Pharmaceutical Research, Vol. 12, No. 5, 1995

Report

Interaction Between Bile Salts and β-Adrenoceptor Antagonists

Martin P. Grosvenor[1,2,3] **and Jan-Erik Löfroth**[1]

Received July 25, 1994; accepted December 21, 1994

The interactions between β-adrenoceptor antagonists and bile salts were investigated by microcalorimetry. Nadolol and oxprenolol interactions with dihydroxy salts could be described by a 1:1 interaction model with the thermodynamic parameters indicating that the drugs were incorporated within the bile salt aggregates. This weak interaction was primarily hydrophobic although electrostatic attraction also played a role. Atenolol and metoprolol did not interact with the dihydroxy salts. None of the compounds interacted with trihydroxy bile salts or with salts below their aggregation concentration. Phase separation resulted when propranolol and alprenolol were present in dihydroxy salt solutions above a certain concentration with the interaction being of a hydrophobic and electrostatic nature. The implications of these results on *in-vivo* drug absorption are discussed.

KEY WORDS: microcalorimetry; calorimetry; bile; association; drugs.

INTRODUCTION

Bile principally consists of different bile salts, cholesterol, phosphatidylcholine and bilirubin. The bile salts contain a rigid ring system which is hydrophilic on the α-face and hydrophobic on the β-face. As a result they are surface active and above a certain concentration form aggregates (1). At low concentrations and salt contents, bile salts form primary aggregates of low aggregation numbers. Aggregation occurs since it is thermodynamically favorable to remove the non-polar parts of the bile salts from the aqueous environment by virtue of the hydrophobic interaction. Increasing the bile or salt concentration results in stepwise aggregation until at high concentrations of both species secondary aggregates are formed by association between the primary aggregates. The forces involved in this association are thought to be due to a combination of hydrogen bonding (1) and hydrophobic interaction (2) between the α-faces of the molecules.

There have been a number of studies as to the effect of surfactants, including bile salts, on G.I. drug absorption. Hydrophobic drugs have been shown to be absorbed more readily due to an increased solubility (3). Absorption of drugs can also be increased by a direct effect of bile salts on membrane permeability (4). On the contrary reduced absorption of drugs has been shown (5–9) which was thought to be due to the formation of a "stable" association complex with bile salts and loss of thermodynamic drug activity. We have investigated the effect of bile salts on ionized molecules using the β-adrenoceptor antagonists as model substances, to obtain information on the molecular characteristics necessary for such an interaction. Previous studies on this class of compounds (6,9) have shown that in general their *in-vivo* absorption has been reduced in the presence of bile although the opposite effect has been shown (11). Microcalorimetry has been used to measure this interaction, being a suitable technique since it does not disturb the equilibrium or introduce experimental artifacts.

MATERIALS AND METHODS

The β-adrenoceptor antagonists atenolol, nadolol hydrochloride and metoprolol tartrate were standard reference materials from Astra Hässle. Propranolol, alprenolol and oxprenolol hydrochlorides were purchased from Sigma Chemical Co., St Louis, USA. Their physicochemical characteristics are given in Table I. The bile salts were purchased from Sigma Chemical Co. as the sodium salts: taurochenodeoxycolate (TCD), glycochenodeoxycholate (GCD), deoxycholate (DC), taurocholate (TC) and glycocholate (GC). They were dried under vacuum at 25°C and stored over dried silica gel prior to use. Solutions of the bile salts and β-adrenoceptor antagonists were made in pH 6.5 10 mM phosphate buffer.

Surface tension measurements of the bile salts and β-adrenoceptor antagonists were carried out using the ring method (Kruss tensiometer K6, Kruss GmbH, Hamberg). Measurements were made at 295 ± 1K in phosphate buffer. The interaction was investigated using a microcalorimeter (model 2277, Thermometric AB, Järfälla, Sweden). 3 ± 0.01 ml aliquots of the buffered bile salt solutions were added to the ampoules of the perfusion/titration units. The buffered β-adrenoceptor antagonist solutions were injected into the ampoules at pre-determined intervals using the precision syringe injector. For comparative purposes 90 mM solutions of the β-adrenoceptor antagonists were used when the bile salts were above their aggregation onset concentration (AOC). The concentrations of bile salt solutions used were 5 mM (TCD, TD, GCD), 12 mM (TC) and 15 mM (GC). Heats of dilution of the bile salt and β-adrenoceptor antagonists were determined and accounted for.

