Aerodynamic Properties and Drug Solubility of Dry Powders Prepared by Spray Drying: Clarithromycin *Versus* its Hydrochloride Salt.

Russo P, Manniello M.D., Simonetti A., Petrone A.M., Porta A., Del Gaudio P., Aquino R.P.

Department of Pharmacy (DIFARMA), University of Salerno, Fisciano (SA) – Italy; (paorusso@unisa.it).

Introduction and Objectives.

The antibiotic therapy for a direct administration to the lung in cystic fibrosis patients has to provide suitable drug availability, possibly in the lower respiratory tract characterized by the presence of thick secretions. Apart from deposition, systemic or local pharmacological activity of an inhalation product depends on drug dissolution into the biological fluids lining the lung. Therefore, one of the crucial step in the therapeutic management of the respiratory disease is the drug solubilization in this site of action. Clarithromycin (CLA; fig.1) is a broad spectrum and a well know macrolide antibiotic usually prescribed particularly for the treatment of respiratory infections, interestingly showing an additional anti-inflammatory effect (Pukhalsky et al., 2004); CLA is characterized by a very poor water solubility (0.33 mg/L). One of the common strategy, for increasing drug solubility in aqueous medium is represented by the production of dry powders in amorphous form using the spray drying technique (Yonemochi et al., 1999). Moreover, CLA has a dimethylamino group, which can be salified for solubility/dissolution improvement (fig.1). Hence, the aim of the present study was to obtain respirable powders of clarithromycin, while improving drug aqueous solubility. Powders were produced with CLA or CLA hydrochloride and characterized in terms of drug content, aerodynamic properties and drug solubility. Finally, in order to assess the effect of the spray-drying process on the antibiotic activity of the engineered particles, microbiological tests were performed.

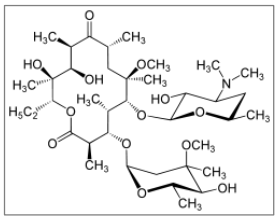

Fig. 1 | Clarithromycin.

Materials and Methods.

Several batches of micronized particles were prepared by spray drying different feed solutions; critical process parameters were solvent composition (isopropyl alcohol/water ratio), drug concentration and pH of the liquid feeds (table 1). Saturated solubility measurements were carried out keeping an excess amount of CLA raw material (RM), CLA spray-dried suspension and hydrochloride spray-dried in phosphate buffer (0.05 M, pH 6.75) at 37°C for 72h. After filtration, the solubility was measured by HPLC method and expressed in mg/ml.

The results were reported as mean of three measurements and standard deviation.

Particle size distribution of Raw Material and engineered particles was determined using a light-scattering laser granulometer, while particle morphology was assessed by scanning electron microscopy (SEM). The in vitro deposition of the micronized powders was evaluate by means of a *Single-Stage Glass Impinger* (SSGI; apparatus A; European Pharmacopoeia 8.0), using a proper device for the aerosolization. The antibacterial assay was carried out in MHB by microdilution method using 96-well microtiter plates. Briefly, 200 µl of 1X107 CFU/ml of *P. aeruginosa* ATCC 27853 were incubated at 37°C with different concentration of drug (4, 6 and 8 µg/ml).

Results and Discussion.

Tab. 1 | Feed solution composition, yield of the spray drying process, fine particle fraction (FPF), emitted dose (ED).

Batch #	Feed solution		Hydrochloride form	Yield %	d_{50} µm (Span)	Fine Particle Fraction % (FPF)	Emitted Dose % (ED)
	Drug Concentration	iPrOH/H_2O (v/v)					
1	3%	50/50	×	60.9 ± 3.0	4.42 (2.24)	30.9 ± 4.2	99.1 ± 4.0
2	5%	30/70	×	71.0 ± 2.8	7.16 (2.41)	23.4 ± 4.5	98.8 ± 4.3
3	3%	70/30	✓	86.0 ± 0.1	3.62 (1.78)	41.0 ± 3.5	97.0 ± 3.8
4	1%	70/30	✓	65.2 ± 4.1	3.25 (1.93)	47.8 ± 3.7	99.7 ± 0.7
5	2%	70/30	✓	70.1 ± 2.8	3.30 (1.89)	50.5 ± 3.1	98.4 ± 1.6
6	2%	60/40	✓	72.5 ± 1.5	3.31 (1.96)	40.2 ± 4.7	96.8 ± 2.2
7	2%	50/50	✓	70.5 ± 1.0	3.25 (2.00)	41.0 ± 1.4	96.0 ± 1.3
8	2%	40/60	✓	73.1 ± 3.5	2.80 (2.29)	42.5 ± 1.7	91.8 ± 4.1
9	2%	30/70	✓	71.5 ± 0.9	3.41 (2.12)	40.0 ± 2.4	95.7 ± 1.2

Morphology and aerodynamic properties of spray-dried particles were strongly dependent on organic solvent concentration as well as on pH of the liquid feeds processed, both influencing drug solubility. Adding clarithromycin to hydroalcoholic mixtures, alkaline feeds (pH~10.5; #1, #2, tab.1) in form of suspensions were obtained.

