Original Article:
Polymeric Microparticles as Alternative Carriers for Antidiabetic Glibenclamide Drug

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ABSTRACT

Background: Glibenclamide is a lipophilic drug widely used in type 2 diabetes treatment. However, its low bioavailability limits its use. Thus, novel formulations should be applied to improve the drug's bioavailability.

Objectives: This study aimed to develop alternative carriers for oral delivery of glibenclamide. For this purpose, two biocompatible polymers, poly(e-caprolactone) and poly(butylene adipate) were formulated as microparticles (MPs) capable of loading the antidiabetic drug.

Methods: In this regard, as microparticle fabrication approach, the modified emulsion solvent evaporation method was applied. Physicochemical evaluation of the prepared microparticles included the examination of their morphology, degradation rate, and thermal properties. Drug entrapment, drug loading, and particle size were also investigated. Simulated intestinal medium and body fluid at 37oC were selected as dissolution media. Differential scanning calorimetry was used to investigate the crystal properties of the microparticles and drugs.

Results: The developed microparticles had sizes between 0.5 and 4 μm. Poly(butylene adipate) based microparticles had a smooth surface, whereas poly(ε-caprolactone) based microparticles showed a porous surface. The DSC thermogram revealed the amorphization of the drug. Hydrolysis results exhibited a very low mass loss, while in vitro release results depicted that the dissolution rate of the prepared microparticles was higher than that of pure glibenclamide demonstrating a prolonged pattern which is ideal for minimizing the daily dose of glibenclamide.

Conclusion: In this study, novel carriers for glibenclamide were successfully prepared with promising future use.

Introduction

Metabolic syndromes belong to a group of disorders, which can increase the risk of heart disease, stroke, and type 2 diabetes. These conditions involve high levels of increased blood pressure, escalated blood sugar, excess body fat around the waist, as well as high cholesterol or triglyceride levels [1, 2]. Diabetes Mellitus (DM) belongs to the chronic and complex diseases. Its main

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characteristic is high blood sugar level or hyperglycemia. Hyperglycemia occurs when insulin secretion cannot compensate for insulin resistance [2, 3]. Many complications can emerge if diabetes cannot be managed, especially in patients suffering from DM. They develop wounds that do not heal normally, and thus, infections might superimpose on them [4-6]. As a result, the management of diabetes with the appropriate drug candidates and adjusting a healthy diet for patients is mandatory.

At the first stage of DM, metformin is the drug of choice. However, if hemoglobin A1c level does not reach the appropriate level within three months, the physician should add a second antidiabetic agent from DPP-4 (Dipeptidyl peptidase-4) inhibitors, thiazolidinediones, sulfonylureas, SGLT2 (sodium-glucose transport protein 2) inhibitors, GLP-1 (Glucagon-like peptide) receptor agonist or basal insulin (stage 2). Numerous studies can be found in the literature showing the hypoglycemic activity of herbal plants that are quite promising [6, 7]. However, clinicians strongly recommend the use of conventional drugs. Glibenclamide (GLI) is a lipophilic antidiabetic drug that belongs to sulfonylureas and is used frequently as a therapeutic strategy in DM. GLI belongs to class II of the Biopharmaceutical Classification System (BCS). It is poorly soluble but can permeate gastrointestinal mucosa. Oral administration of class II drugs is limited because of their low solubility. Thus, increasing their hydrophilicity could enhance their clinical performance and minimize their dose [8]. Besides, an oral drug delivery system that is sustained and controlled can also reduce multiple dosing and improve patient's compliance [9].

Polymeric Microparticles (MPs) have been widely developed as drug delivery systems since they are capable of encapsulating both hydrophilic and hydrophobic molecules [10, 11]. Furthermore, MPs are more stable in the biological environment compared with the liposomes. Biocompatible and biodegradable polymers prepare polymeric MPs in most cases because they can degrade in physiological conditions and, at the same time, release the drug in a controlled manner. Aliphatic polyesters such as Polylactic Acid (PLA) [12], Polyethylene Succinate (PESu), Polyε-Caprolactone (PCL) [13-15] and Polybutylene Adipate (PBAd) [16] belong to such biocompatible substances. All of them are hydrophobic macromolecules which, however, react differently under degradation conditions.

