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Full Length Research Paper

Minor drug-resistant human immunodeficiency virus (HIV)-1 variants in the cellular DNA of Tanzanian women following triple antiretroviral regimen to prevent vertical transmission

Andrea Hauser 1,2, Andrea Kunz 1, Julius Sewangi 3, Stefanie Theuring 1*, Paulina Mbezi 4, Inga Lau 1, Judith Ziske 1, Festo Dugange 5, Stephen Norley 2, Claudia Kuecherer 2 and Gundel Harms 1

1 Institute of Tropical Medicine and International Health, Charité – Universitätsmedizin Berlin, Germany.
2 Robert Koch- Institute, Berlin, Germany.
5 Kyela District Hospital, Ministry of Health and Social Welfare, Dar es Salaam, Tanzania.

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Antenatal zidovudine (AZT), intrapartal nevirapine (NVP) and postpartal lamivudine (3TC)/AZT to prevent mother-to-child transmission of human immunodeficiency virus (HIV)-1 as recommended in 2006 World Health Organization (WHO) guidelines has shown to select HIV-resistant plasma virus in Tanzanian women. During viral replication, HIV integrates into the cellular host genome where resistant strains may remain archived. This study analyzed the dimension of integration of drug-resistant HIV-strains into the host cells as provirus by analyzing corresponding peripheral blood mononuclear cells (PBMCs) for key resistance mutations selected by AZT/NVP/3TC, applying highly-sensitive allele-specific polymerase chain reaction (PCR). HIV-resistance was detected in PBMC-DNA of 10 (28%) and in plasma virus of 15 of 36 women (42%). Most resistance mutations were selected by AZT in comparable proportions in PBMCs (25%) and in plasma virus (22%). In conclusion, antenatal AZT may select for AZT-resistance potentially persisting as provirus, and therefore is likely to negatively impact future treatment options.

Key words: Tanzania, prevention of mother-to-child transmission of human immunodeficiency virus (HIV)-1, triple prophylaxis, HIV-1 drug resistance, peripheral blood mononuclear cells (PBMCs), proviral DNA, minor variants, allele-specific polymerase chain reaction (PCR).

INTRODUCTION

Antiretroviral regimens for the prevention of mother-to-child transmission (PMTCT) of human immunodeficiency
virus (HIV)-1 have a proven efficacy in resource-limited countries. However, the temporary nature of such regimens poses the risk of developing resistance, extensively shown for nevirapine single-dose (NVP-SD) prophylaxis (Arrive et al., 2007). After discontinuation, the absence of selective drug pressure decreases the presence of resistant variants to undetectable levels in plasma (Eshleman et al., 2001). However, any viral variant, including drug resistant variants, that has been replicating for a sufficient length of time integrates into the genome of infected cells and may persist for the life span of the cell (Turriziani et al., 2010). Resistant HIV-strains are thus detectable in peripheral blood mononuclear cells (PBMC) for longer periods than in corresponding plasma samples, which has been shown for patients failing antiretroviral therapy (ART) who had a history of drug resistance (Turriziani et al., 2010; Ellis et al., 2004) as well as for drug naïve women after NVP-SD prophylaxis (Wagner et al., 2010). Previous research demonstrated that the presence of resistant proviruses negatively impacts future treatment options (Jourdain et al., 2010).

Applying a highly sensitive allele-specific real-time PCR (ASPCR), we recently reported the emergence of minor drug-resistant HIV-1 in the plasma of 40% (20/50) of Tanzanian women (Hauser et al., 2012), following the 2006 WHO recommended PMTCT regimen (WHO, 2006). The aim of this substudy was to assess the extent of drug resistance in provirus within the corresponding buffy coat samples and to compare the results to those with paired plasma samples.

