

College of Pharmaceutical Sciences, Manipal, India

Effect of some penetration enhancers on the permeation of glibenclamide and glipizide through mouse skin

S. MUTALIK, N. UDUPA

Received May 13, 2003, accepted May 28, 2003

Prof. Dr. N. Udupa, College of Pharmaceutical Sciences, Manipal-5761109, Karnataka, India
udupa1553@yahoo.com

Pharmazie 58: 891–895 (2003)

The purpose of this investigation was to study the effect of some penetration enhancers on *in vitro* permeation of glibenclamide and glipizide through mouse skin. Ethanol in various concentrations, *N*-methyl-2-pyrrolidinone, transcutol, propylene glycol and terpenes like citral, geraniol and eugenol were used as penetration enhancers. The *in vitro* skin permeation experiments were conducted by both simultaneous application of drug and enhancer solution and by pretreatment of the skin with neat enhancer. At the end of the experiment drug retained in the skin was estimated. The flux values ($\mu\text{g}/\text{cm}^2/\text{h}$) of both drugs significantly ($p < 0.05$) increased in the presence of penetration enhancers, except transcutol and propylene glycol. The glibenclamide flux values ranged from 1.42 ± 0.09 without enhancer, to 18.25 ± 1.21 in a combination of 50% ethanol and 5% eugenol. Glipizide flux values ranged from 3.21 ± 0.51 without enhancer, to 57.21 ± 5.25 in a combination of 50% ethanol and 5% eugenol. Skin retention and solubility of both drugs increased with all penetration enhancers compared to control (except propylene glycol). As the target permeation rates for glibenclamide and glipizide were calculated to be 193.8 and 184.8 $\mu\text{g}/\text{h}$ respectively, the present study showed that the required permeation rates for both drugs could be achieved with the aid of enhancers by increasing the area of application in an appreciable range.

1. Introduction

We previously reported the feasibility of application of transdermal delivery of glibenclamide and glipizide [1]. The enhancing effect of 5% v/v of Tween-20, polyethylene glycol, ethanol and d-limonene on the permeation of glibenclamide and glipizide was studied. Both drugs showed a sufficient permeation rate and negligible skin degradation. In the present study, we investigated the effect of other enhancers like transcutol, propylene glycol (PG), *N*-methyl-2-pyrrolidinone and various terpenes like geraniol, citral and eugenol on *in vitro* skin permeation of glibenclamide and glipizide. In our earlier study, 50% v/v ethanol, when used to solubilise d-limonene, led to a significant increase in the permeation rate of both drugs [1]. Hence, the effect of various concentrations of ethanol on the permeation of glibenclamide and glipizide through mice skin has also been studied.

2. Investigations, results and discussion

As the intrinsic transdermal permeation rate of glibenclamide and glipizide from an aqueous saturated solution was not adequate to meet respective target permeation rates, in our earlier study, we have investigated the permeation enhancing effect of some common and safe enhancers like Tween-20, polyethyleneglycol-400, ethanol and d-limonene. It was found that all the enhancers significantly increased the flux values for both drugs and

d-limonene, a terpene, showed a maximum flux value [1]. The present study was planned to assess the permeation enhancing effect of other putative enhancers including some terpenes. Table 1 and 2 list the permeation parameters, drug retention in the skin and solubility studies of drugs in the first set (simultaneous application of drug and enhancer solution) and in the second set (pretreatment of the skin with neat enhancer) of experiments respectively. In both sets of experiments, all the enhancers, except transcutol and propylene glycol, significantly ($p < 0.05$) increased the flux of both drugs compared to the average flux from the respective controls (without enhancer). For both drugs, the flux values were slightly higher in the second set of experiments where the skin was pretreated with neat enhancer. These observations are in accordance with the earlier findings and clearly demonstrate that both experimental designs lead to similar results [1, 2]. The activity of enhancers increased in the following order: NMP, geraniol, citral, and eugenol. Transcutol exhibited less flux values compared to control. It has been reported that in the presence of transcutol the intercellular lipids are swollen without altering the multiple bilayer structure and these swollen lipids appear to hold the model drugs and there by form an intracutaneous depot for drugs [3]. In that study, the permeation of hydrocortisone and dexamethasone was significantly reduced in the presence of transcutol. In another study transcutol showed a very low flux value with primaquine [4].