In this study, the prepared MPs from PCL or PBAd could load a high amount of GLI and act as oral drug delivery systems. The MPs were studied with Fourier Transform Infrared Spectroscopy (FTIR), revealing interesting interactions between the drug and matrix. Moreover, their morphological formulations were specified using Scanning Electron Microscopy (SEM). Their in vitro degradation and in vitro release were studied in body fluids while Differential Scanning Calorimetry (DSC) was applied for studying their thermal characteristics.

Materials and Methods

Poly-ε-caprolactone, polybutylene adipate, and polyvinyl alcohol (Mw~130.000) were purchased from Sigma-Aldrich. All other reagents were of analytical grade.

Preparation of MPs

The preparation of microparticles was performed via solid-oil-water (s/o/w) modified double emulsification method, as previously reported. [17, 18]. Similarly, 100 mg of polymer, PCL, or PBAd were dissolved in 5 mL of methylene chloride. Then, 10 mg of glibenclamide was added in the case of loaded MPs. The polymeric solution was added dropwise in 20 mL of PVA aqueous solution (1% w/v) and homogenized for 1 min. Afterward, the homogenized solution was inserted in 100 mL water and magnetic stirring until full solvent evaporation. MPs were collected after centrifugation at 8000 rpm for 10 min. They were washed thrice with distilled water to ensure that traces of solvent or PVA have been removed. MPs were finally oven-dried at 35°C and then stored at room temperature.

Characterization techniques

Particle size and morphology evaluation

MPs particle size was measured via Malvern Mastersizer Hydro 2000S (Malvern Instruments, Malvern). MPs were analyzed in water as the dispersion medium. The volumetric mean diameter (4.3) was used to assess the particle size of MPs. The morphology of MPs was observed using a scanning electron microscope (SEM; Zeiss Evo HD 15, Germany). Images were obtained using secondary electrons (topographic contrast) (accelerating voltage of 10 kV).

Drug loading and encapsulation efficiency evaluations

About 20 mg MPs were added to 750 μL of methylene chloride to dissolve the polymer. GLI was extracted using 750 μL of ethanol: Phosphate Buffered Saline (PBS) (80:20) solution after centrifugation at 8800 rpm for 15 min. The solution was filtered and analyzed by HPLC for drug loading, i.e. entrapment efficiency % (EE%).

The drug loading of GLI into the polymeric MPs was calculated using Equation. Microparticles yield was calculated via Equation (1). The drug loading of GLI into the polymeric MPs was calculated according to Equation (2). Finally, Entrapment Efficiency was calculated via Equation (3).

1. $$Microparticles\ yield\ (\%)=\frac{weight\ of\ microparticles}{weight\ of\ polymer\ and\ drug\ fed\ initially}\times 100$$

2. $$Drug\ loading\ content\ (\%)=\frac{weight\ of\ drug\ in\ microparticles}{weight\ of\ microparticles}\times 100$$

3. $$Entrapment\ efficiency\ (\%)=\frac{weight\ of\ drug\ in\ microparticles}{weight\ of\ drug\ fed\ initially}\times 100$$

In vitro release of glibenclamide from the MPs

The study of the in vitro release of MPs was carried out in simulated body fluids and intestinal fluid at 50 rpm. About 0.5 ml of buffer was used to prepare oral suspensions, which added to the dialysis membrane (Spectra/pore, MW of 12-14 kDa) and capped with closures. The bags are then placed in a glass beaker containing 100 mL of release medium kept at 37.0±0.5ºC during the experiment and mechanically stirred (200 rpm). The temperature was maintained at 36±0.5º C to mimic body temperature. At a predetermined time, 1 mL of media was withdrawn and replaced by the same volume of fresh medium. High Liquid Pressure Chromatography (HPLC) analyzed the release and the experiment was performed in triplicate.

Characterization techniques

Fourier transformed infrared spectroscopy

MPs were placed on an ATR crystal. A pressure clamp was used to apply maximum pressure to allow for intimate contact of MPs with the ATR crystal. The spectra study ranged from 4000 to 400 cm–1(FTIR-ATR model FTIR–2000, Perkin Elmer, Germany) [5].

