Prediction of a Stable Microemulsion Formulation for the Oral Delivery of a Combination of Antitubercular Drugs Using ANN Methodology

Snezana Agatonovic-Kustrin,[1,5] Beverley D. Glass,[2] Michael H. Wisch,[3] and Raid G. Alany[4]

Received March 20, 2003; accepted July 9, 2003

Purpose. The aim of this project was to develop a colloidal dosage form for the oral delivery of rifampicin and isoniazid in combination with the aid of artificial neural network (ANN) data modeling.
Methods. Data from the 20 pseudoternary phase triangles containing miglyol 812 as the oil component and a mixture of surfactants or a surfactant/cosurfactant blend were used to train, test, and validate the ANN model. The weight ratios of individual components were correlated with the observed phase behavior using radial basis function (RBF) network architecture. The criterion for judging the best model was the percentage success of the model prediction.
Results. The best model successfully predicted the microemulsion region as well as the coarse emulsion region but failed to predict the multiphase liquid crystalline phase for cosurfactant-free systems indicating the difference in microemulsion behavior on dilution with water.
Conclusions. A novel microemulsion formulation capable of delivering rifampicin and isoniazid in combination was created to allow for their differences in solubility and potential for chemical reaction. The developed model allowed better understanding of the process of microemulsion formation and stability within pseudoternary colloidal systems.

KEY WORDS: rifampicin; isoniazid; ANN; microemulsion formulation.

INTRODUCTION

In 1993 the World Health Organization (WHO) declared tuberculosis (TB) to be a global emergency. The severity of the TB epidemic is such that it kills more youth and adults in the world than any other infectious disease—2 to 3 million people every year, including at least 100,000 children (1). The successful treatment [Directly Observed Treatment System (DOTS) Program] of TB during the continuation phase involves a combination of rifampicin (RIF) and isoniazid (INH) (2). Although adult combination formulations are available, pediatric treatment remains a challenge in terms of compliance, currently involving dosing the children with RIF syrup and INH elixir or, alternatively, using tablets and mixing them with commercially available syrups (3). The differing physicochemical properties of these two drugs, especially in terms of solubility and their reduced stability in combination because of chemical interactions, prompts the need for a formulation that separates these two drugs into two different phases, overcoming both solubility and stability issues. A desired target concentration of these two drugs is 150 mg/15 ml for RIF and 100 mg/15 ml for INH in order to comply with the required dosage regimen in the DOTS program.

Microemulsions, with their long-term stability, low viscosity, transparent and elegant appearance, and ease of preparation, have received recent interest as attractive drug delivery systems. Because of the presence of oil and water as part of the structure, these systems may serve as vehicles for drugs of different solubilities in combination (4,5) and therefore be suitable for the combination of RIF and INH with solubilities in water of 1.82 mg/ml. and 128.8 mg/ml, respectively, stabilized either by surfactant(s) or a surfactant/cosurfactant combination.

The main problem in microemulsion formulations is to identify surfactant combinations that would produce a thermodynamically stable formulation. Thus, it is essential to establish the phase behavior of the particular combination of chosen components. Despite the geometric packing theory and the accumulation of experimental data on microemulsions, their formulation is still highly empirical and time consuming (6,7). Full characterization of such systems is a tedious task and requires a large number of experiments. Accordingly, the aim of the present study was to develop a model to predict and explain phase behavior of complex colloidal systems and thus minimize the required experimental effort.

MATERIALS AND METHODS

Instrumentation and Equipment

A vortex mixer made by Gemmy Industrial Corporation (Taiwan, R.O.C.) was used to homogenize the surfactants, lipid, and aqueous phases. A 1-ml autopipette manufactured by Eppendorf (Germany) was used to pipette all aqueous fractions.

MS-Windows–based ANNs simulator software, Statistica Neural Networks 0.0F (StatSoft Inc., Tulsa, OK) was used to develop a predictive model.