Table 1: Permeation parameters, drug retention in the skin and solubility results of glibenclamide and glipizide in the presence of penetration enhancers

Penetration enhancers	Drug	J _{Max} (µg/cm ² /h)	Permeability coefficient (cm/h)	Amount of drug in skin (µg/mg)	Solubility (mg/ml)
Control (PB alone)	GLB	1.42 ± 0.09	0.143 ± 0.008	0.19 ± 0.07	0.010 ± 0.001
	GPZ	3.21 ± 0.51	0.019 ± 0.002	1.46 ± 0.35	0.165 ± 0.010
Transcutol 5%	GLB	1.30 ± 0.22	0.007 ± 0.001*	0.81 ± 0.09*	0.186 ± 0.012*
	GPZ	2.51 ± 0.21	0.007 ± 0.001*	2.51 ± 0.11*	0.344 ± 0.019*
Propylene glycol 5%	GLB	1.35 ± 0.21	0.043 ± 0.004*	0.17 ± 0.10	0.031 ± 0.002*
	GPZ	2.81 ± 0.45	0.006 ± 0.001*	1.41 ± 0.19	0.477 ± 0.015*
NMP 5%	GLB	4.01 ± 0.77*	0.007 ± 0.001*	0.55 ± 0.12*	0.596 ± 0.110*
	GPZ	11.21 ± 2.01*	0.029 ± 0.002*	2.98 ± 0.19*	0.373 ± 0.029*
Geraniol 5%+ Ethanol 50%	GLB	16.18 ± 3.01*	0.010 ± 0.003*	2.31 ± 0.25*	1.778 ± 0.635*
	GPZ	55.11 ± 4.49*	0.011 ± 0.001*	4.51 ± 0.24*	5.340 ± 1.011*
Citral 5%+ Ethanol 50%	GLB	17.01 ± 2.44*	0.002 ± 0.001*	2.55 ± 0.29*	8.657 ± 1.211*
	GPZ	56.25 ± 5.12*	0.001 ± 0.001*	4.62 ± 0.23*	36.000 ± 2.92*
Eugenol 5%+ Ethanol 50%	GLB	18.25 ± 1.21*	0.003 ± 0.002*	2.81 ± 0.25*	6.592 ± 0.892*
	GPZ	57.21 ± 5.25*	0.002 ± 0.001*	4.75 ± 0.41*	32.040 ± 2.41*
Ethanol 50%	GLB	12.01 ± 1.31*	0.008 ± 0.001*	1.91 ± 0.21*	1.641 ± 0.312*
	GPZ	50.53 ± 4.15*	0.015 ± 0.001	4.31 ± 0.24*	3.310 ± 0.251*

All values are expressed as mean ± SE; n = 3. * p < 0.05, significant compared to control. J_{Max}: maximum flux, PB: phosphate buffer, GLB: glibenclamide, GPZ: glipizide, NMP: N-methyl-2-pyrrolidinone.

Like transcutol, PG showed less permeability than control. Similar results were observed with tamoxifen and it was attributed to less partitioning of drug to the stratum corneum from PG than phosphate buffer [5]. In another study, PG did not show significant improvement in the permeation of haloperidol. This was attributed to the fact that PG is effective at high concentrations and is mostly used as co-solvent for the permeation enhancements [6]. NMP produced a significant increase in the permeation of both drugs. In an earlier study, a series of pyrrolidinones produced significant improvement in the permeation rate of hydrocortisone. It has been reported that pyrrolidinones increase the permeation by fluidizing the lipids along the intercellular lipid domains in the stratum corneum [7]. The terpenes, eugenol, geraniol and citral (5% v/v), in combination with 50% v/v ethanol showed comparatively high permeation rates for both drugs. Many investigators assessed a series of terpenes as percutaneous enhancers [5, 8–11]. Terpenes increased the permeation of solutes by disrupting the highly ordered structure of intercellular lipids and im-

proving the partitioning of solutes in stratum corneum. Enhancement in the permeability of tamoxifen by terpenes was attributed to a large increase in an effective diffusion coefficient, moderate increase in partition coefficient, lipid extraction and macroscopic barrier perturbation [5].

