ELSEVIER

International Journal of Pharmaceutics 184 (1999) 107–114

international journal of pharmaceutics

Powder diffractometric assay of two polymorphic forms of ranitidine hydrochloride

S. Agatonovic-Kustrin *, V. Wu, T. Rades [1], D. Saville, I.G. Tucker

School of Pharmacy, University of Otago, P.O. Box 913, Dunedin, New Zealand

Received 18 November 1998; received in revised form 22 March 1999; accepted 23 March 1999

Abstract

A simple X-ray powder diffractometric method was developed for the qualitative and quantitative assay of the two crystalline modifications of ranitidine-HCl. The main purpose of the present work was to investigate if artificial neural networks (ANNs) could be applied in quantitative X-ray diffractometric analyses. The ANN approach was compared with a conventional mixture design method. The results obtained by the ANN had a smaller standard deviation and relative error and a better precision at lower concentrations. ANNs provide a simple alternative to conventional statistical modelling methods to identify the non-linear relationship without complex equations. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Ranitidine hydrochloride; Polymorphism; Artificial neural network; X-ray powder diffraction

1. Introduction

Polymorphs are different crystalline forms of a drug that may have different physico-chemical properties and biological activities. Since pharmaceuticals, at some stage during the manufacturing process, are organic crystalline materials, polymorphism may affect these products during new drug development and formulation. The existence of different crystal forms impacts on key properties such as shelf-life, vapor pressure, solubility, bioavailability, morphology and density. It is vital to select the polymorph with the preferred properties, and predict problems such as the unwanted crystallisation of other polymorphs. This knowledge is also important for patenting and registration purposes.

X-ray diffractometry (XRD) is a powerful technique for characterising pharmaceutical solids and is widely used for the identification of crystalline solid phases and offers a unique advantage in the quantitative analysis of mixtures (Bartolomei et al., 1997). The X-ray diffractogram of every crystalline form of a compound is unique, making this technique particularly useful for the identification of different polymorphs of a drug. If there is a mixture of crystalline solids, each of these compo-

* Corresponding author.

E-mail address: nena.kustrin@stonebow.otago.ac.nz (S. Agatonovic-Kustrin)

[1] Present address: F. Hoffmann-La Roche, PRNF, 4070 Basel, Switzerland.

PII: S0378-5173(99)00104-0

nents will have a different pattern, independent of the other component in a mixture, making independent analysis feasible. Quantitative analysis of polymorphs from their powder mixture usually requires that at least one high-intensity peak unique to each form is available for intensity measurements. A plot of peak intensity ratio as a function of the weight ratio of the components should result in a straight line (Klug and Alexander, 1974) and has been successfully applied for the quantification of pharmaceutical polymorphs (Doff et al., 1986; Chao and Vail, 1987; Suranarayanan, 1990). Organic compounds show numerous X-ray peaks, and finding the unique peak of each crystal form in a mixture is sometimes not possible. This problem can be solved by analysing integrated intensity (areas under overlapping peaks). The technique is generally able to reveal relative amounts of two crystalline polymorphs from their powder mixture to a sensitivity of 0.5–1% depending on the state of peak overlapping (Tanninem and Yliruusi, 1992).

Advance in computer controlled X-ray powder diffracrometers permits quantitative analysis of multicomponent mixtures using the complete powder diffraction profile rather than a limited amount of low-angle integrated intensity data (Bish and Howard, 1980; Karfunkel et al., 1996; Dinnebier et al., 1997; Smith, 1997).

The aim of this paper was to investigate if neural networks could be applied in quantitative X-ray powder diffractometric analysis. A simple X-ray powder diffractometric method was developed for the qualitative and quantitative assay of the ranitidine-HCl. The method was successfully used to identify, and quantify two modifications of ranitidine-HCl even when the weight fraction of one polymorph in the mixture was as low as 0.01.

