Inhalable N-acetylcysteine Dry Powder Formulations as Potential Adjuvant Treatment for COVID-19

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Introduction

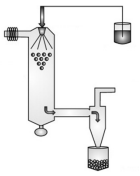
- N-acetylcysteine (NAC), a thiol derivative of the amino acid L-cysteine, is used as an antidote for acetaminophen poisoning. Due to the presence of the sulfhydryl group, NAC acts as an antioxidant, free radical scavenger, mucolytic agent and, among other things, possesses anti-inflammatory and antimycobacterial properties [1-2].
- NAC has been co-formulated with antibiotics for the treatment of *P. aeruginosa* infections in cystic fibrosis patients [3-4].
- NAC has been indicated as adjuvant therapy for COVID-19 [5-6]. *In vitro* and *in vivo* data showed that NAC has effect on respiratory viral infections, increasing antioxidant capacity, interfering with virus replication and suppressing pro-inflammatory cytokine expression in virus infected cells.

Objectives

The aim of this study was the formulation of inhalable high dose NAC spray dried microparticles, with suitable respirability properties. Particular attention was paid to the preparation of the drug solution to be sprayed and to the process conditions. The spray dried powders were characterized in terms of drug content, residual water content, thermal behaviour, particle size distribution, morphology and aerosolization performance.

Methods

NAC spray dried microparticles manufacturing



Buchi Mini Spray Dryer B-290

- NAC and selected excipients dissolved in Milli-Q grade water, acidified with 0.8% w/v of acetic acid
- Solid concentration of the spray dried solutions 1% w/v
- Close loop and nitrogen atmosphere
- Inlet temperature 120 °C, outlet temperature 63 – 67 °C, feed rate 2.0 ml/min, nozzle diameter 0.7 mm, drying air flow 740 L/h

NAC spray dried microparticles characterisation

Drug assay | Thermal behaviour | Residual water content | Particle size distribution | Particle morphology | *In vitro* aerodynamic assessment

HPLC, Shimatzu

DSC/TGA, Mettler Toledo

Spraytec, Malvern

SEM (Zeiss)

Fast screening impactor (FSI, Copley)

Results

Composition of the spray dried microparticles (%, w/v) and process aspirator rate (%) used

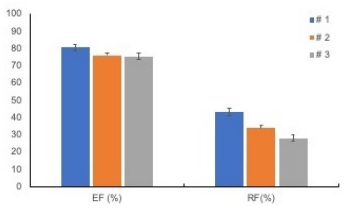
Formulation	NAC	Leucine	PVP	Lactose	Aspirator rate
#1	75	20	5	-	70
#2	75	20	5	-	100
#3	75	20	-	5	70
#4	75	20	-	5	100

Yield of spray drying process and drug content, residual water, melting temperature and enthalpy of fusion of the spray dried powders (n = 3)

Formulation	Yield (%)	Drug content (%)	Residual water (%)	$T_{melting}$ (°C)	Δh_{fusion} (J/g)
#1	71.1	102.6 ± 1.4	3.7 ± 1.3	104.6 ± 0.2	- 86.5 ± 3.4
#2	56.2	97.9 ± 3.6	3.6 ± 0.6	104.5 ± 0.1	- 86.6 ± 1.6
#3	64.0	95.2 ± 3.5	1.5 ± 0.9	105.1 ± 0.1	- 116.4 ± 1.6
#4	75.2	103.9 ± 2.1	1.7 ± 1.1	105.3 ± 0.2	- 106.5 ± 2.6

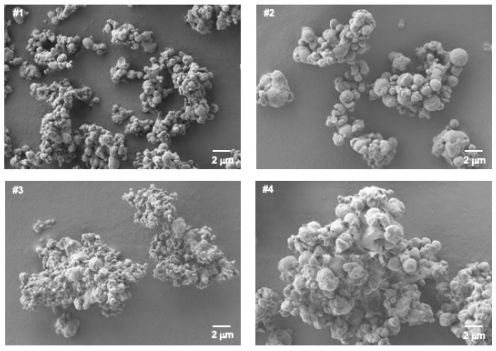
- NAC spray dried powders showed a yield ranging between 56 and 75 %.
- Drug content close to 100% indicated that NAC remains stable during the time in which the drug/excipient solution is sprayed.
- HPLC chromatogram of powder #3 (lowest value of drug content) exhibited the presence of a small peak with a retention time corresponding to an impurity reported in pharmacopoeia. The peak, associate to the impurity, was not present in the chromatogram of powder #4, probably the highest aspiration rate could protect the drug from degradation.
- Residual water was higher in the case of the spray dried powders containing PVP with respect to those containing lactose.
- DSC of the spray dried powders did not evidence a significant change of melting temperature of NAC with respect to the raw material (melting temperature of 110 °C and enthalpy of fusion of 149 J/g). The spray drying process led to a partial amorphization of the drug, more evident in the case of powders containing PVP. The degree of crystallinity was about 58%, 78% and 71%, for powders #1-2, #3 and #4, respectively. The different aspiration rate had a moderate effect only on the lactose-containing spray dried powder.

