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Reverse Micelles of Timolol for Controlled Ocular Delivery

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The reverse micelle is one of many models thought to have properties more nearly resembling the biological cellular environment, than does the traditional dilute-solution biochemical reaction system. The reverse micellar ocular system was prepared using cetyltriammonium bromide, span 60 alone and in combinations in organic solvent of isopropylpal mitate:isopropylmyristate (50:50). The designed systems were characterized for drug content and the process variables that effect the percent drug payload and release profiles of drug. The effect of hydration, temperature and ionic strength on drug pay load on reverse micellar systems had been considered for the present study. Systems were evaluated for in vitro performance. The drug release was recorded to follow approximate first order release kinetics. On the basis of *in vitro* characterization the selected systems were evaluated for *in vivo* activity. It was observed that the micellar systems exhibited prolonged and controlled biological response of timolol maleate.

The reverse micelle is one of many models thought to have properties more nearly resembling the biological cellular environment, than does the traditional dilute-solution biochemical reaction system[1] Most topically applied ophthalmic drugs gain access to their receptors within eye by transcellular diffusion across the cornea. For moderately lipophilic timolol, the corneal epithelium contributes 50% resistance to transport while the stroma and endothelium each contributes 25%[2]. Epithelium being lipoidal in nature may represent a diffusional barrier offering high resistance especially to the hydrophilic drugs[3].

Upon instillation of an opthalmic solution, most of the instilled volume is eliminated from the precorenal area[4]. This loss is mainly attributed to systemic absorption of more than 40% of the instilled ocular dose, drainage of excessive fluid by the naso-lacrimal duct as well as elimination by tears turn over. Upadhyay, *et al.*, (1997) have developed the reverse micelle-lamellar phase transition based depot preparation of rifampicin[5].

Many systems have been developed and advocated to modify optimize the drug response when delivered topically to the eye. The action is improved and duration of action is extended via improved corneal absorption[6]; using soluble gels and emulsions[7,8], hydrophillic ocular inserts[9], ion pair associations[10], viscous vehicles[11], and gelrite R and ion activated ophthalmic vehicles[12].

The ointments, gels of slowly dissolving Lamellae remain in the eye for extended period of time[13,14]. Attempts have been made to improve corneal absorption of timolol using prodrug approach. Timolol lowers intraocular pressure in both normal and myopia in chick[15]. Recently the effect of timolol maleate, has been studied in post photorefractive keratectomy eyes with an elevated intraocular pressure after topical steroid administration[16].

The present study was aimed at occlusion of timolol in a reverse micellar system based on timolol–water/surfactant/

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isopropylpalmitate-isopropylmyristate (50:50) surfactants used were cetyl triammonium bromide (CTAB) and Span 60. The reverse micellar system bearing dissolved aqueous drug solution in a non-polar solvent offers lipophilicity to the drug solution contained in its interior non-polar aqueous pool. The system was designed, developed and characterized for *in vitro* characteristics and *in vivo* ocular hypotensive activity.

MATERIALS AND METHODS

Timolol maleate was obtained as gift sample from Torrent, India Ltd., Ahmedabad, Isopropyl palmitate was purchased from Fluka, cetyl triammonium bromide from Sigma Aldrich, USA, sortbitan monostearate (Span 60) was procured from Robert Johnson, Mumbai and Glucomol R from Torrent, Ahmedabad. All other ingredients were of analytical grade.

Preparation of reverse micellar systems:

An aqueous solution of drug i.e. timolol (5% w/v) was prepared in double distilled water. The drug solution (0.5 ml) was injected by a syringe into an organic pool consisted of isopropyl palmitate: Isopropyl myristate (50:50) and surface active agent or agents under continuous and vigorous stirring. The organic pool was further stirred for I h using a magnetic stirrer at 30±2°.The surface-active agent or system (surface active agents when taken in combination) was contained in the organic bulk. The surface-active agents used were cetyl triammonium bromide CATB and sorbitan monostearate (Span 60)[17] as shown in table 1.

