graz, Austria) on a ID3003 X-ray generator (Rich. Seifert, Ahrensburg, Germany) using a position sensitive detector (PSD –50M, M. Braun, Garching, Germany) and an exposure time of 1–2 h at 25 °C. Reflections were assigned to the different lyotropic liquid crystalline phases using the characteristic spacing ratios (cubic type P (Im3m): $\sqrt{2}$:$\sqrt{4}$:$\sqrt{6}$:$\sqrt{8}$:$\sqrt{10}$:$\sqrt{12}$:$\sqrt{14}$:$\sqrt{16}$:...; cubic type D (Pn3m): $\sqrt{2}$:$\sqrt{3}$:$\sqrt{4}$:$\sqrt{6}$:$\sqrt{8}$:$\sqrt{9}$:$\sqrt{10}$:$\sqrt{11}$:...). From the spacing d of the first reflection observed, the lattice constant a of the respective cubic phase (P or D) was calculated by $a = d \cdot \sqrt{2}$.

3. Results and discussion

3.1. Drug-free dispersion

Homogenization of the drug-free composition led to the formation of a homogeneous, turbid, slightly translucent dispersion with a particle size maximum slightly below 100 nm (Fig. 2). The absence of small angle X-ray reflections indicates a lack of periodic order within the nanoparticles that make up the dispersion (Fig. 3). Earlier investigations revealed a primarily vesicular state of those particles [3,6,8]. Upon autoclaving, the optical appearance of the dispersion turned milky white. A few particle aggregates in the micrometer range could be detected microscopically. LS-PIDS particle size analysis of the autoclaved dispersion revealed a narrow monomodal distribution with a maximum around 250–300 nm (Fig. 2). In small angle X-ray diffraction, distinct reflections compatible with the presence of a P-type cubic phase (lattice constant ~14.4 nm) were observed (Fig. 3).

Table 1
Drugs incorporated into the dispersions (the respective amount of drug was added to the basic composition of 4.4% monoolein, 0.6% poloxamer 407, and 95% water)

Drug		Concentration related to		
		Monoolein + poloxamer 407		Total dispersion
		% (w/w)	% (mol/mol)	% (w/w)
Ubidecarenone	Q_{10}	0.3	0.14	0.015
Tocopheryl acetate	TOC	0.3	0.26	0.015
Miconazole	MCZ	0.3/1/5	0.29/0.97/4.85	0.015/0.05/0.25
Betamethasone-17-valerate	BMV	0.3/1/2 (/5)	0.25/0.85/1.69 (/4.23)	0.015/0.05/0.10 (/0.25)
Chloramphenicol	CAP	0.3/1/2/5	0.37/1.25/2.50/6.25	0.015/0.05/0.10/0.25

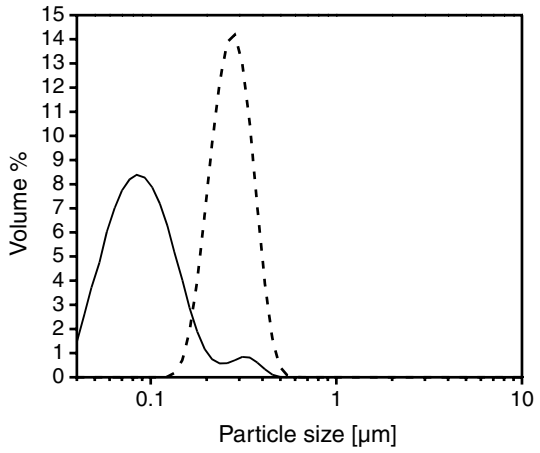

Fig. 2. LS-PIDS particle size distributions of the thermally untreated (solid line) and the autoclaved (dashed line) drug-free dispersion.

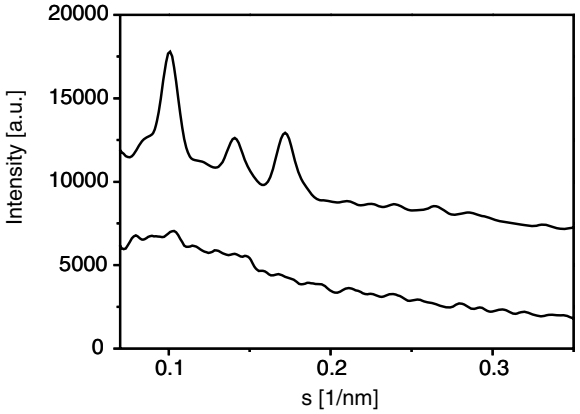

Fig. 3. Small angle X-ray diffractograms of the thermally untreated (bottom) and the autoclaved (top) drug-free dispersion ($s = 1/d$).

