

***This is an author version of the contribution published on****:  
Questa è la versione dell’autore dell’opera:  
 [European Journal of Pharmaceutical Sciences 2013, 50, 56–68, DOI: 10.1016/j.ejps.2013.03.003]*

***The definitive version is available at:*** *La versione definitiva è disponibile alla URL:  
[http://www.sciencedirect.com/science/article/pii/S0928098713000985]*

**Mechanochemical activation of vincamine mediated by linear polymers: assessment of some “critical” steps**

Dritan Hasaa, Beatrice Perissuttia, Mario Grassib, Michele R. Chierottic, Roberto Gobettoc, Valerio Ferrarioa, Davide Lenazd, Dario Voinovicha\*

a Department of Chemical and Pharmaceutical Sciences, University of Trieste, P. le Europa 1, I-34127 Trieste, Italy

b Department of Industrial Engineering and Information Technology, Via Valerio 6/A, I-34127, Trieste, Italy

c Department of Chemistry, University of Torino, Laboratory of NMR Spectroscopy, Via P. Giuria 7, I-10125 Torino, Italy

d Department of Geosciences, University of Trieste, Via Weiss 8, I- 34127 Trieste, Italy

\*Corresponding author:

Prof. Dario Voinovich,

Dept. Chemical and Pharmaceutical Sciences,

University of Trieste

Piazzale Europa 1, 34127, Trieste, Italy

E-MAIL: vojnovic@units.it

TEL : 0039 - 040 - 5583106

FAX : 0039 - 040 - 52572

**Abstract**

The aim of the research was to investigate three “critical steps” that deserve particular attention during the mechanochemical activation of vincamine. The first step consisted in the selection of the best polymeric carrier/most affine stabiliser between linear PVP and NaCMC by using the GRID and the GRID based AutoDock software packages which permit to calculate their surface features and interactions. Moreover, the calculation of the partial and total solubility parameters supported the results obtained by GRID and AutoDock software.

Then, after the selection of linear PVP-K30 as the suitable carrier, the influence of process and formulation variables on the amorphization degree and solubility enhancement was studied, to select the most suitable process conditions and formulation parameters. Subsequently, the best performing samples were widely characterized using XRPD, TEM and SSNMR (including the proton relaxation (1H T1 NMR) time) techniques. These studies highlighted that all the coground samples were nanocrystalline solid dispersions indicating a dramatic difference between the amorphization capacities of linear PVP-K30 and cross-linked PVP, used in previous analogous experiences. In particular, 13C, 15N and 1H T1 NMR data point to a description of the system as a dispersion of nanocrystals in the polymer. In these dispersions vincamine is in a disordered crystalline state due to extensive interactions and contacts with PVP-K30 but the main hydrogen bonding motif characterizing its packing remains. Again, differently from cross-linked PVP, dissolution studies revealed that linear PVP-K30 was able to promote a complete *in vitro* solubilisation of vincamine in some coground samples. What is more important, by using a linear polymer, drug-to-polymer and milling time variables appeared less influent on the solid state and *in vitro* properties of the composites. Finally, stability studies conducted for a period of 1 year highlighted the high physical stability of the selected samples.

**Keywords:**Vincamine, mechanochemical activation, linear polymers, solubility parameters, solid dispersion, physical stability.

**1. Introduction**

Vincamine, a cerebral vasodilator and nootropic drug, possesses interesting properties for use in neurodegenerative conditions (Karpati et al., 2002; Vas and Gulyas, 2005; Vereczkey, 1985). However, pure vincamine has limited solubility (nearly 40 mg/L at pH 7.4 (Hasa et al., 2012a)) due to its complex crystalline structure and lipophilic nature (Mazak et al., 2003). Hence, vincamine shows poor oral bioavailability (Maincent et al., 1986). It is therefore necessary to induce modifications in the physico-chemical properties of vincamine in order to improve its solubility turning vincamine suitable for patient administration.

Among different possible alternatives, the strategy of crystal lattice disruption represents one of the most common approaches for solubility improvement. In fact, the solubilisation phenomenon can be described as the sum of different energetic contributions, one of the most important being represented by the energy necessary to disrupt the crystalline packing (Lipinski et al., 1997).

Mechanochemical activation (MA) is a solvent-free process capable to alter physico-chemical properties of a substance. High amounts of mechanical energy are usually produced in ball mills and are transferred to the loaded crystalline substance. The energy absorbed leads into crystal defects and, subsequently into the formation of nanocrystalline and amorphous phases *via* a complex interplay of several factors (Colombo et al, 2009). Both nanocrystalline and amorphous structures are more prone to dissolution than the crystalline state, due to their higher free energy.

However, the above highly soluble status is metastable, thus likely subjected to re-crystallisation phenomenon. For this reason, MA of a drug is usually performed in presence (cogrinding) of a suitable stabilising agent (carrier), usually a cross-linked polymer, giving origin to nanocrystalline and/or amorphous solid dispersions. Drug/carrier composites offer additional advantages with respect to the pure drug, such as physico-chemical and biopharmaceutical properties, likely to be an interesting drug delivery system for patient’s administration (Colombo et al., 2009; Hsia et al., 1977; Shakhtshneider and Boldyrev, 1999). Moreover, MA is a solvent-free process that is less harmful for the patient health and the environment, and cost-effective for the industry. Nevertheless, despite the aforementioned advantages, the number of patents and therefore the number of commercial products prepared *via* MA process remains low and slowing down in the last years (James et al., 2012). This can be attributed to the scarce knowledge of mechanisms involved in MA process, to the low number of studies in the drug/carrier compatibility direction and to the small number of characterisation techniques used in order to understand the nature of drug/carrier interaction and stabilisation.

The “route” from the initial crystalline drug to a final solid dispersion prepared *via* MA process, possessing adequate physico-chemical and biopharmaceutical properties, can be sketched as a complex pathway with some “critical steps”. The first “critical” step consists in the selection of the optimal carrier on the basis of drug/carrier affinity, since the latter must act as a stabiliser of the former. Therefore, the potential of the carrier to form stable solid dispersions must be proved. It is difficult to find predictive models or techniques for this purpose and the state of the art is still evolving.

The second “critical” step is represented by the MA process itself: studies on process (milling time, bowl loading etc.) and formulation variables influencing the quality of the solid dispersions are always necessary. Pharmaceutical companies are putting a lot of effort to overcome these hurdles in order to design a fully controllable process.

Another “critical” point is the characterisation of the solid dispersions to select the product with the best physico-chemical and biopharmaceutical properties. Full characterisation is a crucial step for understanding the properties of the composites. Therefore, investigating the afore-mentioned “critical” steps is of crucial importance.

In the present research, MA of vincamine was performed in presence of linear polymers, and the above mentioned “critical steps” were considered. Whilst in our previous experience two cross-linked polymers were selected as vincamine carriers in virtue of their different physico-chemical properties (Hasa et al., 2012a), in this study, polivinilpyrrolidone (PVP-K30) and sodium carboxymethyl cellulose, (NaCMC) were considered as carriers and potential stabilisers of the nanocrystalline and amorphous structures of the drug, expected from the MA process.