If we compare our earlier findings of d-limonene (flux values: 19.01 ± 2.14 and 7.26 ± 0.76 µg/cm²/h for glibenclamide in the first and the second set of experiments respectively; 62.97 ± 7.10 and 15.08 ± 1.57 µg/cm²/h for glipizide in the first and the second set of experiments respectively) [1] with the present investigations, the permeation enhancing effect of tested terpenes was increased, although being not significantly different from each other, in the order: geraniol, citral, eugenol, d-limonene. Geraniol has been found to be effective in increasing the permeation of hydrophilic drugs. In earlier studies, geraniol showed high flux values for hydrophilic drugs like diclofenac sodium and 5-fluorouracil [12, 13]. As glibenclamide and glipizide are lipophilic, geraniol could not increase their permeation rate. Similarly, citral could not achieve high flux values either. In general, those terpenes with polar functional groups produce the best improvements in the absorption of hydrophilic drugs, whereas hydrocarbon terpenes, like d-limonene, are more active towards lipophilic drugs [13]. Among the terpenes tested for glibenclamide and glipizide, d-limonene showed high flux values followed by eugenol. Our results are in accordance with an report, where 5% v/v d-limonene in 50% v/v ethanol produced high flux values for tamoxifen followed by 5% v/v eugenol in 50% v/v ethanol [5]. As observed in our earlier results, the combination of 50% ethanol and 5% terpene showed higher flux values for both drugs in comparison with 50% ethanol alone in this study also. This could be attributed to the higher thermodynamic activity in 50% ethanol-buffer solution [14].

Table 3 shows the permeation profile of glibenclamide and glipizide in various volume fractions of ethanol/phosphate buffer, pH 7.4 (PB) system across the mouse skin. In our earlier study, as 50% v/v ethanol, when used to solubilise d-limonene, showed significant increase in the permeation rate of both drugs [1], the effect of various concentrations of ethanol has presently been studied. The steady state permeation rates of both drugs increased as the volume

Table 2: Permeation parameters of glibenclamide and glipizide following pretreatment of skin with neat enhancer

Penetration enhancers	Drug	J _{Max} (µg/cm ² /h)	Permeability coefficient (cm/h)
Control (PB alone)	GLB	1.42 ± 0.09	0.143 ± 0.008
	GPZ	3.21 ± 0.51	0.019 ± 0.002
Transcutol	GLB	1.35 ± 0.21	0.134 ± 0.006
	GPZ	2.68 ± 0.42	0.016 ± 0.002
Propylene glycol	GLB	1.39 ± 0.24	0.137 ± 0.008
	GPZ	2.95 ± 0.51	0.018 ± 0.002
NMP	GLB	4.32 ± 1.01*	0.420 ± 0.053*
	GPZ	11.91 ± 1.25*	0.072 ± 0.003*
Geraniol	GLB	6.01 ± 0.51*	0.605 ± 0.019*
	GPZ	13.01 ± 1.51*	0.078 ± 0.005*
Citral	GLB	6.55 ± 0.58*	0.659 ± 0.019*
	GPZ	13.33 ± 1.13*	0.081 ± 0.002*
Eugenol	GLB	7.01 ± 0.61*	0.705 ± 0.021*
	GPZ	13.91 ± 1.11*	0.084 ± 0.002*

All values are expressed as mean ± SE; n=3. * p<0.05, significant compared to control. J_{Max}: maximum flux, PB: phosphate buffer, GLB: glibenclamide, GPZ: glipizide, NMP: N-methyl-2-pyrrolidinone.

Table 3: Permeation parameters, drug retention in the skin and solubility results of glibenclamide and glipizide in the presence of different concentrations of ethanol

