

## Spray-Dried Powders as Nasal Absorption Enhancers of Cyanocobalamin

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**The aim of this work is to describe and characterize a new spray-drying procedure for the production of nasal powders as an alternative to the conventional freeze-drying method. Cyanocobalamin was chosen as the active ingredient and loaded into five different nonsoluble vehicles with high water absorption ability. Then these hydrated particles were suspended in methylene chloride and spray-dried. Particle size, morphology, true, bulk and tapped density, percentage of compressibility, moisture content, water intake, and drug diffusion were studied and significant differences were obtained depending on the nature of the vehicle. The drying method, either the new spray- or the conventional freeze-drying, was less important. Interestingly, an inverse correlation was found between water uptake and drug diffusion. Microcrystalline cellulose, dextran microspheres, and crospovidone were chosen for an *in vivo* bioavailability study in rabbits. Three other nasal reference formulations and an intravenous solution were also administered. The spray-dried powders showed higher bioavailability than the three nasal reference formulations. The highest absorption enhancement was observed with cellulose microcrystalline powders, which provided a 25% mean absolute bioavailability, followed by crospovidone and dextran microspheres formulations with mean bioavailability values of 14% and 7%, respectively. In conclusion, the new spray-drying method is useful for the production of cyanocobalamin nasal powders.**

**Key words** nasal; bioavailability; spray-drying; cyanocobalamin; cellulose; microsphere

Although nasal administration of drugs has many advantages, it is usually limited by some bioavailability restrictions due to the specific morphological and physiological characteristics of this administration route. To improve nasal absorption of drugs, different chemical enhancers have been used,<sup>1,2</sup> although their side effects usually limit clinical application. Another mechanism to improve nasal absorption is the use of different freeze-dried powders as drug carriers. These vehicles are able to modify tensions in the cytoskeleton or tight-junction proteins by physical mechanisms such as swelling of the vehicle and dehydration of the medium.<sup>3,4</sup> Moreover, bioadhesion of vehicle to the apical cell membrane could also induce conformational changes and increase the residence time of these powders in the nasal cavity, prolonging and improving absorption.<sup>5</sup> Among the materials that have been proposed as nasal enhancers are carboxyvinyl polymers,<sup>6–10</sup> degradable starch microspheres,<sup>5,11</sup> dextran microspheres,<sup>12</sup> cellulose derivatives,<sup>6,13–15</sup> and, more recently, chitosan.<sup>16,17</sup> Most of these water-absorbent and water-insoluble powdered nasal dosage forms have been prepared by freeze-drying. This process produces an intimate union between drug and vehicle because the drug is incorporated into the vehicle as an aqueous solution, which is partly or completely absorbed by the vehicle, depending on whether the volume exceeds the water intake capacity of the powder. Later, when this mixture is freeze-dried and sieved, the resultant powder can present the drug on the surface of the powder particles or in their core, depending on the pore size of the vehicle and the molecular size of the drug. If the pore size is small enough to impair the entrance of the molecules in spite of the entrance of the liquid, the drug is located on the surface and is more accessible to absorption. Meanwhile, if the drug is placed in the core of the particles, its release is slightly impaired. But in both cases drug and vehicle are in close contact and constitute an entity of which enhanced absorption has been documented.<sup>18</sup> Dohi *et al.*<sup>19</sup> noted that an intimate physical mixing in a mortar can produce similar re-

sults to those obtained with freeze-dried powders because most of the drug adheres to the vehicle due to the pressing force of pestle and mortar. A similar enhancement was reported by Provasi *et al.*<sup>20</sup> obtained with a high-energy co-ground process. Therefore an intimate union between drug and vehicle is required to manufacture nasal powders with enhanced absorption.