MATERIALS AND METHODS

Clinical samples and study population

Of 1395 pregnant Tanzanian women recruited within an observational study at Kyela District Hospital (KDH), Mbeya Region, Tanzania, 202 were tested positive for HIV-1 (Kirsten et al., 2011). Of these, 87 treatment-naïve women initiated triple antiretroviral prophylaxis according to the national Tanzanian PMTCT guidelines of 2008 (Tanzania, 2008) and the WHO 2006 recommendations, consisting of antenatal zidovudine (AZT) starting at the 28th week of gestation, NVP-SD at labor onset and AZT/lamivudine (3TC) for one week postpartum (WHO, 2006). Maternal blood samples were collected before initiation of prophylaxis (baseline sample or “individual wildtype” sample) and during follow-up at delivery, 1-2, 4-6 and 12-16 weeks postpartum. EDTA stabilized blood was immediately processed into plasma and buffy coat/ peripheral blood mononuclear cells (PBMCs). The 4-6 and/or 12-16 weeks postpartum buffy coat-samples in addition to the corresponding baseline sample had to be available from those women which were previously analysed for drug-resistance in plasma virus. Informed written consent was obtained from all participants prior to enrolment. The study was approved by the local Mbeya Medical Research and Ethics Committee, the National Institute for Medical Research of Tanzania and the ethical committee of Charité, Universitätsmedizin Berlin, Germany.

Laboratory analysis

For plasma samples with expected low viral loads (<20,000 copies/ml), we previously developed and evaluated seven high-sensitive allele-specific PCR (ASPCR) assays that allow the quantification of key resistance mutations in the viral reverse transcriptase (RT) with a detection limit of <1%. These include two AZT-selected mutations conferring high level resistance of the RT (T215Y, T215F); the low level AZT resistance mutation K70R, which occurs early and transient and thus is indicative for the emergence of AZT-resistance (Boucher et al., 1992); the three most common NVP-selected resistance mutations K103N (codon AAT and AAC) and Y181C; and the most frequently 3TC-selected mutation M184V (Johnson et al., 2013). To compensate for patient specific HIV-variability and thus the impact on real-time PCR’s measurements, baseline samples prior to drug initiation (assumed to be 100% wild-type in drug-naïve women) were used to calculate the true emergence of resistant variants.

Human genomic DNA was isolated from 200 µl buffy coat using the Qiagen QIAamp DNA Blood mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. Cellular HIV-DNA copies (integrated and non-integrated in the human genomic DNA) were quantified by TaqMan real-time PCR of the HIV-1 LTR genomic region (Supplementary Text1). Only samples with at least 10 HIV-DNA copies/µl of the isolated total DNA were further applied to ASPCR assays to guarantee a minimal input of 100 copies/ASPCR and a detection limit of 1%. HIV-DNA amplification and ASPCR assays were conducted using published primers (Hauser et al., 2012) (Supplementary Text 2).

To rule out sample mix-up and to confirm the common origin of plasma virus and provirus, phylogenetic analysis of maternal sequences generated by population-based sequencing was performed (Supplementary Text 3). Statistical analyses were carried out using PASW Statistics 18 (SPSS Inc., Chicago, Illinois, USA). The non-parametric Mann-Whitney U test was used to assess significant differences between two independent samples. The Chi-square test or Fisher’s exact test were used to analyze the independence of categorical variables and for descriptive analysis, median and interquartile ranges (IQR) were calculated. K103N (AAC) mutation and the K103N (AAT) mutation were summed to give the total proportion of viruses carrying the K103N mutation.

RESULTS

Study population

Corresponding baseline and at least one follow-up buffy coat samples with a minimum of 10 HIV-DNA copies/µl total DNA were available from 36 women out of 50 women previously tested for drug-resistance in plasma virus. The median baseline characteristics at initiation of prophylaxis were CD4-cell count of 395 cells/µl (IQR 240-454), a plasma viral load of 21,725 copies/ml (IQR 6,816-60,950) and a cellular HIV-1 load of 92 DNA copies/µg PBMC-DNA (IQR 53-161). Women took AZT during pregnancy for a median of 54 days (IQR 36-75). All women took intrapartal NVP-SD, while intra- and/or post-partal AZT/3TC intake was documented for 32/36 (89%) women. Plasma viral load was reduced to a median of 1,747 copies/ml (IQR 1,265-5,649) at week 1-2 and increased again to 15,123 copies/ml (IQR 8,830-63938) at week 4-6 (Fisher’s exact test: both p<0.005). 67% (24/36) of the women were infected with HIV-1 subtype C and 33% (12/36) with subtype A1.

HIV-resistance mutations detected in PBMC-DNA

Thirty-two buffy coat samples taken after 4 to 6 weeks,
and 11 buffy coat samples taken after 12 to 16 weeks were analysed for the emergence of HIV-resistance compared to the baseline sample (in total, 43 samples/36 women). Quantification of resistant HIV-strains in PBMCs was performed with a median HIV-DNA input of 123 copies/ASPCR (IQR 111-145).