During the first phase of the study, the chemical affinity between vincamine and the two carriers was investigated. To this purpose, the GRID software, AutoDock GRID based software and the solubility parameters and were used in order to evaluate the similarity between vincamine and the linear polymers and to select the adequate carrier capable to induce the formation of a higher degree of structure disorder and to stabilise it for a long period of time. Subsequently, the second critical step was studied by a detailed evaluation of the effects of the process and formulation variables (milling time, drug-to-carrier weight ratio) on both crystalline lattice disruption and solubility enhancement. Then, an in depth examination of the selected coground samples by different solid-state techniques such as transmission electron microscopy (TEM), X-Ray Powder Diffraction (XRPD) and Solid-State NMR Spectroscopy (SSNMR) was carried out. Finally, one year long physical stability studies of the selected composites were carried out.

**2. Materials and methods**

2.1. Materials

Pure vincamine was kindly provided from Linnea SA (Riazzino-Locarno, CH). Polyvinylpyrrolidone K30 (PVP-K30) and Sodium carboxymethyl cellulose (NaCMC) were purchased from BASF (Ludwigshafen, Germany) and Shilling (Milan, Italy) respectively. The other chemicals and solvents of HPLC grade were purchased by Carlo Erba (Milan, Italy).

2.2. Methods

2.2.1. Preparation of physical mixtures (PM)

The vincamine:NaCMC physical mixture (PM) was prepared in a batch of 10 g by manually mixing drug and polymer with a stainless steel spatula for the standardised time of 8 min. Then, 4.9 g were coground in the planetary mill and the rest was used for comparison purposes. While, the vincamine:PVP-K30 physical mixtures were prepared in batches of 25 g. The component weight proportions were the following: 1:2, 1:4 and 1:7 w/w which were manually mixed with a stainless steel spatula for the standardised time of 8 min. Subsequently, the mixtures were divided in 5 quotes. Of those, one part was used as PM for comparison purposes, and the other 4 parts were singularly subjected to mechanochemical treatment for different periods of time (45, 90, 120 and 180 min) using the operating conditions reported in the following paragraph.

2.2.2. Preparation of coground mixtures (Cog)

During the first step of the study two binary systems consisting of vincamine:PVP-K30 and vincamine:NaCMC were prepared in a 1:7 weight ratio (wt ratio) and coground for 180 min based of the previous experience (Hasa et al., 2012a). After the selection of PVP-K30 as optimal polymer, three vincamine:PVP-K30 weight ratios were considered: 1:2, 1:4 and 1:7, and, as milling times: 45, 90, 120 and 180 min. Thus, 12 different composites and 3 physical mixtures were prepared. The grinding process was performed in a planetary mill Fritsch P5 (Pulverisette, Contardi Fritsch s.r.l., Milan, Italy) which was equipped by 4 agate cylindrical grinding chambers (internal height Hv=2.6 cm, internal radius Rv=3.2 cm, internal volume = 27.5 cm3). As grinding media, agate balls having a 2.2 cm diameter were adopted.

In order to maximize the energy transfer from the mill to the loaded powder, *via* the grinding media, the grinding process was optimised according to a previously published mathematical model (Voinovich et al., 2009a). A mean weight of 4.5 g and 4.9 g for the vincamine:PVP-K30 or vincamine:NaCMCsystems respectively were used. The grinding process was stopped every 15 min for the purpose of homogeneously mixing the mass with a stainless steel spatula (Voinovich et al., 2009a, 2009b, Hasa et al., 2012a).

2.2.3. Calculation of three-dimensional solubility parameters

Solubility parameters are calculated on the basis of the cohesive energy density that is the energy necessary to separate the molecules of a solid or a liquid in a sufficient distance to eliminate all the interaction between molecules. Cohesive energy density is then transformed in solubility parameters *via* a series of assumptions and calculations (Hancock et al., 1997). Different authors have proposed different approaches to calculate the solubility parameters. The most used method is a combination between Fedors and Hoftyzer/Van Krevelen methods (Van Krevelen and Hoftyzer, 1990). Moreover, Hansen extended the application of solubility parameters to polar systems permitting a sub-division of the total solubility parameter into three partial parameters: dispersion component (δd), polar component (δp) and hydrogen bonding component (δh, Hansen, 1969). In this work, the three-dimensional solubility parameters for vincamine, PVP-K30 and NaCMC were calculated according to Hansen’s approach using the computer programme SPWin version 2.1 (Breitkreutz, 1998).

2.2.4. X-ray Powder Diffraction Studies (XRPD)

XRPD analyses were performed using the D500 (Siemens, Munich, Germany) diffractometer with a mono-chromate Cu-Kαradiation (1.5418 Å) generated using a secondary flat graphite crystal. Each sample was analysed in duplicate using a current at 20 mA and the voltage was set at 40 kV. The considered 2θ angle ranged from 5 to 35° using a step wise of 0.05° and the counting time was 5 sec/step.

2.2.5. Quantitative analysis by X-Ray Powder Diffraction (QXRPD)

QXRPD analyses were performed in order to quantify the amorphous fraction in the Cog samples prepared. The QXRPD analyses were performed according to a previously published method (Bergese et al., 2002). This method was demonstrated to be suitable for systems formed by a drug dispersed into an amorphous polymer (considering the structured halo in which the crystalline drug peaks are visible). Moreover, it can give reliable information in complex systems formed by crystalline and amorphous drug (Bergese et al., 2004).

2.2.6. Solid-state Nuclear Magnetic Resonance (SSNMR) spectroscopy

SSNMR measurements were run on a Bruker AVANCE II 400 instrument operating at 400.23, 100.65 and 40.56 MHz for 1H, 13C and 15N, respectively. 13C and 15N CPMAS spectra were recorded at room temperature at the spinning speed of 12 and 9 kHz, respectively. Cylindrical 4mm o.d. zirconia rotors with sample volume of 80 µL were employed. A ramp cross-polarization pulse sequence was used with contact times of 3 (13C) or 4 (15N) ms, a 1H 90° pulse of 3.30 µs, recycle delays of 8 s, and 1024 (13C) or 2048-14000 (15N) transients. The two pulse phase modulation (TPPM) decoupling scheme was used with a frequency field of 75 kHz.

13C and 15N scales were calibrated with glycine (13C methylene signal at 43.86 ppm) and (NH4)2SO4 (15N signal at δ=355.8 ppm with respect to CH3NO2) as external standards. Proton spin-lattice relaxation times, 1H T1, were obtained by the 13C-detected Inversion Recovery Cross Polarization technique, where one measures the 13C magnetization that appears through the CP process after relaxation of the 1H magnetization during the time τ.