Characterization of reverse micellar systems, Drug content:

Accurately weighed 1.0 g (1.2 ml) systems were filled into dialysis bags (Sigma, St. Louis, Mo) separately. After closing both the ends with the help of thread, the bags were put in a beaker containing 50 ml of organic phase i.e. isopropyl palmitate:isopropyl myristate (50:50). The whole assembly was kept overnight for equilibrium dialysis. The bags were then taken out and were evaluated by dissolving in the methanol. The solutions were analyzed spectrophotometrically using a Beckman spectrophotometer with appropriate dilution at 295 nm.

Effect of process variable on percent drug loading:

In order to study the effect of ionic strength, monovalent and divalent salts (NaCl and $CaCl_2$) were dissolved in double distilled water separately to obtain 1, 2, 3, 4.....to 12 mmol, solutions were injected into their respective organic pools under continuous and vigorous stirring. The hydration ratio (W_0) was kept constant. Rest of the procedure adopted was the same as described earlier. The solutions were analyzed spectrophometrically at 295 nm timolol content (Table 2 and 3).

Effect of temperature:

Accurately weighed (1.0 g) reverse micellar systems were filled into dialysis bags. After closing the both the ends, each bag was kept in a beaker containing 50 ml. Organic phase at different temperatures ranging from 25 to 60°. The samples from dialysis bags were withdrawn after effective dialysis equilibration and estimated for timolol content (Table 4).

Effect of hydration ratio (W_0):

In the reverse micellar systems different amounts of water i.e. Wo=3 to 30 were injected under constant and

TABLE 1: COMPOSITION OF REVERSE MICELAR SYSTEMS

Product Code	Surface active agent		Timolol maleate		Organic Solvent Isopropyl palmitate: Isopropyl myristate (50:50) G
	CTAB (mM)	Span-60 (mM)	Incorporated (mg)	Determined (mg)	
CTAB.S-1	25	-	25	18.53	20
CTAB.S-2	50	-	25	19.75	20
CTAB.S-3	-	25	25	19.4	20
CTAB.S-4	-	50	25	20.25	20
CTAB.S-5	50	25	25	20.69	20
CTAB.S-6	25	50	25	21.50	20

vigorous stirring. The Wo values for reverse micellar systems were determined by an aqueous nitrate polarity probe method. The method is based on variation in λmax of nitrate ion in organic solvent systems as a function of added water (Wo). At low Wo values, λmax appeared around 309 nm indicating lower strength of interaction between the pool water and nitrate ion. At higher Wo values the strength of the nitrate ion water pool interaction increases and λmax

TABLE 2: EFFECT OF IONIC STRENGTH OF AQUEOUS PHASE ON DRUG LOAD IN REVERSE MICELLAR SYSTEMS

Sodium Chloride (mmol)	CTAB-1	CTAB-2	CTAB-3	CTAB-4	CTAB-5	CTAB-6
1.0	32.5±1.2	41.6±2.1	28.5±1.3	24.8±1.3	30.5±2.2	27.0±3.2
2.0	70.5±2.2	78.0±3.2	45.2±1.9	42.0±2.6	62.0±3.5	46.5±3.6
3.0	83.0±2.8	84.3±2.8	69.4±3.3	61.5±3.3	76.4±2.6	70.5±2.5
4.0	75.2±3.2	79.1±2.0	81.0±2.0	72.1±3.5	84.9±1.8	79.0±1.0
5.0	67.7±1.5	72.9±2.1	86.9±1.6	80.3±2.1	80.4±3.2	86.9±1.5
6.0	61.0±2.0	66.0±2.4	81.2±4.1	88.0±1.4	72.5±2.1	84.0±2.0
8.0	46.5±2.5	52.5±1.5	71.0±3.8	85.0±2.8	60.0±4.0	78.6±2.8
10.0	31.2±4.9	39.0±3.0	63.2±2.9	80.1±3.5	51.0±6.0	72.9±2.8
12.0	15.0±3.0	26.5±3.2	53.5±3.2	75.0±3.0	41.5±4.5	65.5±3.4

Effect of monovalent ion on the drug payload in reverse miceller systems. The bar on the drug payload in reverse micellar systems. The bar on data point indicates mean±s.d. (n=3), Data is expressed as percent payload.

