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Determination of Lactose and Ethanol Diffusion Coefficients in Calcium Alginate Gel Spheres: Predicting Values To Be Used in Immobilized Bioreactors

Sabrina Gabardo, Rosane Rech, and Marco Antônio Záchia Ayub*

BiotecLab, Food Science and Technology Institute (ICTA), Federal University of Rio Grande do Sul State (UFRGS), Av. Bento Gonçalves, 9500, P.O. Box 15090, ZC 91501-970, Porto Alegre, RS, Brazil

ABSTRACT: The diffusion coefficients (D) for lactose and ethanol through calcium alginate gel beads were determined based on the mathematical approach of Fick's second law. Different experimental conditions were tested to evaluate the behavior of the diffusion coefficient of different concentrations of lactose and ethanol in Ca-alginate (3, 4, and 6) % at varying temperatures (298.15, 303.15, and 308.15) K. Results showed that diffusion coefficients were independent from the concentration of lactose and ethanol, as well as from the concentration of Ca-alginate. Diffusion coefficients were affected by temperature, increasing from $4.67 \cdot 10^{-10}$ $m^2 \cdot s^{-1}$ to $6.96 \cdot 10^{-10}$ $m^2 \cdot s^{-1}$ for lactose and from $1.46 \cdot 10^{-10}$ $m^2 \cdot s^{-1}$ to $2.68 \cdot 10^{-10}$ $m^2 \cdot s^{-1}$ for ethanol. These results can be used for the project and scaling up of immobilized bioreactors for the conversion of lactose into ethanol.

INTRODUCTION

Among the numerous techniques for microbial cell immobilization, entrapment in polysaccharide gels of natural origin such as Ca-alginate is one of the most largely used and studied,[1−3] reflecting the importance and impact of this technology for bioprocesses. Entrapment gel techniques present several interesting features including nontoxicity, readiness to form hydrogels under very mild conditions, low cost, and stability even at high temperatures.[4,5] Additionally, cell immobilization in alginate systems enables high cell density and biocatalytic activity, high conversion rates, and smaller reaction time compared to free-cell systems.[6,7] Its main limitation resides in the diffusional barrier of substrates and products through the gel matrix, and the spherical particles resulting from the process of entrapment will pose a mass transfer resistance through the matrix that can lead to ill-diffusion of substrates and/or the building-up of inner concentrations of toxic metabolic products such as ethanol.[7−13] Therefore, the understanding of the transport properties of gel matrixes is important to predict whether substrates can freely enter the sphere and products can be permeated to the medium.[14]

The influence of the diffusion coefficient, or diffusivity, on the rate of reactions of the biocatalyst will affect its efficiency and should be known to assist the optimization of the bioprocesses, and it is important to know how the properties of the immobilizing material affect the transport of various solutes.[4]

Immobilized bioreactors are gaining importance in industrial applications, especially in those where aeration is not required; substrates are soluble sugars such as lactose, and products are excreted as primary metabolites as ethanol. For these processes, the diffusion coefficient (D) is the fundamental parameter required for the quantitative analyses of mass transfer for a successful microbial cell immobilization.[15]

The literature has reported on the diffusion of small molecules such as glucose, lactose, and ethanol, when in solutions surrounding several gel supports.[2,5,9,11] In this work we propose to investigate the diffusion coefficient of such molecules, measuring them from within Ca-alginate beads, which closely resembles the mechanisms of mass transfer in immobilized bioreactors. We measured the diffusion coefficient (D) for lactose and for ethanol in Ca-alginate gel beads using a methodology based on the mathematical models of Fick's law and evaluated the effects of solute concentration, Ca-alginate concentration, and temperature on the diffusion coefficients. These variables were chosen because they are important when devising a process to convert lactose into ethanol in immobilized bioreactors.

MATERIALS AND METHODS

Preparation of Gel Beads. Alginate gel beads were formed by dripping sodium alginate solution (3 %, 4 %, or 6 % w/v) through a needle of 2.1 mm diameter (14 G) using a peristaltic pump into a flask containing 0.1 M $CaCl_2$ solution at 308.15 K. The beads were gently agitated for 30 min and washed twice with distilled water at 277.15 K. The diameters of 100 randomly selected beads were measured using a caliper rule. The mean volume of the beads was calculated as the mean of the arithmetic diameter considering a perfect sphere.