2.2.7. Dissolution kinetic tests (DKT)

For the DKT, 150 cm3 of a pH 7.4 buffer (0.2 M KH2PO4/0.2 M NaOH) was used at 37°C. In this dissolution medium, the solubility at equilibrium of pure vincamine was 41.6 mg/L as previously reported (Hasa et al., 2012a). In the present study, the *in vitro* dissolution tests were used as a prediction of the *in vivo* performance of each product. Therefore, sink conditions were not maintained during dissolution tests in order to build up the super-saturation, to mimic conditions in the gastro-intestinal tract and to allow possible events such as nucleation, crystallization and precipitation (Sun et al., 2012).Hence, at time zero, a suitable amount of sample (as a pure vincamine, PM or coground sample) to give 12.6 mg of vincamine was added to the dissolution medium and each DKT lasted 180 min. In these tests, uniformity conditions were ensured by using an impeller (rotational speed 200 rpm). Instead, the determination of vincamine concentration was performed by using a fibre-optic apparatus (HELLMA, Milano, Italy), which was connected to a spectrophotometer (ZEISS, Germany, wavelength 268.49 nm). This technique, also used in our previous studies (Hasa et al., 2011, Hasa et al., 2012a), allows an *in situ* determination of concentration of a substance without perturbing the dissolution environment and often overcomes the problem connected to drug concentration measurements in the presence of generated solid particles. However, in this study linear polymers were used as vincamine carriers. Consequently, monitoring drug concentrations by using an *in-situ* fibre-optic probe without proper precautions may lead to an overestimation of drug concentration due to several causes including Tyndall effect (Van Eerdenbrugh et al., 2011). Accordingly, in the present research the scattering effect due to super-saturation phenomenon, occurring at every wavelength, was eliminated by a difference between the absorbance measured at 270.19 nm (which is vincamine maximum absorption) and that measured at 400.49 nm where vincamine did not absorb. Moreover, a further subtraction from the resulting absorbance was performed to take into account the Tyndall effect: preliminary DKT of the two polymers (PVP-K30 and NaCMC) were performed by recording absorbance values at the same time intervals as those performed for the selected composites. The absorbance value observed at each time interval of DKT of pure polymers was then subtracted to that obtained during DKT of the coground samples. The samples were tested in triplicate and the results were expressed as mean ± S.D.

2.2.8. Screening of *in vitro* equivalent composites using the FDA bioequivalence limits.

In order to make a comparison among the Cog samples, the FDA bioequivalence limits (FDA, 2003) were adopted and used for the *in vitro* solubilisation experiments. Firstly, the area under the curve of solubilised concentration vs. time from t=0 min to the last measuring point (180 min, AUC0-180) was calculated and used as a parameter to evaluate the degree of activation of the samples. The results are listed in Table 3 (as AUC0-180 ± S.D.; *n*=3) for all the Cog samples and PM.

Subsequently, in all the samples prepared, the higher AUC value was recognised and indicated as AUCmax. Further, the ratio (indicated asZ) between the AUC of each formulation and AUCmax was calculated as following:



(1)

For all the Z ratios, the associated uncertainty (indicated as dz*)* was calculated with the classical formula of the relative error of a ratio (Taylor, 1997):

 (2)

where dAUCformulation and dAUCmax signify the S.D. of the AUCformulation and AUCmax values, respectively.

2.2.9. Transmission Electron Microscopy (TEM)

Prior to analysis the selected samples were fixed in 1 % osmium tetroxide for 12 hour at 4°C and embedded in Dow Epoxy Resin (DER 332). Subsequently, ultrathin sections were cut by an ultratome Leica Ultracut UCT8 (Leica, Mikrosysteme Aktiengesellschaft, Wirn, Austria), double stained with uranyl acetate and lead citrate and examined with a Philips EM 208 transmission electron microscope (Philips, Eindhoven, the Netherlands) with an accelerating voltage of 100 kV. Images were acquired using an Olympus Morada CCD camera.

2.2.10. Aging Studies

The XRPD and DKT analyses of the best performing composites were repeated every 4 months for a period of 12 months in order to check possible re-crystallization and/or crystal growth phenomena. Throughout the study the samples were reserved at 25°C in desiccators.

**3. Results and Discussion**

**3.1. Selection of carrier**

As previously mentioned, solid dispersions represent potential drug delivery systems to improve dissolution rate of poorly soluble drugs such as vincamine (Hasa et al., 2012a). Moreover, when the solid dispersion is produced *via* a solvent-free and cost-effective process such as MA, it becomes attractive to pharmaceutical companies i.e. for marketing. However, due to some limitations (e.g. physical instability and drug/carrier incompatibility), the number of marketed solid dispersions produced *via* MA is very small (James et al., 2012). Therefore, studies in this direction set themselves apart by their importance.

In the present study, in order to evaluate vincamine:PVP-K30 and vincamine:NaCMC compatibility/affinity some analyses were carried out.

Firstly, the three-dimensional contour surfaces of vincamine, PVP-K30 and NaCMC (monomer units) were generated using a GRID mapping (Goodford, 1985).

In this study GRID was used to pinpoint hydrophilic and hydrophobic three-dimensional zones in the molecules and monomers under study. This was made possible by using two different probes: WATER that calculates the molecule regions where it is possible to position a water molecule including also proton acceptor and/or donor sites (Goodford, 1985), and DRY to locate the apolar molecular regions. The results, reported in Fig. 1, clearly indicate that VIC and PVP-K30 possess mainly hydrophobic regions, whereas only small portions are water zones. On the contrary, NaCMC possesses wide hydrophilic regions.

We speculate that similarity among three dimensional zones favours drug/carrier interaction.

If we assume that during MA, in presence of a carrier, each vincamine molecule “detached” from the crystalline lattice is in contact with a carrier monomer unit, then must establish week intermolecular interactions with the carrier and the contact zone between drug molecules and the monomer must be energetically favourable in order to form a stable system. This statement may be also valid in case of vincamine molecules at the nanocrystal surface. Hence, the similarity of molecular zones becomes crucial since it indicates an interaction site with favourable energy and, consequently a higher probability that vincamine molecules will establish intermolecular interactions. A higher similarity between vincamine and PVP-K30 will suggests a higher possibility of interaction/stabilisation of the two materials.

In the case of vincamine/NaCMC, the three-dimensional similar regions are only a minor part of the overall structure. Thus the interactions between these two molecules will be possible only in limited three-dimensional positions.

Moreover, the interaction between the drug and the monomer of each polymer was estimated by performing molecular docking simulation.

The docking simulation estimates the interaction energy between two molecules (drug and polymer in this case); in order to evaluate which preparation (drug and polymer) has the best affinity.

As docking algorithm AutoDock version 4 was used (Morris et al., 2009). The algorithm is a grid-based docking methodology. This means that the physical chemical features (i.e. hydrophobicity, hydratability, H-bond capabilities, etc.) of each molecule were previously calculated by performing a grid mapping (Goodford, 1985) and the molecules were approached each other by considering the grid mapping previously performed (Fig. 1).

During the docking procedure all the molecule flexibilities were taken into account, so that, no conformational possibility restriction was applied. The docking procedure was performed using the Lamarckian genetic algorithm (Morris et al., 1998) by generating 250,000 different energy evaluations for each generation of the genetic algorithm. The number of generation was set to 100 for each docking calculation. The best 10 poses for each docking experiment were selected and scored by means of a semi-empirical free energy force field based (Huey et al., 2007). Moreover, the interaction energy of each docking pose was calculated. The best pose for each docking experiment is always shown in Fig. 1.

Although the best poses of each polymer were similar, the interaction energy calculated for the best pose of the binary system vincamine:PVP:K30 is -1.83 kcal/mol and -0.58 kcal/mol for the vincamine:NaCMC system.

This result clearly indicates that the drug will interact significantly better with PVP-K30. The reason of this difference in terms of interaction energy is clearly due to the hydrophobic nature of the drug: PVP-K30 is more hydrophobic than NaCMC. Therefore, PVP-K30 is able to establish more favourable interactions with the drug molecule.