TABLE 3: EFFECT OF IONIC STRENGTH OF AQUEOUS PHASE ON THE DRUG PAYLOAD IN REVERSE MICELLAR SYSTEMS

Sodium Chloride (mmol)	CTAB-1	CTAB-2	CTAB-3	CTAB-4	CTAB-5	CTAB-6
1.0	83.0±0.8	84.5±0.8	38.5±3.1	27.2±4.4	64.0±3.8	40.5±2.6
2.0	77.9±3.5	81.3±1.6	75.1±2.6	56.0±3.2	85.0±2.8	78.1±3.1
3.0	69.5±1.9	73.8±2.7	86.0±1.8	79.5±2.8	83.2±4.0	87.0±2.0
4.0	59.8±3.0	64.5±4.1	84.0±3.5	88.0±1.0	74.5±2.5	85.9±1.2
5.0	50.0±5.0	55.0±3.0	73.2±2.8	86.0±2.0	63.0±3.9	80.0±2.5
6.0	40.5±1.9	47.0±3.0	62.4±3.0	80.6±3.6	53.3±1.8	70.4±2.8
8.0	30.9±2.0	32.4±3.1	50.2±2.9	67.2±2.5	41.0±4.0	58.2±4.2
10.0	11.2±2.5	19.6±4.5	41.0±4.0	58.9±3.0	30.5±3.2	51.0±2.9
12.0	1.2±1.8	7.4±3.5	29.5±3.4	52.7±2.0	18.8±4.1	41.0±4.0

Effect of divalent ion on the drug payload in reverse micellar systems. The results were represented as mean±s.d. (n=3). Data is expressed as percent payload.

value shifted around 302.05 nm as seen in bulk water. The effect of hydration rate on percent drug payload was recorded (Table 5)[17].

In vitro drug release rate:

In vitro release rate of timolol from reverse micellar systems for corneal drug availability was determined using Franz diffusion cell. The pH of lachrymal fluid and the blinking rate of the eyes were the factors taken into consideration and were simulated. The receptor compartment contained 20 ml of artificial tear solution (ATS, pH 7.4). One gram (1.2 ml) of the system was kept in donor compartment over a cellophane membrane (PD 215, Dupont de Nemours) separating the system from receptor compartment. The diffusion cell was kept in a water bath maintained at 37±0.5°. The metabolic to and fro shaker of the bath was used at a rate of 22 movements per min. 2 ml sample was withdrawn from the receptor compartment at hourly intervals for 14 h. The volume was replaced by an artificial tears solution (pH 7.4). Samples were analyzed spectrophotometrically at 295 nm for timolol content.

In vivo activity:

On the basis of *in vitro* drug release profile, the systems CTAB.S-1 and CTAB.S-6 that demonstrated better release profile as compared to other systems were selected for *in vivo* evaluation. *In vivo* biological response of reverse micellar systems on the induced ocular hypertension was studied by measuring the change in ocular tension of six male albino rabbits (3.0-3.5 kg). The dose applied to rabbit eye in all the cases was equivalent to 35.0 µg of timolol. The left eye of the rabbits was used for test while the right served as a control.

Both the eyes of the rabbits were anaesthetized by instillation of 4-5 drops of 1% xylocaine solution. After two minutes intraocular pressure of normal eyes was recorded, then 0.5 ml of 5% hypertonic solution of sodium chloride was injected intravenously into the left eye to develop conjunctival edema. Immediately after development of edema the intraocular pressure was measured using a Tonometer (Zur- Bentzung des Schioetz- Germany). One drop of each system was then instilled into the left eye separately and intraocular tensions were measured at different time intervals. The anti hypertensive effect of the micellar systems was compared with effect of aqueous eye drop Glucomol R (Table 6 and 7).

RESULTS

Drug content in the reverse micellar systems was determined by the equilibrium dialysis method. The drug content estimated in various reverse micellar systems ranges between 74.16±0.5% to 86.0±1.5% based on the amount of drug added. Low ionic strength (NaCl) favors reverse micellar solubilization of aqueous drug solution and that in turn drug payload as compared to higher ionic strength ($CaCl_2$). In the case of low ionic strength, the drug entrapment was observed to increase with increase in ionic strength. At 12 mmol NaCl solution decreased percent drug payload for systems CTAB.S-1 to CTAB.S–6 was noted respectively (Table 2). Similarly at higher ionic strength ($CaCl_2$) the equilibration was attained relatively earlier. However, there was a discernible decrease in total percent drug payload at 12 mmol, $CaCl_2$ solution for systems CTAB.S-1 to CTAB.S-6 (Table 3). Hence the optimum ionic strength for maximum drug payload was 3 mmol.