Differential Scanning Calorimetry

MPs (8 mg) were sealed in aluminum pans. Then, they were heated above polymers and drug melting point at a heating rate of 10 ºC/min. The samples were held at that temperature for 5 min, and then they were cooled at 300oC/min. A Perkin Elmer, Pyris Diamond Differential Scanning Calorimeter (DSC), was used [5].

In vitro hydrolysis of PCL and PBAd MPs

In vitro hydrolysis studies were performed using SBF (pH 7.4). The specific amount of MPs was placed in glass tubes, which were incubated at 37±1º C in an oven for five days. Every two days, tubes were taken off; MPs powder was centrifuged and washed with deionized water and finally, oven-dried. Then MPs were weighted until reaching the constant weight. Degradation degree was calculated from the mass loss [19].

HPLC analysis

Drug content and release rate were calculated using an HPLC system consisted of a gradient pump and a UV (Ultra Violet) detector supplied by Agilent 1100. The column used was C18 (5 µm, 150 x 4.6 mm). The samples were analyzed at 230 nm with 1 mL/min at 25°C flow rate. The mobile phase consisted of a mixture of 0.1% orthophosphoric acid: acetonitrile: methanol (20:50:30). The applied retention time was 2.75 min. Validation of HPLC method consisted of partial linearity, the Limit of Detection (LOD), the Limit of Quantitation (LOQ), precision, accuracy and specificity, selectivity, and stability [20].

Results

Morphology and size characterization of MPs by SEM and DLS

MPs morphological characteristics were evaluated by SEM. Neat PCL MPs present a spherical shape with an average diameter of 0.5-4 µm (Figure 1). SEM images demonstrated that PCL MPs surface has slight imperfections since pores are visible, which probably resulted from the water droplets trapped at the interior of MPs. These droplets can be evaporated during drying, which leaves empty spaces. Similar observations have been reported by Filippousi et al. study [10]. Neat PBAd MPs (microparticles based on poly [butylene adipate]) present a slightly smaller size than PCL MPs. Figure 2 shows the MPs loaded with GLI. It can be said that drug incorporation enlarges MPs size. Besides, PCL MPs loaded with GLI also had a porous structure due to the rigorous solvent evaporation.

Furthermore, according to DLS measurement (Figure 3), MPs size ranged between 0.29 and 4 µm, with a mean particle size of 3.5 µm. It can be said that most MPs demonstrated a desirable size of 1.5 µm. The results of DLS are similar to those obtained from SEM.

Preliminary studies, FT-IR and DSC

PCL and PBAd spectra exhibit similar bands as aliphatic polyesters, i.e. at 1740 and 1730 cm-1 correlating with the carbonyl stretching C=O, whereas they are 2944 and

