Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmaceutics and Biopharmaceutics 58 (2004) 615–619
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Development of an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspension technology

Jan Möschwitzer[a], Georg Achleitner[b], Herbert Pomper[b], Rainer H. Müller[a,*]

[a]*Department of Pharmaceutical Technology, Biotechnology and Quality Management, Free University of Berlin, Berlin, Germany*
[b]*Fresenius Kabi Austria GmbH, Graz, Austria*

Received 6 January 2004; accepted in revised form 9 March 2004

Available online 15 June 2004

Abstract

Omeprazole is a proton pump inhibitor, which is used for the treatment of peptic ulcers, reflux esophagitis and Zollinger-Ellison syndrome. It is a poorly soluble, chemically labile drug with a high degradation rate in aqueous media. The aim of this study was to show the feasibility of omeprazole stabilization using the DissoCubes® technology and to find optimal production parameters for a stable, highly concentrated omeprazole nanosuspension. The high performance liquid chromatography analysis has proved the predominance of the nanosuspension produced by high pressure homogenization in comparison to an aqueous solution. Even 1 month after production no discoloration or drug loss was recognizable when the nanosuspension was produced at 0 °C. As a result it can be stated that the production of nanosuspensions by high pressure homogenization is suitable for preventing degradation of labile drugs.

Keywords: Omeprazole; DissoCubes®; High pressure homogenization; Poorly soluble drugs; High performance liquid chromatography; Nanoparticles

1. Introduction

Omeprazole is a substituted benzimidazole that selectively inhibits the gastric proton pump of the parietal cells. It is used for the treatment of peptic ulcers, reflux esophagitis and the Zollinger-Ellison syndrome. In some cases an intravenous omeprazole formulation is needed, especially for the treatment of patients in intensive care. Normally these patients receive a freshly prepared aqueous omeprazole solution via perfusion. Due to their chemical instability, available formulations should be used within 6 h. Omeprazole is a lipophilic, weak base with $pK_{a1} = 4.2$ and $pK_{a2} = 9.0$; it is chemo- and thermolabile and rapidly degraded and discolored when exposed to acidic media and warm temperature [1]. The free base is only poorly soluble in water (82.3 mg/l) [2].

One approach for the stabilization of chemically labile molecules in solution is to transform the soluble molecules into insoluble molecules (pro-drugs) and produce a suspension. In case of poorly soluble molecules, such as omeprazole, it is not necessary to make a chemical modification since a suspension is generated by using a high drug concentration in a small amount of water. Omeprazole is soluble in water up to a concentration of 0.008%; in this study drug concentrations up to 10% were used resulting in a suspension. Of course, to yield an intravenously injectable product, a nanosuspension rather than a macrosuspension needs to be produced. A nanosuspension is defined as a dispersion of drug nanocrystals (< 1000 nm) in an outer liquid phase. Production technologies currently used are pearl milling (Nanosystems/Elan [3]) and high pressure homogenization either in water by Skypharma [4] and Baxter [5] or alternatively in mixtures of water with water miscible liquids or non-aqueous media, such as liquid polyethylene glycols or oils (Pharmasol [6]). Depending on the procedure used the nanosuspensions appear under the different trade names NanoCrystals™, NANOEDGE™ and DissoCubes®, respectively.

Because of its chemical lability omeprazole was chosen as drug candidate for this study. The aim of this study was to evaluate the DissoCubes® technology with respect to omeprazole stabilization and to find optimal production parameters for a stable, highly concentrated omeprazole

* Corresponding author. Department of Pharmaceutical Technology, Biotechnology and Quality Management, Free University of Berlin, Kelchstrasse 31, 12169 Berlin, Germany. Tel.: +49-30-83850678; fax: +49-30-83850616.
E-mail address: mpharma@zedat.fu-berlin.de (R.H. Müller).

0939-6411/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2004.03.022

nanosuspension. In contrast to the available products our intravenous formulation is easy to produce in one step using the Dissocubes® technology and has shown a very good chemical stability over the observed period.

2. Materials and methods

2.1. Materials

The drug omeprazole was provided by Fresenius Kabi (Graz, Austria). Poloxamer 188 was purchased from BASF (Ludwigshafen, Germany), sodium bicarbonate from Caelo (Hilden, Germany), phosphoric acid from Merck (Darmstadt, Germany) and di-sodium hydrogen phosphate from Fluka Chemie AG (Buchs, Switzerland).