1.1. Artificial neural networks

Artificial neural network theory has been explained previously (Zupan and Gasteiger, 1991), as has an example of how to apply this theory to quantitative chemical analysis (Bos and Weber, 1991). Artificial neural networks (ANNs) have been used mostly in pattern recognition problems and modelling, so they should be applicable in deciphering the pattern in diffraction data from polymorphic mixtures.

For many years linear modelling was the most commonly used technique based on the modelling of one single variable at the time. Modelling problems in analytical chemistry are not this simple, however. The problems are often non-linear, numerous constraints apply in the optimisation problem, and besides, the single-factor-at-a-time strategy does not take into account factor interactions. Multivariable experimental design can overcome the problems with interaction effects and non-linear estimation can be used to compute the relationship between several independent variables and a dependent variable. The application of ANNs is another method for modelling complicated systems. In particular, neural networks attempt to model non-linear functions with large numbers of variables.

Although there are many types of ANNs, the one that predominates in the area of pattern recognition is the feed-forward, back-propagation network. In this type of algorithm, information from various sets of inputs is fed forward through the ANN to make predictions that are compared with known values (training data). The error in the prediction is propagated back through the system to modify the interneuron connections to minimise the error in the predictions. This process is continued with multiple training sets until the error is minimised across many sets.

Evaluating the performance of the network on the training data may not produce the best results. If a network is left to train for too long, it will overtrain, memorise the training data and will lose the ability to generalise. Thus, three types of data sets are used:

training data: used to train network;
test data: used to monitor the neural network performance during training; and
validation data: used to measure the performance of a trained application.

Each type of data has a corresponding error.

2. Experimental

2.1. Materials

The two polymorphic forms of ranitidine-HCl hydrochloride (Form 1 (Ch.-B 560018) and Form 2 (A.-Nr. 32005)) were obtained from Dolorgiet Pharmaceuticals, St. Augustin, Germany. Binary mixtures were made from these two polymorphic forms with different proportions. The weight factions of Form (II) in the mixtures were as follows: 0, 1, 2, 5, 10, 20, 30, 50, 70 and 100% w/w.

2.2. Measurement of the XRD profiles

For XRD calibration analysis, 360 mg of binary mixtures of ranitidine-HCl powder were compacted as tablets (∅16 mm) under a mass of 2 tons. Triplicate tablets were prepared and each tablet was scanned three times. The XRD scans were performed on a Philips wide angle X-ray powder diffractometer with X-ray generator (PW 1130/00) and goniometer (PW1050, Philips, Almelo, The Netherlands). A copper target X-ray (wavelength 1.541Å CuKα) tube was operated at a power of 40 kV, 30 mA. The automatic divergence slit was set at 1° for the X-ray beam and at 0.1° for the receiving scintillation detector. The scans were carried out at a step size of 0.04° and counting time for 0.5 s/step within the ranges of 7–48° (2θ).

X-ray diffractograms were recorded digitally by a scintillation counter and graphed by the software program Microsoft® Excell 5/95. Intensities of six characteristic peaks extracted from the spectra from both forms were analysed.

2.3. ANN software and network topology

The MS-Windows based artificial neural network software, NNMODEL Version 1.404 (Neural Fusion) was used.

A standard feed-forward network, with back-propagation learning rule and with a single hidden layer architecture was chosen. A single hidden layer was used for simplicity, and because there is little evidence to suggest that a larger number of hidden layers improves performance (Hornik et al., 1989; Ripley, 1996).

The peak heights in the diffractogram as a function of the fraction of the polymorphic form in the mixture of the two polymorphic forms was emulated by 90 input/output data sets, with six inputs (three peak heights at 17.04°2θ, 21.9°2θ, 24.8°2θ, for Form 1 and 20.02°2θ, 23.3°2θ, 27.4°2θ, for Form 2), one hidden layer and one output neuron (percentage of Form 2). The number of hidden neurons, an adjustable parameter, was optimised. The ANN was trained with four to 12 hidden neurons and from zero to 1000 training cycles and performance was tested after each addition of a neuron.

At the start of the training run, both weights and biases were initialised with random values. During training, 20% of the data were used as the test set and was back-propagated through the network to evaluate the trained network.