THEORY

The interaction can be represented by

$$\mathrm{D} + \mathrm{BS} \Leftrightarrow \mathrm{DBS} \qquad (1)$$

where D is the β-adrenoceptor antagonist and BS is the bile salt existing as an aggregate. Eq. (1) describes a 1:1 interaction and assumes that there is no interaction between the drug and bile salt monomer. It has been found to satisfactorily describe the data obtained in this study without the need to resort to higher interaction orders. It does not assume any particular stoichiometry for the complex.

The equilibrium constant K is defined by

$$\frac{[\mathrm{DBS}]}{([\mathrm{D}]_t - [\mathrm{DBS}])([\mathrm{BS}]_t - [\mathrm{DBS}])} \qquad (2)$$

where [DBS] is the complex concentration and $[\mathrm{D}]_t$ and $[\mathrm{BS}]_t$ are the total antagonist and bile salt aggregate concentra-

---

[1] Drug Delivery Research, Astra Hässle AB, S-43183, Mölndal, Sweden.

[2] To whom correspondence should be addressed.

[3] Present address Elan Corporation plc, Athlone, Ireland.

Table I. Physicochemical Characteristics of the β-Adrenoceptor Antagonists[a]

Drug	M	pKa	Log P
Atenolol	266.3	9.3	0.23
Nadolol	309.4	9.7	0.71
Metoprolol	278.3	9.7	2.15
Oxprenolol	265.3	9.6	2.18
Alprenolol	249.3	9.6	2.61
Propranolol	259.3	9.5	3.65

[a] M is the molecular weight of the amine bases.

tions. The cumulative heat flow due to complex formation, Q, is given by Eq. (3)

$$Q = \Delta H \frac{[DBS]}{[BS]_t} \tag{3}$$

where ΔH is the reaction enthalpy. Elimination of [DBS] from Eq. (3), using Eq. (2) leads to

$$Q = \frac{\left([D]_t + [BS]_t + \frac{1}{K} - \left(\left([D]_t + [BS]_t + \frac{1}{K}\right)^2 - 4[D]_t[BS]_t\right)^{1/2}\right)\Delta H}{2[BS]_t} \tag{4}$$

Eq. (4) can be solved for the heat resulting from a series of injections by non-linear least-squares regression to yield values for the enthalpy and equilibrium constant.

RESULTS AND DISCUSSION

The AOC values from Table II were used in Eq. (4) to determine the bile salt monomer concentration present as aggregates. Aggregation numbers of 2 for GC and TC and 6 for the dihydroxy bile salts were used (1). Measurement of the surface tension of nadolol and propranolol showed that both compounds were weakly surface active. Whilst nadolol showed no self-association at concentrations up to 100 mM, propranolol formed aggregates at 80 mM. This value compared with 95 mM (water) (11) and 98 mM (30 mM salt, pH 4.5) (12). In addition metoprolol and oxprenolol are surface active (11). Thus all these β-adrenoceptor antagonists contain both hydrophobic and hydrophilic regions which if they are sufficiently large and separate would indicate that an interaction with bile salt aggregates could occur.

Atenolol was not incorporated into the aggregates of either the di- or tri-hydroxy bile salt aggregates as evidenced by a constant endothermic enthalpy per injection. This result was as expected since atenolol was the most hydrophilic β-adrenoceptor antagonist and would have a stronger tendency to remain within the aqueous phase than the hydrophobic environment of the bile salt aggregates. In addition the group at the aromatic ring 1-position is strongly hydrophilic and appeared to prevent insertion of the molecule within the aggregate, as also observed for metoprolol.

Yamaguchi (9) showed that 50 and 250 mM sodium cholate had no effect on the absorption of atenolol in an *in situ* rat study. It has, however, been shown (6) that the absorption of atenolol was reduced by 30% when co-administered with bile acids. As a consequence of our result, in which no interaction was found, the above result suggests that the reduced absorption could have occurred by an indirect mechanism with bile salts either releasing a third component which interacted with atenolol or altering the luminal/microvilli environment such that the formation of ionized atenolol was suppressed.