Solvents and Reagents

Cremophor RH (polyoxyl 40 hydrogenated castor oil) and sorbitol were donated by the Druggists Group Research Unit (Pharmacare-Lennon, RSA). Miglyol 812 (caprylic/capric acid triglycerides), imwitor 308 and imwitor 742 (glyceryl monostearate and glycerol mono- and dicaprylate/caprate, respectively) were donated by Hüls (South Africa), brij 97 [polyoxyethylene (10) oleyl ether] was purchased from Sigma (St. Louis, MO), and crillet 3 [polyoxyethylene(20)sorbitane monostearate] donated by Croda (South Africa). All aqueous fractions were prepared using double-distilled deionized water obtained from a Milli-RO15 water purification system, manufactured by Millipore (Woburn, MA).

Artificial Neural Network

ANNs are computer programs designed to simulate the way in which the human brain processes information. ANNs

[1] School of Pharmaceutical, Molecular and Biomedical Science, University of South Australia, North Terrace, Adelaide 5000, Australia.
[2] School of Pharmacy and Molecular Sciences, James Cook University, Townsville, Queensland 4811, Australia.
[3] Department of Chemistry, Rhodes University, Grahamstown 6140, South Africa.
[4] Division of Pharmacy, The University of Auckland, Auckland, New Zealand.
[5] To whom correspondence should be addressed. (email: nena.kustrin@unisa.edu.au)

0724-8741/03/1100-1760/0 © 2003 Plenum Publishing Corporation

learn (or are trained) through experience with appropriate learning exemplars, not from a preprogrammed set of rules. The behavior of a neural network is determined by the transfer functions of its neurons, by the learning rule, and by the architecture itself. The two most commonly used neural network architectures are multilayer perceptrons (MLP) and radial basis function networks (RBF). The only difference is the way in which hidden units combine values coming from preceding layers in the network. An MLP models the response function using the composition of sigmoid functions. A radial basis function network (RBF) (8) has a hidden layer of radial units, each modeling a Gaussian bell-shaped response surface, peaked at the center and descending outwards. Detailed descriptions of the ANN models have been published (9–13).

Methodology

Microemulsions are graphically represented as stability areas in triangular phase diagrams (14), where each triangular corner designates a certain component. Pseudoternary phase diagrams were constructed by titrating a series of mixtures of lipid (miglyol 812) and surfactants or surfactant/cosurfactant, with water at room temperature. Surfactants or surfactant/cosurfactant ratios of 9:1, 7:3, 5:5, 3:7, and 1:9 were prepared. Water was added to each fraction in 2.5% w/w portions, followed by agitation on a vortex mixer with the mixture allowed to stand and stabilize. The phases formed were visually assessed after each addition, and only stable single-phase regions were recorded and the ternary and pseudoternary phase diagrams mapped. Phases were classified as isotropic, liquid crystalline, or coarse emulsion. Isotropic regions in each phase diagram that are transparent fluid microemulsions and mixed micellar solution regions in areas of low oil or low water composition are labeled as ME. Liquid crystalline regions are designated as LC, being defined as birefringent gel-like phases, tending to be regions of transition between high-lipid- and high-water-content phases. Coarse emulsions that proved stable with time are designated by EM. The phase diagrams were constructed with the top apex representing 100% water content, the left apex representing 100% surfactant/cosurfactant, and the right apex representing 100% lipid.

Analyzed samples representing the 20 different phase diagrams provided 4680 input-output data sets for the ANN. Each sample was labeled according to the proportions of surfactant blend, HLB, oil, and water in the mixture and matched with the nature of the phase structure found for that composition. Samples were coded as +1 to signify the presence of a particular system and –1 to indicate its absence. Thus, a region consisting of ME would have values of +1, –1, –1; pure LC was –1, +1, –1; EM was –1, –1, +1; whereas two phases consisting of LC and EM were categorized as –1, +1, +1. A perfectly trained network should recover such values for perfect phase classification. The HLB number, percentage of oil, water, and surfactants or surfactant/cosurfactant blend were used as the inputs for the ANN. The outputs were the three different outcomes: ME, LC, and EM.

Samples having HLB values of 9.25, 11.55, and 13.85, obtained with different ratios of imwitor 742 and crillet 3 (5:5, 3:7, 1:9), were not used during the model development but were used to validate the developed model and evaluate its prediction abilities. The rest of the data were used as a working data set. Before each training run, a working data set was randomly divided into training (80%) and testing (20%) data sets. During training, the performance of the ANN was evaluated with a testing data set. The training set was used to train the network, and the testing set was used to determine the level of generalization produced by the training set and to monitor overtraining of the network, each with corresponding root mean squared (RMS) error. For an unbiased estimate of the generalization error, the ANN was presented with a validation data set that was not used at all during the training process.