An increase in temperature was noted to increase the micellar solubilization of aqueous drug solution up to optimum temperature. For a system CTAB.S-1 the drug payload recorded at 35° was 87.0±1.8% whilst the system at higher temperature of 60° could entrap 35.0±6.1% of timolol. Hence, optimum temperature for maximum entrapment of drug in the system CTAB-1 was considered to be 35°. In the similar manner optimum temperatures for systems CTAB.S-2, CTAB.S-3, CTAB.S-4, CTAB.S-5 and CTAB.S-6 noted to be 45, 35, 45, 50 and 50° respectively. The percent payload estimated for various systems at optimum temperature (Table 4).

An increase in the hydration ratio resulted in to gradual increase in drug solubilization. However, beyond the optimum Wo the increase in the Wo causes decrease in the drug payload or entrapment (Table 5). At Wo=30 the percent drug payload for the systems CTAB.S-1 to CTAB.S-6 was recorded respectively. The optimum Wo values for these systems were 18, 24, 12, 18 and 15 respectively (Table 5).

The release rate constant (k) calculated for the preparations found to be 0.1429 h^{-1}, 0.0857 h^{-1}, 0.0607 h^{-1}, 0.050 h^{-1} and 0.1079 h^{-1} respectively for the systems CTAB.S-1to CTAB.S-6. The observations reveal that maximum retardation was offered by the system CTAB.S-4, while the maximum fastest and uniform release was observed in the case of system CTAB.S-1 and CTAB.S-6 which were considered to be effective and desired drug delivery for

TABLE 4: EFFECT OF TEMPERATURES ON THE DRUG PAYLOAD IN REVERSE MICELLAR SYSTEMS

Temp (°)	CTAB.S-1	CTAB.S-2	CTAB.S-3	CTAB.S-4	CTAB.S-5	CTAB.S-6
25	41.0±2.5	43.5±3.8	53.0±1.9	44.2±2.3	39.5±3.1	51.4±3.5
30	63.5±2.0	62.0±1.2	74.0±2.4	63.5±3.2	58.2±3.6	63.0±1.0
35	87.0±1.8	72.1±2.3	85.0±1.3	74.2±3.8	71.0±4.2	69.5±1.9
40	81.4±1.7	79.0±3.2	78±2.0	82.5±2.6	80.5±3.9	75.2±2.6
45	75.0±2.8	86.3±1.6	72.0±10.4	88.2±1.8	84.0±3.2	79.1±3.6
50	62.5±2.0	80.0±2.1	68.4±2.7	76.5±2.0	87.0±1.9	83.0±2.0
55	51.8±4.0	47.5±2.2	47.5±2.2	68.5±1.5	83.5±1.2	81.5±2.3
60	35.0±6.1	60.5±1.5	30.4±1.5	55.0±2.7	78.8±2.2	63.9±4.0

Effect of temperature on the drug payload in reverse micellar systems. The results were represented as mean±s.d. (n=3). Data is expressed as percent payload.

ocular anti-hypertensive activity (Glaucoma) (Table 6 and 7).

DISCUSSION

The reverse micellar systems were prepared by the method reported by Brochette *et al.* (1988)[19]. It was observed that the surface-active agents when added in concentration above their respective critical micelle concentration tend to form reverse micelles. The formation of reverse micelles was however, confirmed by an aqueous nitrate polarity probe method. The latter is essentially based on absorption maxima that are different in polar and non-polar solvent. The exhibition of maxima that corresponds to the non-polar water reveals the formation of reverse micelles. The selection of reverse micellar system was made on the basis of various parameters studied including drug content.

