Desalination and Water Treatment
www.deswater.com

27 (2011) 114–119
March

1944-3994/1944-3986
doi: 10/5004/dwt.2011.2068

Facilitated transport of valine through bulk liquid membranes containing Aliquat 336: A kinetic study

Gerardo León[a,*], María Amelia Guzmán[b]

[a]*Departamento de Ingeniería Química y Ambiental. Universidad Politécnica de Cartagena. 30203 Cartagena. Spain*
Tel. +34 968 325669; email: gerardo.leon@upct.es
[b]*Dirección Territorial de Comercio y Delegación del ICEX., Paseo Alfonso X el Sabio s/n. 30008, Murcia, Spain*

Received 31 May 2010; Accepted 8 December 2010

ABSTRACT

This paper describes a kinetic study, in optimal conditions, of the facilitated counter-transport of valine through bulk liquid membranes using tricapryl methyl ammonium chloride (Aliquat 336) as carrier and chloride as counter-ion. Recovery close to 80% has been obtained after 24 h. The transport kinetic was analysed by means of a model involving two consecutive irreversible first order reactions. The rate constants of the extraction and stripping reactions were determined by numerical analysis. Good agreement between the model and experimental data was observed. A maximum flux of valine transport through the bulk liquid membrane of 0.052/h was obtained.

Keywords: Valine; Facilitated transport; Bulk liquid membranes; Aliquat 336; Kinetics

1. Introduction

α-Amino acids are the main structural components of proteins and enzymes, both of which are decisive products for human activity. At present, the commercial importance of α-amino acids is based on the wide number of applications they have in the food, pharmaceutical and chemical fields [1].

The production of α-amino acids is mainly based on chemical and biochemical synthesis, the latter usually involving microbial fermentation or enzymatic synthesis. They are obtained at low concentrations in dilute aqueous solutions and their separation from the fermentation broths or protein hydrolyzates is rather difficult. For this, a succession of filtration, crystallisation, with numerous concentration steps, and, in some cases, additional complementary ion exchange steps and absorption treatments, are required [2]. More effective separation and concentration processes would bring down the cost of the overall production process.

To this end, membrane technology may supply interesting solutions, because it has been used with successful results in separation processes of a diverse nature. For example, the use of pressure driven membrane processes [3–9] and liquid membranes processes [10–16] has been described as being useful for the separation and purification of amino acids. The main interest of liquid membranes is related with the possibility of combining both the extraction and the striping operations into only one step [17]. In this paper, this technique is used to recover valine from aqueous solutions.

Valine (2-amino-3-methyl-butanoic acid) is one of the twenty common amino acids that make up human and animal proteins, and is considered an essential amino acid because the human body is unable to manufacture it naturally. At an industrial level, it is mainly produced by fermentation processes [18–20]. As one of the branched-chain amino acids (leucine, isoleucine and valine), it is considered critical to glucose metabolism, protein synthesis, and regulation of the immune system. Valine has also been described as being involved in muscle metabolism and growth, tissue repair, maintenance of

*Corresponding author.

Presented at the VII Ibero-American Conference on Membrane Science and Technology (CITEM 2010), April 11–14, 2010, Sintra, Portugal

nitrogen balance in the body and exercise recovery time [21–23]. This crucial influence on the proper functioning of muscle tissues has led to growing interest in this amino acid with endurance athletes, body builders, and others simply looking to stay healthy.

Like all α-amino acids with an uncharged residue, valine exists in three forms in solution depending on the pH. Although it may have no net charge, this amino acid always contains at least one charged moiety.

$$\underset{pH<3}{Val^{+}} \quad \Leftrightarrow \quad \underset{3<pH>9}{Val^{+/-}} \quad \Leftrightarrow \quad \underset{pH>9}{Val^{-}}$$

This ever-present charge greatly diminishes the solubility of the amino acid in non-polar solvents and its separation using liquid membranes needs the presence in the organic phase of an agent to carry the amino acid from the feed aqueous solution to the product aqueous solution across the non-polar membrane.

Several carriers have been described for the separation of valine by liquid membranes, including crown ethers [12], D2EHPA [24], Aliquat 336 [13,25] and calix[6] arene [16]. In this study, we use Aliquat 336 as carrier and chloride ions as counter ions. The three long alkyl chains of Aliquat 336 prevent its solubility in the aqueous feed and product phases. The carrier is charged and it complexes with the counter ion to maintain electroneutrality in the membrane phase. In order to transport valine in the anion form and to use an ion chloride gradient as the only driving force of valine transport, both aqueous phases (feed and product) are maintained above pH 9.