HIV-resistance mutations were detected in 11 follow-up buffy coat samples of 10/36 (28%) women. AZT-selected mutations were identified in the cellular HIV-DNA of 9/36 (25%) women (four with the K70R mutation, five with T215Y/F +/- K70R). NVP-selected mutation (K103N) was found in the HIV-DNA of 3/36 (8%) women, and 3TC-selected mutation (M184V) was found in one woman (3%). Three women carried dual-resistant HIV-strains in their PBMCs: either selected by AZT (K70R) and NVP (K103N) or by AZT (T215Y) and 3TC (M184V) (Table 1). Women in whom AZT-resistance mutations were detected in HIV-DNA tended to have had a longer antenatal AZT intake, but this was not statistically significant (68 versus 49 days, Mann-Whitney U-test p=0.11).

Proportions of HIV-resistance mutations in PBMC compared to plasma

The previous ASPCR analysis of corresponding week 4-16 plasma samples revealed resistant plasma virus in 17/54 samples (35 week 4-6 samples, 19 week 12-16 samples) constituting to 15/36 (42%) women. In 8/36 (22%) women, resistance was selected by AZT (five women with K70R and three with T215Y/F), in 5/36 (14%) women selected by NVP (K103N and/or Y181C) and in 2/36 (6%) women selected by 3TC (M184V) (Hauser et al., 2012).

The frequencies of resistant HIV-variants detected in PBMCs were not significantly different from those detected in plasma virus (11/43 versus 17/54; Fisher's exact test p=0.67). Additionally, the proportions of women in whom resistant HIV was selected by AZT/NVP/3TC were similar for both blood compartments (AZT (T215Y/F): p=0.7; AZT (K70R): p=1.0; NVP: p=0.7; 3TC: p=1.0 using Fisher's exact test; Figure 1). In detail, in six women, all but two plasma virus mutations were also identified in HIV-DNA from PBMCs collected at the same or later time points. In three of these six women, an additional resistance mutation was detected in the viral DNA genomes. In two of them this additional resistance mutation was selected by the same drug as the present one, thus not leading to an additional drug resistance. In four women, resistant HIV-variants were identified exclusively in the PBMCs, whereas in eleven women, resistant variants were detected in plasma samples only.

Table 1. Drug resistant HIV-variants in plasma and PBMCs detected by ASPCR.

<table>
<thead>
<tr>
<th>No</th>
<th>HIV-1 subtype</th>
<th>Antenatal AZT-intake (days)</th>
<th>Plasma</th>
<th>4-6 weeks p.d.</th>
<th>12-16 weeks p.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Delivery-2weeks p.d.</td>
<td>Plasma</td>
<td>PBMC</td>
</tr>
<tr>
<td>046</td>
<td>C</td>
<td>40</td>
<td>wt</td>
<td>3.7% T215F</td>
<td>wt</td>
</tr>
<tr>
<td>081</td>
<td>C</td>
<td>57</td>
<td>-</td>
<td>19% K103N*</td>
<td>-</td>
</tr>
<tr>
<td>093</td>
<td>A1</td>
<td>105</td>
<td>wt</td>
<td>-</td>
<td>wt</td>
</tr>
<tr>
<td>098</td>
<td>C</td>
<td>91</td>
<td>wt</td>
<td>-</td>
<td>wt</td>
</tr>
<tr>
<td>130</td>
<td>A1</td>
<td>33</td>
<td>12% K70R*</td>
<td>wt</td>
<td>1.5% K70R</td>
</tr>
<tr>
<td>02#</td>
<td>C</td>
<td>77</td>
<td>0.7% M184V</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11#</td>
<td>C</td>
<td>92</td>
<td>wt</td>
<td>2.7% K70R</td>
<td>1.2% K70R</td>
</tr>
<tr>
<td>10#</td>
<td>A1</td>
<td>95</td>
<td>wt</td>
<td>4.9% K70R</td>
<td>5.5% K70R</td>
</tr>
<tr>
<td>16#</td>
<td>C</td>
<td>49</td>
<td>wt</td>
<td>36% K103N*</td>
<td>4.0% K70R</td>
</tr>
<tr>
<td>13#</td>
<td>C</td>
<td>32</td>
<td>wt</td>
<td>3.9% T215Y</td>
<td>wt</td>
</tr>
</tbody>
</table>

*Sample not available according to inclusion criteria; wt: No resistance mutation detected; *Mutation also detected by Sanger sequencing; p.d.: Post delivery.
HIV-resistance was found in neither the plasma viruses nor the PBMCs of fifteen women (Table 1).