2.2. Preparation of nanosuspension

An 8.4% sodium bicarbonate solution was prepared; 1% Poloxamer 188 was added to this solution and dissolved. The drug powder was dispersed in the aqueous phase using an Ultra Turrax T25 equipped with an 18 G rotor (Jahnke & Kunkel, Staufen, Germany) for 1 min at 9500 rpm. The obtained pre-mix was homogenized using a Micron LAB 40 (APV Systems, Unna, Germany). At first, 2 cycles at 150 bar and 2 cycles at 500 bar as premilling steps were applied, then 40 cycles at 1500 bar were run to obtain the nanosuspension. Production was performed at different temperatures.

2.3. Particle size analysis

The particle size analysis was performed by photon correlation spectroscopy (PCS) using a Zetasizer 4 (Malvern Instruments, Malvern, UK) and laser diffractometry (LD) using the Mastersizer E (Malvern Instruments). PCS yields the mean particle diameter of the suspension and the polydispersity index (PI). The PCS mean diameter is a light intensity weighted size and the PI is a measure of the width of the particle size distribution. The LD yields a volume distribution. Before measurement, the samples had to be diluted with a satured omeprazole–sodium bicarbonate solution to obtain a suitable concentration for measurement and to avoid dissolution of the particles.

2.4. HPLC-analysis

The omeprazole concentrations were determined by using an high performance liquid chromatography (HPLC) method. The mobile phase was based on the method of the European Pharmacopoeia: 1.4 g of di-sodium hydrogen phosphate were added to 1000 ml MilliQ-water and the pH was adjusted with phosphoric acid to 7.6. Seventy-three parts of this buffer were mixed with 27 parts of HPLC-grade acetonitrile. Flow rate was 1 ml/min; the UV detector was operated at a wavelength of 280 nm. A Eurosphere 100, C18, 5 μm column was used in the HPLC hardware from Kontron Instruments (Germany). The nanosuspension samples were prepared by diluting 10 μl of nanosuspension to 10 ml with mobile phase. A freshly prepared standard was checked with every run. To compare the stability of the nanosuspension with the omeprazole solution a reference specimen was prepared by dissolving an exact amount (5 mg) of omeprazole in 8.4% sodium bicarbonate solution. The concentration of this reference was assayed directly without dilution.

3. Results and discussion

3.1. Process parameters—temperature

The degradation of omeprazole is easily recognizable by discoloration of the solution/nanosuspension. At the beginning of these studies the homogenization was performed at room temperature and the batch was cooled down to room temperature between each cycle. This leads to a colored nanosuspension, caused by the degradation of the thermolabile omeprazole. The degradation was so obvious by the change in color that no chemical analysis need to be performed to evidence the degradation. For the DissoCubes® technology [4] production at temperatures of 0 °C and even below (e.g. −20 °C) is described to process chemically labile compounds. For example, homogenization of azadicarbonamide at room temperature leads to degradation and formation of carbon dioxide recognizable by a foamy product. Processing at lower temperatures prevents this. In order to avoid the degradation of omeprazole during production the nanosuspensions were prepared at 0 °C and the samples were cooled down to 0 °C between each cycle. As a result the product appears white (Fig. 1).

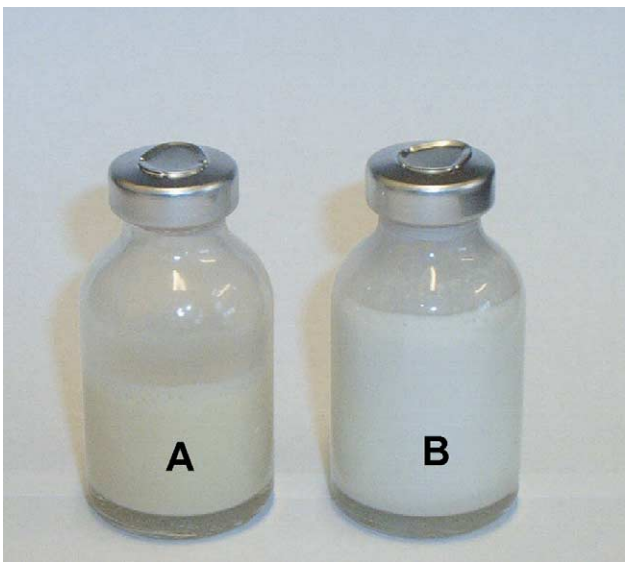

Fig. 1. Discoloration during production process. (A) Production at room temperature, (B) production at 0 °C.

