

Optimizing Insulin-loaded Nanoparticles with INForm

Nanoemulsions are good options for delivering drugs with poor aqueous solubility. Ease of preparation and scale-up, stability and increased bioavailability are all features of such formulations.

Control of particle size and polydispersity, while ensuring that sufficient drug is both encapsulated and released, is imperative if nanoparticles are to be used successfully as delivery systems.

However, systems like these are complex and often show non-linear relationships which can make modelling by traditional techniques (like statistics) difficult for the non-expert. Now, neural networks can be used to develop good models quickly, and these can be used to optimize nano-particle properties and behaviour.

This study reports the use of **INForm**, based on neural networks and genetic algorithms, for modelling and optimization of nanoparticle properties.

Insulin-loaded Nanoparticles

Insulin-loaded nanoparticles have been made and studied by D Attivi and coworkers (*Drug Dev Ind Pharm* **31** 179-189 (2005)), using an oil-in-water-in-oil emulsion process.

A central composite experimental design was used in their data collection, with controlled variations allowed in three factors:

- ratio of poly(epsilon caprolactone) to Eudragit RS, referred to as PCL/RS ratio
- the volume of the PVA aqueous solution
- pH of the aqueous PVA solution).

The five properties that were measured were the particle size (nm), polydispersity index, zeta potential, amount of entrapped insulin, and the amount of released insulin after 7 hours. The aim is to make small particles, with small polydispersity, containing controlled amounts of insulin which is released within a desired time frame.

Attivi *et al* examined their results using response surface methodology, using the graphics produced in order to develop an optimum formulation. Good models could be developed for all properties except zeta potential, where an adequate model was created.

In the present study, the data of Attivi *et al* was used in **INForm**, which uses neural networks for modelling. The values for the repeats especially those at the centre of the design were averaged, giving 17 unique experiments. 15 of these were used for training the models, with 2 withheld for testing the models' predictivity. A separate model was developed for each of the 5 properties.

This data set has also been 'mined' using **FormRules** – a separate application note discusses the models and rules developed in that study.

Developing Neural Network Models

The RPROP backpropagation algorithm (which requires no user-specified parameters) was used in training, with asymmetric sigmoid transfer functions between the different layers. Because data were sparse, only 2 nodes were used in the hidden layer of the neural network.

ANOVA statistics were used to assess the quality of the models. These were similar to or slightly better than the ANOVA values from the statistical models, as shown in Table 1 below.

Property	R ²	R ² Stats Model
Particle size	0.97	0.91
Polydispersity	0.83	0.86
Zeta potential	0.68	0.57
Entrapped insulin	0.88	0.81
Released insulin	0.81	0.91

Table 1. Training parameters and ANOVA R² value for the various property models, compared to statistical treatment of Attivi *et al*.

Results

Particle size

Attivi *et al* used response surfaces to conclude that particle size depended primarily on the PCL/RS ratio and pH, a finding that was confirmed by the data mining study using **FormRules**. The models developed by **INForm** show similar behaviour, as illustrated in Figure 1. pH is a 'hidden variable' in this plot, and it can

be seen that changing the volume of PVA has little effect on the particle size.

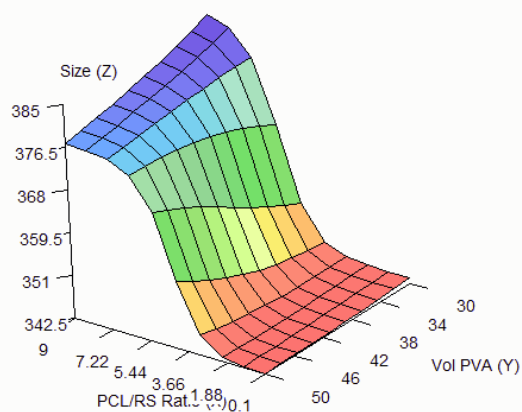


Figure 1. Particle size as a function of PCL/RS ratio and volume PVA, pH in mid range

Decreasing the pH (which can be studied interactively using **INForm's** graphical Explorer) shows the results given in Figure 2.

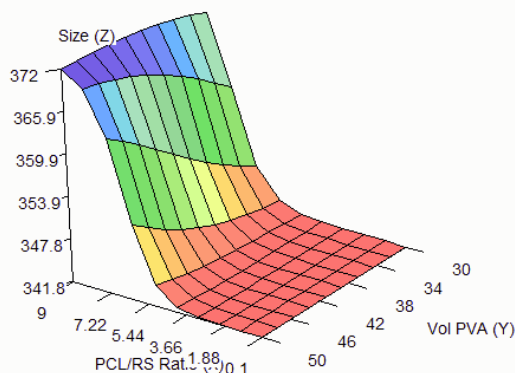


Figure 2. Particle size as function of PCL/RS ratio and volume PVA, pH at minimum value

Polydispersity

The response surfaces for polydispersity showed very non-linear behaviour. As for size, volume PVA played only a small role, with PCL/RS ratio and pH being much more important.

Optimization

Attivi *et al* were interested in finding a formulation in which small nanoparticles, with just a small degree of polydispersity, entrapping at least 25 IU of insulin per 100 mg polymer, and releasing at least 4 IU after 7 hours. They used response surfaces to try to deduce the conditions which would give this.

In the present study, the genetic algorithms in **INForm** were used to find an optimum. This required a "fitness function" to be defined, in which the importance of each of the properties is specified, together with the desired values.

This is shown in the partial screenshot in Figure 3. Here, we have specified that the size is the most important property, and that it should be less than 400 nm. Polydispersity should be less than 0.21 (almost at the minimum value of 0.20 seen in the experimental data). With the values we have specified, zeta potential can take any value. Entrapped insulin should be above 28.5, and released insulin above 4 IU.

Property	Weight	Min	Mid1	Mid2	Max	Desirability Function
1 Size	10.00	337.00	400.00	400.00	659.00	Down
2 Polydispersity	9.00	0.20	0.21	0.21	1.00	Down
3 Zeta Potential	1.00	30.00	39.50	39.50	49.00	Flat
4 Entrapped insulin	7.00	24.00	28.50	28.50	33.00	Up
5 Released insulin	8.00	1.68	4.00	4.00	6.00	Up

Figure 3. Information needed to set up "fitness function" for genetic algorithm optimizer

This could be largely achieved with a PCL/RS ratio of 0.5, volume PVA about 30 (the minimum value in the experimental set) and a pH of 3. All properties reached their target values except polydispersity, which was 0.24 instead of the desired 0.21. This is within the calculated range, though, as Figure 4 shows.

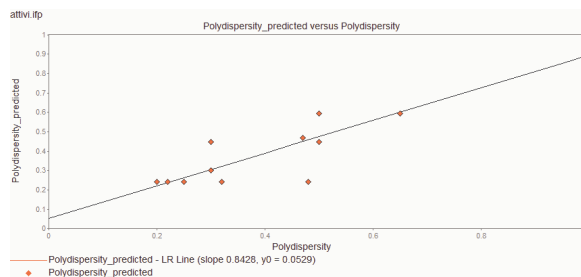


Figure 4. Predicted vs actual values for polydispersity

Trying various trade-offs showed that it was not possible to achieve a very low degree of polydispersity while still meeting the requirement that at least 4 IU of insulin be released within 7 hours.

Conclusions

INForm's neural networks developed models at least as good as those from statistics, showing that the neural network could cope well with non-linearities that are hard to model statistically.

More importantly, it was considerably easier to find an optimum using **INForm's** genetic algorithms, rather than using response surfaces to try to deduce an optimum.

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