DISCUSSION

In the present study, resistant HIV-1 strains in maternal PBMCs were identified in 28% of HIV-infected Tanzanian women 3-15 weeks after cessation of WHO 2006 transmission prophylaxis. NVP-resistance was detected in the HIV-DNA of 8% of the women, a considerably lower frequency than the 52.3% of women analysed 6 weeks after NVP-SD by Loubser et al. (2006). The efficacy of a seven day AZT/3TC-tail after NVP-SD exposure in order to reduce NVP-resistance has often been demonstrated for plasma virus (Farr et al., 2010) and also seems to apply to cellular HIV-DNA.

However, the antenatal mono-AZT administration resulted in the presence of proviral AZT-resistance mutations in one quarter of the women (25%), although the median antenatal AZT intake of 54 days (7.5 weeks) was shorter than recommended in the WHO 2006 guidelines (12 weeks). Prolongation of antenatal AZT intake to 26 weeks, as advocated by the WHO 2010 guidelines (WHO, 2010), may further increase the development of AZT-resistance mutations. Although the exact rate at which HIV-infected CD4+ cells return to a resting state as memory cells remains unclear, it is assumed that at least a proportion of the resistant proviruses will be archived in the host genome as a "latent reservoir" (Lambotte et al., 2004; Turriziani et al., 2010) and persist for the life span of these cells (Alexaki and Wigdahl, 2008). Under the selective pressure of subsequent treatments, latently infected cells can reactivate the production of resistant virus to release resistant virus (Alexaki and Wigdahl, 2008; Turriziani et al., 2007, 2010). Wind-Rotolo et al. (2009) detected replication-competent proviruses carrying resistance mutations in re-activated PBMCs from 8% of women six months after NVP-SD treatment. Women with detectable NVP-resistance in their HIV-DNA were significantly more likely to experience virologic failure at initiation of a treatment involving NVP (Jourdain et al., 2010).

In the present study, the proportions of women with AZT, 3TC and/or NVP-resistance mutations in HIV from both blood compartments were comparable. This may reflect the ongoing process of viral replication after clearance of the drugs (Wind-Rotolo et al., 2009) and is supported by a 10-fold increased plasma viral load (viral rebound) one month after cessation of antiretroviral regimen and by the fact that HIV-resistance mutations in the plasma viruses were also found in HIV-DNA from PBMCs of six women. Viral rebound in the presence of resistant variants may enhance the integration of resistant genomes into the cellular reservoir.

Infection of cells by resistant plasma virus could not be confirmed for 11 women whose HIV-resistance mutations declined to minor (<5%) or undetectable levels in plasma. In these women, the mutant virus seemed to be rapidly replaced by wild-type variants, presumably through the
re-emergence of archived wild-type virus (Turriziani et al., 2010). Vice versa, HIV-resistance detected exclusively in HIV-DNA from buffy coat samples (four women) may be the result of viral infection and presumable integration at early stages of the prophylaxis regimen. This might explain the exclusive presence of the AZT resistance mutations in the HIV-DNA of three women. These women had taken antenatal AZT for 40, 91 and 105 days (Table 1), potentially allowing the selection and integration of AZT resistance mutations. The K103N mutation (detected in PBMCs only of one woman) has been reported to be detectable for longer periods and at higher frequencies in PBMC HIV than in plasma RNA (Saladini et al., 2010).

Such discrepancies in the occurrence and persistence of drug resistance mutations between plasma RNA and PBMC DNA have been reported in several studies, demonstrating that the PBMC compartment does not necessarily reflect the plasma compartment (Saladini et al., 2010; Turriziani et al., 2010; Wind-Rotolo et al., 2009). While plasma samples provide information on actively replicating viruses (Turriziani et al., 2010), cellular HIV-DNA reflects both actively and latently infected cells (Wirden et al., 2011). Due to the low concordance of 46.7% between HIV-DNA of viral load suppressed patients and previous RNA RT-genotypes, Wirden et al. (2011) concluded that archived mutated DNA is difficult to reach.