At a particular ionic strength for each, preparing the periodic estimation of drug indicated a slow increase in percent drug payload with time however, following equilibrium

TABLE 5: EFFECT OF HYDRATION OF RATIO (WO) ON THE DRUG PAYLOAD IN REVERSE MICELLAR SYSTEM

Value of Wo	CTAB.S-1	CTAB.S-2	CTAB.S-3	CTAB.S-4	CTAB.S-5	CTAB.S-6
3	26±2.0	18.4±3.1	58.0±1.6	34.0±1.5	22.5±2.3	32.4±1.4
6	38.5±1.8	29.0±4.0	75.2±3.8	46.5±1.9	33.2±3.1	49.0±1.2
9	49.0±2.6	41.2±2.8	69.1±2.9	69.5±3.6	49.5±4.0	62.5±4.6
12	65.5±1.1	50.4±1.9	58.4±4.4	79.8±2.1	66.0±2.4	76.0±2.3
15	71.6±0.8	62.0±3.4	45.5±5.5	65.2±2.8	74.2±1.3	85.5±1.5
18	79.0±1.5	70.5±1.5	32.0±3.0	50.0±2.0	83.0±2.5	71.0±3.0
21	64.5±2.5	77.0±1.7	20.4±3.6	36.0±3.0	75.8±2.2	51.2±4.7
24	48.5±2.5	80.5±2.4	12.5±1.5	24.0±2.2	65.0±3.0	36.5±1.4
27	36.0±3.8	73.0±3.0	6.5±3.0	15.0±4.0	52.2±2.5	25.5±2.5
30	27.8±4.0	57.9±4.1	2.7±1.3	10.2±1.7	41.0±5.0	17.8±2.1

Effect of hydration ratio (Wo) on the drug payload in reverse micellar systems. The results were represented as mean±s.d. (n=3). Data is expressed as percent payload.

no further increase in percent drug payload or entrapment was observed. Beyond optimum ionic strength further increase in ionic strength causes decrease on percent drug payload.

Possible explanation for the observations shown in Tables 2 and 3 can be given as (a) the polar head of the reverse micelles from the charge interior which can absorb opposite ions from the added salt in the free or hydrated form, (b) water is an associated solvent and the individual molecule of water associated with each others by hydrogen bonding and (c) when a salt is added in low concentration the water pool has a power to dissolve the drug. Additional amount of salt (divalent) diverts the power of solvation and the charge interaction with the interior polar groups thus widening of gaps occurs between the adjacent polar heads which facilitates the leaching of drug into organic bulk[20].

The relation was noted (Table 4) to be true up to a particular temperature for each system beyond which instead of increased percent drug payload a decrease was recorded. This could presumably be attributed to the reasons that substantial increase in temperature provides kinematic energy too, could possibly destabilize the micellar system and mobilize the drug molecules from water pool to the organic bulk.

It could possibly be accounted for by the fact that the volume of reverse micellar solubilized water increase that in turn increase the amount of the percent drug payload. Further increase in the aqueous phase resulted into disarrangement of the monolayers leading to the leaching of drug into the organic bulk in which drug was moderately soluble[21].

It could possibly be accounted for by the fact that the volume of reverse micellar solubilized water increase in turn increase the amount of the percent drug payload (Table 5)[1]. Further increase in the aqueous phase resulted into disarrangement of the monolayers leading to the leaching of drug into the organic bulk in which drug was moderately soluble[21].

The reverse micellar systems were studied for their *in vitro* drug release profile. The Table-6 shows an approximate first order release profile. On the basis of *in vitro* release

TABLE 6: INTRAOCULAR PRESSURE-TIME PROFILES OF TIMOLOL AQUEOUS EYE DROPS *IN VIVO*

Time (min)	Change in IOP (mm Hg)
00	00
15	6.2±1.8
30	12.0±1.5
45	16.6±2.2
60	18.5±1.4
75	20.2±0.9
90	18.4±2.0
105	13.3±2.7
120	11.4±1.0
150	7.0±1.7
180	3.5±2.0

Change in intraocular pressure-time profiles of timolol *in vivo* in rabbit's eye. The results were represented as mean±s.d. (n=3)

TABLE 7: INTRAOCULAR PRESSUE TIME PROFILE OF REVERSE MICELLAR SYSTEM OF TIMOLOL *IN VIVO*

Time(min)	Change in IOP (mm Hg)	
	CTAB.S-1	CTAB.S-6
00	00	00
30	7.2±1.3	4.1±2.0
60	12.5±2.2	7.9±1.5
90	17.5±1.0	12.2±2.7
120	19.4±1.3	16.6±1.4
150	20.5±1.5	19.5±1.2
180	18.0±1.0	20.1±1.0
210	14.5±2.5	20.3±1.7
240	8.5±1.7	19.1±2.5
270	4.4±2.8	18.0±3.0
300	-	15.8±1.6
330	-	12.0±3.0
360	-	7.5±2.0