3.2. Process parameters—cycle numbers

The power density (W/m^3) is a factor determining the achievable dispersity of the dispersions, i.e. the fineness of the drug nanocrystals. The power density P_V is defined as the energy W dissipated in the homogenization volume V related to the time t :

$$P_V = \frac{W}{tV} \quad (1)$$

Based on this equation, the factors determining P_V are the homogenization pressure and the width of the homogenization gap. To produce nanocrystals that are as small as possible, a pressure of 1500 bar was applied. The width of the homogenization gap under these conditions is approximately 25 μm. Another factor determining the fineness of the product is the time during which the particles are homogenized, i.e. the time they are subjected to diminution. The resistant time in the homogenization gap is extremely short, because of the high streaming velocity of the dispersions (up to 1000 m/s). In order to obtain longer diminution time it is necessary to run several processes through the homogenizer (homogenization cycles.) A typical number of homogenization cycles reported for nanosuspensions is between 10 and 20, depending on the softness or hardness of the drug to be processed.

To obtain an ultrafine nanosuspension with a low number of homogenization cycles it is recommended to start with a raw material as small as possible, i.e. micronized product. In addition it has to be considered that the homogenization gap at 1500 bar is approximately 25 μm; that means if there are too many large particles they might block the homogenization gap. Fig. 2 (left) shows the size distribution of the raw material. The bulk population is in the range of approximately 2–20 μm; however there is also a pronounced fraction in the range 50–180 μm. The Mastersizer E has different lenses covering different size ranges, i.e. for 0.1–80 and 0.5–180 μm. The small lens was applied in this study, because we were also interested in the submicron fraction. To avoid a blockage of the homogenization gap a pre-milling at lower pressures of 150 and 500 bar was performed. The homogenization gap is larger at lower pressure, thus allowing even larger crystals to be processed. In this pre-milling step these crystals are reduced in number and size allowing a further subsequent processing at the highest pressure of 1500 bar with the smallest gap (smallest homogenization volume, does highest power density).

At first 20 homogenization cycles were run to produce the nanosuspension; however, it was found that the PCS diameters were still in the range 900–1000 nm. Therefore the cycle number was increased; the optimal number of cycles was found to be between 35 and 40 yielding PCS diameters of about 500 nm and a PI of approximately 0.30. Fig. 3 shows the decrease in particle size as a function of cycle numbers for three batches produced. There is a batch-to-batch variation in the PCS diameters up to 20 homogenization cycles. However, above 25 cycles the diameters are very well reproducible. The three batches produced possess a mean PCS diameter between 598 and 603 nm (Fig. 3).

Fig. 2 (right) shows the LD size distribution of the nanosuspension produced with 40 homogenization cycles. The peak maximum is clearly below 1 μm and particles up to 5 μm are detectable. When looking at the size distribution curves it has to be borne in mind that it is a volume distribution. Therefore, sizes found with this method are clearly higher than ones obtained with PCS