Diffusivity Tests. The solutions used in these sorption kinetics experiments were prepared using bidistilled water and calibrated volumetric flasks and balances. The accuracy of their composition was within ± 0.1 %. Diffusivity experiments were carried out in two steps. In the first, different solutions of lactose [(0.07, 0.15, or 0.22) $mol \cdot kg^{-1}$] and ethanol [(0.54 or 1.08) $mol \cdot kg^{-1}$] were used to evaluate the impact of the solution concentration on the diffusion coefficient (D) of these molecules within 4 % Ca-alginate beads at 303.15 K. In the second set of experiments, the influence of the temperature (298.15, 303.15, or 308.15) K and alginate concentration (3 %, 4 % or 6 %) were

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evaluated on the diffusion coefficient, while fixing lactose and ethanol concentrations at (0.15 and 0.54) mol·kg^{-1}, respectively. For each test, five beads with a diameter of 3.8 mm were placed into lactose (250 mL) or ethanol (10 mL) solutions at the tested temperatures and were kept constant using an accurate thermostat (± 0.1 K) in a water bath. The spheres were maintained at constant and gentle agitation to avoid external mass transfer resistance. Sphere samples were taken along the duration of the experiments (1200 s) to calculate the diffusion coefficient, and the spheres were dissolved into 1 mL of sodium citrate buffer 0.1 M (pH 6.2) into microcentrifuge tubes and vortex agitated by 5 min to accelerate their breakup. All experiments were run in duplicate.

Diffusion Coefficient Calculations. In this research Fick's second law was used for the calculation of diffusivity assuming that the Ca-alginate beads are perfectly spherical and homogeneous with radial diffusion of solute in the particle.

Fick's second law for the case of diffusion through a particle is given by:

$$\frac{\partial C}{\partial t} = D\,\frac{\partial^2 C}{\partial r^2} \tag{1}$$

where C is the solute concentration, D is the diffusion coefficient, t is the time, and r is the radial distance from the center of the bead.

For a spherical geometry (gel particle of radius R), eq 1 leads to:[16]

$$\frac{\partial C}{\partial t} = \frac{1}{r^2}\cdot\frac{\partial}{\partial r}\left[D\cdot r\cdot\frac{\partial C}{\partial r}\right] \tag{2}$$

If D is assumed to be constant and also assuming that the mass transfer resistance through the film surrounding the beads is negligible, the transient concentration change of the solute in the gel sphere can be expressed as:[17]

$$\frac{C_t - C_\infty}{C_0 - C_\infty} = \frac{6}{\pi^2}\sum_{n=1}^{\infty}\left[\frac{1}{n^2}\exp\left(-\frac{n^2\cdot\pi^2\cdot D\cdot t}{R^2}\right)\right] \tag{3}$$

where C_t is the solute concentration in the gel bead as function of time, C_∞ is the maximal solute concentration in bulk solution, C_0 is the initial solute concentration, and R is the radius of the gel bead.

For sufficiently large values of time t, eq 3 can be approximated by the first term of the series:

$$\frac{C_t - C_\infty}{C_0 - C_\infty} = \frac{6}{\pi^2}\exp\left(-D\left(\frac{\pi}{R}\right)^2 t\right) \tag{4}$$

The system is defined by the following initial and boundary conditions:

$$C = 0; \quad t = 0, \quad 0 \le r < R$$

$$C = C_\infty; \quad t \ge 0, \quad r = R$$

$$\frac{\partial C}{\partial r} = 0; \quad t \ge 0, \quad r = 0$$

The diffusion coefficient D is obtained by plotting the natural logarithm of the first term of eq 4 against t, with $D(\pi/R)^2$ as the slope.

Table 1. Diffusion Coefficients for Lactose and Ethanol as a Function of Solution Concentration in 4 % Ca-Alginate Beads at 303 K

solution concentration lactose/ethanol	D_{lact}	D_{EtOH}
mol·kg^{-1}	10^{-10} m^2·s^{-1}	10^{-10} m^2·s^{-1}
0.07/0.54	6.32 ± 1.33	1.46 ± 0.26
0.15/1.08	4.84 ± 0.46	1.28 ± 0.39
0.22	4.62 ± 0.14	*a*

[a] Not tested.