Table II. Aggregation Onset Concentrations of Different Bile Salts in pH 6.5 10 mM Phosphate Buffer at 295 K

Bile salt	Aggregation onset concentration (mM)
Taurochenodeoxycholate	0.3
Glycochenodeoxycholate	1.4
Taurodeoxycholate	2.8
Taurocholate	8
Glycocholate	9

The cumulative heat flows for the interactions between nadolol and dihydroxy bile salts are illustrated in Fig 1. The experimental data were fitted to Eq. (4) with the resultant thermodynamic parameters being given in Table III. The uncertainties of the fitting parameters were obtained from the diagonal of the error matrix. As the molar ratio of drug:bile salt was increased, the number of available interaction sites decreased, which resulted in a reduced exothermic enthalpy per injection. The observed enthalpy was considered to be due to changes in hydrogen bonding and van der Waals' forces. The entropy increase was thought to be due to two effects; the release of counterions, initially more or less associated with the charged aggregates, from the environment of the ionized molecules into the bulk and the release of hydration water around the charged species and non-polar regions.

Whereas the interaction with TCD and GCD at 298K and pH 6.5 was both enthalpically and entropically favorable that with TD was only enthalpically driven. There were clear trends in the enthalpy and entropy values resulting from the interaction which followed the hydrophobicity of the bile salts. However ΔG was negative and of a similar magnitude for the three salts indicating a common interaction mechanism and its enthalpy-entropy compensation (13,14). The

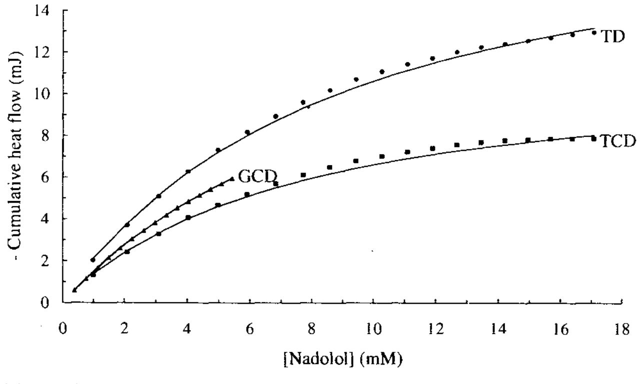

Fig 1. Cumulative Heat of Interaction between Nadolol and Dihydroxy Bile Salts (solid line is data fit to equation (4)).

Table III. Apparent Thermodynamic Data for the Interaction Between Nadolol and Dihydroxy Bile Salts[a]

Bile Salt	pH	T (K)	$-\Delta H$ (kJmol^{-1})	K (M^{-1})	$-\Delta G$ (kJmol^{-1})	ΔS (JK^{-1}mol^{-1})
TCD	6.5	298	5.75 ± 0.24	146 ± 10	12.35 ± 0.16	22.16 ± 0.28
			5.93 ± 0.25	130 ± 9	12.06 ± 0.17	20.58 ± 0.26
			5.09 ± 0.11	137 ± 5	12.19 ± 0.09	23.83 ± 0.07
TCD	10[b]	298	29.25 ± 0.80	191 ± 9	13.01 ± 0.11	−54.50 ± 2.31
			24.90 ± 1.37	193 ± 18	13.04 ± 0.22	−39.80 ± 4.61
TCD	6.5	310	21.35 ± 0.28	83 ± 2	11.39 ± 0.06	−32.12 ± 0.71
			23.65 ± 0.28	72 ± 1	11.02 ± 0.04	−40.73 ± 0.76
GCD	6.5	298	7.39 ± 0.10	84 ± 2	10.98 ± 0.06	12.05 ± 0.14
			6.46 ± 0.08	96 ± 2	11.31 ± 0.05	16.28 ± 0.11
			6.78 ± 0.12	92 ± 2	11.20 ± 0.06	14.83 ± 0.20
TD	6.5	298	23.10 ± 0.70	105 ± 5	11.53 ± 0.12	−38.81 ± 1.93
			21.02 ± 0.12	118 ± 6	11.82 ± 0.12	−30.89 ± 0.01
			26.47 ± 0.59	89 ± 3	11.12 ± 0.08	−51.50 ± 1.71

[a] 10 mM phosphate buffer.

[b] 10 mM carbonate buffer.

reason for the unfavorable entropy decrease for TD was unclear although it was the most hydrophilic of the dihydroxy bile salts and other cases exist where an interaction is solely enthalpy driven (14). The magnitude of the equilibrium constant was similar for both TD and GCD, although a stronger interaction was indicated with TCD. These results were also broadly in line with the bile salt hydrophobicities (Table II).