2.4. Conventional statistical modelling method

An often used statistical modelling method is response surface methodology (RSM, or polynomial regression). Mixture experiments represent a special class of experimental design. In a general mixture problem the measured response is assumed to depend only on the relative proportions of the ingredients present in the mixture and not on the amount of the mixture (Cornell, 1990). No matter how many ingredients are present in a mixture their sum must equal 100%. Because one component must be used to make up the total of 100%, the number of independent variables is one less than the number of components in the mixture. This is known as the mixture constraint, and the types of response surfaces that may be fitted are more restricted to allow for the relationship between the factor levels (Clarke and Kempson, 1997). A general form of the quadratic mixture model with two independent variables (components) is:

$$y = c_1x_1 + c_2x_2 + c_{12}x_1x_2$$

where x_1 and x_2 are weight fractions of the different forms, $x_2 = 1 - x_1$, c_1, c_2 and c_{12} are estimates of model parameters, and y is the dependent variable such as the peak height. Thus, six models were fitted, one at each of the six analytic diffraction peaks.

The models were simplified by a backward stepwise regression so that only significant terms ($P < 0.05$) were included in them.

2.5. Method validation

In order to test the predictions of the ANN and traditional statistical modelling, six additional experiments with four replicates were performed for use as a validation data set. The factor levels of the input variables were within the range of the training experimental data. The average relative error for each value (ERR%; Murtoniemi et al., 1994) was used to examine the best generalisation ability of the models.

3. Results and discussion

XRD is a powerful technique for the identification of crystalline solid phases, but there are numerous sources of error in quantitative XRD. X-ray lines are affected by preferred orientation of the particles in the sample. Variation in particle size can have a significant influence on the peak shape. It will affect the maximum peak intensity but will not affect the integrated intensity (Cullity, 1978) of peak (area under the curve). The presence of overlapping peaks makes the determination of integrated intensity impossible and maximum intensity is then used for the quantitation. Grinding could minimise preferred orientation by reducing the particle size. However, grinding was avoided because it could induce polymorphic transitions. We wanted to keep the method as simple as possible and to directly analyse the powdered samples with minimal pretreatment. Differences in the position and intensities of the peaks may not be attributed to preferred orientation of crystals, but different arrangements of ranitidine hydrochloride molecules in the crystalline lattice of each form. XRD patterns of the two polymorphs were sufficiently distinct to characterise each crystalline form.

The powder X-ray diffractograms of Form 1 and Form 2 showed characteristic diffractions at 17.04, 21.9, 22.5, 24.8°2θ and 20.02, 23.3, 27.4°2θ, respectively, for identification. From these, peaks at 17.04, 21.9, 24.8°2θ, and 20.02, 23.3, 27.4°2θ were chosen as the quantitative assay since they showed no, or only minor, interference with the diffraction signals from those of the other form (Fig. 1).

The final regression models obtained with RSM for peak heights versus weight fractions were linear for Form 1 and a quadratic mixture model for Form 2 (Table 1):

Significant interaction terms in models for Form 2 peaks (y_4–y_6) revealed that the concentration of Form 1 in a mixture influences the relationship between Form 2 concentration and peak height. All six models were used to predict con-