RESULTS AND DISCUSSION

The initial goal of this study was to map as many microemulsion systems as possible (Table I). This had to be limited because of the number of combinations, given the components at hand. The triglyceride oil miglyol 812 has proven effective as the dispersed phase in microemulsion formulations (15) and was selected as the lipid of choice in the phase mapping because of the solubility profiles of RIF (10.76 mg/ml) and INH (0.32 mg/ml) and for its accepted use in oral pharmaceutical preparations. The selection of the surfactants to be used to identify microemulsion regions was the criterion that was given the most attention. All the surfactants used in this study were nonionic in nature, used for their lack of toxicity and predisposition toward the formation of stable emulsion systems. The systems mapped indicated the necessity of a cosurfactant to stabilize the interfacial film and to introduce the degree of flexibility required for microemulsification to occur with the exceptions of imwitor 308 and imwitor 742 in a combination with crillet 3, which could form microemulsion regions without the need for a cosurfactant.

The synergistic effect of these two agents is displayed in the pseudoternary phase diagrams constructed in Fig. 1. The combined effect of the two surfactants is manifested by a large area of existence of the isotropic region ME. The size of the region increases as the crillet 3 composition increases, with the largest and most dilatable microemulsion region in

Table I. Systems Used for ANNs Training with Miglyol 812 as Lipid

Surfactants (HLB)	Mixing ratio	HLB number
Cremophor RH (15) + sorbitol (15.5)	9:1	15.05
	7:3	15.11
	5:5	15.20
	3:7	15.31
	1:9	15.43
Inwitor 308 (6) + crillet 3 (14.9)	9:1	6.90
	7:3	8.70
	5:5	10.50
	3:7	12.30
	1:9	15.30
Inwitor 742 (3.5) + crillet 3 (14.9)	9:1	4.65
	7:3	6.95
	5:5	9.25
	3:7	11.55
	1:9	13.85
Brij 97 (11.4) + sorbitol (15.5)	9:1	11.70
	7:3	12.35
	5:5	13.09
	3:7	13.94
	1:9	14.93