To confirm that the interaction was due to incorporation within the aggregates rather than on their surface, the interaction with TCD was studied at 310K. This resulted in a solely enthalpy driven reaction with $\Delta C_p = -1409$ JK^{-1}mol^{-1}. The negative value indicated the transfer of non-polar groups into a non-aqueous environment (15) which, in this case, was the aggregate interior. Its magnitude showed the extremely hydrophobic environment of the interior since ΔC_p for the transfer of benzene to β-cyclodextrin has been determined as only -268 JK^{-1}mol^{-1} (16). The reduction in equilibrium constant, which resulted from the relative changes in ΔH and ΔS, confirmed the hydrophobic nature of the interaction. The changes in ΔG, ΔS and ΔC_p were in agreement with other results e.g. transfer of liquid hydrocarbons to water (17) and incorporation of alkanols within cyclodextrin cavities (18).

At pH 10 the equilibrium constant increased indicating the formation of a more "stable" complex. At this pH the hydrophobicity of nadolol was greater than at pH 6.5 due to suppression of ionization. Since an unionized molecule could be inserted more completely within the aggregate than an ionized one, due to the absence of electrostatic attraction at the surface, a stronger complex would be expected. The increased equilibrium constant indicated that both hydrophobic and electrostatic contributions were important in the interaction although ionization of nadolol was not essential for incorporation within the aggregates.

In solutions <AOC a low, constant endothermic enthalpy per injection resulted indicating a weak interaction probably of an electrostatic nature such as ion-pair attraction (19). Nadolol was not solubilised within the trihydroxy bile salt aggregates as evidenced by a constant endothermic enthalpy per injection.

The hydrophobicity of oxprenolol and alprenolol was intermediate to that of nadolol and propranolol so effects common to those drugs would be expected (Fig 2). Alprenolol exhibited phase separation with the dihydroxy salts at drug:bile salt ratios of 0.6 (GCD), 0.8 (TCD) and 1.3 (TD) whilst oxprenolol did not produce separation.

Thermodynamic parameters resulting from the oxprenolol interaction are given in Table IV. The most striking observations were the large exothermic enthalpic contribution to the free energy term and the unfavourable entropy change. These values contrasted sharply to those for nadolol where the interaction was promoted by both entropic and enthalpic factors. Surprisingly oxprenolol interacted less strongly with TCD than nadolol since it would be expected that the more hydrophobic compound would have the greatest tendency to be incorporated within the aggregate. However, whilst log P values predict the overall hydrophilic/hydrophobic balance of a molecule they do not describe the size of these regions or whether they are sufficiently separate for large regions to have different properties. From our interpretation of the results, the presence of distinct hydrophilic and hydrophobic regions appeared to be a more important requirement for incorporation within the aggregates than the log P value of the molecule. No phase separation or incorporation was noted for either compound with the trihydroxy bile salts.

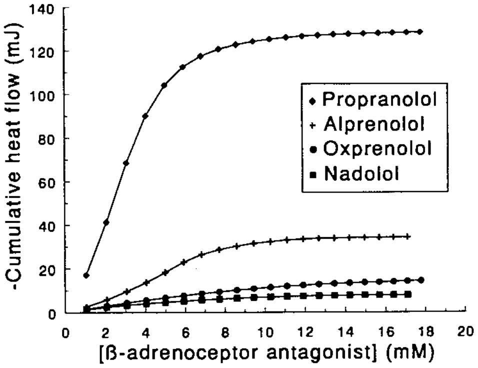

Fig 2. Cumulative Heat of Interaction between β-Adrenoceptor Antagonists and TCD.

Table IV. Apparent Thermodynamic Data for the Interaction Between Oxprenolol and TCD at 298K in pH 6.5 Phosphate Buffer

$-\Delta H$ ($kJmol^{-1}$)	K (M^{-1})	ΔG ($kJmol^{-1}$)	$-\Delta S$ ($JK^{-1}mol^{-1}$)
12.66 ± 0.51	76 ± 5	10.73 ± 0.16	−6.47 ± 1.18
14.15 ± 0.65	73 ± 5	10.63 ± 0.16	−11.83 ± 1.65

Metoprolol was not incorporated within either the di- or trihydroxy bile salt aggregates as shown by the constant endothermic enthalpy. In addition no phase separation occurred. Metoprolol has a similar log P value to oxprenolol which, however, exhibited phase separation in a TCD solution >AOC. Clearly attachment of the group at the aromatic ring 1-position prevented incorporation since it produced steric hindrance and a hydrophilic area within the hydrophobic region.