Penetration enhancers	Drug	J_{Max} ($\mu\text{g}/\text{cm}^2/\text{h}$)	Permeability coefficient (cm/h)	Amount of drug in skin ($\mu\text{g}/\text{mg}$)	Solubility (mg/ml)
Control (PB alone)	GLB	1.42 ± 0.09	0.143 ± 0.008	0.19 ± 0.07	0.010 ± 0.001
	GPZ	3.21 ± 0.51	0.019 ± 0.002	1.46 ± 0.35	0.165 ± 0.010
Ethanol 20%	GLB	$6.55 \pm 1.31^*$	$0.098 \pm 0.004^*$	$0.65 \pm 0.12^*$	$0.066 \pm 0.011^*$
	GPZ	$21.21 \pm 3.24^*$	$0.031 \pm 0.001^*$	$3.25 \pm 0.45^*$	$0.676 \pm 0.101^*$
Ethanol 30%	GLB	$8.55 \pm 1.27^*$	$0.029 \pm 0.006^*$	$1.01 \pm 0.24^*$	$0.332 \pm 0.101^*$
	GPZ	$33.44 \pm 3.01^*$	$0.028 \pm 0.002^*$	$3.51 \pm 0.51^*$	$1.248 \pm 0.211^*$
Ethanol 40%	GLB	$10.92 \pm 1.22^*$	$0.026 \pm 0.002^*$	$1.45 \pm 0.19^*$	$0.420 \pm 0.099^*$
	GPZ	$42.12 \pm 3.55^*$	$0.018 \pm 0.001^*$	$4.01 \pm 0.31^*$	$2.593 \pm 0.281^*$
Ethanol 50%	GLB	$12.01 \pm 1.31^*$	$0.008 \pm 0.001^*$	$1.91 \pm 0.21^*$	$1.641 \pm 0.312^*$
	GPZ	$50.53 \pm 4.15^*$	$0.015 \pm 0.001^*$	$4.31 \pm 0.24^*$	$3.310 \pm 0.351^*$
Ethanol 60%	GLB	$13.02 \pm 2.12^*$	$0.007 \pm 0.001^*$	$2.25 \pm 0.31^*$	$1.811 \pm 0.301^*$
	GPZ	$51.76 \pm 4.91^*$	$0.012 \pm 0.001^*$	$4.44 \pm 0.29^*$	$4.612 \pm 0.914^*$
Ethanol 70%	GLB	$15.21 \pm 2.11^*$	$0.006 \pm 0.001^*$	$2.36 \pm 0.29^*$	$2.421 \pm 0.410^*$
	GPZ	$53.25 \pm 5.11^*$	$0.011 \pm 0.001^*$	$4.55 \pm 0.41^*$	$5.084 \pm 1.021^*$
Ethanol 80%	GLB	$11.01 \pm 1.55^*$	$0.005 \pm 0.001^*$	$1.75 \pm 0.27^*$	$2.221 \pm 0.392^*$
	GPZ	$44.54 \pm 4.25^*$	$0.009 \pm 0.001^*$	$4.05 \pm 0.33^*$	$4.761 \pm 0.992^*$

All values are expressed as mean \pm SE; n = 3. * p < 0.05 compared to control. J_{Max} : maximum flux, PB: phosphate buffer, GLB: glibenclamide, GPZ: glipizide.

fraction of ethanol increased, reaching a maximum at 70% v/v ethanol in PB, and then decreasing with further increase in ethanol concentration. Ethanol is one of the most commonly used skin permeation enhancers [15]. Ethanol used as a part of a co-solvent system has been observed to increase the permeation of a wide range of drugs through human and animal skin [16]. A linear relationship between the skin permeation of nitroglycerin and the transdermal flux of ethanol has been reported, which implies that ethanol penetrates through the skin and changes the permeation properties of the skin. It is also suggested that when less than 70% ethanol is used, permeation of both ethanol and drug is enhanced by hydration of entire stratum corneum [16, 17]. As high concentrations of ethanol were used in the present study, the local concentration of ethanol would have probably been high in the stratum corneum and viable tissues, which may have led to an increase in the solubility of drugs in the skin thereby causing an increase in drug flux [18]. Some researchers have demonstrated an ethanol concentration dependent enhancement mechanism in which ethanol altered the lipid component at low fractions, whereas new pores formed in the stratum corneum at higher ethanol fractions [19]. The flux was increased as the concentration of ethanol was increased up to 70% v/v. However, when 80% v/v ethanol is used, the outer layer of the stratum corneum is substantially dehydrated, and this dehydrated portion increases barrier properties to the permeation of ethanol and drug [16]. Accordingly in the present study, the flux values for both drugs were reduced with 80% v/v ethanol. As ethanol concentration was increased, the increase in the flux was associated with the decrease in the permeability coefficients. Similar results were observed with testosterone and its derivatives where permeability coefficient values decreased from 1.08 ± 0.43 (20% v/v ethanol) to 0.04 ± 0.01 (70% v/v ethanol) although the flux values were increased from 0.49 ± 0.19 (20% v/v ethanol) to 2.69 ± 0.69 (70% v/v ethanol) [16].

Skin retention of both drugs was found to increase as a function of flux values, except for transcutol. High transdermal flux generally results in the retention of larger drug quantity in the skin [20]. The present results indicate that the amount of the drug retained in the skin is related to the

transdermal flux, supporting a report where the skin concentration after topical application of piroxicam was related to its flux across the skin [21]. But transcutol, as it is capable of forming an intracutaneous depot of the drug, showed high skin retention of the drugs in spite of low flux values. In an earlier study, in the presence of transcutol, the skin retention of dexamethosone and hydrocortisone was high, although the flux values were low [3].