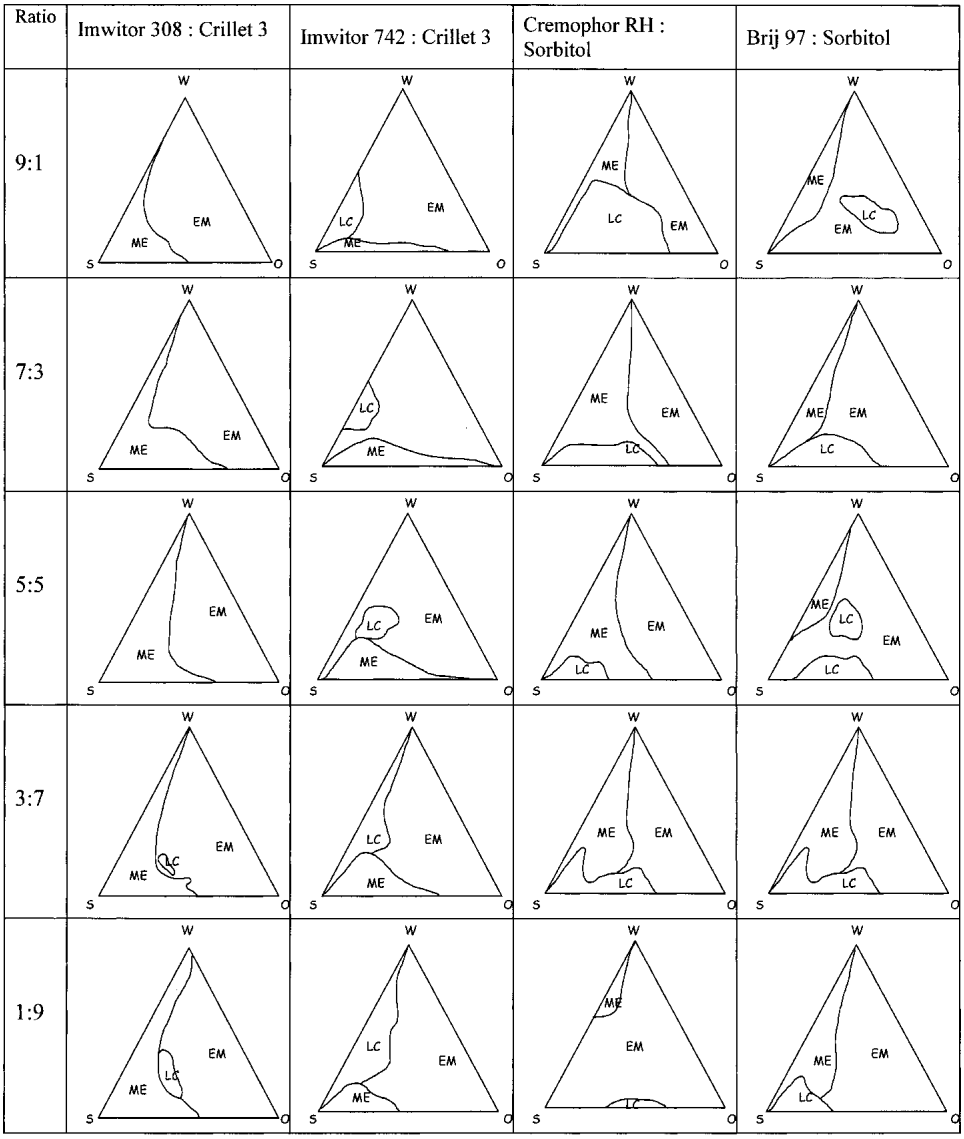

Fig. 1. Pseudoternary phase diagrams.

5:5 ratio capable of solubilizing up to 25–30% miglyol 812. The liquid crystalline region is small, only emerging at a high crillet 3 content. The usefulness of a lecithin/alcohol combination as surfactant/cosurfactant combination has been also investigated. It forms a microemulsion over a range of low to high surfactant and lipid content; however, dilutability proved to be a problem. Microemulsions that incorporate a cosurfactant into the interfacial film tend to be unstable in that they are destroyed on dilution. The cosurfactant partitions into the interfacial film, the water, and oil phases; hence, on dilution, the cosurfactant leaves the interface, entering the continuous phase to restore the equilibrium, which destroys the film. This was particularly demonstrated for the ovothin 200 and imwitor 742 microemulsions produced. In the case of ovothin:ethanol systems, a transparent isotropic region was formed at high ovothin composition relative to ethanol. Decreasing the ovothin content resulted in a reduced lipid-solubilizing capacity. Ovothin on its own is too lipophilic to form a stable microemulsion, tending to form lamellar phases and bilayers over a limited range. A liquid crystalline region emerged at low water content, decreasing in size as the ethanol content increased. The maximum miglyol 812 incorporated into the microemulsion of 15% occurred at the high ovothin:ethanol composition (9:1). Another negative feature is the use of ethanol as a cosurfactant, with limitations placed on its use in oral pharmaceutical preparations for a pediatric population, restricting the application of this lecithin system. Lecithin, being a surfactant of natural origin, is unstable itself, with oxidation of the acyl chains and hydrolysis of the polar head a problem from a formulation stability point of view (16).

Data obtained from the 20 phase diagrams (Fig. 1) were fed to the ANN, and the network structure was optimized, comparing the predictions obtained from several high-scoring ANN models (Table II). The criterion for judging the best model was the percentage success of the model prediction. The best model was the RBF network with a hidden layer of 100 neurons, thus forming 4-100-3 network architectures. The model successfully regenerated the EM region and correctly classified data points in the ME region. However, it failed to predict LC phase, with most of the mistakes confined to the

Table II. Performance of the Network Sets

Network type	Number of data sets	Inputs	Hidden units	Root mean square (RMS) error		
				Training	Testing	Verification
RBF	4860	4	149	0.622	0.627	0.663
RBF	4680	4	118	0.424	0.504	0.778
RBF[a]	4680	4	100	0.639	0.635	0.597
RBF	4680	4	90	0.435	0.512	0.799
RBF	4680	4	78	0.438	0.516	0.774
RBF	2340	4	118	0.424	0.504	0.778
RBF	2340	4	108	0.417	0.506	0.796
RBF	2340	4	104	0.417	0.506	0.788
RBF	2340	4	90	0.434	0.512	0.799
RBF[a]	2340	4	78	0.455	0.510	0.778

[a] Selected network model.