Phase separation occurred between propranolol and the bile salts at molar ratios given in Table V. The ratios followed the bile salt hydrophobicities indicating that a hydrophobic phase could be formed. Once the aqueous solubility of this phase had been exceeded, phase separation resulted. Addition of salt increased the molar ratio, probably by reducing the interaction between the charged groups through electrostatic screening. Increasing the pH to 8.3 produced a similar result by reducing the concentration of ionized species. It appeared that the interaction was initiated by electrostatic attraction between propranolol cations and bile salt anions with the hydrophobic effect being responsible for the subsequent phase separation.

For these systems Eqs. (1)–(4) were no longer applicable so that the data could not be interpreted in terms of the entropy and equilibrium constant. The plateau region of the cumulative heat flow curves (Fig 3) corresponded to the occupation of all interaction sites and occurred at similar propranolol concentrations for all bile salts indicating that the hydrophobicity of propranolol masked differences between the bile salt hydrophobicities. The interaction with GCD was less enthalpy driven than with TCD or TD, although it was unknown whether this would be compensated by an increased entropic contribution. The interaction heat flows were, however, ~10 times those observed for nadolol and were due to the growth in hydrophobic regions which would be expected to continue beyond the phase boundary. Comparison of Figs 1 and 3 showed that the plateau region occurred at a lower concentration of propranolol than nadolol. This indicated that incorporation of propranolol within the aggregates was favoured more than nadolol presumably due to its strongly hydrophobic aromatic ring system.

Table V. Molar Ratio of Propranol:Bile Salt Required for Phase Separation in pH 6.5 Phosphate Buffer

Bile Salt	Ionic Strength (mM)	Molar Ratio
TCD	10	0.34
	60[a]	0.46
	110[a]	0.57
	160[a]	0.62
	10[b]	0.63
GCD	10	0.32
TD	10	0.50
TC	10	1.14
GC	10	1.20

[a] buffer + 50, 100, 150 mM salt.
[b] pH 8.3 buffer.

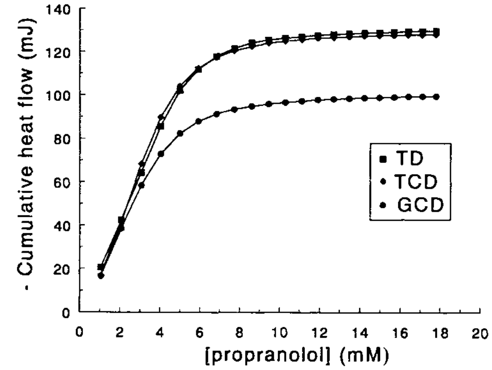

Fig 3. Cumulative Heat of Interaction between Propranolol and Dihydroxy Bile Salts.

This behaviour is typical of more general surfactant-surfactant systems in which oppositely charged species are present (20). In such systems at low salt concentrations, associative phase separation occurs which can be reduced or eliminated by increasing the salt content. Addition of an excess of either surfactant will lead to re-solubilization of the complex. This re-solubilization behaviour has not been observed for propranolol since its AOC is high and aggregation number low (12).

CONCLUSIONS

In the absence of an interaction, excluding that of an ion-pair nature, we suggest that bile salts would have no direct effects on the *in-vivo* absorption of β-adrenoceptor antagonists. Thus the absorption of atenolol and metoprolol should be unaffected by bile salts. However whilst nadolol and oxprenolol were incorporated into the dihydroxy aggregates it is unlikely that their absorption would be reduced since the complexes were weak and should dissociate fast enough to allow the free concentration of drug to be maintained during longitudinal transport in the intestine. A decreased absorption of propranolol and alprenolol, however, would be expected in the presence of bile if the molar ratio at which phase separation occurred was achieved. We suggest that whilst the first pass effect is the primary cause of the low bioavailability of propranolol the interaction with bile may also exert an influence on its absorption under such conditions.

It is our view that other basic drugs, if they contain a hydrophobic region sufficiently large and separate from a hydrophilic one, will interact with bile salts, particularly the dihydroxy ones, in a similar way. The strength of this association will determine whether the drug's *in-vivo* absorption is reduced.

ACKNOWLEDGMENTS

Financial support of MPG by Astra Hässle is acknowledged.