*Please insert Fig.1.*

Another approach to study and reasonably predict drug/carrier affinity or incompatibility between vincamine and the linear carriers consists in using the solubility parameters. This represents an interesting way to predict miscibility between two substances including low molecular weight materials and polymers. Solubility parameters have been successfully used in the pharmaceutical field to predict miscibility and stability of solid dispersions (Hancock et al., 1997). Generally, solubility parameters have been used to predict miscibility and stability of amorphous solid dispersions prepared *via* processes that eliminate the drug crystalline variable e.g. melt extrusion process (Forster et al., 2001). In the present study, we proposed to extend the use of the solubility parameters also in the case of solid dispersions prepared *via* the MA process. It must however be pointed out that, in this case, the presence of the initial crystalline structure of vincamine does not allow to predict whether a completely amorphous solid dispersion can be formed or not; solubility parameters can be instead useful to predict the stability and the occurrence of a massive crystal disruption.

Three-dimensional solubility parameters of vincamine, PVP-K30 and NaCMC were calculated according to Hansen’s approach using the group contribution method by the computer program SPWin version 2.1 (Breitkreutz, 1998). Hence, for convenience, the molecular structures of vincamine and monomeric units of PVP-K30 and NaCMC are reported in Fig. 2. The solubility parameters are listed in Table 1.

*Please insert Fig 2*

*Please insert Table 1*

From Table 1 it is evident that the δd values are similar in magnitude in all three materials. While concerning δp, Vincamine possesses a value only similar to that of NaCMC. This is quite reasonable considering the fact that the unit of PVP-K30 is quite small (molar volume=81.20 cm3/mol) thus the polarity of the C=O group becomes more relevant (Fig. 2). On the other hand, while vincamine and PVP-K30 possess a very similar δh. this parameter is drastically different in the case of NaCMC. In fact, NaCMC possesses many OH groups in its chemical structure (Fig. 2) that can explain its very high value of δh. What is more important, the δtot of vincamine and PVP-K30 is almost identical (the difference is about 1.22 (J/cm3)1/2), while vincamine and NaCMC posses a different (about 8.13 (J/cm3)1/2) total solubility parameter. In a very interesting study (Greenhalgh et al., 1999), it was stated that binary systems having a total solubility parameter difference less than 7 units are likely to form a eutectic mixture. Conversely, when the difference is greater than 15 units the two materials are completely immiscible. The range between 7-15 units represent grey zone. Thus, from Table 1 parameters’ differences, vincamine is likely to be more affine to PVP-K30 than NaCMC. These results are in agreement with those obtained using GRID and docking analyses.

Subsequently, in order to demonstrate the validity of the theoretical studies performed, some preliminary cogrinding tests were performed. Vincamine was processed for 180 min in presence of PVP-K30 and then NaCMC in a 1:7 wt ratio. The milling times and the wt ratio values were selected on the basis of the previous experience carried out with cross-linked polymers (Hasa et al., 2012a). These conditions corresponded to the highest activation/ maximum crystal lattice disruption of the samples. After the preparation, the two Cog samples were analysed by XRPD in terms of stability and in terms of amorphization degree.

*Please insert Fig 3.*

From the preliminary results reported in Fig. 3, a greater affinity of vincamine with PVP-K30 than NaCMC can be noted. In fact, in the XRPD pattern of freshly prepared Cog sample vincamine:PVP-K30 the diffraction peaks appeared broad with a dramatic intensity reduction indicating a large degree of crystal defects in the structure of vincamine. The activated structures appeared completely stable after two months (Fig. 3, right). Conversely, in the pattern of the freshly prepared vincamine:NaCMC Cog sample the diffraction peaks were more intense and persistent despite the presence of a small part of vincamine in amorphous form. However, after two months of storage, the diffraction peaks in the vincamine-NaCMC Cog peaks increased in intensity and became similar to those in the PM, indicating a low stability of the sample and, once again, the lower affinity between these two materials.

These analyses confirmed previous theoretical assumptions and permitted a reliable selection of the optimal polymeric carrier to be used for the formation and stabilization of highly activated vincamine structures *via* MA. The next step was to examine the MA process and to study the influence of formulation and process variables on the solid state and dissolution performances of the formed solid dispersions.

**3.2. Screening of variables**

After the selection of PVP-K30 as a suitable carrier, vincamine was coground in presence of the polymer at three drug-to-polymer wt ratios and four different milling times, selected on the basis of the preliminary trials and the previous experience (Hasa et al., 2012a). Thus, twelve different composites were prepared. Drug-to-polymer wt ratio and milling time are often the most important variables that influence the solid-state features and the dissolution properties of solid dispersions prepared *via* MA (Hasa et al., 2012a, Hasa et al., 2011). Hence, in order to evaluate their influence on the solid-state features of the 12 composites prepared, the crystallinity degree was calculated according to a previously published method (Bergese et al., 2002) and the results are reported in Table 2.

*Please insert Table 2.*

From Table 2 it can be observed that the amorphous degree slightly increased as the milling time increased. Drug-to-polymer weight ratio did not markedly influence the formation of amorphous arrangements. In fact, the Cog samples vincamine:PVP-K30 1:2 wt ratio and vincamine:PVP-K30 1:7 wt ratio treated for 180 min have the same amorphous percent content. These results are different from those obtained when cross-linked PVP was used. In that case, using the longest milling time and a high amount of polymer, a complete crystal lattice disruption and a whole X-ray amorphous product was obtained (Hasa et al., 2012a).

Subsequently, in order to observe how the solid state features influence the *in vitro* behaviour, all the Cog samples were subjected to DKT.

In order to make a comparison among the Cog samples, the FDA bioequivalence limits were adopted. FDA guidance suggests that two compounds/formulations can be considered bioequivalent if the ratio of their AUCs is included in the 0.80 – 1.25 interval (FDA, 2003). This evaluation parameter is proposed for *in vivo* studies; however we demonstrated that it can be successfully used also for *in vitro* experiments. Indeed, in the previous study we observed that the *in vitro* equivalent composites were also bioequivalent (Hasa et al. 2012a). Hence, using this evaluation parameter it is possible to select from the *in vitro* studies the composites likely to be bioequivalent. The calculation procedure is reported in the “methods” section (paragraph 2.2.8.).

All the Z ± dz values are listed in Table 3 and represented graphically in Fig. 5. In order to have a more restrictive selection only the coground formulations having a *Z* value higher than 0.85 (instead of 0.80) were considered for further examinations.

*Please insert Table 3.*

*Please insert Fig. 4.*

Looking at Table 3, the sample vincamine:PVP-K30 1:7 wt ratio coground for 180 min (formulation 15) showed the maximum AUC value (AUCmax). Other two samples resulted to be equivalent to this formulation: vincamine:PVP-K30 1:7 wt ratio coground for 120 min and vincamine:PVP-K30 1:4 wt ratio coground for 180 min (formulations number 14 and 10 respectively, Fig. 4). Instead, the Cog sample vincamine:PVP-K30 1:7 wt ratio treated for 90 min (formulation number 13, Fig. 4) resulted borderline. The equivalent Cog samples had similar vincamine percentages in amorphous form (see Table 3) especially formulations number 10 and 15.