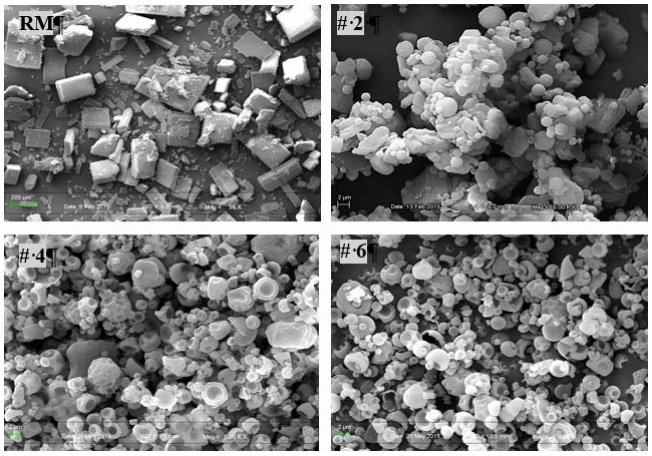

Fig. 2 | SEM images of Clarithromycin Raw Material (RM); spray-dried spherical particles obtained at pH 10.50 (#2) and wrinkled particles obtained at pH 6.50 (#4, #6).

The resulting spray-dried powders showed good process yield, but unsatisfactory aerodynamic properties, due to a high particle size (tab. 1) and the presence of residual drug crystals mixed to spherical particles (fig.2; #2). With the aim to obtain clear feeds in form of solutions, we tested clarithromycin in its hydrochloride salt form, obtained lowering pH values of feed solutions (pH 6.5).

Micronized salified powders showed higher process yield and very interesting FPF values, thanks to smaller and wrinkled particles (fig.2 #4, #6; tab.1).

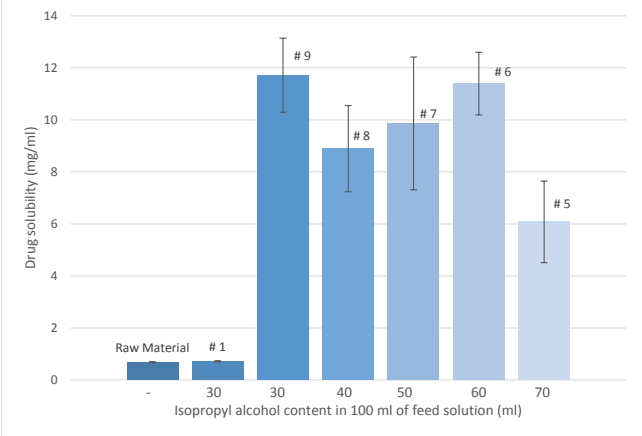

Fig. 3 | Raw material and spray-dried powder solubility in phosphate buffer (pH 6,75).

Moreover, water solubility of spray-dried powders was strongly influenced by clarithromycin form. Powders obtained from alkaline feed suspensions showed lower solubility in a phosphate buffer 0.05 M, pH 6.75 (fig.3, # 1).

A substantial increase in drug solubility was obtained, at the same conditions, with powders dried from feed solution containing clarithromycin hydrochloride (fig.3 #9-#5).

To verify the ability of the produced formulations to was performed. Three different drug concentrations were tested in a multi-well plate.

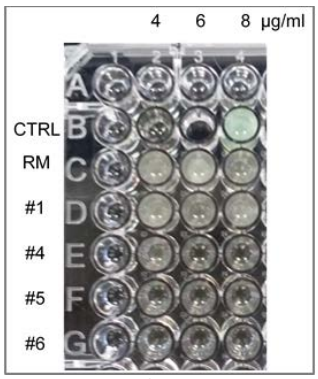

Fig. 4 | Microbiological assay of Clarithromycin raw material (RM) and spray-dried powders against *P. aeruginosa*.

Clarithromycin raw material (RM, line C, figure 4) and #1 (line D, figure 4) showed a lower activity against *P. aeruginosa* growth compared to #4, #5, #6, containing the hydrochloride form. This different behavior against *P. aeruginosa* may be due to the lower CLA solubility; at higher concentration, clarithromycin precipitates in RM and #1 wells, becoming unavailable for antibiotic purpose.

Conclusions.

Clarithromycin inhalable powders containing the drug in its hydrochloride form showed good aerodynamic properties and higher water solubility. Thanks to a fine-tuning of the process parameters and liquid feed composition, no excipients were necessary to obtain respirable powders. The spray drying process of CLA hydrochloride not only preserved antimicrobial activity, but also, increasing drug solubility, improved drug efficacy against *P. aeruginosa*.

References.

Pukhalsky, A. L., Shmarina, G. V., Kapranov, N. I., Kokarovtseva, S. N., Pukhalskaya, D., & Kashirskaja, N. J. (2004). Anti-inflammatory and immunomodulating effects of clarithromycin in patients with cystic fibrosis lung disease. *Mediators Inflamm*, *13*(2), 111-117. doi: 10.1080/09629350410001688495

Yonemochi, E., Kitahara, S., Maeda, S., Yamamura, S., Oguchi, T., & Yamamoto, K. (1999). Physicochemical properties of amorphous clarithromycin obtained by grinding and spray drying. *Eur J Pharm Sci*, *7*(4), 331-338.