Glibenclamide and glipizide show low solubility in PB; but the addition of penetration enhancers (5% v/v) increased the solubility significantly (p < 0.05). The solubility of both drugs in several binary ethanol-PB cosolvent systems was higher than that of in both the pure solvents. The highest solubility in an ethanol-PB binary system was observed with 70% v/v ethanol. Similar results were observed by Krishnaiah et al. with nicardipine hydrochloride who attributed this observation to cosolvency effects [22]. In the present study, drug suspensions have been tested and hence drug solubility might not play any role in permeation enhancement as maximum thermodynamic activity is reached as soon as the solubility limit is exceeded. If drugs are suspended in solvents and the solvents do not affect the skin barrier, the skin permeation rate of the drugs should be constant irrespective of the nature of the solvent [23]. Therefore the penetration enhancing effect could mainly be attributed to the alteration of skin properties by enhancers, which was further supported by the results of a second set of experiments.

The target permeation rates for glibenclamide and glipizide were calculated to be 193.8 and 184.8 $\mu\text{g}/\text{h}$ respectively [1]. The flux values obtained with the aid of enhancers indicate that the target permeation rates for both drugs can be achieved within an appreciable range of application area.

The present study shows that, ethanol alone (50–70% v/v) or in combination with a terpene can be used as solvent system in reservoir type transdermal systems and the terpene tested can be used alone as penetration enhancer in matrix type transdermal systems. In earlier studies 60–70% v/v ethanol was used in reservoir type transdermal systems of several drugs [24–26]. Based on these results, the development of matrix and reservoir type transdermal systems are under progress.

3. Experimental

Glibenclamide and glipizide were gifts from Modi-Mundi Pharma, India. Citral, geraniol, eugenol, *N*-methyl-2-pyrrolidinone (NMP) were purchased from Sigma Chemical Company, USA. Transcutol was generously supplied by Colorcon, Asia Pacific Pte Ltd., Singapore. All the other chemicals used were of reagent grade. Membrane for the permeability studies was the dorsal section of full thickness skin from Swiss albino mice, 6–8 weeks old, whose hair had been previously removed with an electric clipper.

3.1. *In vitro* skin permeation studies

In vitro skin permeation experiments were conducted as described earlier using vertical type diffusion cells having a receptor compartment capacity of 20 ml [1]. In brief, the excised skin was mounted on the diffusion cell and the receiver was filled with 20 ml of phosphate buffer (PB). Three ml drug suspension in PB, with or without 5% v/v penetration enhancer, was placed in the donor compartment and sealed with Parafilm[®]. To observe the effect of different concentrations of ethanol, the donor compartment contained 3 ml of drug suspension in PB and varying concentrations of ethanol ranging from 20–80% v/v. The sample solution was withdrawn from the receptor compartment at regular intervals and assayed. For geraniol, citral and eugenol, the donor compartment contained 3 ml drug suspension in PB containing 5% v/v terpene and 50% v/v ethanol. Fifty percent ethanol was used to solubilise the terpenes [27]. A permeation study with 50% v/v ethanol alone was also conducted. In order to confirm the permeation activity enhancers, a second set of experiments was conducted [2]. The skin was mounted on the diffusion cell. This time, 100 µl of the appropriate penetration enhancer alone was applied to the skin for 2 h. Subsequently the residual enhancer was removed from the skin and drugs were applied as aqueous enhancer free suspensions (3 ml). The experiment was then continued as in the first set. At the end of the permeation experiment, the amount of the drug retained in the skin was determined [28].

3.2. Solubility studies

An excess amount of drug was added to PB, with or without penetration enhancer. The solution was immersed in a shaking water bath and allowed to equilibrate. After 24 h, the saturated solution was assayed after appropriate dilution with PB [16].

In all the experiments, the drug concentration in aqueous solution was determined spectrophotometrically at a maximum wavelength of 300 and 274 nm for glibenclamide and glipizide respectively, after filtering through a 0.45-µm membrane filter (Nulge Nunc, UK). Statistical significance was analyzed by Student's *t*-test. Difference below the probability level 0.05 was considered to be statistically significant.