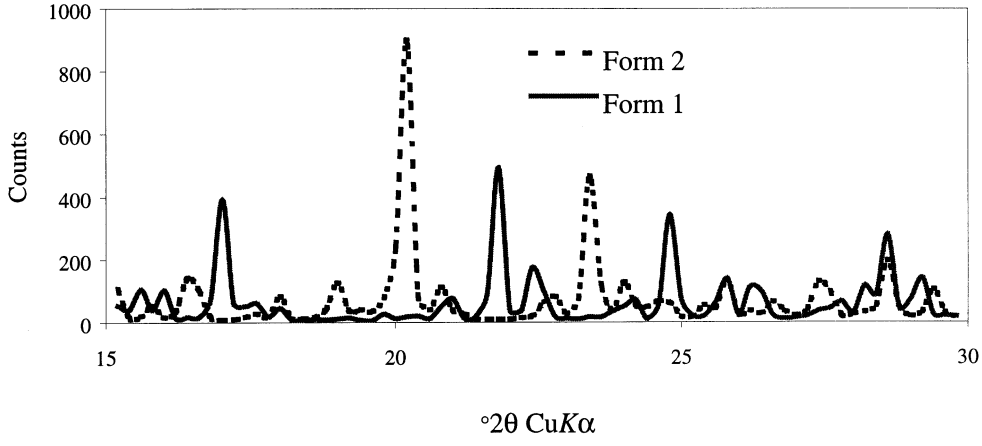

Fig. 1. X-ray diffractograms of two polymorphic forms of ranitidine-HCl.

Table 1
Summary results of regression for estimated models

Peak height[a]	Parameter	Parameter estimate	St. error	R
Y_1	c_1	23.689	0.3017	
	c_2	4.205	0.4220	0.995
Y_2	c_2	33.296	0.2643	
	c_1	4.156	0.3696	0.998
Y_3	c_1	23.934	0.1898	
	c_2	2.837	0.2655	0.995
	c	6.449	1.467	
Y_4	c_2	69.714	1.875	
	c	0.598	0.079	0.989
	c_1	3.213	0.524	
Y_5	c	29.997	0.670	
	c_{12}	0.161	0.028	0.991
	c_1	7.865	0.219	
Y_6	c_2	14.973	0.280	
	c_{12}	0.063	0.012	0.996

[a] y_1–y_6 are peak heights at 17.04°2θ, 21.9°2θ, 24.8°2θ, 20.02°2θ, 23.3°2θ, 27.4°2θ, respectively.

centrations from the selected peak heights and an averaged value was compared with the ANN's prediction.

For the ANN the error in the training values decreased as the number of hidden neurons was increased. By increasing the number of hidden neurons, the ANN more closely followed the topology of the training set data. However, exceeding an optimum number of hidden neurons results in tracing the training pattern too closely and the system was overtrained, and exhibits poor prediction for unseen data. The best results and lowest mean square root error for the test set was obtained with ten hidden neurons and 400 training cycles. More hidden neurons and training cycles did not improve the generalisation ability as the testing error started to increase, i.e. model was overtrained (Sarle, 1995).

The results of the predictions of the compositions of unknown samples (validation set) by RSM are shown in Table 2 and by ANN in Table 3. Although the differences between RSM and ANN methods are not great, the ANN predictions show a smaller standard deviation and relative error especially at lower concentrations of active form.

Table 2
Accuracy and precision in RSM predictions

Percentage of Form 2	Averaged prediction	Range[a]	SD[a]	ERR (%)[b]
1.00	7.79	6.29–10.49	1.92	679.40
2.00	10.31	8.92–11.96	1.27	415.72
25.00	28.65	28.53–29.54	0.86	14.62
40.00	43.15	42.19–44.45	1.02	7.87
60.00	62.17	60.80–63.64	1.25	3.62
80.00	81.19	78.80–83.59	1.99	1.49

[a] Range and standard deviation for the replicates at each percentage of Form 2 ($n = 4$).
[b] ERR(%) = ((Predicted − Actual)/Actual) × 100.

Comparison of the two methods was done by a least square fitting method (Massart et al., 1988). The results predicted by the RSM and ANN were plotted against measured weight fraction of Form 2 (Fig. 2). The intercept is a measure of method bias, while the deviation of the slope from unity is a measure of proportional error. A proportional systematic error leads to a change in a slope, so that the difference between slope and unity gives an estimate of the proportional error. The regression equations were: $RSM = 7.04 + 0.918x$ and $ANN = 0.340 + 0.999x$; the correlation coefficient was 0.999 for both methods indicating highly correlated results. For the RSM model, intercepts ($t_{(4)} = 10.13$; $t_{tab} = 2.776$) was significantly different from zero indicating a method bias and the slope was not equal to unity ($t_{(4)} = 12.95$; $t_{tab} = 2.776$) specifying a constant systematic error. For the ANN model the intercept was not significantly different from zero ($t_{(4)} = 0.71$) and the slope was equal to unity ($t_{(4)} = 0.23$).