In agreement with previous observations [7,8], the particles in the dispersion thus undergo an ultrastructural transformation process from a non-cubic into a cubic state upon autoclaving, which is accompanied by a characteristic change in visual appearance, a shift in the particle size distribution, and the appearance of typical small angle X-ray reflections. The mechanism of this transformation has not been fully elucidated yet. Obviously, it includes fusion of the vesicular particles into larger structures at elevated temperature. According to earlier results, the sample needs to reach a temperature that allows formation of an isotropic phase by the amphiphilic components of the dispersion in order to achieve considerable ultrastructural transformation of the nanoparticles. Upon cooling after heat treatment, the larger (isotropic) particles formed in the heat are assumed to transform into the P-type cubic structure (the equilibrium state at room temperature) [8].

3.2. Dispersions loaded with different model drugs

Different poorly water-soluble model drugs were incorporated into the dispersions to study the compatibility of the transformation process upon autoclaving with the presence of incorporated substances. The effects of drug loading on the particle sizes and the lattice constant of the cubic phase resulting after autoclaving are summarized in Figs. 4 and 5.

The incorporation of 0.3% ubidecarenone or tocopheryl acetate did not affect the general behavior of the dispersions (non-autoclaved or autoclaved) compared to an unloaded dispersion. The originally homogeneous, turbid, slightly translucent dispersions with a particle size around 100 nm and without X-ray reflections transformed into milky white (or slightly yellowish in the presence of ubidecarenone) dispersions of larger particle size which displayed small angle X-ray reflections characteristic of a P-type cubic phase. Higher concentrations of these drugs were not investigated since preliminary investigations in 4.6% monoolein dispersions stabilized with 0.4% poloxamer 407 had indicated that 1% ubidecarenone formed

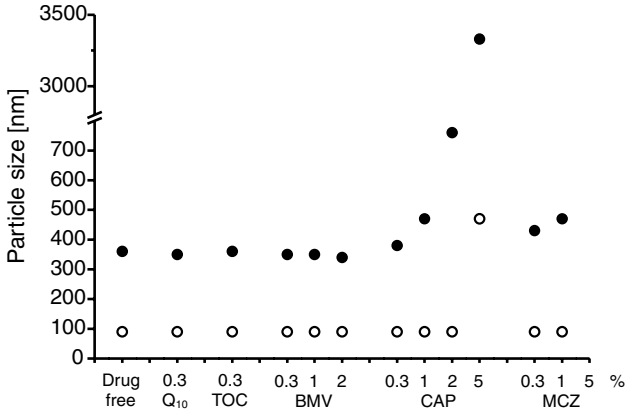

Fig. 4. Median particle size of the non-autoclaved (open symbols) and of the autoclaved (full symbols) monoolein/poloxamer 407 dispersions loaded with different amounts of model drugs (Q_{10}, ubidecarenenone; TOC, tocopheryl acetate; BMV, betamethasone-17-valerate; CAP, chloramphenicol; MCZ, miconazole). For the non-autoclaved dispersion with 5% miconazole, the particle size could not be determined.

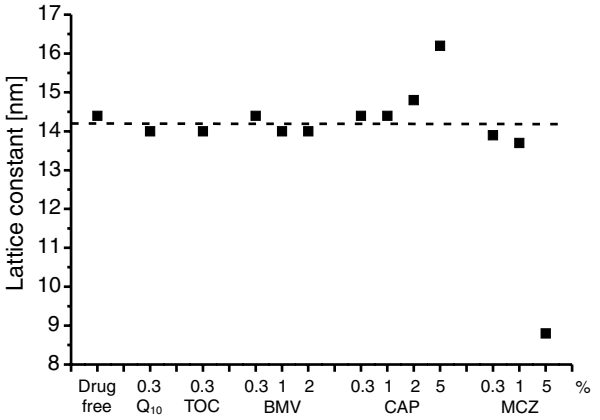

Fig. 5. Lattice constant of the P-type cubic phase in autoclaved dispersions (5% MCZ: D-type cubic phase of semisolid supernatant). For abbreviations, see Fig. 4. The line is drawn as a guide to the eye.

drug crystals already during equilibration of the coarse dispersion in the preparation procedure and since phase separation of excess tocopheryl acetate would be difficult to detect due to the liquid state of this substance, respectively.

Also upon loading with 0.3%, 1% or 2% betamethasone-17-valerate no influence on the homogenized or autoclaved dispersions was observed. Five percent of this drug could, however, not be incorporated in the dispersions as it did not dissolve completely in the monoolein/poloxamer melt even after increasing the temperature to 80 °C.