Interestingly, the Cog sample vincamine:PVP-K30 in a 1:2 wt ratio treated for 180 min (formulation number 5, Table 3 and Fig. 4) having the same content of vincamine in amorphous form (Table 3) did not result equivalent *in vitro*. This anomalous *in vitro* dissolution behaviour can be ascribed to the different polymer concentrations in these two Cog samples: being PVP-K30 a water soluble polymer, its presence in higher concentrations may improve vincamine wettability for example by increasing the contact surface between dissolution media and drug. A further possibility of relevance is also the presence of vincamine in different solid-state packing: although the percentages of amorphous vincamine in the two samples are the same, the crystalline fraction (that probably exists as a nanocrystalline form) may differ in terms of crystal dimension. In this study we do not posses sufficient information to *in depth* investigate in this direction.

Therefore, from the screening analysis, the systems that presented the optimal combination between the formation of high amounts of amorphous vincamine and high solubilisation performance were considered for further considerations. The following four Cog samples meet these requirements: vincamine:PVP-K30 1:7 wt ratio coground for 90, 120 and 180 min and vincamine:PVP-K30 1:4 wt ratio coground for 180 min. For the sake of brevity, here below these samples are named Cog formulations 13, 14, 15 and 10 respectively according to Table 3 and Fig. 4.

**3.3. Characterisation of coground samples**

3.3.1. Solubilisation kinetics and TEM analysis of the selected samples

Mean solubilisation profiles of pure vincamine, PM and selected composites are reported in Fig. 5. In the 3h analysis, pure vincamine showed a solubilisation kinetic typical of a poorly soluble drug: the solubilisation rate was slow and the amount of drug solubilised was far below the saturation solubility (Cs) value. In the case of PM, a modest solubilisation improvement could be observed (Fig. 5), attributable to an improvement of drug wetting and localized solubilisation by the hydrophilic PVP-K30.

*Please insert Fig. 5.*

On the other hand, the four selected Cog samples showed similar dissolution behaviour: rapid solubilisation followed by a plateau that was maintained for all the period of analysis (Fig. 5). The plateau concentration value in the case of Cog formulations 10 and 15 corresponds to a *quasi* total vincamine solubilisation (the maximum concentration achievable is 84 mg/L). In the Cog formulation 13 and 14 the plateau value approximately corresponds to 80% and 88% of solubilised vincamine. The dissolution behaviour of the Cog samples seems to be more compatible with the contemporaneous presence of nanocrystalline and amorphous form. In fact, no super-saturation phenomenon (an indicator of the presence of large amount of amorphous structures) can be observed. Hence, the dissolution behaviour of Cog samples prepared using water soluble PVP resulted remarkably different from those prepared using insoluble cross-linked PVP. In the previous experience, the amorphous solid dispersion containing vincamine deeply penetrated in the cross-linked PVP network showed an *in vitro* solubilisation behaviour characterized by a super-saturation phenomenon and only a partial solubilisation of the drug amount subject to the analysis (Hasa et al., 2012a).

TEM analysis revealed that the crystalline phase is of nanometre scale and similar in all the selected samples (Fig. 6). However, in the case of Cog samples 10 and 15 (both coground for 180 min) the nanocrystals appeared slightly smaller than in the other Cog samples and, probably, with a narrower size distribution (data not available). This might explain the total solubilisation of the drug amount in the dissolution medium (Fig. 5).

*Please insert Fig. 6.*

3.3.2. XRPD analysis of the selected samples

The XRPD patterns of pure vincamine, pure PVP-K30, PM and selected Cog samples are reported in Fig. 7. As expected, in the XRPD pattern of original vincamine different sharp and intense signals (for example at 8.10, 10.50, 12.65, 14.05, 17.60, 23.15° of 2θ) were observed in the range of analysis (Fig. 7a), indicating the highly crystalline and complex structure of vincamine. On the other side, pure PVP-K30 pattern was characterised by a total absence of diffraction signals, and by the presence of two halos at 13° and 21° of 2, typical of amorphous materials (Fig. 7h).

PM patterns were simply the sum of pure vincamine and PVP-K30 patterns and the reduction of peaks intensity with respect to pure vincamine can be attributed to the PVP-K30 dilution effect (Fig. 7b and d). In the patterns of the selected Cog samples, a dramatic reduction of peaks intensity and a general broadening of these reflections were observed. These phenomena are compatible with the presence of highly disordered structures (intensity reduction) and the presence of a huge number of defects in the nanocrystalline structure (peak broadening). Moreover, no peak shifts and no formation of new diffraction peaks can be observed indicating that no polymorphic phases are formed during MA in presence of PVP-K30.

*Please insert Fig. 7.*

*3.3.3. SSNMR analysis*

Owing to a multinuclear (13C and 15N) and multiparametric (chemical shift, linewidth and 1H T1) approach, SSNMR spectroscopy represents an effective and reliable tool for characterising the interaction degree between components in both homogeneous and heterogeneous mixtures. It can easily afford information on weak interactions, such as hydrogen bonding, and on the local environment at each specific site, thus on the crystal packing of a molecule (Braga et al., 2006) Furthermore, 1H T1 relaxation analysis allows estimating the homogeneity or heterogeneity of a mixture of two or more components over a nanometre scale providing a reliable comparison with TEM image.

Concerning 13C and 15N SSNMR we refer to the previously reported (Hasa et al., 2012a) assignment which for clarity is summarized in Table 4. The atom labelling is depicted in Fig. 2.

The 13C CPMAS NMR spectra of the selected Cog formulation 10, 13, 14 and 15 compared with those of PM compounds and pure vincamine and PVP-K30 are shown in Fig. 8.

The 13C CPMAS spectra of the PM systems (vincamine:PVP-K30 1:4 and 1:7 wt ratio) are the weighted superimposition of the pure component spectra indicating the lack of drug-carrier interactions. No changes have been observed for the C14 resonance (82.6 ppm) which is the most sensitive to vincamine packing modifications since directly involved in the N···H-O hydrogen bond (Fig. 8b and d). Spectra of coground samples, on the other hand, suggest the formation of strong drug-polymer interactions as highlighted by the large broadening of the vincamine signals. This phenomenon has been related to a wide range of isotropic chemical shifts, arising from a distribution of chemical environments for the same carbon belonging to different drug molecules. This can be due either to a reduction of the particle size (which leads to an increase of the surface/volume ratio and thus of the drug-polymer interactions) and/or to the vincamine molecules in an amorphous state. The presence of dynamic motions, as the origin of the signal broadening, has been ruled out by running spectra at different spinning speeds or decoupling frequencies which did not show any interference phenomena (Long et al., 1994).

The broadening degree does not seem to be related either to the grinding time (90, 120 and 180 min) or to the reagent ratio (1:4 or 1:7 p/p), that is the largest interaction degree is reached already after 90 min.

It is well known that the 15N chemical shift is a reliable probe of the protonation state of nitrogen atoms (Braga et al., 2007). In the case of vincamine the N4 resonance is sensitive to the nitrogen atom environment, *i.e.* deprotonated (~ 4 ppm), involved in hydrogen bond (~ 12 ppm) or protonated (~19 ppm) (Hasa, et al., 2012b). In the samples under study no shifts are observed for the two nitrogen atoms in both coground and PM samples (Fig. 9), indicating that even if disordered or amorphous the N···H-O hydrogen bond interaction is preserved.