ME-LC boundary. This could be because of the insufficient training data sets from the LC region. Also, the liquid crystalline regions were small in comparison to microemulsion or coarse emulsion regions, and the network was unable to learn the pattern from limited data points. Moreover, the LC region is a multiphase region (Fig. 2), tending to be a region of transition between high lipid and high water content phases. In order to further evaluate the usefulness of ANN in building the model and understand why it fails to predict the LC region for the cosurfactant-free system, we developed a second model based only on data from the ternary phase diagrams with imwitor 308 or imwitor 742 and crillet 3 surfactant blends. HLB values of 6.95, 9.25, and 11.55 were used to validate the model. The best RBF model had 4-78-3 network architecture (Table III). The system again predicted the presence of a small LC region for only the higher HLB values. It became clear that the initial classification of phase behavior might be wrong or that the HLB value used to describe surfactant/cosurfactant properties may not be sufficient in predicting microemulsion stability and phase behavior. From a microstructure perspective, a ME may have high water content, in which water is the continuous medium [oil-in-water (o/w) ME] or have high lipid content in which oil is the continuous medium [water-in-oil (w/o) ME], and finally there is water and oil bicontinuous ME, in which almost equal amounts of water and oil exist (17). The type of emulsion formed as well as the transition from o/w to w/o ME is dependent on the phase ratio of the aqueous and lipid phases, together with the type, concentration, and preferred solubility of the emulsifying agents used. Transition can be accomplished via bicontinuous ME or via the LC phase (Fig. 3) (18). When added to a three-component system (oil–surfactant–water), cosurfactants induce the formation of both w/o and o/w microemulsions, destabilize the liquid-crystalline phase, and extend the isotropic region to higher surfactant concentrations. The transition from a w/o microemulsion into an o/w microemulsion happens gradually and continuously (bicontinuous phase) without any phase separation. For the cosurfactant-free systems having a mixture of surfactants, transition occurs through the LC phase (19). In the absence of a cosurfactant, a lower amount of water could be solubilized by the ME formed by the surfactant blend and the oil, suggesting the formation of a w/o microemulsion. The major advantage of these ME systems is their low aqueous resistance to dilution and ability to be diluted without loss of integrity. On dilution with water this w/o ME system undergoes a phase transition into highly ordered LC systems and forms lamellar liquid crystals, typical of a lipophilic surfactant blend favoring long-range ordered packing. The formation of a lamellar liquid crystalline phase in the case of surfactant/cosurfactant blend was prevented by the inclusion of the cosurfactant. Incorporation of sorbitol as cosurfactant in a system containing cremophor RH or brij 97 inhibits the tendency of the cosurfactant-free system to form ordered LC systems and promotes the formation of balanced ME. Hence, elucidation of the microstructure and evaluation of the rheologic properties of the reported colloidal systems as well as their physiologic compatibility should be addressed.

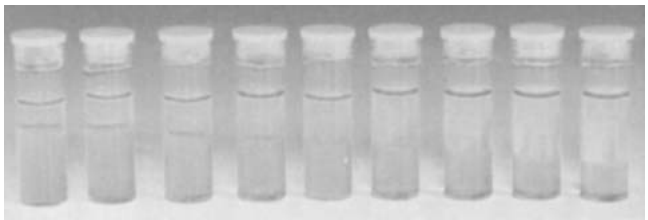

Fig. 2. Multiphase microemulsion (upper phase) and lamellar liquid crystalline (lower phase) systems from the LC region of the system.