Valine transport is illustrated in Fig. 1. The chloride-carrier complex ($C^{+}Cl^{-}$) diffuses from the bulk membrane phase to the feed/membrane interface, where chloride ion is exchanged for valine. The valine-carrier complex ($C^{+}Val^{-}$) thus formed diffuses through the membrane to the membrane/product interface, where valine is exchanged for chloride and liberated in the product phase. The chloride-carrier complex formed begins a new separation cycle. The mechanism of valine ion transport is therefore a coupled counterion transport, with valine and Cl^{-} travelling in the opposite direction.

The object of this paper is to obtain the optimal Aliquat 336 and ion chloride concentrations for maximum valine counter-transport through the described bulk liquid membrane and to study the kinetics of the valine transport process at those optimal concentrations.

2. Experimental equipment and procedure

The experimental studies were carried out applying the bulk liquid membrane technique, using a stirred transfer Lewis type cell with bulk liquid membrane layered above the feed and product phases (Fig. 2). Twenty five millilitres of a 0.1 M solution of D,L-valine in NaOH (pH 11) was used as feed phase. The membrane phase (25 ml) was made up of solutions of different concentrations of Aliquat 336 in kerosene. Sodium chloride solutions of different concentrations in NaOH (pH 11) were used as product phase (25 ml). The pH was measured using a Crison micro pH 2000 pH-meter (Crison S.A., Barcelona, Spain). The respective areas of both feed/membrane and membrane/product interfaces were 3.2 cm^2. The stirring speed was 200 rpm and all experiments were carried out at 25°C.

To study the influence of carrier concentration on valine removal, solutions of Aliquat 336, 0.25% (0.006186 M), 0.5% (0.012372 M), 1% (0.024744 M), 2% (0.049488 M) and 4% (0.098976 M) in kerosene were used as membrane phase and 2M sodium chloride solution in NaOH (pH = 11) was used as product phase. To study the influence of counter-ion concentration on the removal of valine, solutions of sodium chloride 0.25 M, 0.5 M, 1 M, 2 M and 4 M in NaOH (pH = 11) were used as product phase

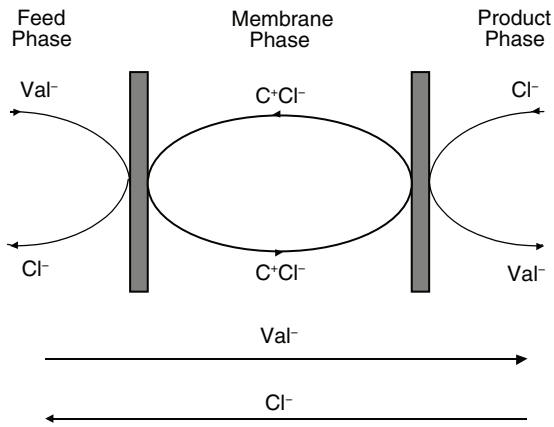

Fig. 1. Diagram of the facilitated transport of valine using Aliquat 336 as carrier and chloride as counterion.

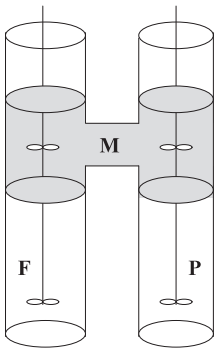

Fig. 2. Schematic representation of the experimental system (F, feed phase, M, membrane phase, P, product phase).

and 1% (0.02474 M) Aliquat 336 solution in kerosene was used as membrane phase. Each experiment lasted 24 h.

Valine concentrations in both feed and product phases were measured by UV spectrophotometry, using an UNICAN UV2 equipment, following the OPA method [26] and measuring the absorbance of the reaction product between valine and *o*-ftaldialdehyde, in the presence of mercaptoethanol, at 340 nm. The valine concentration in membrane phase was established from the material balance.