Cumulative percent drug release *in vivo* of timolol from reverse micellar systems. The results were represented as mean±s.d. (n=3)

profiles the systems CTAB.S-1 and CTAB.S-6, were selected for *in vivo* study. *In vivo* activity of the ocular systems (test) were compared with an aqueous eye drop, the reverse micellar systems showed increased bioavailability i.e. CTAB.S-1 (2.0-2.5 fold) and CTAB.S-6 (2.25-2.50 fold) (Table 7). The reverse micellar systems induced a lower level of ocular pressure than the aqueous solution. The biological response observed for reverse micellar systems (CATB.S-6) was most prominent and prolonged than aqueous eye drops[20].

The time corresponding to peak biological response was significantly delayed, but intensity of the biological response was maximized in the case of reverse micellar systems. This demonstrates an initial slow absorption followed by a controlled absorption of the drug from the reverse micellar systems. There have been no objective sign of damage or corneal abrasions or intraocular inflammation. It was evident from this study that reverse micellar system could control the release of relatively water-soluble drugs like timolol to the precorneal area for the effective treatment of Glaucoma via lipophilicity and increased corneal contact time.

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REFERENCES

1. Chattopadyay, S.K., Toews, K.A., Butt, S., Barlett, R. and Brown, H.D., **Cancer Biochem. Biophys.,** 1997, 15, 245.
2. Lee, V.H. and Robinson, J.R., **J. Ocul. Pharmacol.**, 1986, 2, 67.
3. Mitra, A.K. and Mikkelson, T.J., **J. Pharm. Sci.,** 1988, 77, 771.
4. Chari, S.S., Makoid, M.C., Brikson , S.P. and Robinson, J. R. , **J. Pharm. Sci.,** 1974, 63, 333.
5. Upadhyay, A.K., Omray, L.K., Khopade, A.J. and Jain, N.K., **J. Pharmazi.**, 1997, 52, 961.
6. Gurny, R., **Pharm. Acta Helv.,** 1981, 56, 130.
7. Bamba, M., Puisieux, F., Marty, J.P. and Carstensen J.T., **Int. J. Pharm.,** 1979, 2, 307.
8. March, W.F., Stewart, R.M., Mandrel, A.C. and Bruce, L.A., **Arch. Opthalmol.**, 1982, 100, 1270.
9. Coury, A.J., Cahalam, P.T., Jevne, A.H., Prerrault, J.J. and Kallok, M.J., **Med. Devices Diagnostics,** 1984, 6, 28.
10. Davis, S.S., Tomilinson, E. and Wilson, C.G., **Brit. J. Pharmacol.,** 1978, 64, 444.
11. Zaki, I., Fitzerald, P., Hardy, J.G. and Wilson, C.G., **J. Pharm. Pharmacol.,** 1986, 38, 463.
12. Rozier, A., Mazuel, C., Grove, J. and Plazonet, B., **Int. J. Pharm.,** 1989, 57, 163.
13. Patton, T.F. and Robinson, J.R., **J. Pharm. Sci.,** 1975, 64, 1312.
14. Bensinger, R. Shin, D.H., Kass, M.A., Podos, S.M. and Becker, B., **Invest. Opthal.,** 1976, 15, 1008.
15. Schmidt, K.L., Abbott, M., Humphries, M., Pyne, K. and Wildsoet, C.F., **Exp. Eye Res.,** 2000, 70, 659.
16. Nagy, Z.Z., Szabo, A., Krueger, R.R. and Suveges, I., **J. Cataract Refract. Surg.,** 2001, 27, 1018.
17. Kumar, V.V and Raghunathan, P., **Lipid,** 1986, 21, 764.
18. Leucata, S.E., **Int. J. Pharm.,** 1989, 54, 71.
19. Brochette, P., Petit, C. and Pileni, M.P., **J. Physical Chem.,** 1988, 92, 3505.
20. Jiang, Y.B. and Jin, M.G., **Spectrochim. Acta.** 2000, 56, 623.
21. Creagh, A.L., Prausnitz, J. M. and Blanch, H. W., **Enzyme Microb. Technol.,** 1993, 15, 383.