Contrary wise, it was concluded that using proviral DNA for HIV-genotyping is an adequate and even preferable approach to monitor resistance development following discontinuation of antiretroviral PMTCT interventions. The prolonged persistence of resistance mutations in the cellular DNA compared to plasma RNA makes PBMCs a useful resource to estimate the total spectrum and “resistance potential”. This knowledge is of critical importance for the success of subsequent long-term therapy, especially in those countries that lack the resources to carry out genotypic resistance testing.

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Conflict of interest

Authors have none to declare.

References


Full Length Research Paper

Optimization of a novel tablets formulation using D-optimal mixture design

Jesus Rafael Rodríguez-Amado1*, Ariadna Lafourcade-Prada1, Julio César Escalona Arranz1, Humberto Morris Quevedo2, Antonio Iraizoz Colarte3 and Jose Carlos Tavares Carvalho4

1Department of Pharmacy, University of Orient, Santiago of Cuba, Cuba.
2Biotechnological Studies Center, University of Orient, Cuba.
3Pharmaceutical Technology Department, Food and Pharmacy Institute, University of Havana, Cuba.
4Laboratorio de Pesquisa en Farmacos. Universidade Federal de Amapá, Brasil.

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This study involved the obtaining of a new tablet formulation of Tamarindus indica L., using a D-optimal mixture design. Tablets were obtained by wet granulation. Compressibility, angle of repose, flowability, particle size and humidity were the responses for the granules evaluation, while hardness, disintegration time and friability were responses for the tablets. The desirability function was applied for tablets optimization. All responses were adjusted to statistical models. Polyvinylpyrrolidone is the excipient that more affects all responses. The D-optimal design allowed selecting the optimal proportions of the excipients: lactose monohydrate 36.54%, microcrystalline cellulose 30.00% and polyvinylpyrrolidone 6.93%.

Key words: Mixture design, tablets, tamarind, soft extract, desirability.

INTRODUCTION

Tamarindus indica L. or tamarind, as commonly known, is a medium-sized tree, which belongs to the Caesalpinaceae family. The leaves composition of this plant include proteins, fatty acids, carbohydrates, tannins, flavonoids, essential oils, volatile compounds and some micro elements like calcium, magnesium, iron, phosphor, copper, sulphur, sodium and potassium. Tamarind leaves are rich in flavonoids as orientine, isoorientine, vitexine, and isovitexine. Polyphenols like apigenine, ferulic acid and caffeic, luteoline, rutine and vicenine are present too (Dehesa et al., 2006). Vitamin A and C are also present (El-Siddig et al., 2006). Tamarind is a medicinal plant that has been used for centuries in Cuba. It is the most useful plant for liver disorders treatment. Their leaves have hepatoprotective activity, associated with the presence of polyhydroxylated compounds; with majority being polyphenols and flavonoids (Jouyex et al., 1995).

The extracts of the leaves of this plant have protective activity against the arteriosclerosis induced by a hypercholesterolemic diet. A clinical study, suggests that the extracts of tamarind leaves improves levels of total cholesterol and reduces the diastolic pressure (Martineiro et al., 2006; Iftekhar et al., 2006). The leaves of this plant has a broad spectrum of antimicrobial activity, because of they are potential source of new classes of antibiotics that could be useful for infectious diseases (Doughary,
The aqueous extract of tamarind leaves has hepatoprotective activity against acute intoxication induced by acetaminophen, showing a liver regenerative effect (Pimple et al., 2007; Mahesh et al., 2010; Meher and Das, 2013). The tamarind soft extract is a brownish viscose syrup, with natural odor of fresh fruit. This one contains not less than 63 and not more than 67% of total solids. Soft extract contain 6.75% of polyphenols expressed as tannic acid. Tablets are the most manufactured pharmaceutical form around the world and probable the most investigated ones.

However, the development of a new formulation of tablets is a very complex work that depends on both formulation factors and manufacturing process. The complete knowledge of the factors affecting the development process of pharmaceutical tablets is very important for the development of a new formulation. Once these factors are identified, they could be mathematically modelled and allowed to predict the behaviour of most important properties of the product.

The development of any pharmaceutical form necessitate the complete study of different levels of excipients used in various formulations, in order to identify the exact proportion of what maximizes quality and stability of the final product.

The aim of this work was to obtain a new tablet formulation, starting from the soft extract of the leaves of *T. indica* L., by using a D-optimal mixture design.

**MATERIALS AND METHODS**

**Soft extract**

Firstly, fluid extract was prepared by percolation (Escalona et al., 2010). Posteriorly soft extract is obtained by concentration in a vacuum evaporation system (KIKI WERKE GMBH & Co. Germany) at 42±2°C, until reduce the volume from 4 to 1 ml.