REFERENCES

1. D. M. Small. Size and Structure of Bile Salt Micelles. *Adv. Chem. Ser.* 84:31-52 (1968).
2. N. A. Mazer, M. C. Carey, R. F. Kwasnick and G. B. Benedek. Quasielastic Light-Scattering Studies of Aqueous Biliary Lipid Systems. Size, Shape and Thermodynamics of Bile Salt Micelles. *Biochemistry* 18:3064-3075 (1979).
3. T. R. Bates, M. Gibaldi and J. L. Kanig. *Nature* 210:1331-1333 (1966).
4. M. Gibaldi and S. Feldman. Mechanisms of Surfactant Effects on Drug Absorption. *J. Pharm. Sci.* 59:579-589 (1970).
5. W. W. Faloon, I. C. Paes, D. Woolfolk, H. Nankin, K. Wallace and E. N. Haro. Effect of Neomycin and Kanamycin upon Intestinal Absorption. *Ann. N. Y. Acad. Soc.* 132:879-887 (1966).
6. S. G. Barnwell, T. Laudanski, M. Dwyer, M. J. Story, P. Guard, S. Cole and D. Attwood. Reduced Bioavailability of Atenolol in Man: the Role of Bile Acids. *Int. J. Pharm.* 89:245-250 (1993).
7. F. G. Poelma, R. Breäs and J. J. Tukker. Intestinal Absorption of Drugs. III. The Influence of Taurocholate on the Disappearance Kinetics of Hydrophilic and Lipophilic Drugs from the Small Intestine of the Rat. *Pharm. Res.* 7:392-397 (1990).
8. T. Kimura, K. I. Inui and H. Sezaki. Differences in Effects on Drug Absorption between Dihydroxy and Trihydroxy Bile Salts. *J. Pharmacobio.-Dyn.* 8:578-585 (1985).
9. T. Yamaguchi, C. Ikeda and Y. Sekine. Intestinal Absorption of a β-adrenergic Blocking Agent Nadolol. I. Comparison of Absorption Behaviour of Nadolol with those of other β-Blocking Agents in Rats. *Chem. Pharm. Bull.* 34:3362-3369 (1986).
10. M. R. Gasco, M. Trotta and M. Eandi. The Influence of Bile Salts on the Absorption *in vitro* and *in vivo* of Propranolol. *J. Pharm. Biomed. Anal.* 2:425-439 (1984).
11. D. Attwood and S. P. Agarwal. The Surface Activity and Self-Association of Some β-Adrenoceptor Blocking Agents in Aqueous Solution. *J. Pharm. Pharmacol.* 31:392-395 (1979).
12. D. N. Elliott, P. H. Elworthy and D. Attwood. An Investigation of the Properties of Propranolol Hydrochloride in Aqueous Solution. *J. Pharm. Pharmacol.* 25:118P (1973).
13. R. Lumry and S. Rajender. Enthalpy-Entropy Compensation Phenomena in Water Solutions of Proteins and Small Molecules: a Ubiquitous Property of Water. *Biopolymers* 9:1125-1227 (1970).
14. P. Gilli, V. Ferretti, G. Gilli and P. A. Borea. Enthalpy-Entropy Compensation in Drug-Receptor Binding. *J. Phys. Chem.* 98: 1515-1518 (1994).
15. N. Nichols, R. Sköld, C. Spink and I. Wadsö. Thermochemistry of Solutions of Biochemical Model Compounds. 6. Alpha- and Omega-dicarboxylic acids, diamines and diols in Aqueous Solution. *J. Chem. Thermodyn.* 8:993-999 (1976).
16. I. Gomez-Orellana and D. Hallen. The Thermodynamics of the Binding of Benzene to β-Cyclodextrin in Aqueous Solution. *Thermochimica Acta* 221:183-193 (1993).
17. D. Hallen, A. Schön, I. Shehatta and I. Wadsö. Microcalorimetric Titration of α-Cyclodextrin with some Straight-chain Alkanols at 288.15, 298.15 and 308.15 K. *J. Chem. Soc. Faraday Trans.* 88:2859-2863 (1992).
18. P. L. Privalov and S. J. Gill. The Hydrophobic Effect: A Reappraisal. *Pure & Appl. Chem.* 61:1097-1104 (1989).
19. E. L. Tan, J. C. Liu and Y. W. Chien. Characterization of Ion-Pair Formation between Ionized Indomethacin and Cationic Surfactants. *J. Disp. Sci. Technol.* 14:609-624 (1993).
20. B. Lindman, A. Khan, E. Marques, M. Miguel, L. Piculell and K. Thalberg. Phase Behaviour of Polymer-Surfactant Systems in Relation to Polymer-Polymer and Surfactant-Surfactant Mixtures. *Pure & Appl. Chem.* 65:953-958 (1993).