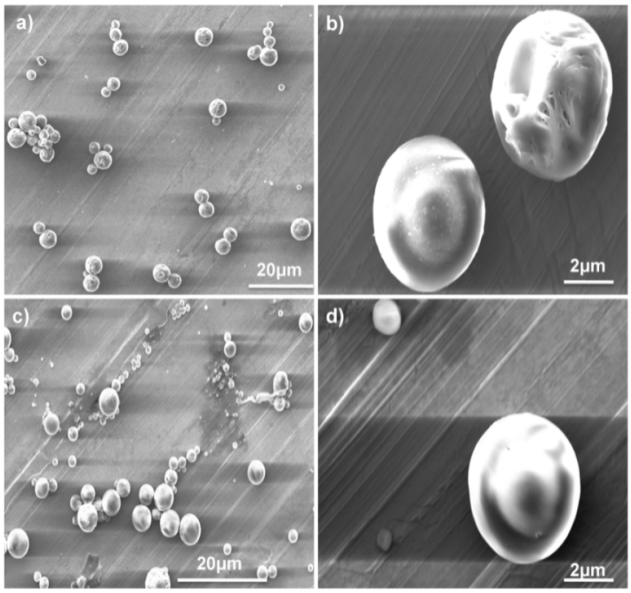

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Figure 1. SEM images

A. PCL (Mag=1.00 KX); B. PCL (Mag=10.00 KX); C. PBAd (Mag=1.5 KX); and D. PBAd (Mag=10.00 KX)

2965 cm-1 at the stretching vibration of -CH_2. GLI spectrum presents the following bands at 1720, 1671 (C=O stretching), 1517 (CH_2 bending), 1342, 1300 (SO_2 asymmetric stretching), 1244, 1158 (C–N stretching). FTIR spectroscopy studies depicted the successful loading of GLI into the polymeric MPs since bands of GLI found to MPs spectrum. In addition, interactions between carriers and drugs have not been revealed since bands located in the same wave number indicating the stability of the products [21-23] (Figure 4A, 4B).

The physical state of the developed MPs was also studied by DSC (Figure 5A), considering that crystallinity may result in different release patterns from polymeric systems [15, 16, 21]. It has been reported that PCL is a semi-crystalline polyester with melting Temperature

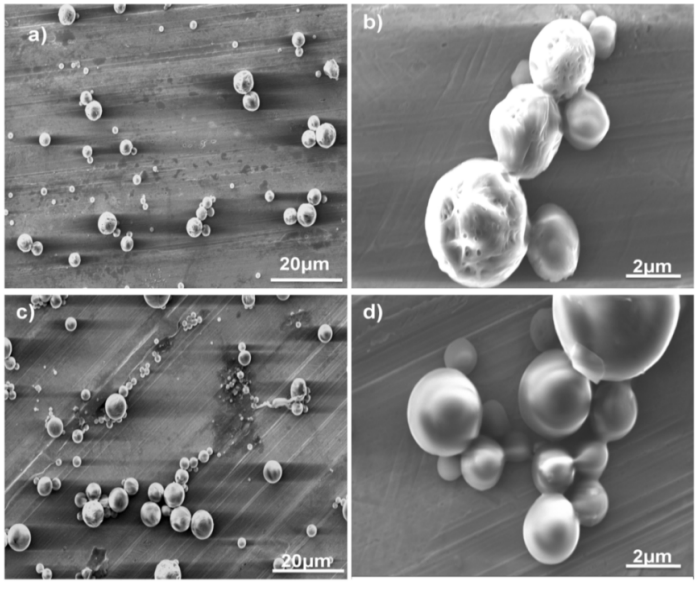

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Figure 2. SEM images

A. PCL+GLI (Mag=1.00 KX); B. PCL+GLI (Mag=10.00 KX); C. PBAd + GLI (Mag=1.5 KX); and D. PBAd + GLI (Mag=10.00 KX)

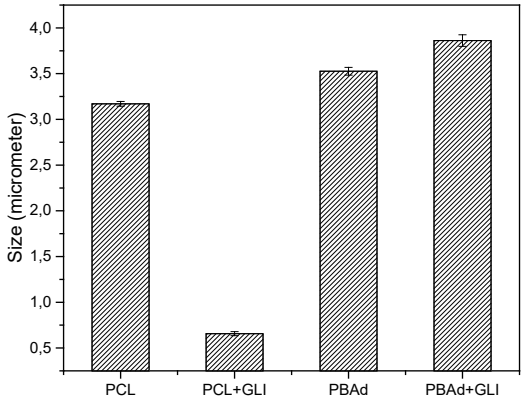

Figure 3. Mean size of MPs

(Tm) at 53.82º C [15], whereas PBAd also presents semi-crystalline characteristics with low Tg value at -55º C and Tm at 55, 85-60º C [19]. GLI pure drug presents a sharp endothermic peak at 174.78° C similar to previously reported studies [24]. Based on Figure 5B, after drug encapsulation polymers exhibit their crystal properties, the results are similar to already published data. However, the absence of GLI melting point demonstrated amorphous drug dispersion into the MPs. This fact led to a higher dissolution rate of GLI in the MPs.

In vitro hydrolysis studies

In vitro hydrolysis study is essential when polymeric systems are designed. It has been widely reported that aliphatic polyesters can break down to non-toxic monomeric hydroxy acids when they come in contact with human body fluids [19]. Additionally, degradation is strongly consistent with the drug release mechanism. In most cases, it is also associated with drug diffusion or matrix erosion. Consequently, degradation studies have been accomplished at 37oC for 5 days in the SBF medium to check whether degradation behavior is correlated with GLI dissolution behavior (Figure 6).