**Excipients**

The microcrystalline cellulose (Microcell MC-101, Blanver, Brazil), and lactose monohydrate (Contero Excipient, New Zealand) are the most used excipients in the formulation of tablets and capsules (Kashif et al., 2014; Rani and Begum, 2014). The polyvinylpyrrolidone (Kollidon 25, Basf, Germany) is a binder used for formulating tablets because, binder contributes to a better tablet disintegration (Lachman et al., 1981; Dineshmohan et al., 2014). The colloidal silicon dioxide (Aerosil® V-200, Degusa, Belgium) was selected because it provides good flowability to powders and granules, and for providing protection against excess humidity (Lachman et al., 1981; Gibson, 2006). The croscarmellose sodium (AcDisol, Blanver, Brazil) is very useful as tablet disintegrant, and the magnesium stearate (Derive SA, Germany) is one of the most lubricants used in the manufacture of tablets (Lachman et al., 1981).

**Experimental design**

The excipients proportion restrictions used for tablets formulation are listed in Table 1. The mixtures of active ingredient and excipient were made according to the 14-run D-optimal mixture design. This kind of design is particularly useful in a restricted region because of maximization of the volume of each ingredient in a k-dimensional space. In this way, you can use only the interest excipients, keeping constant the quantities of the rest of the formulation ingredients (Gabrielson et al., 2002). The total amount of the mixture was held constant. The relative amounts of the different excipient varied according to the mixture design (Table 2).

**Granules and tablets preparation**

Wet granulation method, using absolute alcohol as solvent was used to prepare granules and tablets. The mixture of excipients was made, in each case, in the proportions described in Table 2. Drying was done in an oven Nova Ethics (Brazil), at 42°C for 1 h. The granules were mixed with the rest of the excipients using a turbula mixer. Tablets of 600 mg with the equivalent to 20% of soft extract, as total solids were prepared. The compression was made in an eccentric tablet machine (Manesty, UK), using flat-faced bevelled punches, 12.7 mm on diameter, at a compression force of 80 MPa.

**Physicomechanical properties of the granules**

**Compressibility index**

For bulk (*D₀*) and tapped (*D₁*) densities evaluation, an appropriate amount of granules were placed in a 100 ml graduated cylinder. The volume reading directly from the cylinder was used to calculate the bulk density according to the mass/volume ratio. For tapped density, the cylinder was tapped 1000 times using a tap density analyser (Erweka SVM1, Germany). Compressibility index (CI) was calculated using the following expression:

\[ CI = ((D₁ - D₀)/D₀)\times100 \]

**Flow rate**

Flow ability is the capability of a mass powder or granules to flow through a hopper. Granules flow rate was measured by pouring a mass (m) of granules through a glass funnel (wall of 45°) having a round orifice of 8.0 mm (d). The funnel’s outlet was separated 100 mm respect to a horizontal surface. Flow rate was calculated using the equation:

\[ Fr = m/0.785.d^2.t \]

Where *m* is the granules mass expressed in grams, *t* is the time that mass of granules takes to flow through the funnel in seconds; *d* is the diameter of the orifice of funnel in cm (Iraizoz et al., 1992).

**Angle of repose**

Angle of repose was evaluated by discharging a mass of granules through a glass funnel of internal diameter 8 mm that is hold at constant distance from the horizontal surface. The height (h) of the heap formed and the radius (r) of the cone base were measured (USP, 2012). The angle of repose (*Aᵣ*) was calculated using the following equation:

\[ Aᵣ = \tan^{-1}(h/r) \]

**Particle size characterization**

In all cases, the particle size was performed by applying a shaking siever with a set of sieves; 800, 630, 450, 250, 125 µm apertures.
Particle size distribution was verified.

**Residual humidity**

The residual humidity was performed by the gravimetric method, using a gravimetric balance (Sartorius, SA 325, Germany) (USP, 2012). Measures were made in triplicate.

**Technological properties of tablets**

**Hardness**

Tablets hardness was evaluated using an Erweka strength tester (Offenbach, Germany). The mean value of 10 determinations was reported (USP, 2012).

**Disintegration time**

Disintegration time was measured according to the British Pharmacopoeia (BP, 2010) in a disintegration tester (PTZ1; Pharmatest GmbH, Hinesburg, Germany). Deionised water at 37±2°C was used as immersion medium. For each formulation, six randomly selected tablets were tested. All measurements were made in triplicate.