3. Results and discussion

For practical reasons, reduced concentrations of valine in the feed (R_f), membrane (R_m) and product (R_p) phases were used, being $R_f + R_m + R_p = 1$ ($R_f = C_{ft}/C_{f0}$, $R_m = C_{mt}/C_{f0}$ and $R_p = C_{pt}/C_{f0}$, where C_{f0} is the valine initial concentration in the feed phase and C_{ft}, C_{mt} and C_{pt} are the valine concentrations in the feed, membrane and product phases at time t, respectively).

The removal of valine from aqueous solutions at different carrier concentrations in the membrane phase and different counter-ion concentrations in the product phase is shown in Fig. 3. An increase in carrier concentration in the membrane phase leads to an increase in valine removal from the feed phase, particularly for carrier concentrations of under 1%, although the increase is much less pronounced for carrier concentrations over 1%. Similarly, an increase in counterion concentration in the product phase leads to an increase in valine removal from the feed phase, especially for a counterion concentration of under 2 M, but the increase is not so pronounced for a counterion concentration of over 2 M.

In order to study the kinetics of the process, variations of reduced concentrations in feed, membrane and product phases with time were determined using a 1% v/v (0.0247 M) Aliquat 336 concentration in the membrane phase and 2 M chloride ion concentration in the product phase. In these conditions, valine recovery in product phase close to 80% was obtained after 24 h. The results showed that R_f decreased monoexponentially with time, R_p followed a monotonically increasing sigmoided type curve, and R_m time dependence presented a maximum at six hours of extraction (Fig. 4).

These results suggest that valine transport obeys the kinetic laws of two consecutive irreversible first-order reactions [27,28], the extraction (rate constant, k_1) and the stripping (rate constant, k_2) reactions, $J_{f/m}$ and $J_{m/p}$ being fluxes through the feed/membrane and membrane/product interfaces, respectively. This kinetic approach has been used to describe the facilitated transport of different chemical species through bulk liquid membranes [29–32] and can be described according to the following equations [27,33–35]:

$$\frac{dR_f}{dt} = -k_1 R_f \equiv J_{f/m} \quad (1)$$

$$\frac{dR_m}{dt} = k_1 R_f - k_2 R_m \quad (2)$$

$$\frac{dR_p}{dt} = k_2 R_m \equiv J_{m/p} \quad (3)$$

Integration of these differential equations gives

$$R_f = \exp(-k_1 t) \quad (4)$$

$$R_m = \frac{k_1}{k_2 - k_1}\left[\exp(-k_1 t) - \exp(-k_2 t)\right] \quad (5)$$

$$R_p = 1 - \frac{1}{k_2 - k_1}\left[k_2 \exp(-k_1 t) - k_1 \exp(-k_2 t)\right] \quad (6)$$

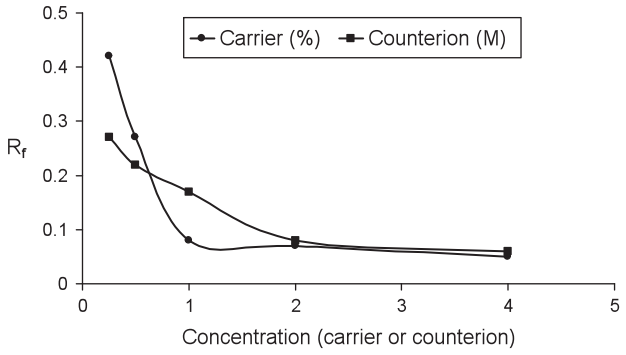

Fig. 3. Valine removal from feed phase at different carrier and counterion concentrations.

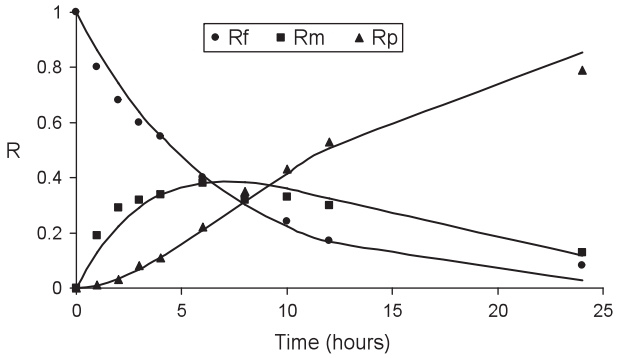

Fig. 4. Time dependence of R_f in feed phase, R_m in membrane phase and R_p in product phase (Aliquat 336 concentration in membrane phase 1% v/v. NaCl concentration in product phase 2 M; Points, experimental values; Line, model values).