It can be said that PCL MPs degrade slower than PBAd MPs. This conclusion is rational since PCL is used for the controlled release systems. Furthermore, PBAd demonstrates a higher hydrolytic rate due to its low glass transition (-55º C) and low Tm, which is relatively close to degradation temperature. It has been referred that this property could enable macromolecular chains to present higher mobility. Consequently, this can lead to higher water penetration inside MPs and easier hydrolyze the ester bonds of PBAd.

Drug uptake, drug loading, and in vitro release studies

Table 1 presents MPs yield (%), drug loading (%), and entrapment efficiency (%). MPs yield was 83.77% for PCL+GLI and 84.51% in the case of PBAd+GLI, which is relatively high. Drug loading values were 8.27% and 8.55% for PCL+GLI and PBAd+GLI, respectively.

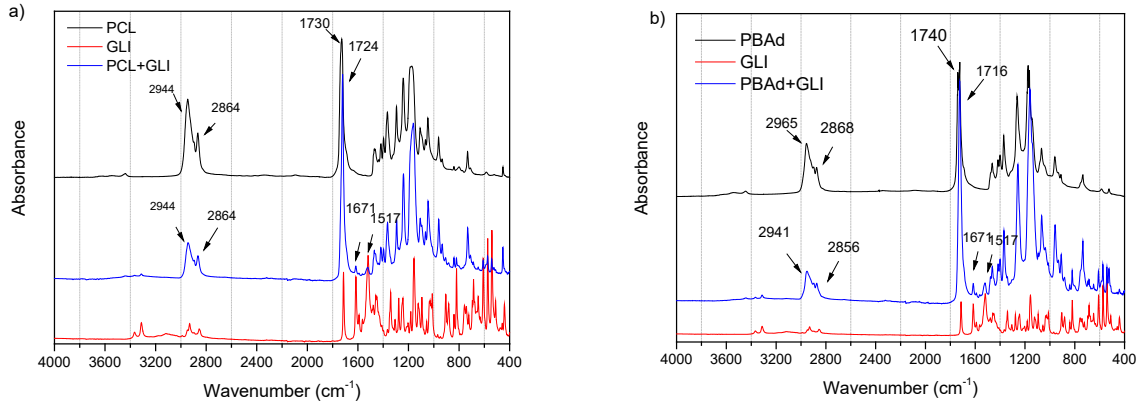

Figure 4. FTIR studies

A. neat PCL and PCL+GLIMPs; B. PBAd and PBAd+ GLIMPs

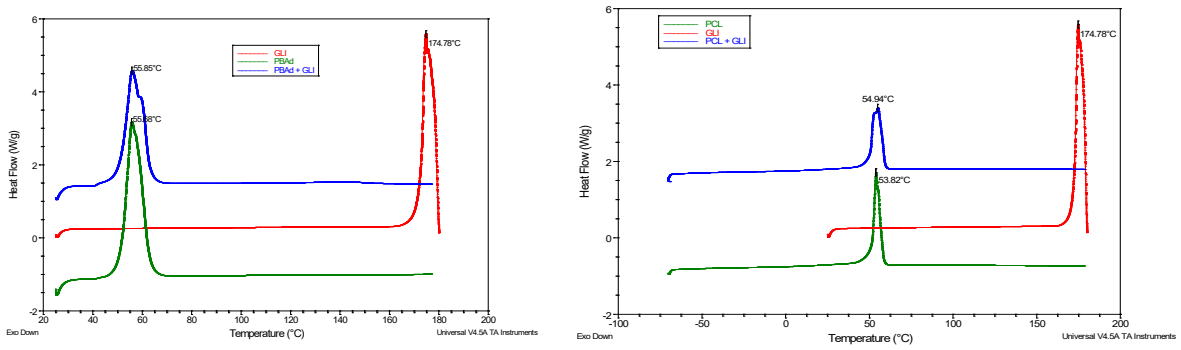

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Figure 5. Differential scanning calorimeter studies
A. neat PCL and PCL+GLIMPs GLI; B. PBAd and PBAd+ GLIMPs and pure GLI