*Please insert Table 4.*

*Please Insert Fig. 8.*

*Please insert Fig. 9.*

*1H T1 relaxation times*

The degree of mixing between two components can be easily probed through the measurement of proton spin-lattice relaxation times in the laboratory frame (1H T1) under high-resolution experimental conditions. Indeed, in a simplified model, when protons belong to the same phase or to different phases characterised by small domains (average dimensions < 100 Å) they behave as a single integral entity via spin diffusion (i.e. transfer of spin energy among nuclei through homonuclear dipolar couplings faster than the transfer of energy to the lattice). This results in longitudinal relaxation rates of protons within the molecule which all tend to the same value. On the other hand, different relaxation times can be measured for protons belonging to different domains when these domains have average linear dimensions greater than about 100 Å. This approach, although simplified since does not consider all parameters (in particular structure and dynamic) influencing the spin diffusion, is useful for these systems where the mobility of the vincamine molecules could be ruled out (*see above*).

1H T1 relaxation times were measured exploiting the high resolution of the 13C spectra, by means of the Inversion Recovery CPMAS experiment where signals of vincamine and PVP-K30 are clearly observable and distinguishable. The results are reported in Table 5. In the experiments performed on the pure components (vincamine and PVP-K30) a single T1 value was measured for all the protons (5.2 and 1.5 s, respectively), as expected. In PM samples, both with 1:4 and 1:7 wt ratio, two very different 1H T1 values (5.4/5.2 s for vincamine and 1.4/1.2s for PVP-K30) have been measured in agreement with the heterogeneous nature of the mixture. These samples were characterised by only few and weak drug-polymer interactions and the reagents remained still pure preserving their packing. On the other hand, in all the coground samples, a single relaxation time was measured for all the protons both of vincamine and PVP-K30. These results clearly indicate that drug and polymer were intimately mixed in the ground mixtures with domain average dimensions smaller than about 100 Å.

*Please insert Table 5.*

3.3.4. Physical stability studies

The drug physical stability in the selected Cog samples was monitored by repeating the XRPD and DKT analyses every 4 months for a period of 1 year. These two techniques were chosen because of their complementary nature. Moreover, the crystalline (and amorphous) percentage of the 1 year XRPD pattern was calculated according to the previously published method (Bergese et al., 2002).

The selected Cog samples resulted stable upon 1 year of storage, as it can be seen from the XRD patterns (Fig. 10), from the calculated crystalline content (Table 6) and from the dissolution profiles (Fig. 10). Only the Cog formulation 10 showed a small increase of the diffraction XRPD peaks intensity (Fig. 10a), corresponding to an increase of 1.5% of the crystalline percentage (Table 6). However, DKT of the fresh and 1 year stored Cog 10 samples were superimposable. Since Cog formulation 10 differs from the other three selected samples for its lower polymeric content, this aspect probably plays a role in the solid-state stability of nanocrystalline and/or amorphous vincamine.

*Please insert Fig. 10.*

*Please insert Table 6.*

**4. Conclusions**

This study represents a successful application of mechanochemical activation for preparing stable binary solid dispersions of vincamine in presence of linear polymers with the aim of improving its oral bioavailability. The use of linear polymers instead of classical cross-linked polymers lead to coground samples having dissolution performances and solid state features remarkably different from those obtainable *via* a MA process mediated by cross-linked polymers. The process and formulation variables in this case are of minor relevance on the characteristics of the samples. Despite the above difference from the previous studies, the step-by-step investigation of the mechanochemical process permitted to obtain vincamine activated composites also with linear polymers; in particular this was done paying attention to the selection of the suitable carrier/stabilizer for the drug, to the operating and formulation parameters and, finally, to the characterisation of the coground samples. The binary vincamine/PVP-K30 composites, consisting of a dispersion of drug nanocrystals in the polymer, were highly *in vitro* bioavailable and physically stable for at least one year. This fact was probably due to extensive interactions and contacts with PVP-K30 and to the maintenance of the main hydrogen bonding motif characterising vincamine packing also in the disordered crystalline state of the composites.

**Acknowledgements**

The authors thank Linnea (Locarno, CH) for the kind gift of the active ingredient used in this study.

**References**

Bergese, P., Colombo, I., Gervasoni, D., Depero, L.E., 2002. Assessment of the x-ray diffraction-absorption method for quantitative analysis of largely amorphous composites. J. Appl. Cryst. 36, 74-79.

Bergese, P., Colombo, I., Gervasoni, D., Depero, L.E., 2004. Melting of nanostructured drugs embedded into a polymeric matrix. J. Phys. Chem. B. 108, 15488-15493.

Braga, D., Grepioni, F., Polito, M., Chierotti, M.R., Ellena, S., Gobetto, R., 2006. A solid-gas route to polymorph conversion in crystalline [Fe-II(η5-C5H4COOH)2]. A diffraction and solid-state NMR study. Organomet. 25, 4627-4633.

Braga, D., Maini, L., Fagnano, C., Taddei, P., Chierotti, M.R., Gobetto, R., 2007. Polymorphism in crystalline cinchomeronic acid. Chem. Eur. J. 13, 1222-1230.

Breitkreutz, J., 1998. Prediction of intestinal drug absorption properties by three-dimensional solubility parameters. Pharm. Res. 15 (9), 1370-1375.

Colombo, I., Grassi, G., Grassi, M., 2009. Drug Mechanochemical Activation. J. Pharm. Sci. 98 , 3961-3986.

Food and Drug Administration CDER, Revision 1, 2003. Guidance for Industry, Bioavailability and Bioequivalence Studies for Orally Administered Drug Products-General Considerations. <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070124.pdf> (accessed on March 30, 2012).

Forster, A., Hempenstall, J., Tucker, I., Rades, T., 2001. Selection of ecxipients for melt extrusion with two poorly water-soluble drugs by solubility parameter calculation and thermal analysis. Int. J. Phar. 226, 147-161.

Goodford, P.J., 1985. A computational procedure for determining energetically fovorable binding sites on biologically important macromolecules. J. Med. Chem. 28, 849-857.

Hancock, B.C., York, P., Rowe, R.C., 1997. The use of solubility parameters in pharmaceutical dosage form design. Int. J. Pharm. 148, 1-21.

Hansen, C.M., 1969. The universality of the solubility parameter. Ind. Eng. Chem. Res. 8, 2-11.

Hasa, D., Perissutti, B., Chierotti, M.R, Gobetto, R., Grabnar, I., Bonifacio, A., Dall’Acqua, S., Invernizzi, S., Voinovich, D., 2012a. Mechanochemically induced disordered structures of vincamine: the different mediation of two cross-linked polymers. Int. J. Pharm. 436, 41-57.

Hasa, D., Voinovich, D., Perissutti, B., Bonifacio, A., Grassi, M., Franceschinis, E., Dall’Acqua, S., Speh, M., Plavec, J., Invernizzi, S., 2011. Multidisciplinary approach on characterizing a mechanochemically activated composite of vinpocetine and crospovidone. J. Pharm. Sci. 100, 915-932.

Hasa, D., Perissutti, B., Dall’Acqua, S., Chierotti, M.R., Gobetto, R., Grabnar, I., Cepek, C., Voinovich, V., 2012b. Rationale of using Vinca minor Linne dry extract phytocomplex as a vincamine’s oral bioavailability enhancer, Eur. J. Pharm. Biopharm. http://dx.doi.org/10.1016/j.ejpb.2012.11.025

Hsia, D.C., Kim, C.K., Kildsig, D.O., 1977. Determination of energy change associated with dissolution of a solid. J. Pharm. Sci. 66, 961-965.

Huey, R., Morris, G.M., Olson, A.J., Godsell, D.S., 2007. A semiempirical free energy force field with charge-based desolvation. J. Comput. Chem. 28, 1145-1152.