The statistics of results were analyzed with analysis of variance (ANOVA) using the software *Statistica* 7.0 (StatSoft, USA).

Analytical Determinations. Lactose concentration was determined by the dinitrosalicyclic acid (DNS) method for reducing sugars described by Chapplin and Kennedy.[18] Ethanol concentration was determined by gas chromatography using a gas chromatograph (CG-14B Shimadzu, Tokyo, Japan) with a flame ionization (FID) detector using a capillary column DB-1 with a 30 m length and a 0.25 mm internal diameter. The column temperature was constant at 313.15 K, and the temperatures of both injector and detector were 523.15 K. To quantify the amount of ethanol, a calibration curve was constructed varying ethanol concentration and using a fixed amount of *n*-propanol as the internal standard.

RESULTS AND DISCUSSION

Effects of the Solution Concentration on the Diffusion of Lactose and Ethanol. There are few studies evaluating the effect of solution concentrations in diffusion coefficients of molecules into gel beads such as spheres of calcium alginate. In this study, diffusion coefficients for lactose (D_{lact}) and ethanol (D_{EtOH}) were calculated for different solution concentrations, and the results are presented in Table 1.

Despite the fact that values of the diffusion coefficients show small increments with lactose and ethanol concentrations, the statistical analysis shows that diffusion coefficients of lactose and ethanol are not affected by solution concentration in 4 % Ca-alginate beads at 303.15 K (Table 1). These results are in agreement with studies performed with different molecules and supports where no influences of the solute concentration on the diffusion coefficient were found: glucose and maltose in polyacrilamide gels;[2] glucose and ethanol in calcium alginate gels;[19] and lactose in κ-carragen gels.[9]

The profiles of the lactose and ethanol concentration in the gel beads are presented in Figures 1 and 2, respectively. Due to the great concentration gradient, mass transfer was faster at the beginning of the process and then reached equilibrium after 1000 s [(0.06, 0.14, and 0.19) mol·kg^{-1} for lactose solutions of (0.07, 0.15, and 0.22) mol·kg^{-1}; (0.34 and 0.60) mol·kg^{-1} for (0.54 and 1.08) mol·kg^{-1} of ethanol solutions]. Dembczynski and Jankowski[14] studied diffusion coefficients for different sugars, among which is lactose, in Ca-alginate membrane liquid-core capsules at temperature of 303.15 K, using the model described by Crank[20] and obtained values between (4.51 and 4.64·10^{-10}) m^2·s^{-1}. Chai et al.,[5] using a different mathematical approach than the one used in this research, reported a diffusion coefficient of 5.5·10^{-10} m^2·s^{-1} for lactose in 1.2 % intrahollow calcium alginate microcapsules at a temperature of 298.15 K. For ethanol, diffusion coefficients amounted to 1.46·10^{-10} m^2·s^{-1} (Figure 2).

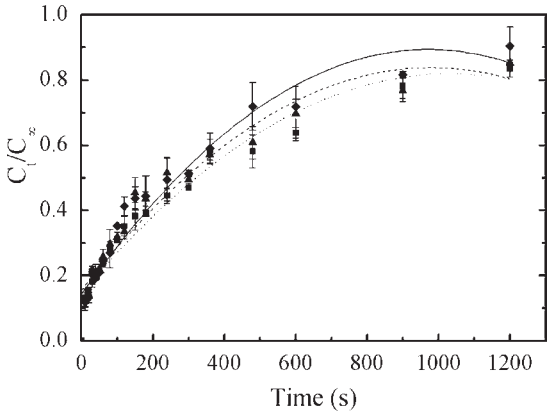

Figure 1. Lactose diffusion in calcium alginate beads as a function of time. Conditions were Ca-alginate 4 % at 303.15 K; lactose concentration solutions of 0.07 (—♦—); 0.15 (· · · ·■· · · ·); and 0.22 (---▲---) mol·kg^{-1}. Bars represent the experimental uncertainty, while lines represent the polynomial fitting.