Chloramphenicol at 0.3%, 1%, and 2% did not affect the non-autoclaved dispersions which remained of small particle size and without X-ray reflections. In the autoclaved dispersions, the particle sizes increased distinctly with drug concentration and a slight increase in the lattice constant of the cubic phase was observed. Chloramphenicol (5%) could only be completely dissolved in the monoolein/poloxamer 407 melt by increasing the temperature to 80 °C for a short time. In contrast to the drug-free dispersion or dispersions with lower amounts of this drug, the sample with 5% chloramphenicol was already milky white after homogenization with particle sizes mainly around 0.47 μm and X-ray reflections of a P-type cubic phase (lattice constant: 16.2 nm). Autoclaving shifted the maximum of the particle size distribution to above 3 μm, the X-ray reflections remaining essentially unchanged.

Miconazole at 0.3% and 1% had no influence on the properties of the non-autoclaved dispersions. Autoclaving of these systems led to slightly (0.3% drug) or distinctly larger (1% drug) particle sizes compared to the drug-free dispersion. The lattice constant as derived from X-ray diffraction decreased slightly. Miconazole could be incorporated at 5% into the monoolein/poloxamer 407 melt already at 60 °C without leaving crystalline residues. After homogenization, the dispersion appeared visually similar to that of the drug-free control or the samples with lower miconazole content and did not display X-ray reflections. A reliable particle size distribution could not be determined due to strong variations between single runs. Autoclaving of this dispersion led to phase separation into an almost clear subnatant covered by a turbid, semisolid mass that displayed X-ray reflections of the D-type cubic phase (lattice constant 8.8 nm). The formation of D-type cubic phase upon autoclaving may be the cause for the phase separation as there are indications that particles of this phase are difficult to stabilize in the monoolein/poloxamer 407/water system (Wörle et al., unpublished).

Drug crystals could be detected in none of the drug-loaded dispersions with polarized light microscopy, indicating complete solubilization of the incorporated substances by the vesicular as well as the cubic phase particle dispersions.

At low concentration (0.3%), none of the selected drugs thus had pronounced influence on the dispersions whereas with increasing drug concentrations different effects were observed. Depending on the type of drug no effects (betamethasone-17-valerate), increasing particle size of the autoclaved dispersions (chloramphenicol, miconazole) or even phase separation upon autoclaving (5% miconazole) were observed. Interestingly, the properties of the vesicular dispersions were virtually unaffected by drug incorporation in most cases. Only upon incorporation of 5% chloramphenicol a pronounced alteration, the formation of particles of cubic phase already without additional heat treatment, was observed. Although slight alterations of the lattice constant occurred in dispersions containing higher concentrations of chloramphenicol and miconazole, no influence on the general presence of cubic phase was found for successfully stabilized dispersions. In particular, no shift into a lamellar or reverse hexagonal phase (which were, e.g., formed in monoolein/water bulk systems upon addition of lidocaine–HCl or lidocaine, respectively [10]) was observed.

The exact causes for the different influence of the incorporated drugs during heat treatment remain largely unknown at present. It may be speculated that the more hydrophobic substances, ubidecarenone, tocopheryl acetate, and betamethasone valerate, reside primarily in the lipophilic part of the bilayer (as previously suggested for vitamin K_1 [11]) or in the interior of the particles of isotropic phase formed during autoclaving, respectively, and thus do not affect the fusion process. Chloramphenicol and miconazole, on the other hand, exhibit surface active properties [12,13]. They are, therefore, also expected to influence the more hydrophilic part of the bilayer or the surface or the particles of isotropic phase, respectively. This way, they might interfere with the fusion process during heat treatment and induce the formation of larger particles (as well as of cubic phase already upon homogenization of the system with 5% chloramphenicol). Different localizations of the drug molecules within the bilayer might also explain the differences in the swelling behavior of the cubic phase: Drugs, which are assumed to reside within the bilayer, do not exhibit a noticeable effect on the lattice parameter (which might also be due to low concentration in case of tocopheryl acetate and ubidecarenone). In contrast, hydrophilic parts of the chloramphenicol and miconazole molecules are probably oriented towards the hydrophilic portion of the bilayer where they can exert an effect on hydration (and thus the lattice parameter) when present at higher concentration. Concentrations of 5% of these two substances also seem to have an influence on the geometry of packing within the bilayer which induces the transformation into the D-type cubic phase in the case of miconazole upon autoclaving and the formation of the cubic state already during homogenization of the chloramphenicol-loaded particles.

In conclusion, the ultrastructural transformation from non-cubic to cubic nanoparticles does also take place in the presence of foreign compounds such as drugs. A slight to pronounced increase in particle size of the autoclaved dispersions may, however, have to be taken into consideration for some of the systems under investigation here. Our study also shows that the drug incorporation capacity of

the systems is often quite low. This is not unexpected as the incorporated drug molecules are not simply dissolved in an unorganized matrix but have to fit into the delicate structure of the vesicular or cubic particles. Future investigations will have to show how far the concentration of monoolein/poloxamer can be increased to enhance the drug carrier capacity of the dispersions.

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