These equations show that the time dependence of R_f is monoexponential and the time dependence of both R_m and R_p is biexponential.

R_m reaches a maximum, the time at which this occurs being obtained from $dR_m/dt = 0$.

$$t_{max} = \frac{\ln\left(\frac{k_1}{k_2}\right)}{k_1 - k_2} \quad (7)$$

the value of R_m at that time being

$$R_{m\ max} = \left(\frac{k_1}{k_2}\right)^{-\frac{k_2}{k_1 - k_2}} \quad (8)$$

Combining Eqs. (7) and (8) gives the following relationship.

$$k_2 = \frac{ln\left(\frac{1}{R_{m\ max}}\right)}{t_{max}} \quad (9)$$

First order time differentiation of Eqs. (4) to (6) leads to the final form of the flux equations [27].

$$\frac{dR_f}{dt} = -k_1 \exp(-k_1 t) \quad (10)$$

$$\frac{dR_f}{dt} = \frac{k_1}{k_2 - k_1} - \left[\exp(-k_1 t) - \exp(-k_2 t)\right] \quad (11)$$

$$\frac{dR_p}{dt} = \frac{k_1 k_2}{k_2 - k_1}\left[\exp(-k_1 t) - \exp(-k_2 t)\right] \quad (12)$$

By substituting the expression of t_{max} given for Eq. (7) in Eqs. (10) to (12), maximum fluxes can be obtained [27]

$$\left[\frac{dR_f}{dt}\right]_{max} = -k_1\left(\frac{k_1}{k_2}\right)^{-\frac{k_1}{k_1 - k_2}} = J_{f/m}^{max} \quad (13)$$

$$\left[\frac{dR_m}{dt}\right]_{max} = 0 \quad (14)$$

$$\left[\frac{dR_p}{dt}\right]_{max} = k_2\left(\frac{k_1}{k_2}\right)^{-\frac{k_2}{k_1 - k_2}} = J_{m/p}^{max} \quad (15)$$

$$-\left[\frac{dR_f}{dt}\right]_{max} = +\left[\frac{dR_p}{dt}\right]_{max} \Rightarrow -J_{f/m}^{max} = +J_{m/p}^{max} \quad (16)$$

Table 1
Rate constants for extraction (k_1) and stripping (k_2) processes and maximum flux (J_{max}) for the counter transport of valine using Aliquat 336

k_1/h	k_{2m}/h	k_{2p}/h	$k_{2(average)}$/h	J_{max}/h
0.149	0.140	0.129	0.135	0.052

Numerical analysis, by non-linear curve fitting, of the experimental results shown in Fig. 4 permits the rate constants of the kinetic process to be determined (Table 1). The values of k_1 are directly obtained by iteration from Eq. (4). This value is introduced as a constant value in Eqs. (5) and (6). An initial value of k_2 is obtained from Eq. (9), then introduced in Eqs. (5) and (6) and iterated. Two values of the rate constant k_2 are obtained, one derived from Eq. (5), k_{2m}, and the other derived from Eq. (6), k_{2p}. Good agreement between k_{2m} and k_{2p} constants was observed. Maximum flux (J_{max}), calculated from Eqs. (13) or (15), is also included in Table 1.

Model curves of time dependence of R_f, R_m and R_p, calculated from Eqs. (4) to (6) are also shown in Fig. 4. Good agreement between the experimental and model data can be observed.

4. Conclusions

This paper has described a kinetic study of the recovery of valine from dilute aqueous solutions by bulk liquid membrane, using a facilitated counter-transport mechanism. Aliquat 336 was used as carrier and chloride ions as counter ions. The results show that using a 1% v/v Aliquat 336 concentration in the membrane phase and 2 M chloride ion concentration in the product phase, most of the valine can be recovered from its aqueous solution by this technique, with an efficiency of close to 80%. A kinetic study of the process was also carried out. The transport kinetics was analysed by means of a model involving two consecutive irreversible first order reactions the extraction and the stripping reactions. The rate constants of these reactions were determined by numerical analysis. Good agreement between the model and the experimental data can be observed. A maximum flux of valine transport through the bulk liquid membrane of 0.052/h was obtained at these optimal conditions.

Acknowledgements

We would like to thank Mr. A. Guzmán and Mr. J.A. López for the manufacture of the experimental cell.