Spray-drying is an alternative process that is cheaper and faster than freeze-drying and has been employed previously to prepare nasal particles from soluble polymers.<sup>21,22</sup> The aim of the present study was to develop a novel spray-drying method to manufacture nasal powders suitable for different water-insoluble and water-absorbent vehicles and an allowing intimate union between drug and vehicle. Cyanocobalamin was chosen as the model drug for nasal administration. The *in vitro* physical characteristics of microcrystalline cellulose (MC), dextran microspheres (DM), hydroxypropyl cellulose (HP), croscarmellose sodium (CC), and crospovidone (CP) spray-dried powders are studied and compared with those of the corresponding freeze-dried powders to demonstrate that spray-drying can be a suitable alternative to freeze-drying. The *in vivo* characteristics of spray-dried MC (SD-MC), spray-dried DM (SD-DM), and spray-dried CP (SD-CP) as potential nasal absorption enhancers are studied and compared with those of three other nasal formulations and one intravenous formulation.

### MATERIALS AND METHODS

**Chemicals, Reagents, and Animals** Cyanocobalamin (Merck), DM (Sephadex<sup>®</sup> G25 Fine, Sigma), MC (Avicel<sup>®</sup> PH-101, FMC), CC (Ac-Di-Sol<sup>®</sup>, FMC), CP (Polyplasdone<sup>®</sup> XL, ISP Technologies), HP (LH-31, Shin-Etsu Chemical), and inhalation lactose (Pharmatose<sup>®</sup> 325M DMV International) were of pharmaceutical grade. The other chemicals employed were of analytical or pharmaceutical grade and were purchased from Panreac (Spain). Male New Zealand

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White rabbits weighing 2–2.5 kg were used in the *in vivo* study. Animals were allowed free access to food and water before the experiments, and the “Principles of Laboratory Animal Care” were adhered to.

**Preparation of Spray-Dried Powders** One milliliter of an aqueous solution of cyanocobalamin (1% w/v) was mixed with 1 g of the vehicles MC, DM, CP, HC, or CC to obtain a uniform distribution of the liquid in the solid bed. Then the mixture was dispersed in 400 ml of methylene chloride per gram of each powder and sprayed in a Büchi® B191 Mini Spray-Drier at an inlet temperature of 150 °C. The suspension was fed to the drier at a rate of 11 ml/min. An air flow rate of 800 l/h and an aspiration vacuum of 35 mbar were used. To compare with the previously described spray-dried particles, a pure aqueous solution of cyanocobalamin (1% w/v) was spray-dried in the same conditions.

**Preparation of Freeze-Dried Powders** Freeze-dried powders were prepared by adding 1 ml of an aqueous solution of cyanocobalamin (1% w/v) to 1 g of the vehicle. This mixture was frozen at –40 °C for 10 h in a Telstar® L-3 freeze-drier, then a vacuum was applied to obtain a pressure of 0.05 mbar. Temperature was increased from –40 °C to 0 °C in a 37 h cycle, and after 2 h at this temperature, it was increased to 30 °C over 6 h. The freeze-dried solid was sieved through a 0.1 mm mesh to obtain the primary particles.

**Determination of Particle Size and Morphology** Particle size was measured in a Galai® Cis-1 “Time of Flight” apparatus as the mean of six determinations with powder particles suspended in a Tween 80 (0.1% w/v) saline solution to avoid particle aggregation. The impact of the manufacturing processes or process conditions on particle morphology was studied with a scanning electron microscope (JEOL®, JSM 6400, CAI, UCM).

**Determination of Particle Density** True density was measured in triplicate using a helium pycnometer (Multivolume 1305 Micromeritics®). Poured ( $D_0$ ) and tapped ( $D_f$ ) density was measured in triplicate using a 10-ml graduated cylinder to measure the volume occupied by 3 g of powder, as described by Cornaz *et al.*<sup>23</sup> A Pharma-Test® PT-TD apparatus was employed to determine tapped density. Percentage compressibility as a measure of flowability was determined according to the equation reported by Staniforth<sup>24</sup>: %compressibility =  $100 \cdot (D_f - D_0) / D_f$ .

**Determination of Powder Moisture** Moisture was measured in triplicate in 100-mg samples according to the Karl-Fischer method in a Methrom® Karl-Fischer apparatus.