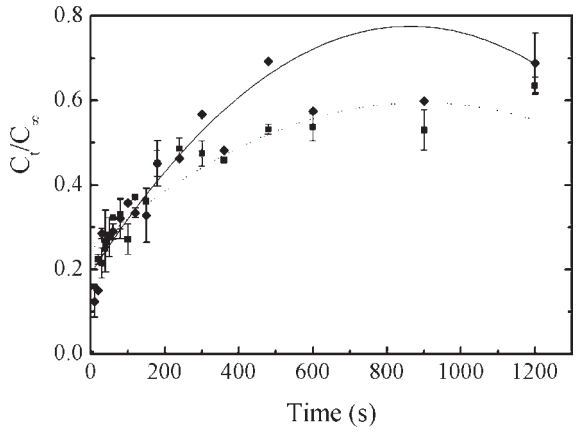

Figure 2. Ethanol diffusion in calcium alginate beads as a function of time. Conditions were Ca-alginate 4 % at 303.15 K; ethanol concentration solutions of 0.54 (—♦—) and 1.08 mol·kg^{-1} (· · · ·■· · · ·). Bars represent the experimental uncertainty, while lines represent the polynomial fitting.

This value is lower than those reported by other authors.[11,19,21,22] This can be explained by the different methodology used in our work, where the ethanol concentration was measured inside the Ca-alginate beads, while in the cited studies the ethanol concentration was measured in the solution surrounding the beads. During preliminary experiments it was noticed that there is a quick and substantial loss of ethanol from the solution by evaporation. Therefore, in our research, tests to determine ethanol diffusion coefficient were made in closed systems to prevent this problem. This fact might account for the differences of results from other studies, in which the portion of the ethanol evaporated in the diffusivity were included in the calculations. In our work, the values of the diffusion coefficient for ethanol represent the real amount of ethanol through the sphere and might be considered as a more realistic figure.

Effects of the Alginate Concentration and Variation of Temperature on the Diffusivity of Lactose and Ethanol. To investigate the effects of gel concentration and temperature on the diffusion coefficients of lactose and ethanol, experiments were carried out using Ca-alginate beads with 3 %, 4 % or 6 % alginate concentration and temperatures of (298.15, 303.15, or 308.15) K. The solutions of lactose and ethanol were constant, at 0.15 mol·kg^{-1} and 0.54 mol·kg^{-1}, respectively, and the results can be seen in Table 2. For lactose, the ANOVA shows that diffusion coefficient values were significantly affected by temperature, while alginate concentration had no influence in D_{lact}: a small variation of D_{lact} ($6.32 \cdot 10^{-10}$ m^2·s^{-1} to $6.96 \cdot 10^{-10}$ m^2·s^{-1}) was observed when increasing the concentration of Ca-alginate beads from 3 % to 6 % at 308.15 K, but a significant variation ($4.67 \cdot 10^{-10}$ m^2·s^{-1} to $6.96 \cdot 10^{-10}$ m^2·s^{-1}) was verified with increasing temperatures from (298.15 to 308.15) K in gel beads of 6 %, for instance. The same behavior was observed for other temperatures and gel concentration (Table 2). The values of D_{lact} obtained for the gel beads are smaller than values for the diffusion coefficients of lactose measured in aqueous solutions reported by Ribeiro et al.,[23] as expected, showing the physical barrier represented by the gel structure. Meanwhile, for ethanol, the ANOVA showed that neither alginate concentration nor temperature had any significant effects on D_{EtOH}. These results are contrasting with findings from other authors, who generally reported reducing diffusivities for increasing concentrations of Ca-alginate. For instance, Hacimusalar and Mehmetoglu[22] investigated the ethanol diffusion at 298.15 K in different concentrations of Ca-alginate [(2, 3, and 4) %] and found that diffusivity decreased inversely with gel concentration (8.6 to $10.8 \cdot 10^{-10}$ m^2·s^{-1}); likewise, Hannoun and Stephanopolous[19] reported that the diffusion coefficient of ethanol in (1, 2, and 4) % Ca-alginate decreased in the same way. Their calculations were based on lag-time analysis. Although statistically not significant according to ANOVA, there can be seen a small positive influence of temperature on diffusion coefficients, when temperature goes from (298.15 to 308.15) K (Figure 4). This fact could suggest that a higher range in temperature might indicate significant variations on diffusivity, which is, however, of less relevance since microbial cultivations are always run in the range of the tested temperature, especially for yeasts in the case of ethanol production [(298.15 to 303.15) K].