Symbols

C	—	concentration (M)
R	—	reduced concentration (C_t/C_0)
k_1	—	rate constant of extraction reaction (per h)
k_2	—	rate constant of stripping reaction (per h)
$J_{f/m}$	—	flux through feed/membrane interface (per h)
$J_{m/p}$	—	flux through membrane/product interface (per h)
J_{max}	—	maximum flux (per h)
R_m^{max}	—	maximum value of Rm
t_{max}	—	time of R_m^{max} (h)

Subscripts

f	—	feed
m	—	membrane
p	—	product
0	—	initial
t	—	time t

References

[1] I. Escudero, M.O. Ruiz and J.M. Benito, Recovery of α-phenylglicine by micellar ultrafiltartion using organic membranes in a stirred cell, Desalination, 200 (2006) 327–329.0

[2] K. Aida, I. Chibata, K. Nakayama, K. Takinami and H. Yamada, Biotechnology of Amino Acids Production, Elsevier, Amsterdam, (1986).

[3] T. Tsuru, T. Shutou, S.I. Nakao and S. Kimura, Peptide and amino acid separation by nanofiltration membranes, Sep. Sci. Technol., 29 (1994) 971–984

[4] A. Garem, G. Daufin, J. Maubois and J. Léonil, Selective separation of amino acids with charged inorganic nanofiltration membrane: effect of physicochemical parameters on selectivity, Biotechnol. Bioeng., 54 (1997) 291–302.

[5] C. Martin-Orue, S. Bouhallab and A. Garem, Nanofiltration of amino acid and peptide solutions: mechanisms of separation, J. Membrane. Sci., 142 (1998) 225–233.

[6] J.M.K. Timmer, M.P.J. Speelmans and H.C. Van der Horst, Separatioon of amino acids by nanofiltration and ultrafiltration membranes, Sep. Purif. Technol., 14 (1998) 133–144.

[7] Y. Shim and S. Chellam, Steric and electrostaic interactions govern nanofiltration of amino acids, Biotechnol. Bioeng., 98 (2007) 451–461.

[8] Z. Kovacs and W. Samhaber, Nanofiltration of concentrated amino acid solutions, Desalination, 240 (2009) 78–88.

[9] P. Bourseau, L. Vandanjon, P.Jaouen, M. Chaplain-Derouiniot, A. Massé, F. Guérard, A. Chabeaud, M. Fouchereau-Péron, Y. Le Gal, R. Ravallec-Plé, J.P. Bergé, L. Picot, J.M. piot, I. Batista, G. Thorkelsson, C. Delannoy, G. Kakobsen and I. Johansson, Fractionation of fish protein hydrolysates by ultrafiltration and nanofiltration: impact on peptidic populations, Desalination, 244 (2009) 303–320.

[10] H. Itoh, M.P. Thien, T.A. Hatton and D.I.C. Wang, A liquid emulsion membrane process for the separation of amino acids, Biotechnol. Bioeng., 35 (1990) 853–860.

[11] R. Molinari, L. De Bartolo and E. Drioli, Coupled transport of aminoacids through a supported liquid membrane. I. Experimental optimization, J. Membrane. Sci., 73 (1992) 203–215.

[12] L. Mutihac, R. Mutihac and H.J. Buschmann, Liquid membrane transport of supramolecular complexes of some amines and amino acids with macricyclic ligands, J. Inclus. Phenom. Mol., 23 (1995) 167–174.

[13] M. Matsumoto, T. Ohtake, M. Hirata and T. Hano, Extraction rates of amino acids by an emulsion liquid membrane with try-n-octylmethylammonium chloride, J. Chem. Technol. Biotechnol., 73 (1998) 237–242.

[14] P. Dzygiel, P. Wieczorek, L. Mathiasson and J.A. Jönsson, Enrichment of amino acids by supported liquid membrane extraction using Aliquat 336 as carrier, Anal. Letter, 31 (1998) 1261–1274.

[15] R.S. Juang and Y.Y. Wang, Amino acid separation with D2EHPA by solvent extraction and liquid surfactant membranes, J. Membrane. Sci., 207 (2002) 241–252.

[16] P. Raizada, V. Vyas and U. Sharma, Liquid membrane extraction and transport of amino acids using calix[6]arene, Indian J. Chem. Technol., 17 (2010) 267–273.