**Determination of *in Vitro* Water Intake** The ability of the spray-dried powders to absorb water by capillarity was evaluated using a Franz cell (12.5 ml) with a lateral capillary graduated tube in which the volume of liquid absorbed was measured.<sup>23</sup> The tests were carried out in triplicate with samples of 50 mg placed on a Millipore filter NY11 for 3.5 min and with purified water as liquid.

**Determination of *in Vitro* Drug Diffusion** The release kinetics of cyanocobalamin samples were studied in triplicate with a Franz cell<sup>25</sup> (Crown Glass). Powders and microspheres 30 mg were placed on a Millipore filter NY11. As diffusion medium, 12.5 ml of phosphate buffer, pH 6.0, R2 (Ph. Eur., 3rd ed.) was used. This diffusion medium was added only to the receptor chamber of the cells, kept at  $37.0 \pm 1.0$  °C and maintained in gentle agitation by means of

a magnetic stirrer. A sample of 625  $\mu$ l was drawn every 5 min until complete diffusion or a plateau was reached, and this volume was immediately replaced with thermostated fresh buffer. Samples were measured spectrophotometrically at 361 nm, and the amount extracted in each sample was taken into account to calculate the cumulative diffusion.

**Nasal Bioavailability Studies** Each formulation was administered to three or four restrained, non anaesthetized conscious rabbits. The nasal administration of the spray-dried powders (SD-MC, SD-CP, or SD-DM) was carried out with a nasal insufflator marketed for the nasal administration of sodium cromoglycate (Rinoflador, Fisons, Spain). Ten milligrams of each powder (1% w/w of cyanocobalamin) containing a dose of 100  $\mu$ g of cyanocobalamin was inserted into a hard gelatin capsule (#3) and delivered into the nasal cavity of the rabbits through one nostril. A physical mixture obtained with spray-dried cyanocobalamin and inhalation lactose (Cy-L) was also administered in the same way as spray-dried powders and used as a reference formulation. Intravenous and nasal administration of a liquid dosage form, 100  $\mu$ l of a 0.1% w/v cyanocobalamin aqueous solution (equivalent to a 100  $\mu$ g cyanocobalamin dose), was carried out through the marginal vein of the left ear and in the nasal cavity. The nasal aqueous solution was administered nasally as drops with a micropipette and as a spray by means of the marketed nasal sprayer that delivers an approximate volume of 100  $\mu$ l (Beconase® nasal spray, Glaxo, Spain).

Blood samples (3 ml) were collected in glass tubes by puncture of the marginal vein of the right ear immediately prior to and at 15, 30, 45, 60, 90, 120, 180, and 240 min after nasal administration and prior to and at 5, 10, 30, 45, 60, 90, and 120 min after intravenous administration. To obtain a better characterization of the concentration–time profile after intravenous administration, the first sampling time points were set sooner. The serum was separated after the blood had been stored for one week at 4 °C and analyzed immediately afterward. Serum cyanocobalamin was assayed by radioimmunoassay (RIA) with Magic® B12 [<sup>57</sup>Co]/Folate [<sup>125</sup>I] No-Boil Radioassay (Chiron Diagnostics).

The pharmacokinetic parameters area under the concentration–time curve from 0 to 120 min ( $AUC_{0-120}$ ), peak concentration ( $C_{max}$ ), and time to reach the peak concentration ( $t_{max}$ ) after both intravenous and intranasal administration of cyanocobalamin were calculated independently of kinetic models by the trapezoidal rule and from the raw data. The absolute bioavailability following intranasal administration was determined by dividing the intranasal  $AUC_{0-120}$  by the intravenous  $AUC_{0-120}$ .

**Statistical Analysis** These calculations were carried out using the computer program Statgraphics Plus 3.0 (Statistical Graphics). Comparisons were carried out by one-way analysis of variance (ANOVA) and Newman-Keuls multiple range tests to determine the different mean values when ANOVA found statistically significant differences or Fisher's least significant differences to detect tendencies when ANOVA did not find statistically significant differences.