The relative precision of the two methods was determined by comparing the variances for replicate analyses of single samples at various weight fractions of Form 2. The estimated variances were similar ($F_{3,3} = 1.052–1.214$: $F_{tab} = 9.28$), except at 1 and 2% (Table 4). In other words, both methods have the same precision, except at lower concen-

Table 3
Accuracy and precision in ANNs predictions

Percentage of Form 2	Averaged prediction	Range[a]	SD[a]	ERR (%)[b]
1.00	0.78	0.25–1.15	0.43	22.58
2.00	1.99	0.67–3.96	0.72	0.49
25.00	26.19	24.94–27.16	0.95	4.77
40.00	41.15	40.21–42.42	1.00	2.86
60.00	59.74	58.35–61.23	1.26	0.45
80.00	79.98	77.36–82.58	1.177	0.03

[a] Range and standard deviation for the replicates at each percentage of Form 2 ($n = 4$).
[b] ERR(%) = ((Predicted − Actual)/Actual) × 100.

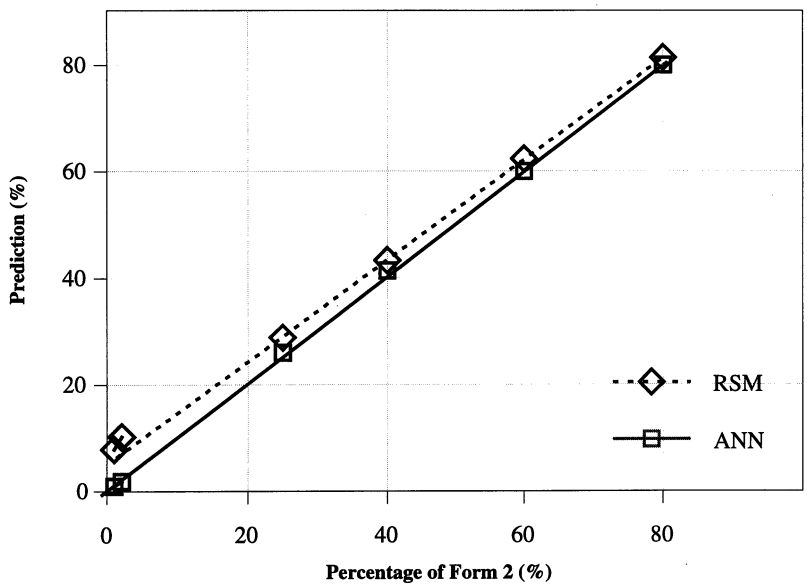

Fig. 2. Predicted concentrations by the RSM and ANN plotted against measured percentage of Form 2.

Table 4
The precision of the respective methods determined by comparing the variance for replicate analyses

Percentage of Form 2	Variance		F-ratio[a]
	RSM	ANN	
1.00	8.855	0.141	62.75
2.00	0.162	1.201	7.44
25.00	2.026	2.460	1.21
40.00	0.479	0.456	1.05
60.00	0.647	0.661	1.02
80.00	1.853	2.180	1.18

[a] $F_{3,3} = 9.28$ (10% of two sided test).

trations where the ANN method gave better results.

With the ANN model it was possible to predict the Form 2 concentration with good accuracy and precision even at 2%.

4. Conclusion

The used ANN model predicted concentrations precisely, accurately and with minimal bias through a wide range of two ranitidine-HCl polymorphic forms in mixture.

Conventional RSM required the specification of a polynomial function for each peak to be regressed. These equations could be considered as six independent models. The ANN modelled all six peak heights simultaneously. Besides, the polynomial regression analyses were dependent on predetermined statistical significance levels and less significant terms were eliminated from the models.