Table 2. Calculated Diffusion Coefficients for Lactose (D_{lact}/(10^{-10} m^2·s^{-1})) and Ethanol (D_{EtOH}/(10^{-10} m^2·s^{-1})) in Ca-Alginate Beads as a Function of Alginate Concentration and Temperature

molecule	Ca-alginate (%)	T (K)		
		298.15	303.15	308.15
lactose	3	5.16 ± 0.07	5.52 ± 0.73	6.32 ± 0.85
ethanol		1.99 ± 0.09	2.13 ± 0.63	2.68 ± 0.32
lactose	4	4.73 ± 0.23	4.84 ± 0.46	6.10 ± 0.81
ethanol		1.84 ± 0.12	1.46 ± 0.26	2.67 ± 0.68
lactose	6	4.67 ± 0.07	5.19 ± 0.61	6.96 ± 0.13
ethanol		1.53 ± 0.09	2.25 ± 0.13	2.44 ± 0.11

The kinetics of lactose diffusion as a function of calcium alginate concentration and temperature are presented in Figure 3. Profiles of lactose diffusion at different Ca-alginate concentrations were similar, showing that this variable did not affect the diffusion of this sugar within the gel beads. However, the influence of temperature over lactose diffusion clearly shows a direct effect on mass transfer, increasing accordingly the diffusivity. Chai et al.[5] studied the diffusivity of several substances,

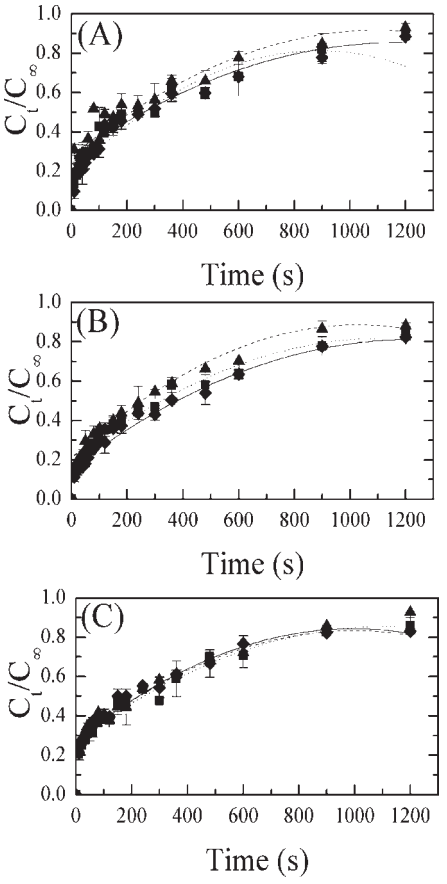

Figure 3. Lactose diffusion in calcium alginate beads of (A) 3 %, (B) 4 %, and (C) 6 % at different temperatures: 298.15 K (—♦—), 303.15 K (· · · ·■· · · ·), 308.15 K (---▲---). Bars represent the experimental uncertainty, while lines represent the polynomial fitting.

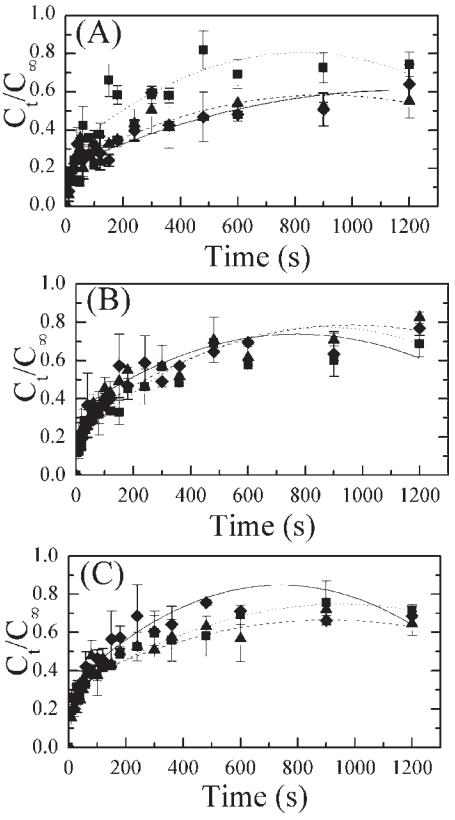

Figure 4. Ethanol diffusion in calcium alginate beads of (A) 3 %, (B) 4 %, and (C) 6 % at different temperatures: 298.15 K (—♦—), 303.15 K (· · · ·■· · · ·), 308.15 K (---▲---). Bars represent the experimental uncertainty, while lines represent the polynomial fitting.