James, S.L., Adams, C.J., Bolm, C., Braga, D., Collier, P., Friscic, T., Grepioni, F., Harris, K.D.M., Hyett, G., [Jones](http://www-jmg.ch.cam.ac.uk/cinf/rig/recent/wj.html), W., Krebs, A., Mack, J., Maini, L., Orpen, A.G., Parkin, I.P., Shearouse, W.C., Steed, J.W., Waddell, D.C., 2012. Mechanochemistry: opportunities for new and cleaner synthesis. Chem. Soc. Rev*.* 41, 413-447.

Karpati, E., Biro, K., Kukorelli, T., 2002. Investigation of vasoactive agents with indole skeletons at Richter Ltd. Act. Pharm. Hung. 72, 25-36.

Lipinski, C.A., Lombardo, F., Dominy, B.W., Feeney, P.J., 1997. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Del. Rev. 1, 179-189.

Long, J.R., Sun, B.Q., Bowen, A., Griffin, R.G., 1994. Molecular Dynamics and Magic Angle Spinning NMR. J. Am. Chem. Soc. 116, 11950-11956.

Maincent, P., Le Verge, R., Sado, P., Couvreur, P., Devissaguet, J.P., 1986. Disposition Kinetics and oral bioavailability of Vincamine loaded polyalkyl cyanoacrilate nanoparticles. J. Pharm. Sci. 75, 955-958.

Mazak, K., Vamos, J., Nemes, A., Racz, A., Noszal, B., 2003. Lipophilicity of vinpocetine and related compounds characterised by reverse-phase thin-layer chromatography. J. Chromat. A 996, 195-203**.**

Morris, G.M., Godsell, D.S., Halliday, R.S., Huey, R., Hart, W.E., Belew, R.K., Olson, A.J., 1998. Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. J. Comput. Chem. 19, 1639-1662.

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Godsell, D. S., Olson, A. J., 2009. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. J. Comput. Chem. 16, 2785-2791.

Nogami, H., Nagai, T., Youtsuyanagi. T., 1969. Dissolution phenomena of organic medicinals involving simultaneous phase changes. Chem. Pharm. Bull. 17, 499–509.

Shakhtshneider, T.P., Boldyrev, V.V., 1999. Mechanochemical synthesis and mechanical activation of drugs, in: Boldyreva, E., Boldyrev V., (Eds.), Reactivity of molecular solids, Wiley, Chichester, pp, 271–311.

Sun, D.D., Ju, T.R., Lee, P.I., 2012. Enhanced kinetic solubility profiles of indomethacin amorphous solid dispersions in poly(2-hydroxyethyl methacrylate) hydrogels. Eur. J. Pharm. Biopharm. 81, 149-158.

Taylor, J.R., 1997. An Introduction to Error Analysis: The Study of Uncertainties in Physical Measurements. University Science Books, Sausalito, CA.

Van Eerdenbrugh, B., Alonzo, D.E., Taylor, L.S., 2011. Influence of particle size on the ultraviolet Spectrum of particulate-containing solutions: implications for in-situ concentration monitoring using UV/Vis fiber-optic probes. Pharm. Res. 28, 1643-1652.

Van Krevelen, D. W., Hoftyzer, P.J., 1990, Properties of polymers: their estimation and correlation with chemical structure, 3rd ed., Elsevier: Amsterdam, 1990

Vas, A., Gulyas, B., 2005. Eburnamine derivatives and the brain. Med. Res. Rev. 25, 737-757.

Vereczkey, L., 1985. Pharmacokinetics and metabolism of vincamine and related compounds. Eur. J. Drug Metab. and Pharmacokinet. 10, 89-103.

Voinovich, D., Perissutti, B., Grassi, M., Passerini, N., Bigotto, A., 2009a. Solid state mechanochemical activation of Silybum Marianum dry extract with betacyclodextrins: characterization and bioavailability of the coground systems. J. Pharm. Sci. 98, 4119–4129.

Voinovich, D., Perissutti, B., Magarotto, L., Ceschia D., Guiotto P., Bilia, A.R., 2009b. Solid State Mechanochemical Simultaneous Activation of the Constituents of the Silybum marianum Phytocomplex with Crosslinked Polymers. J. Pharm. Sci. 98, 215-228.

**Table 1**. Total and partial solubility parameters of vincamine, PVP-K30 and NaCMC calculated using SPWin version 2.1 programme (Breitkreutz, 1998).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Substance** | **d (J/cm3)1/2** | **p (J/cm3)1/2** | **h (J/cm3)1/2** | **tot (J/cm3)1/2** |
| Vincamine | 21.85 | 5.29 | 12.40 | 25.68 |
| PVP-K30 | 20.44 | 13.67 | 9.28 | 26.90 |
| NaCMC | 22.51 | 7.70 | 24.02 | 33.81 |

δtot - total solubility parameter

δd - contribution of dispersion component

δp - contribution of polar component

δh - contribution of hydrogen bonding component

**Table 2**. The calculated crystalline and amorphous percentages of each Cog sample.

|  |  |  |  |
| --- | --- | --- | --- |
| **Vincamine:PVP-K30 wt ratio** | **Milling time**  **(min)** | **Xcr**  **(%)** | **Xa**  **(%)** |
| **1:2** | 45 | 72.9 | 27.1 |
| 90 | 68.0 | 32.0 |
| 120 | 52.4 | 47.6 |
| 180 | 34.6 | 65.4 |
| **1:4** | 45 | 65.2 | 34.8 |
| 90 | 65.4 | 34.6 |
| 120 | 54.8 | 45.2 |
| 180 | 35.7 | 64.3 |
| **1:7** | 45 | 54.8 | 45.2 |
| 90 | 53.4 | 46.6 |
| 120 | 50.0 | 50.0 |
| 180 | 36.8 | 63.2 |

Xcr - Crystalline degree expressed as percentage (%)

Xa - Amorphous degree expressed as percentage (%)

**Table 3.** AUC±S.D.; n=3 and z±dz values for the vincamine:PVP-K30 Cog samples and PM mixtures.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **vincamine:PVP k30** | | | | |
| ***N*** | **X1**  **(min)** | **X2**  **(wt ratio)** | **AUC0-180**  **(mg/L\*h)**  **(mean ± S.D.)** | **z ± dz**  **(mean ± S.D.)** |
| **1** | 0  (PM) | 1:2 | 30.10 ± 0.12 | 0.12 ± 0.003 |
| **6** | 1:4 | 32.23 ± 0.63 | 0.13 ± 0.004 |
| **11** | 1:7 | 32.29 ± 1.35 | 0.13 ± 0.007 |
| **2** | 45 | 1:2 | 78.73 ± 3.67 | 0.33 ± 0.017 |
| **7** | 1:4 | 112.98 ± 8.34 | 0.47 ± 0.037 |
| **12** | 1:7 | 143.60 ± 4.32 | 0.59 ± 0.024 |
| **3** | 90 | 1:2 | 83.87 ± 1.27 | 0.35 ± 0.011 |
| **8** | 1:4 | 107.20 ± 6.78 | 0.44 ± 0.030 |
| **13** | 1:7 | 203.51 ± 2.37 | 0.84 ± 0.024 |
| **4** | 120 | 1:2 | 88.60 ± 1.99 | 0.37 ± 0.013 |
| **9** | 1:4 | 141.22 ± 9.54 | 0.58 ± 0.042 |
| **14** | 1:7 | 217.71 ± 5.03 | 0.90 ± 0.032 |
| **5** | 180 | 1:2 | 95.22 ± 6.80 | 0.39 ± 0.030 |
| **10** | 1:4 | 230.14 ± 10.90 | 0.95 ± 0.052 |
| **15** | 1:7 | **241.87 ± 6.41a** | 1.00 ± 0.037 |