**Friability**

Tablets friability was measured as the percentage of weight loss of 20 tablets tumbled in a friabilator (Pharma Test, model TTSR-A (Germany). After 4 min of rotation at 25 rpm, the dust of tablets was removed, and the percentage of weight loss was calculated (Gibson, 2006; USP, 2012).

**Data analysis**

All responses for granules and tablets characterization were treated using Design Expert software (Version 6.0.1, Stat-Ease, Inc. Minneapolis, USA). The best-fitted mathematical models for each response were selected. To select the model that best describes the variability of response depending on the factors used (quantities of excipients), the following criteria were taken into account; the highest adjusted R-squared value ($R^2$) and the predicted R-squared ($Q^2$). For the selection of the model was also considered the different minor between $R^2$ and $Q^2$, the minor value of the sum of squares of the predicted error (PRESS) and the test of lack of fit without statistical significance ($p>0.05$). The number of experimental points (14) was enough to adjust the response until special cubic model (Gabrielson et al., 2002).

## RESULTS AND DISCUSSION

### Characterization of granules

A D-optimal mixture design was carried out to evaluate the granules behaviour as a function of selected excipient proportion. Table 3 shows the results of the experiments.

### Table 1. Excipients proportion restrictions.

<table>
<thead>
<tr>
<th>Excipients</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (%)</td>
</tr>
<tr>
<td>Lactose monohydrate</td>
<td>31.50</td>
</tr>
<tr>
<td>Microcrystalline cellulose</td>
<td>30.00</td>
</tr>
<tr>
<td>Polyvinylpyrrolidone</td>
<td>3.50</td>
</tr>
</tbody>
</table>

### Table 2. D-optimal mixture design for evaluating granules and tablet formulations.

<table>
<thead>
<tr>
<th>Run</th>
<th>LM (%)</th>
<th>MCC (%)</th>
<th>PVP (%)</th>
<th>Partial (%) LM+MCC+PVP</th>
<th>CSD (%)</th>
<th>CCNa (%)</th>
<th>MS (%)</th>
<th>TSE (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34.50</td>
<td>33.75</td>
<td>5.25</td>
<td>73.50</td>
<td>20.00</td>
<td>1.50</td>
<td>3.5</td>
<td>1.50</td>
<td>100.00</td>
</tr>
<tr>
<td>2</td>
<td>35.75</td>
<td>32.50</td>
<td>5.25</td>
<td>73.50</td>
<td>1.50</td>
<td>3.50</td>
<td>1.50</td>
<td>20.00</td>
<td>100.00</td>
</tr>
<tr>
<td>3</td>
<td>36.50</td>
<td>30.00</td>
<td>7.00</td>
<td>73.50</td>
<td>1.50</td>
<td>3.50</td>
<td>1.50</td>
<td>20.00</td>
<td>100.00</td>
</tr>
<tr>
<td>4</td>
<td>40.00</td>
<td>30.00</td>
<td>3.50</td>
<td>73.50</td>
<td>1.50</td>
<td>3.50</td>
<td>1.50</td>
<td>20.00</td>
<td>100.00</td>
</tr>
<tr>
<td>5</td>
<td>31.50</td>
<td>35.00</td>
<td>7.00</td>
<td>73.50</td>
<td>1.50</td>
<td>3.50</td>
<td>1.50</td>
<td>20.00</td>
<td>100.00</td>
</tr>
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<td>35.00</td>
<td>35.00</td>
<td>3.50</td>
<td>73.50</td>
<td>1.50</td>
<td>3.50</td>
<td>1.50</td>
<td>20.00</td>
<td>100.00</td>
</tr>
<tr>
<td>7</td>
<td>31.50</td>
<td>35.00</td>
<td>7.00</td>
<td>73.50</td>
<td>1.50</td>
<td>3.50</td>
<td>1.50</td>
<td>20.00</td>
<td>100.00</td>
</tr>
<tr>
<td>8</td>
<td>37.50</td>
<td>32.50</td>
<td>3.50</td>
<td>73.50</td>
<td>1.50</td>
<td>3.50</td>
<td>1.50</td>
<td>20.00</td>
<td>100.00</td>
</tr>
<tr>
<td>9</td>
<td>34.00</td>
<td>32.50</td>
<td>7.00</td>
<td>73.50</td>
<td>1.50</td>
<td>3.50</td>
<td>1.50</td>
<td>20.00</td>
<td>100.00</td>
</tr>
<tr>
<td>10</td>
<td>35.00</td>
<td>32.50</td>
<td>3.50</td>
<td>73.50</td>
<td>1.50</td>
<td>3.50</td>
<td>1.50</td>
<td>20.00</td>
<td>100.00</td>
</tr>
<tr>
<td>11</td>
<td>36.50</td>
<td>30.00</td>
<td>7.00</td>
<td>73.50</td>
<td>1.50</td>
<td>3.50</td>
<td>1.50</td>
<td>20.00</td>
<td>100.00</td>
</tr>
<tr>
<td>12</td>
<td>37.88</td>
<td>31.25</td>
<td>4.38</td>
<td>73.50</td>
<td>1.50</td>
<td>3.50</td>
<td>1.50</td>
<td>20.00</td>
<td>100.00</td>
</tr>
<tr>
<td>13</td>
<td>33.25</td>
<td>35.00</td>
<td>5.25</td>
<td>73.50</td>
<td>1.50</td>
<td>3.50</td>
<td>1.50</td>
<td>20.00</td>
<td>100.00</td>
</tr>
<tr>
<td>14</td>
<td>40.00</td>
<td>30.00</td>
<td>3.50</td>
<td>73.50</td>
<td>1.50</td>
<td>3.50</td>
<td>1.50</td>
<td>20.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