[17] A.M. Sastre, A. Kumar, J.P. Shukla and R.K. Singh, Improved techniques in liquid membrane separations: An overview, Sep. Purif. Meth., 27 (1998) 213–298.

[18] T. Herman, Industrial production af amino acids by coryneform bacteria. J. Biotechnol., 104 (2003) 155–172.

[19] K. Todorov, T. Georgiev and A. Ratkov, Mathematical identification of l-valine fed-batch fermentation process, J. Biotechnol., 136 (2008) S481–S482.

[20] J. Holátko, V. Elišáková, M. Prouza, M. Sobotka, J. Nešvera and M. Pátek, Metabolic engineering of the L-valine biosynthesis pathway in *Corynebacterium glutamicum* using promoter activity modulation, J. Biotechnol., 139 (2009) 203–210.

[21] R.A. Bassit, L.A. Sawada, R.F.P. Bacurau, F. Navarro, E. Martins, R.V.T. Santos, E.C. Caperuto, P. Rogeri and L.F.B.P. Costa, Branched-chain amino acid supplementation and the immune response of long-distance athletes, Nutrition 18 (2002) 376–379.

[22] N.A. Ratamases, W.J. Kraemer, J.S. Volek, M.R. Rubin, A.L. Gómez, D.N. French, M.J. Sharman, M.M. Mcguigan, T. Scheett, K. Häkkinen, R.U. Newton and F. Dioguardi, The effects of amino acid supplementation on muscular performance during resistance training overreaching. J. Strength Condit. Res., 17 (2003) 250–258.

[23] H. Portier, J.C. Chatard and E. Filaire, Effects of branched-chain amino acids supplementation on physiological and psychological performance during an offshore sailing race. Eur. J. Appl. Physiol., 104 (2008) 787–794.

[24] M.H. Yi, S.J. Nam and S.T. Chung, Separation of L-valine by anionic carrier mediated transport in a supported liquid membrane, Korean. J. Chem. Eng., 14 (1997) 263–269.

[25] P. Deblay, M. Minier and H. Renon, Separation of L-valine from fermentation broths using a supported liquid membrane, Biotechnol. Bioeng., 35 (1990) 123–131.

[26] V.J.K. Svedas, I.J. Galaev, I.L. Borisov and I.V. Berezin, The interaction of amino acids with o-phthaldialdehyde: A kinetic study of spectrophotometric assay of the reaction product, Anal. Biochem., 101 (1980) 188–195.

[27] M. Szpakowska and O.B. Nagy, Membrane material effect on copper coupled transport through liquid membranes, J. Membrane Sci., 64(1–2) (1991) 129–143.

[28] M. Szpakowska and O.B. Nagy, Non-steady state vs. steady state kinetic analysis of coupled ion transport through binary liquid membranes, J. Membrane. Sci., 76 (1993) 27–38.

[29] C. Aydiner, M. Kobya and E. Demirbas, Cyanide ions transport from aqueous solutions by using quaternary ammonium salts through bulk liquid membranes, Desalination, 180 (2005) 139–150

[30] G. León and M.A. Guzmán, Kinetic study of the effect of carrier and stripping acid concentration on the facilitated transport of cobalt through bulk liquid membranes, Desalination 184 (2005) 79–87.

[31] G. Muthuraman, T.T. Teng, C.P. Leh and I. Norli, Use of bulk liquid membranes for the removal of chromium (VI) from aqueous acidic solution with tri-n-butylphosphate as carrier, Desalination 249 (2009) 884–890.
[32] F.T. Minhas, S. Memon and M.I. Bhanger, Transport of Hg(II) through bulk liquid membrane containing calix[4]arene thioalkyl derivative as carrier, Desalination, 262 (2010) 215–220.
[33] D. He, M. Ma, and Z. Zhao, Transport of cadmium ions through a liquid membrane containing amine extractants as carriers, J. Membrane Sci. 169 (2000) 53–59.
[34] G. León and M.A. Guzmán, Facilitated transport of cobalt through bulk liquid membranes containing D2EHPA as carrier. Kinetic study of the influence of some operational variables. Desalination and Water Treatment, 13 (2010) 267–273.
[35] G. León and M.A. Guzmán. Facilitated transport of copper through bulk liquid membranes containing different carriers. Compared kinetic study. Desalination, 223 (2008) 330–336.