## RESULTS AND DISCUSSION

Depending on the solubility of the drug in the liquid medium to be spray-dried, different types of interactions be-

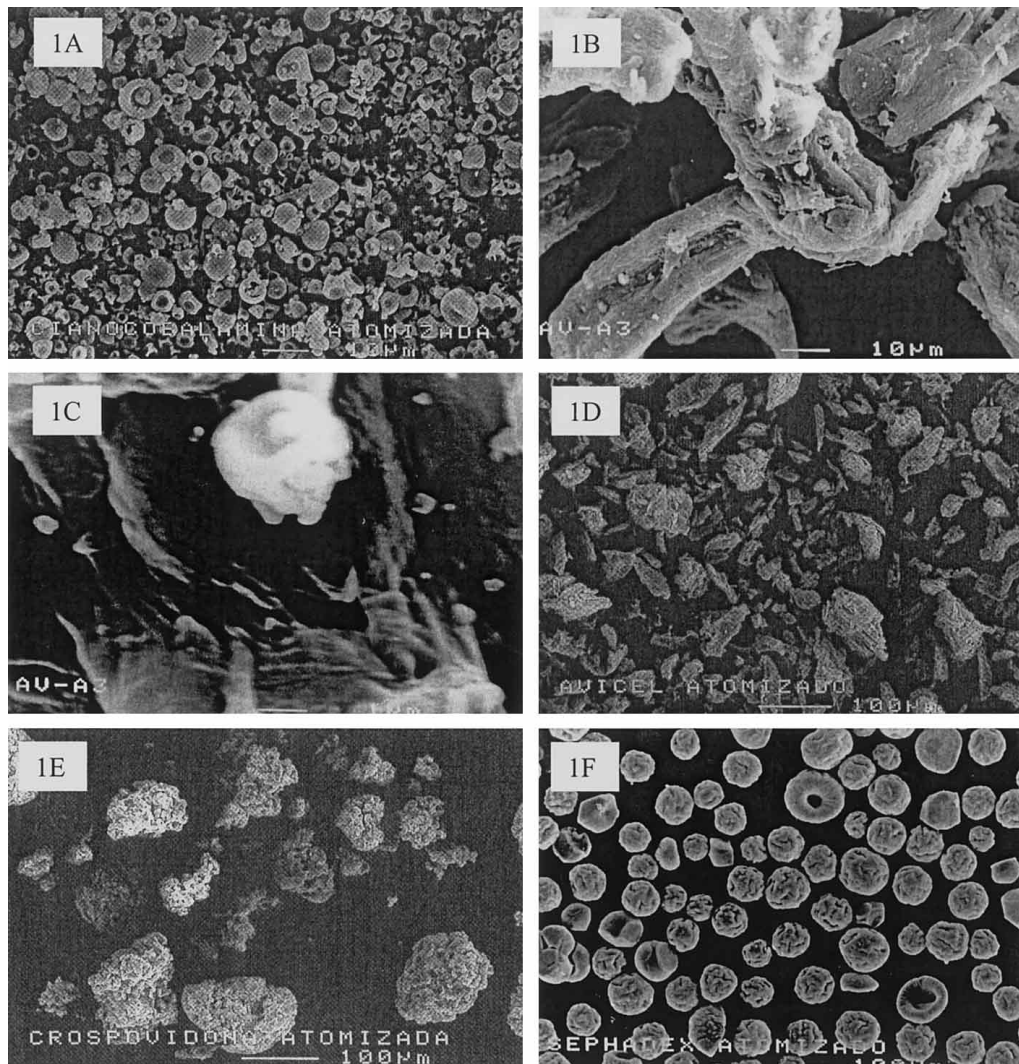


Fig. 1. Scanning Electron Micrographs of Cyanocobalamin Particles Obtained from an Aqueous Cyanocobalamin Solution (A), SD-MC Particles Obtained from an Aqueous Suspension (B), and the Following Spray-Dried Particles: SD-MC (C and D), SD-CP (E) and SD-DM (F) Obtained from a Methylene Chloride Suspension