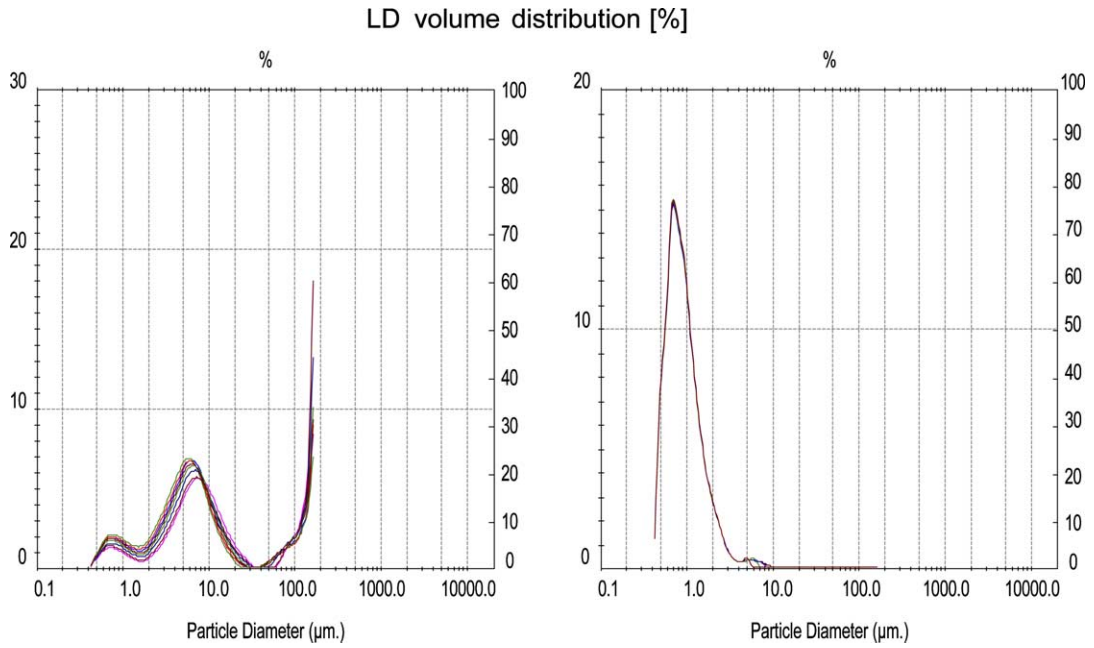

Fig. 2. Particle size distribution: (left) raw material, (right) after 40 homogenization cycles.

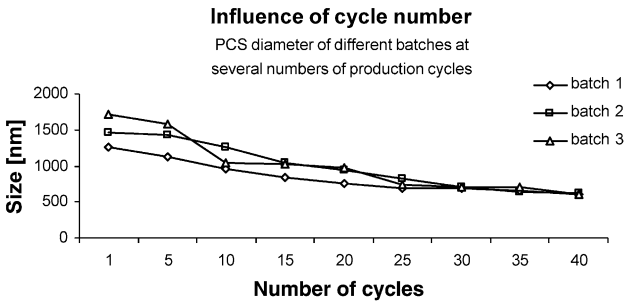

Fig. 3. Influence of applied cycles in regard to PCS diameter.

(different measuring principle). Compared to the size distribution of the raw material (Fig. 2 left) the analysis nicely shows the efficiency of the high pressure homogenization.

3.3. Physical stability

The concentration of the produced nanosuspensions ranges from 1 to 10 percent; all nanosuspensions show a good physical stability with a moderate size increase during 3 days of monitoring (Table 1). Day 0 was the day of production; day 1 was one day after production. On day 1, even a slight decrease in PCS diameters was observed. This could be attributed to some limited dissolution of particles because drug nanocrystals possess a higher saturation solubility compared to drug microparticles. This can be explained by an increased dissolution pressure below a size of approximately 1–2 μm, the theoretical background being the Calvin equation [7].

The size increase monitored during different days of storage of course indicates that these nanosuspensions will not possess a long-term stability of 2 years. However, a long-term stable formulation was not the aim of this study, the study was focused on the chemical stability. To obtain physically long-term stable nanosuspensions it would now be necessary to perform a routine formulation screening to optimize types and concentrations of surfactants or surfactant mixtures. Physical stability of nanosuspensions for up to 3 years has been reported [8], which is explained to

Table 1
Mean PCS diameters and polydispersity index (PI) of several batches produced at 0 °C as a function of 3 days storage time

Day	Batch							
	A		B		C		D	
	Size	PI	Size	PI	Size	PI	Size	PI
0	598	0.344	497	0.323	600	0.296	619	0.388
1	557	0.326	536	0.457	529	0.324	571	0.369
3	518	0.281	634	0.398	653	0.202	648	0.358

Dispersion media: A, B—0.001 M sodium bicarbonate solution; C, D—8.4% sodium bicarbonate solution. Drug concentrations: A, B, C—5%; D—10%.

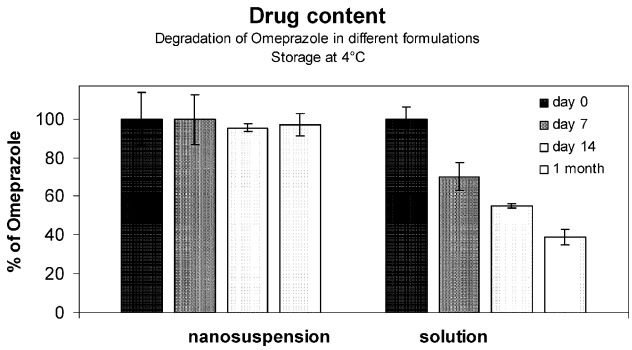

Fig. 4. Drug content in different formulations.

be due to the absence of Ostwald ripening. Ostwald ripening does not occur due to two reasons. Firstly, the drugs are poorly soluble anyway thus leading to only very little changes in the dissolved concentration during the preparation. Secondly, the particles are relatively homogenous in size thus avoiding larger differences in the saturation solubility between differently sized crystals.