The visual assessment of the stability of the potential formulations was assessed by observing homogeneity. Any sign of phase separation or cracking would not allow the formulation to maintain integrity over the length of the stability study. The formulations were observed as being stable or not at 25°C and 60°C over a 2-week period. Systems with imwitor 308 and crillet 3 as surfactant combination proved to be the most successful, incorporating 25–30% miglyol 812, an intermediate quantity of surfactant and maintaining homogeneity on dilution. The effect of the more lipophilic imwitor 742 relative to imwitor 308 was demonstrated by the reduced ability of the imwitor 742 system to produce microemulsion regions incorporating sufficient lipid to act as a lipophilic drug carrier that is dilutable. The composition of this stable formulation was water (21.06%), miglyol 812 (23.68%), imwitor 308 (27.63%), and crillet 3 (27.63%). This formulation maintained homogeneity, with no color or appearance changes over the period of assessment, remaining a clear liquid o/w microemulsion, and maintained integrity on excess dilution with water. The formulations produced for this stability study remained physically stable for the entire 10-week trial period,

Table III. Success of the RBF Models in Characterizing the Phase Behavior

Number of data sets	HLB	Predicted phase (%)			Observed phase (%)		
		ME	LC	EM	ME	LC	EM
4680	9.25	29	0	71	27	8	65
	11.55	35	0	65	27	15	58
	13.85	36	2	61	14	23	63
2340	6.95	39	0	63	28	8	67
	9.25	40	0	61	27	13	61
	11.55	16	18	64	14	23	63

maintaining homogeneity and clarity at all temperatures and conditions.

The composition of the final formulation—water (21.06%), miglyol 812 (23.68%), imwitor 308 (27.63%), and crillet 3 (27.63%)—included rifampicin 150 mg/ml and isoniazid 100 mg/ml. RIF stability was improved, as anticipated, by its being isolated in the internal lipid phase of the o/w microemulsion. Solubilization of the RIF in the lipid droplets and lipophilic chains of the surfactants comprising the surfactant/cosurfactant interfacial film surrounding the lipid droplets increases the quantity of RIF that can be incorporated into the formulation while protecting it from oxidative degradation to which it is sensitive. The incorporation of RIF into this internal phase also decreases its contact with INH and drug–drug interaction. The microemulsion used in the formulation comprised a high surfactant content (>50%), water, and miglyol 812, permitting the dilution of this o/w microemulsion, although at high water content, on dilution, the system probably reverts to a mixed micellar solution, with the lipid and water content allowing both separation and solubilization of RIF and INH in combination resulting in an acceptable formulation. The formulation maintained homogeneity, with no color or appearance changes over the period of assessment, remained a clear liquid o/w microemulsion, and maintained integrity on excess dilution with water. Preliminary stability results indicate that RIF retained an adequate therapeutic concentration in the formulation of over 90% at 25°C for 6 weeks.

The developed ANN model clearly suggested higher stability of microemulsion regions for the cosurfactant-free system and provided valuable information into possible future improvements of the microemulsion formulation.

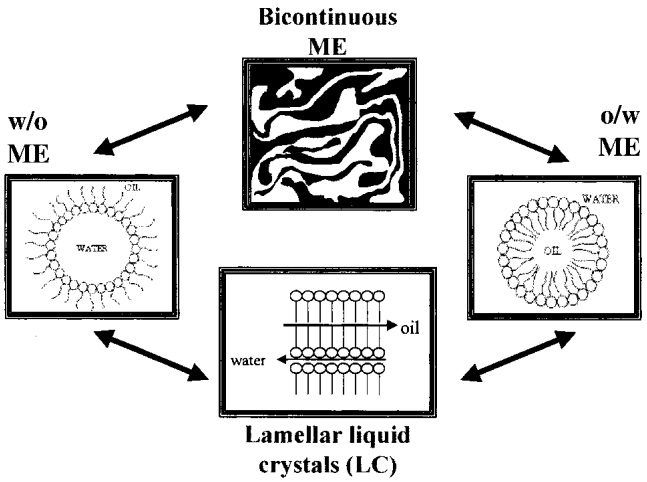

Fig. 3. Possible phase transitions in microemulsion systems.