tween drug and the nonsoluble vehicle can be obtained. Figure 1 shows the morphology of different spray-dried particles. Figure 1A shows a typical hollow, round appearance of pure cyanocobalamin obtained after spray-drying of a water solution. When cyanocobalamin is mixed with different vehicles and then dried, various types of dried particles can be obtained depending on the solvent medium. If water is used, cyanocobalamin is dissolved in it and thus all drops to be dried will contain drug, although the nonsoluble vehicle may not necessarily be contained in them. Depending on the presence of vehicle particles in the drops, two populations of particles may be formed. Some particles can be the vehicle with the loaded drug, while other particles can be composed of pure drug. Figures 1B and C show this effect for an SD-MC formulation. Some of the particles in Figs. 1B and C are large and fibrous, corresponding to loaded MC, and others are smaller and more spherical, corresponding to pure cyanocobalamin. As these two different types of particles present very different sizes and shapes they should have different deposition patterns in the nasal cavity. Therefore drug absorption cannot be efficiently enhanced because this effect

is limited to the vicinity of the enhancer vehicle particles responsible for tight-junction opening. To avoid the production of these two populations of particles a new method is proposed. In the first stage, the drug is incorporated into the vehicle as a solution, taking advantage of the water intake and swelling of the vehicle. Then the drug-vehicle particles are dispersed in methylene chloride, in which the drug is insoluble. This will avoid drug leakage from the vehicle particles. Methylene chloride is suitable for use in the spray-drier because it has a low risk of explosion and its toxicologic characteristics are well known.<sup>26,27</sup> Figures 1D—F show the morphology of SD-MC, SD-CP, and SD-DM particles, respectively with cyanocobalamin obtained with methylene chloride as solvent. Small spherical particles of pure cyanocobalamin are visible in none. The appearance of all these types of spray-dried particles is very similar to the initial raw materials and the freeze-dried particles (results not shown), with the exception of DM, which after freeze-drying and spray-drying has a surface appearance different from that of the initial smooth surface of the raw material. This effect has been reported previously.<sup>18,23</sup>

Table 1. Mean Values and Standard Deviation in Parentheses of the Following Powder Properties: Size (Volume Median Diameter), Moisture, True Density, Percentage Compressibility, and Water Intake, of Raw Vehicles, Freeze-Dried (FD) and Spray-Dried (SD) Powders

Powder type	Size ( $\mu\text{m}$ )	Moisture (%)	True density ( $\text{g}/\text{cm}^3$ )	Percentage compressibility (%)	Water intake ( $\text{ml}/\text{g}$ )
DM	48.7 (2.8)	5.6 (0.5)	1.49 (0.006)	8.1 (0.1)	3.4 (0.2)
FD-DM	48.0 (1.2)	2.2 (0.3)	1.42 (0.010)	12.9 (0.4)	3.6 (0.1)
SD-DM	50.3 (2.2)	1.8 (0.2)	1.46 (0.007)	12.6 (0.9)	3.3 (0.2)
MC	204.2 (16.2)	4.3 (0.1)	1.60 (0.016)	28.7 (0.9)	3.3 (0.1)
FD-MC	199.6 (15.2)	4.5 (0.1)	1.55 (0.006)	30.6 (0.5)	2.7 (0.1)
SD-MC	201.9 (10.5)	2.6 (0.03)	1.55 (0.006)	33.6 (0.9)	2.7 (0.1)
HP	203.6 (10.0)	5.5 (0.2)	1.46 (0.008)	33.7 (0.9)	6.1 (0.2)
FD-HP	201.5 (16.5)	6.5 (0.4)	1.46 (0.006)	38.3 (0.2)	4.5 (0.3)
SD-HP	190.1 (14.3)	4.5 (0.1)	1.42 (0.009)	38.6 (0.5)	5.4 (0.3)
CC	37.5 (10.6)	6.0 (0.1)	1.59 (0.012)	25.4 (0.5)	6.9 (0.4)
FD-CC	36.7 (13.9)	4.5 (0.3)	1.59 (0.119)	29.7 (0.1)	9.1 (0.5)
SD-CC	38.6 (8.0)	2.7 (0.3)	1.53 (0.160)	16.1 (0.5)	6.9 (0.1)
CP	226.9 (12.4)	11.3 (0.8)	1.21 (0.005)	12.8 (0.8)	3.8 (0.2)
FD-CP	224.3 (7.3)	12.1 (1.0)	1.24 (0.007)	28.6 (1.4)	3.7 (0.1)
SD-CP	200.1 (20.4)	8.0 (0.2)	1.23 (0.008)	28.3 (0.8)	3.9 (0.2)