In vitro release studies were performed using two release media. For the first 10 hours, PBS was used (pH=6.8) to simulate intestinal conditions, and after 24 hours, its pH changed to 7.4 to simulate body fluids. GLI is a hydrophobic drug with low solubility in body fluids. So to improve the dissolution of the drug, polymeric MPs were prepared. Figure 6 shows the dissolution profiles of GLI from the MPs. The cumulative percentage release of GLI at pH 6.8 from PCL and PBAd MPs were 4.316% and 4.663%, respectively, after 10 hours. When the simulation medium changed to pH=7.4, the release rate got significantly higher. Almost 70% of the drug was released after 13 days. Thus, the objective of the controlled release was achieved (Figure 7).

Conclusions

In this work, preliminary studies were conducted on polymeric MPs from PCL and PBAd as oral carriers

Table 1. MPs yield (%), drug loading (%), and entrapment efficiency (%) characteristics

Sample	Mean±SD		
	MPs Yield	Drug Loading	Entrapment Efficiency
PCL+GLI	83.77±2.19	8.27 ±0.78	68.7±0.95
PBAd+GLI	84.51±1.63	8.55±0.24	72.3±1.09

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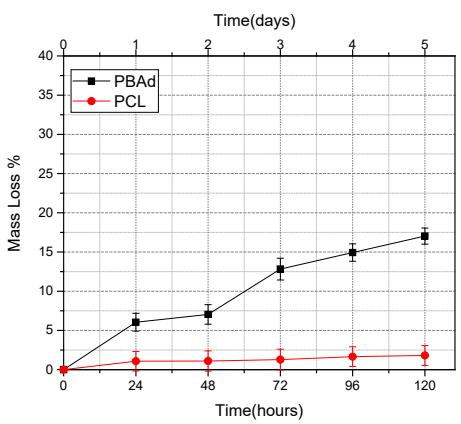

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Figure 6. In vitro hydrolysis studies of PCL and PBAd microparticles in PBS (37°C)

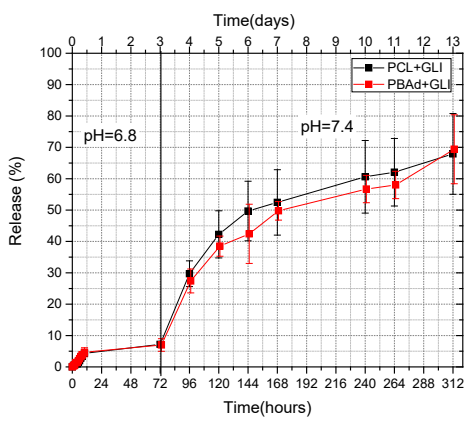

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Figure 7. In vitro release studies of GLI from PCL and PBAd MPs

of glibenclamide. The developed formulations were of micron size as SEM and DLS studies exhibited. SEM images depicted that drug loading slightly enlarged the size of the MPs. GLI loaded MPs demonstrated stability, considering that FT-IR spectroscopy did not reveal interactions between the carriers and the drug. DSC studies indicated that the drug was in the amorphous state due to the absence of GLI melting point for both PCL and PBAd carriers. Hydrolysis studies showed that PBAd MPs had a higher degradation rate than PCL MPs. Furthermore, MPs yield and drug entrapment were relatively high, whereas in vitro release was higher than that of pure GLI. To conclude, the developed MPs showed interesting characteristics and could be an alternative option for antidiabetic drug carriers.

Ethical Considerations

Compliance with ethical guidelines

There was no ethical considerations to be considered in this research.

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Authors' contributions

Conceptualization: Panoraia I. Siafaka, Ioannis D. Karantas, Neslihan Üstündağ Okur, Hatice Yeşim Karasulu; Methodology and investigationall, writing – original draft, writing – review & editing: All authors; Supervision: Neslihan Üstündağ Okur, Hatice Yeşim Karasulu.

Conflict of interest

The authors declared no conflict of interest.

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