3.4. Improvement of chemical stability

To show the advantage of the DissoCubes® technique for increasing the chemical stability, HPLC analysis was performed. The drug content of an omeprazole solution in 8.4% sodium bicarbonate solution and of a nanosuspension was tested over a 1 month period. Based on the maximum solubility of omeprazole (free base) in water (8.23 mg/100 ml) [2], a solution containing about 5 mg/100 ml was tested. Despite storage at optimal conditions (at 4 °C/no light) a significant loss of omeprazole and discoloration was identifiable in the solution. In contrast, the omeprazole nanosuspensions demonstrated an excellent stability. Stored at the same conditions (4 °C/no light) the drug omeprazole nanosuspensions showed no significant alteration in drug content and in color over the monitored period (Fig. 4). In the literature this phenomenon is explained by two effects. Firstly the molecules of the surface stabilizer could shield the chemical compound or secondly the crystalline structure in a nanoparticulate sized formulation results in higher drug stability [9]. The basic advantage of the homogenization process for producing nanosuspensions is saving of time, avoidance of contamination with erosion from milling pearls and microbiological problems [10]. In the case of a labile drug such as omeprazole, homogenization at low temperature proved to be superior. Another advantage of this system is that it is easy to adapt from a laboratory scale to an industrial scale.

4. Conclusions

The possibility of protecting omperazole from degradation by using the DissoCubes® technique was

investigated. This technique is suitable for producing particulate drug formulations in order to protect chemical labile drugs from degradation. The drug nanosuspensions are easy to produce and, show excellent chemical stability compared to drug solutions. It is possible to produce highly concentrated nanosuspensions, which are chemically stable and protected from degradation.

References

[1] D. Castro, M.A. Moreno, S. Torrado, J.L. Lastres, Comparison of derivative spectrophotometric and liquid chromatograpic methods for the determination of omeprazole in aqueous solutions during stability studies, J. Pharm. Biomed. Anal. 21 (1999) 291–298.

[2] SRC PhysProp Database, Internet available.

[3] G. Liversidge, IIR Drug Delivery Partnerships™ Meeting, Workshop 'Nanotechnology—solid particles, lipids and nanocomplexes', Cologne/Germany, 11–13 June, 2003.

[4] R.H. Müller, K. Mäder, K. Krause, Verfahren zur schonenden Herstellung von hochfeinen Micro-/Nanopartikeln, PCT Application PCT/EP00/06535, Germany, 2000.

[5] J.E. Kipp, J.C.T. Wong, M.J. Doty, C.L. Rebbeck, Microprecipitation method for preparing submicron suspensions, United States Patent, 6, 607,784 (2003).

[6] R.H. Muller, R. Becker, B. Kruss, K. Peters, Pharmaceutical nanosuspensions for medicament administration as systems with increased saturation solubility and rate of solution, United States Patent, 5, 858, 410 (1999).

[7] C. Nyström, Dissolution properties of poorly soluble drugs: theoretical background and possibilities to improve the dissolution behaviour, in: R.H. Müller, S. Benita, B. Böhm (Eds.), Emulsions and Nanosuspensions for the Formulation of Poorly Soluble Drugs, medpharm Scientific Publishers, Stuttgart, 1998, pp. 143–148.

[8] K. Peters, Nanosuspensions—a novel formulation principle for poorly soluble drugs, PhD thesis, Free University Berlin, Berlin, 1999.

[9] E. Liversidge, L. Wei, Stabilization of chemical compounds using nanoparticulate formulations, United States Patent No. 0054042 A1 (2003).

[10] R.H. Müller, O. Kayser, C. Jacobs, DissoCubes—a novel formulation for poorly soluble and poorly bioavailable drugs, in: M.J. Rathborn, J. Hadgraft, M.S. Roberts (Eds.), Drugs and the Pharmaceutical Sciences, Modified-Release Drug Delivery Technology, vol. 126, 2003, pp. 135–149.