Keycode: MC (microcrystalline cellulose), DM (dextran microspheres), CP (crospovidone), CC (croscarmellose), HP (hydroxypropyl cellulose), FD (freeze-dried) SD (spray-dried).

Table 1 shows some of the different *in vitro* characteristics of the spray-dried particles. It is clear that the results vary depending mainly on the nature of the vehicle.

In terms of differences in size, it can be pointed out that during the spray-drying process some of the coarsest particles cannot be transported by air and fall to the bottom of the drying chamber. In the same experiment, the finest particles are lost with the exhausted air, as has been described previously.<sup>28)</sup> As a consequence, the volume median diameter only varies in some powders in which the loss of fine particles does not compensate for the loss of coarse particles. This variation lacks any practical consequence because of its small magnitude, reaching statistical significance only in the case of CP. This effect can be considered as an advantage since it eliminates some of the finest particles that can reach the alveoli and it also provides a slightly more uniform particle size distribution.

Flow characteristics of the different powders were studied by means of the percentage of compressibility (Table 1). Percentage compressibility varies depending on the material between free-flowing powders such as DM and very poor flowing powders such as HP. The water intake and drug diffusion performance of the different spray-dried particles are shown in Table 1 and Fig. 2, respectively. These parameters are closely related to the nasal absorption enhancement because water intake is responsible for tight-junction opening while drug diffusion is responsible for drug release, although some other parameters, such as nasal deposition pattern or bioadhesion characteristics, may also be of great importance in the *in vivo* performance of the formulations. Spray-drying and freeze-drying have a similar effect on the water intake ability of MC, DM, and CP but not of that of HP and CC. Freeze-drying increases the water intake of CC and decreases that of HP more than spray-drying. An interesting inverse relationship has been observed between water uptake and drug diffused at every sample time point for spray- and freeze-dried products, as shown in Fig. 3. The higher the water intake, the lower the drug diffusion property. For example, FD-MC and SD-MC formulations show the highest drug diffusion and the lowest water intake, and the opposite effect is observed with FD-CC. It appears that for these vehicles when the water is

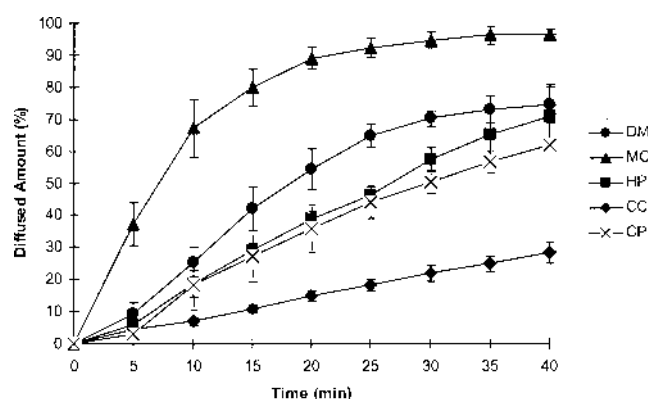


Fig. 2. Cyanocobalamin Diffusion Profile from Different Spray-Dried Powders

▲, SD-MC; ●, SD-DM; ×, SD-CP; ◆, SD-CC; ■, SD-HP.