Acknowledgements: Authors are grateful to Council for Scientific and Industrial Research (CSIR), India for providing Senior Research Fellowship

to Mr. S. Mutalik. They are thankful to Modi-Mundi Pharma, India for supplying glibenclamide and glipizide as gift samples. They are also thankful to Ms. Chetana M and Ms. Sulochana B for help and cooperation.

References

- Mutalik, S.; Udupa, N.: *Pharmazie* **57**, 838 (2002)
- Ruland, A.; Kreuter, J.; Rytting, J. H.: *Int. J. Pharm.* **103**, 77 (1994)
- Panchagnula, R.; Ritschel, W. A.: *J. Pharm. Pharmacol.* **43**, 609 (1991)
- Mayorga, P.; Puisieux, F.; Couarraze, G.: *Int. J. Pharm.* **132**, 71 (1996)
- Zhao, K.; Singh, J.: *J. Pharm. Sci.* **6**, 771 (2000)
- Vaddi, H. K.; Wang, L. Z.; Ho, P. C.; Chan, S. Y.: *Int. J. Pharm.* **212**, 247 (2002)
- Godwin, D. A.; Michniak, B. B.; Player, M. R.; Sowell, J. W.: *Int. J. Pharm.* **155**, 241 (1997)
- Williams, A. C.; Barry, B. W.: *Int. J. Pharm.* **74**, 157 (1991)
- Moghim, H. R.; Williams, A. C.; Barry, B. W.: *J. Pharm. Pharmacol.* **50**, 955 (1998)
- Cornwell, P. A.; Barry, B. W.; Bouwstra, J. A.; Gooris, G. S.: *Int. J. Pharm.* **127**, 9 (1996)
- Morimoto, Y.; Wada, Y.; Seki, T.; Sugiyabashi, K.: *Biol. Pharm. Bull.* **1**, 134 (2002)
- Cornwell, P. A.; Barry, B. W.; in: Scott, R. C.; Guy, R. H.; Hadgraft, J.; Bodde, H. E. (Eds): *Prediction of percutaneous penetration: Methods, Measurements, Modelling*. p. 394, IBC Technical Services, London 1991
- Arellano, A.; Santoyo, S.; Martin, C.: *Ygartua: Int. J. Pharm.* **130**, 141 (1996)
- Obata, Y.; Takayama, K.; Maitani, Y.; Machida, Y.; Nagai, T.: *Biol. Pharm. Bull.* **16**, 312 (1993)
- Williams, A. C.; Barry, B. W.: *Crit. Rev. Ther. Drug Carrier Systems* **9**, 305 (1992)
- Kim, M. K.; Lee, C. H.; Kim, D. D.: *J. Pharm. Pharmacol.* **52**, 369 (2000)
- Berner, B.; Mazzenga, G. C.; Otte, J. H.; Steffens, R. J.; Juang, R. H.; Ebert, C. D.: *J. Pharm. Sci.* **78**, 402 (1989)
- Catz, P.; Friend, D. R.: *Int. J. Pharm.* **58**, 93 (1998)
- Ghaneum, A. H.; Mahmoud, H.; Higuchi, W. I.; Rohr, U. D.; Borsadia, S.; Liu, P.; Fox, J. L.; Good, W. R.: *J. Contr. Rel.* **6**, 75 (1987)
- Santoyo, S.; Ygartua, P.: *Eur. J. Pharm. Biopharm.* **50**, 245 (2000)
- Doliwa, A.; Santoyo, S.; Ygartua, P.: *Int. J. Pharm.* **229**, 37 (2001)
- Krishnaiah, Y. S. R.; Satanaarayana, V.; Karthikeyan, R. S.: *J. Pharm. Pharmaceut. Sci.* **2**, 124 (2002)
- Higuchi, T.: *J. Soc. Cosmetic. Chem.* **11**, 85 (1960)
- Altenburger, R.; Rohr, U. D.; Kissel, T.: *Pharm. Res.* **8**, 1238 (1998)
- Rohr, U. D.; Altenburger, R.; Kissel, T.: *Pharm. Res.* **6**, 877 (1998)
- Kim, M. K.; Zhao, H.; Lee, C. H.; Kim, D. D.: *Int. J. Pharm.* **219**, 51 (2001)
- Gao, S.; Singh J.: *Int. J. Pharm.* **154**, 67 (1997)
- Hashiguchi, T.; Yasutake, T.; Manako, T.; Otagiri, M.: *Int. J. Pharm.* **158**, 11 (1997)