The ANN methodology provided a good alternative to the polynomial regression method to identify the non-linear relationship as these complex non-linear relationships were investigated without complex equations. A further step will be to use the whole pattern rather than selected peak intensities to achieve greater accuracy and to minimise problems of preferred orientation and overlapping X-ray lines.

Acknowledgements

The authors wish to thank D.J. Wall, Geology Department, University of Otago, for technical support and advising during data collection.

References

Bartolomei, M., Ramusino, M.C., Gheti, P., 1997. Solid-state investigation of fluocinolone acetonide. J. Pharm. Biomed. Analysis 15, 1813–1821.

Bish, D.L., Howard, S.A., 1980. Quantitative phase analysis using the Rietveld method. J. Appl. Cryst. 21, 86–92.

Bos, M., Weber, H.T., 1991. Comparison of the training of neural networks for quantitative X-ray fluorescence spectrometry by a genetic algorithm and backward error propagation. Anal. Chim. Acta 247, 97–105.

Chao, R.S., Vail, K.C., 1987. Polymorphism of 1,2-dihydro-6-neopentyl-2-oxonicotinic acid: characterization, interconversion, and quantitation. Pharm. Res. 4, 429–432.

Clarke, G.M., Kempson, R.E., 1997. Introduction to the Design and Analysis of Experiments. London, New York: Arnold; Wiley.

Cornell, J.A., 1990. Experiments with Mixtures: Design, Models and the Analysis of Mixture Data, 2nd ed. Wiley, New York.

Cullity, B.D., 1978. Elements of X-ray Diffraction. Addison–Wesley, Reading, MA.

Dinnebier, R.E., Olbrich, F., van Smaalen, S., Stephens, P.W., 1997. Ab initio structure determination of two polymorphs of cyclopentadienylrubidium in a single powder pattern. Acta Cryst. B53, 153–158.

Doff, D.H., Brownen, F.L., Corrigan, O.I., 1986. Determination of α-impurities in the β-polymorph of inosine using infrared spectroscopy and X-ray powder diffraction. Analyst 111, 179–182.

Hornik, K., Stinchcombe, M., White, H., 1989. Multilayer feedforward networks are universal approximators. Neural Networks 2, 359–366.

Karfunkel, H.R., Wu, Z.J., Burkhard, A., Rihs, G., Sinnreich, D., Buerger, H.M., Stanek, J., 1996. Crystal packing calculations and rietveld refinement in elucidating the crystal structures of two modifications of 4-amidinoindanone guanylhydrazone. Acta Cryst. B52, 555–561.

Klug, H.P., Alexander, L.E., 1974. X-ray Diffraction Procedures. Wiley, New York.

Massart, D.L., Vanderginste, B.G.M., Deming, S.N., Michote, Y., Kaufman, L., 1988. Chemometrics: A Textbook. Elsevier, Amsterdam, p. 33.

Murtoniemi, E., Yliruusi, J., Kinnunen, P., Merkku, P., Leiviska, K., 1994. The advantages by the use of neural networks in modeling the fluidized bed granulation process. Int. J. Pharm. 108, 155–163.

Ripley, B.D., 1996. Pattern Recognition and Neural Networks. Cambridge University Press, Cambridge.

Sarle, W.S., 1995. Stopped Training and Other Remedies for Overfitting, Proceedings of the 27th Symposium on the Interface. SAS Institute, Cary, NC.

Smith, D.K., 1997. Evaluation of the detectability and quantification of respirable crystalline silica by X-ray powder diffraction methods. Powder Diffraction 12, 200–227.

Suranarayanan, R., 1990. Determination of the relative amounts of α-carbamazepine and β-carbamazepine in a mixture by powder X-ray diffractometry. Powder Diffraction 6, 155–159.

Tanninem, V.P., Yliruusi, J., 1992. X-ray powder diffraction profile fitting in quantitative determination of two polymorphs from their powder mixture. Int. J. Pharm. 81, 169–177.

Zupan, J., Gasteiger, J., 1991. Neural networks: a new method for solving chemical problems or just a passing phase? Anal. Chim. Acta 248, 1–30.