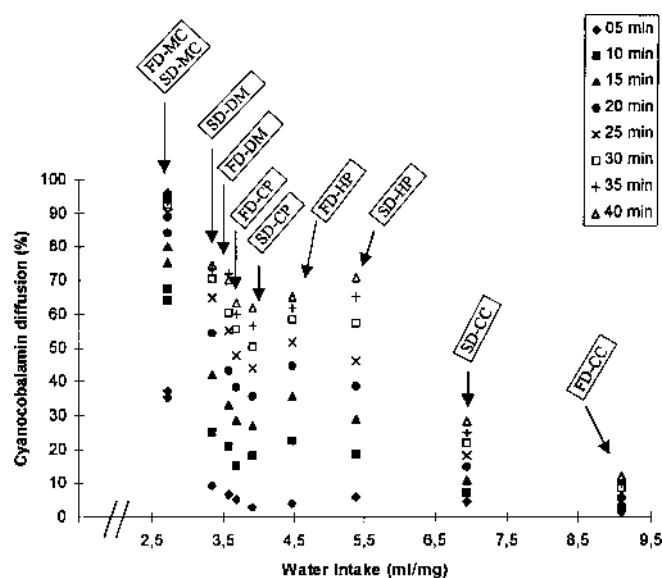


Fig. 3. Diffused Cyanocobalamin at Different Time-Points versus Water Intake of the Nasal Powders Prepared with Different Vehicles and Manufactured Either by Freeze-Drying (FD-Particles) or Spray-Drying (SD-Particles)

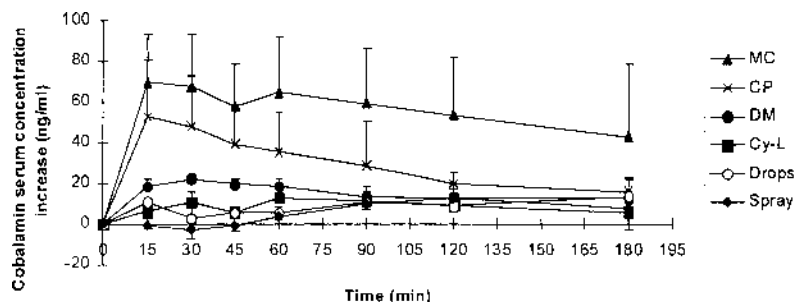


Fig. 4. Cobalamin Serum Concentration Profile after the Administration of Six Different Nasal Formulations of Cyanocobalamin (Mean and Standard Deviation)

▲, SD-MC; ●, SD-DM; ×, SD-CP; ◆, spray; ○, drops; ■, Cy-L (physical mixture of cyanocobalamin and lactose).

being intaken the drug release is impaired. The results in Fig. 3 suggest that for most of these systems the drug resides on or near the surface of the particle and that the process of release is due to desorption rather than diffusion from the interior of the particles.

To study the *in vivo* enhancement effect of spray-dried particles, SD-MC, SD-CP, and SD-DM formulations were chosen to compare their bioavailability characteristics with those of three other reference nasal formulation plus one intravenous formulation. Figure 4 shows the mean cobalamin serum profile of the six nasal formulations: three spray-dried powders; one physical mixture; and two cyanocobalamin nasal solutions (drops and spray). The nasal solution was administered as nasal drops and as a nasal spray to determine if the route of administration affects the absorption process. In our experimental conditions, neither the nasal solution in drops nor in spray were able to increase the basal level of serum cobalamin in rabbits to a statistically significant level. This means that either the cyanocobalamin is hardly absorbed by the nasal route when it is administered without absorption enhancers or that due to the lack of any viscosity-enhancing agent these formulations were not retained in the nasal cavity for long enough to allow their absorption. These agents are usually incorporated into the formulation to increase its residence time in the nasal cavity, but they might also enhance nasal absorption by other mechanisms such as tight-junction opening. To avoid this rare, but possible, effect, these agents were not included in the liquid formulations. Recently, a nasal bioavailability of 5% has been reported by van Asselt *et al.*<sup>29</sup> in humans after administration of a hydroxocobalamin solution containing a viscosity-enhancing agent.