*N* – Formulation number

*X1* – milling time (min) (t=0 corresponds to the physical mixtures)

*X2* – drug-to-polymer weight ratio

AUC0-180 –Area under the curve of solubilised concentration vs. time, from t=0 min to the last measuring point (180 min)

Z ± dz – the *Z* ratio and associated uncertainty *dz* calculated from equation (3) and (4), respectively

**a** Cog sample having AUCmax

**Table 4.** 13C and 15N chemical shifts with relative assignment of pure vincamine and PVP-K30 (Hasa et al., 2012a).

|  |  |  |  |
| --- | --- | --- | --- |
| **vincamine** | | | **PVP-K30** |
| **13C δ** | **carbon** | **Note** | **13C δ** |
| 7.5 | 21 | CH3 | 18.4 |
| 15.3 | 18 | CH2 | 31.5 |
| 21.3 | 6 | CH2 | 35.9 sh |
| 24.8 | 20 | CH2 | 42.5 |
| 28.9 | 17 | CH2 | 176.1 |
| 36.0 | 16 | Cq |  |
| 44.0 | 15 | CH2 |  |
| 45.3 | 19 | CH2 |  |
| 49.1 | 5 | CH2 |  |
| 51.8 | 23 | OCH3 |  |
| 60.1 | 3 | CH |  |
| 82.6 | 14 | Cq |  |
| 106.0 | 7 | Cq |  |
| 111.6 | 12 | CH |  |
| 118.6 | 11 | CH |  |
| 119.6 | 9 | CH |  |
| 120.1 | 10 | CH |  |
| 129.2 | 8 | Cq |  |
| 130.0 | 2 | Cq |  |
| 134.1 | 13 | Cq |  |
| 173.2 | 22 | C=O |  |
| **15N δ** | **nitrogen** | **Note** | **15N δ** |
| 12.0 | 4 | N···H-O | 104.4 |
| 117.2 | 1 | N | - |

**Table 5.** 1H Spin-Lattice Relaxation Times, T1 (s), measured for pure reagents (vincamine and PVP-K30), PM samples (PM 1:4 and 1:7 wt ratios) and coground mixtures 10, 13, 14 and 15 using high resolution techniques as described in the Material and Methods section.

|  |  |  |
| --- | --- | --- |
| **Compound** | **vincamine signals 1H T1** | **PVP-K30 signals 1H T1** |
| vincamine | 5.2 | - |
| PVP-K30 | - | 1.5 |
| PM 1:4 wt | 5.4 | 1.4 |
| 10 | 2.1 | 1.9 |
| PM 1:7 wt | 5.2 | 1.6 |
| 13 | 2.0 | 1.8 |
| 14 | 2.1 | 1.9 |
| 15 | 2.0 | 1.9 |

**Table 6.** Crystalline percentages calculated according to Bergese et al., 2002, for the fresh and 1 year stored Cog samples.

|  |  |  |
| --- | --- | --- |
| Sample | Crystalline percentage | |
| Fresh | 12 months |
| Cog formulation 10 | 35.7 | 37.2 |
| Cog formulation 13 | 53.4 | 53.2 |
| Cog formulation 14 | 50.0 | 50.1 |
| Cog formulation 15 | 36.8 | 36.8 |

**figures**

****

**Fig. 1**. Grid mapping representations of vincamine (up), PVP-K30 and NaCMC monomers (middle). The yellow surfaces indicate the hydrophobic areas while the blue surfaces indicate the hydrophilic zones. In the Bottom the best pose for each docking experiment is reported: left- best docked pose of PVP-K30 in cyan sticks mode; right- best docked pose of NaCMC in pink sticks mode; drug is always represented in sticks mode with green carbons**.**



**Fig. 2**. Chemical structures of vincamine (A), PVP-K30 monomer (B) and NaCMC monomer (C).



**Fig. 3**. Left: XRPD patterns of physical mixture vincamine:NaCMC 1:7 wt ratio (a), fresh Cog 180 min vincamine:NaCMC 1:7 wt ratio (b), Cog 180 min vincamine:NaCMC 1:7 wt ratio one month after preparation (c) and Cog 180 min vincamine:NaCMC 1:7 wt ratio two months after preparation. Right: XRPD patterns of physical mixture vincamine:PVP-K30 1:7 wt ratio (a), fresh Cog 180 min vincamine:PVP-K30 1:7 wt ratio (b), Cog 180 min vincamine:PVP-K30 1:7 wt ratio one month after preparation (c) and Cog 180 min vincamine:PVP-K30 1:7 wt ratio two months after preparation.



**Fig 4**. Graphical representation of the z ± dz values calculated using equations (1) and (2), respectively. The samples are numbered according to Table 3. In dotted line the limit of *in vitro* equivalence (0.85) is expressed.



**Fig. 5**. Solubilisation kinetics of pure vincamine (♦), Cog formulation number 13 (▲), Cog formulation number 14 (∆), Cog formulation number 10 (■) and Cog formulation number 15 (🞏). PMs vincamine:PVP-K30 1:4 wt ratio (+) and 1:7 wt ratio (○) showed superimposable solubilisation kinetics. Pure vincamine solubility at equilibrium (Cs) is indicated by a dotted line.



**Fig. 6**. TEM image of different crystalline clusters in the specimen of Cog formulation number 10 (A), Cog formulation number 13 (B), Cog formulation number 14 (C) and Cog formulation number 15 (D).



**Fig. 7**. XRPD patterns of pure vincamine (a), PM vincamine:PVP-K30 1:4 wt ratio (b), Cog formulation 10 (c), PM vincamine:PVP-K30 1:7 wt ratio (d), Cog formulation 13 (e), Cog formulation 14 (f) and Cog formulation 15 (g) , pure PVP-K30 (h).

****

**Fig. 8**. 13C (100 MHz) CPMAS spectra of pure vincamine (a), PM vincamine:PVP-K30 1:4 wt ratio (b), Cog formulation 10 (c), PM vincamine:PVP-K30 1:7 wt ratio (d), Cog formulation 13 (e), Cog formulation 14 (f), Cog formulation 15 (g), and pure PVP-K30 (h) recorded at the spinning speed of 12 kHz.



**Fig. 9**. 15N (40 MHz) CPMAS spectra of pure vincamine (a), PM vincamine:PVP-K30 1:4 wt ratio (b), Cog formulation 10 (c), PM vincamine:PVP-K30 1:7 wt ratio (d), Cog formulation 13 (e), Cog formulation 14 (f), Cog formulation 15 (g), and pure PVP-K30 (h) recorded at the spinning speed of 9 kHz.



**Fig. 10**. XRPD patterns and DKT as inserts at time 0 (fresh), and 12 months after the preparation of Cog formulation 10 (A), Cog formulation 13 (B), Cog formulation 14 (C) and Cog formulation 15 (D).



GRAPHICAL ABSTRACT