- The *in vitro* aerodynamic assessment of NAC spray dried powders showed that the emitted fraction (EF, %) was more than 75% of the metered dose, upon actuation of the device. The respirable fraction (RF, %) was 28%, 33% and 43% for the spray dried powders #3, #2 and #1, respectively. In case of powder #4, a RF of about 15% was obtained, as it could be expected from the results of particle size distribution and the morphology exhibited in the SEM image.



Comparison of EF (%) and RF (%) for the spray dried powders #1, #2 and #3

- SEM images evidenced rounded and wrinkled microparticles that tend to agglomerate. The images of lactose-containing spray dried microparticles (powders #3 and #4) showed the presence of particles fused together forming aggregates.



- Powders #1-3 showed D_{v50} values between 3.18 – 3.40 mm. The D_{v50} value of powder #4 was higher (7.33 mm), due to the presence of aggregates, as also highlighted by a D_{v90} value of 16.55 mm and by the SEM image.

Particle size distribution of the spray dried powders (n = 3)

Formulation	D_{v10} (µm)	D_{v50} (µm)	D_{v90} (µm)	Span
#1	1.33 ± 0.11	3.18 ± 0.22	7.05 ± 0.24	1.81 ± 0.10
#2	1.35 ± 0.03	3.40 ± 0.51	7.38 ± 0.33	1.92 ± 0.21
#3	1.34 ± 0.01	3.25 ± 0.18	7.32 ± 0.12	1.95 ± 0.01
#4	1.41 ± 0.05	7.33 ± 0.54	16.55 ± 1.67	2.11 ± 0.23

Conclusions

The spray dried process allowed the preparation of powders containing high dose of N-acetylcysteine. The combination of NAC solution in acid environment and the spray drying process in close loop under nitrogen atmosphere protected the drug from degradation during the process. The aspirator rate had a moderate effect on the characteristics of the obtained powders. Otherwise, lactose decreased the residual water content of the spray dried powders, while PVP reduced the aggregation of spray dried microparticles and increased the *in vitro* respirability of the powders. The aerosol performance of powder #3 can be considered adequate as RF was higher than 40%.

Future work will include the development of a design of experiments to optimise the spray drying process parameters with the aim to further improve the aerosol performance of the NAC powders.

References

[1] Samuni Y, Goldstein S, Dean O M, Berk M: *The chemistry and biological activities of N-acetylcysteine*, Biochim Biophys Acta 2013; 1830: pp 4117-4129.

[2] Blasi F, Page C, Rossolini G M, Pallecchi L, Matera M G, Rogliani P, Cazzola M: *The effect of N-acetylcysteine on biofilms: implications for the treatment of respiratory tract infections*, Resp Med 2016; 117: pp 190-197.

[3] Lababidi N, Kissi E O, Elgaher W A M, Sigal V, Haupenthal J, Scwarz B C, Hirsch A K H, Rades T, Schneider M: *Spray-drying of inhalable, multifunctional formulations for the treatment of biofilms formed in cystic fibrosis*, J Control Rel 2019; 314: pp 62-71.

[4] Manniello M D, Del Gaudio P, Aquino R P, Russo P: *Clarithromycin and N-acetylcysteine co-spray-dried powders for pulmonary drug delivery: a focus on drug solubility*, Int J Pharm 2017; 533: pp 463-469.

[5] De Flora S, Balansky R, La Maestra S: *Rationale for the use of N-acetylcysteine in both prevention and adjuvant of COVID-19*, FASEB J 2020; 34: pp 13185–13193.

[6] Wong K K, Lee S W H, Kua K P: *N-acetylcysteine as adjuvant therapy for COVID-19 – A perspective on the current state of the evidence*, J Inflamm Res 2021; 14: pp 2993-3013.