The physical mixture of cyanocobalamin and lactose was employed as a reference for the same route and same pharmaceutical form. If a high concentration of cyanocobalamin in some locations of the nasal mucosa membrane facilitates nasal absorption, after the administration of this powder a higher serum profile than those observed with the aqueous solutions would be expected. However, a similar profile was found, which confirms the difficulties of cyanocobalamin absorption without absorption enhancers.

The previous three reference formulations allow comparison of the enhancing properties of the spray-dried powders. The serum cobalamin concentration–time profiles after the nasal administration of SD-DM, SD-CP, and SD-MC were significantly different from the basal value and from the ref-

Table 2. Pharmacokinetic Parameters of Three Cyanocobalamin Nasal Spray-Dried Powder Formulations (SD-MC, SD-CP, SD-DM) and One Intravenous (IV) Solution (Mean and 95% Confidence Interval or Mode and Range)

Formulation	$AUC_{0-120}$ (ng·min/ml)	$C_{max}$ (ng/ml)	$t_{max}$ (min)
SD-MC	6988.9 (1812–12165)	72.9 (24.88–120.9)	15 (15–30)
SD-CP	4072.6 (102.0–8043)	67.1 (41.53–108.6)	15 (15–30)
SD-DM	1916.5 (1732–2101)	22.3 (3.436–41.25)	30 (30)
IV solution	27860.1 (22441–33280)	472.3 (312.9–631.5)	—
ANOVA $F$ ( $p$ -value)	$F=2.6303$ ( $p=0.1406$ )	$F=2.7118$ ( $p=0.1343$ )	—

erence formulations. Table 2 shows the mean values and the 95% confidence interval of  $AUC_{0-120}$ ,  $C_{max}$ , and  $t_{max}$  after the nasal administration of these three spray-dried nasal powders and the intravenous administration of an equal dose of cyanocobalamin in aqueous solution. These results confirm the enhancing properties of the water-insoluble and water-absorbent vehicles, demonstrating that CP, to our knowledge used here for the first time as enhancer, is a suitable nasal absorption-enhancing vehicle since it shares the insolubility and absorption properties of MC and DM. In addition, these results show that MC is more active than CP as a nasal absorption enhancer and that the latter is more active than DM. The absolute mean bioavailability of cyanocobalamin in rabbits are: 25.1%, 14.6%, and 6.9% for SD-MC, SD-CP, and SD-DM, respectively. The surprisingly low enhancing effect observed with SD-DM could be due to its inadequate deposition on the nasal cavity of rabbits, where a very humid environment in the external end is observed. Although *in vivo* results cannot easily be correlated to *in vitro* characteristics it is clear that the highest bioavailability was obtained with the large and poor flow of SD-MC and SD-CP particles, while the smaller, free-flowing spherical SD-DM provides the lowest bioavailability. Furthermore, SD-MC particles have the highest diffusion rates although the effect of this factor is not so clear on bioavailability because SD-DM has a similar or better diffusion than SD-CP, but its bioavailability is lower.

In conclusion, this new spray-drying method for water-insoluble and water-absorbent vehicles produces solid dried particles with similar physical characteristics to those ob-

tained by freeze-drying. Therefore this novel spray-drying method can be considered as an alternative to the conventional freeze-drying process. From the present study it is clear that most of the vehicle physical characteristics are not modified in the drying process. The properties of the final powders are more related to the nature of the vehicle than to the drying procedure.

*In vivo* experiments show that cyanocobalamin nasal absorption in the rabbit model is very low and almost a negligible amount is absorbed when administered without absorption enhancers. Nevertheless, the incorporation of cyanocobalamin in water-insoluble and water-absorbent particles by means of spray-drying significantly increases the bioavailability of the drug. In our experimental conditions, the best results were obtained with SD-MC particles as a cyanocobalamin carrier and nasal absorption enhancer, achieving a 25% mean absolute bioavailability.

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