CONCLUSION

The results of this study suggest the formulation of a ME capable of delivering rifampicin and isoniazid at the desired target concentration for the treatment of children during the continuation phase of tuberculosis. This formulation thus provides an answer to the problems of combining two drugs with different solubilities, and because of their separation into the oil and water phases, an improved stability is predicted. ANN modeling allowed better understanding of the process of microemulsion formation and stability within ternary and pseudoternary colloidal systems. This type of methodology can be applied to evaluation of the surfactant/cosurfactants for pharmaceutical formulations to minimize experimental effort.

ACKNOWLEDGMENTS

The authors wish to acknowledge the financial support of the Universities of South Australia, James Cook University and Rhodes University, the National Research Foundation (NRF), South Africa, and Pharmacare-Lennon, Port Elizabeth, South Africa. The authors also wish to recognize the contribution of Drs. Matthew Worthington, Darryl Whittaker, and Lawrence Penkler of Pharmacare-Lennon for their input into the choice of lipids and surfactant/cosurfactant mixtures.

REFERENCES

1. *World Health Organisation TB Report 1998*, World Health Organisation Press Office, Geneva, 1998.
2. *South African Medicines Formulary (4th ed.)*, Department of Pharmacology, UCT, Pioneer Press, 1997, pp. 260–270.
3. MIMS. Medical Specialities, Times Media. *Pretoria* **39**:289–293 (1999).
4. M. J. Lawrence. Microemulsions as drug delivery vehicles. *Curr. Opin. Colloid Interface Sci.* **1**:826–832 (1996).
5. M. Malmsten. Microemulsions in pharmaceuticals. In P. Kumar, K. L. Mittal (eds.), *Handbook of Microemulsion Science and Technology*, Marcel Dekker, New York, 1999.
6. D. Attwood and G. Ktistis. A light scattering study on oil in water microemulsions. *Int. J. Pharm.* **52**:165–171 (1989).
7. P. P. Constantinides and P. J. Scalart. Formulation and physical characterization of water in oil microemulsions containing long- versus medium-chain glycerides. *Int. J. Pharm.* **158**:57–68 (1997).
8. J. Moody and C. J. Darkin. Fast learning in networks of locally-tuned processing units. *Neural Comput.* **1**:281–294 (1989).
9. S. Haykin. *Neural Networks: A Comprehensive Foundation.* Macmillan, New York, 1994.

10. C. Bishop. *Neural Networks for Pattern Recognition*. Oxford University Press, Oxford, 1995.
11. M. Egmont-Petersen, J. L. Talmon, and A. Hasman. Assessing the importance of features for multi-layer perceptrons. *Neural Networks* **11**:623–635 (1998).
12. G. Bologna, and C. Pellegrini. Three medical examples in neural network rule extraction. *Phys. Med.* **13**:183–187 (1997).
13. T. F. Rathbun, S. K. Rogers, and M. P. DeSimio. MLP iterative construction algorithm. *Neurocomputing* **17**:195–216 (1997).
14. J. Kreuter. *Colloidal Drug Delivery Systems*, Marcel Dekker, NewYork, 1994.
15. R. Aboofazelia, N. Patela, M. Thomasb, and M. J. Lawrencea. Investigations into the ormation and characterization of phospholipid microemulsions. IV. Pseudo-ternary phase diagrams of systems containing water-lecithin-alcohol and oil; the influence of oil. *Int. J. Pharm.* **125**:107–116 (1995).
16. M. Trotta, M. Gallarate, F. Pattarino, and M. E. Carlotti. Investigation of the phase behaviour of systems containing lecithin and 2-acyl lysolecithin derivatives. *Int. J. Pharm.* **190**:83–89 (1999).
17. D. Attwood. Microemulsions. In H. Kreuter (ed.), *Colloidal Drug Delivery Systems*, Marcel Decker, New York, 1994, p. 31.
18. R. G. Alany, T. Rades, S. Agatonovic-Kustrin, N. G. Davies, and I. G. Tucker. Effect of alcohols and diols on the phase behaviour of quaternary systems. *Int. J. Pharm.* **196**:141–145 (2000).
19. R. G. Alany, N. G. Davies, I. G. Tucker, and T. Rades. Characterising colloidal structures of pseudoternary phase diagrams formed by oil/water/amphiphile systems. *Drug Dev. Ind. Pharmacy* **27**:31–38 (2001).