including lactose, in Ca-alginate microcapsules of (0.8, 1.0, and 1.2) % and concluded that the concentration of the gel beads did not affect the diffusivity, with very similar diffusion coefficients for any tested gel concentration. Dembczynski and Jankowski[14] investigated diffusion coefficients of glucose, fructose, sucrose, and lactose in a Ca-alginate membrane liquid-core capsules and verified that diffusivities were not influenced by the variation in alginate concentration. In contrast, Oyaas et al.[24] observed a decrease in lactose diffusivity when increasing alginate concentrations from (1 to 4) %. Hacimusalar and Mehmetoglu[22] reported a similar behavior for glucose diffusion coefficients for different alginate concentrations [(2 and 3) %] at 298.15 K. These authors also investigated the influence of temperature [(293.15 to 308.15) K] on glucose diffusion in 3 % Ca-alginate beads showing increased diffusion coefficients, with values changing from (4.52 to $7.38 \cdot 10^{-10}$) $m^2 \cdot s^{-1}$, respectively. According to Amsden and Turner,[4] the sequence of the monomers in the molecule (mannuromic and guluronic acid) and the composition of the alginate gel (low or high content of these different monomers) determine the properties of the gel formed, consequently affecting the diffusion of solutes. This author suggested that gels with higher guluronic acid would impose greater diffusivity barrier than those of higher contents of mannuromic acid.

The kinetics of ethanol diffusivity in calcium alginate beads at different gel concentrations and temperatures is presented in Figure 4. It appears that the diffusion of ethanol is not affected by either gel concentration or temperature.

Diffusion mechanisms depend on energy of activation (E), which are molecular specific, to allow diffusion, and this can be achieved with increasing temperature. The effect of temperatures on diffusion coefficients of small molecules at constant pressure can be represented by an Arrhenius-type equation:[24,25]

$$D = D_0 \exp\left(-\frac{E}{RT}\right) \quad (5)$$

where D_0 is the pre-exponential factor, E is the activation energy of diffusion, R is the gas constant, and T is the Kelvin temperature.

The values of the activation energy calculated for lactose and ethanol were 21.8 $kJ \cdot mol^{-1}$ and 28.5 $kJ \cdot mol^{-1}$, respectively. Oyaas et al.[24] found that the energy of activation for lactose in 2 % Ca-alginate beads was approximately 20.0 $kJ \cdot mol^{-1}$. Hacimusalar and Mehmetoglu[22] investigated the diffusion coefficient of glucose in Ca-alginate gel beads, varying temperatures of (293.15 to 308.15) K, and observed that an increase in temperature led to increments of the diffusion rate and calculated the activation energy for glucose as 24 $kJ \cdot mol^{-1}$. Yankov[2] observed the behavior of diffusion coefficient for glucose and maltose in polyacrylamide gels at different temperatures [(293.15 to 333.15) K] and found activation energies of 14.7 $kJ \cdot mol^{-1}$ and 13.8 $kJ \cdot mol^{-1}$ for glucose and maltose, respectively.

CONCLUSIONS

Mass transfer mechanisms are the core issue in the design of bioprocesses using immobilized cell bioreactors. With the increasing interest in the conversion of agro-industrial residues, such as whey, into ethanol, the comprehension of diffusivity characteristics of lactose and ethanol in Ca-alginate matrixes could help to develop efficient bioreactor systems. In this work the diffusion coefficients (D) of small molecules, such as lactose and ethanol, from within Ca-alginate gel beads based on the Fick's second law were evaluated. Important variables for bioprocesses such as temperature and gel and solution concentrations were tested, with only the first being of importance for diffusivity.

AUTHOR INFORMATION

Corresponding Author

*E-mail address: mazayub@ufrgs.br (M.A.Z. Ayub). Tel.: +55 51 3308 6685; fax: +55 51 3308 7048.

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