MCC, microcrystalline cellulose; LM, lactose monohydrate; PVP, polyvinylpyrrolidone; CSD, colloidal silicon dioxide; CCNa, sodium croscarmellose; MS, magnesium stearate; TSE, tamarind soft extract.
Table 3. Physicomechanical properties of the granules. The shown data represent triplicates ± SD.

<table>
<thead>
<tr>
<th>Run</th>
<th>Compressibility index (%)</th>
<th>Angle of repose (°)</th>
<th>Flow rate (g/cm².s)</th>
<th>Residual humidity (%)</th>
<th>Particle size(µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23.22±1.28</td>
<td>29.90±1.69</td>
<td>11.90±0.82</td>
<td>2.20±0.12</td>
<td>216.33±7.22</td>
</tr>
<tr>
<td>2</td>
<td>21.12±1.33</td>
<td>28.89±0.32</td>
<td>12.20±1.14</td>
<td>1.99±0.14</td>
<td>210.3±16.34</td>
</tr>
<tr>
<td>3</td>
<td>15.34±1.02</td>
<td>24.65±0.96</td>
<td>13.02±0.25</td>
<td>2.70±0.21</td>
<td>212.3±19.14</td>
</tr>
<tr>
<td>4</td>
<td>19.01±1.55</td>
<td>30.90±0.79</td>
<td>9.20±1.20</td>
<td>2.00±0.20</td>
<td>215.14±19.87</td>
</tr>
<tr>
<td>5</td>
<td>16.26±0.94</td>
<td>27.36±1.68</td>
<td>12.21±0.61</td>
<td>2.80±0.33</td>
<td>199.23±15.14</td>
</tr>
<tr>
<td>6</td>
<td>21.98±0.74</td>
<td>32.13±0.87</td>
<td>7.36±0.96</td>
<td>1.85±0.16</td>
<td>200.12±13.25</td>
</tr>
<tr>
<td>7</td>
<td>17.25±0.85</td>
<td>27.12±0.97</td>
<td>12.68±0.35</td>
<td>2.64±0.47</td>
<td>209.67±21.10</td>
</tr>
<tr>
<td>8</td>
<td>23.05±2.29</td>
<td>31.21±0.98</td>
<td>8.25±0.17</td>
<td>2.10±0.27</td>
<td>209.45±22.32</td>
</tr>
<tr>
<td>9</td>
<td>18.23±0.96</td>
<td>25.52±2.36</td>
<td>12.73±0.55</td>
<td>2.50±0.29</td>
<td>222.23±13.45</td>
</tr>
<tr>
<td>10</td>
<td>19.12±1.48</td>
<td>31.56±2.29</td>
<td>7.90±0.88</td>
<td>2.10±0.25</td>
<td>232.17±19.63</td>
</tr>
<tr>
<td>11</td>
<td>14.55±0.64</td>
<td>24.65±2.08</td>
<td>13.03±0.99</td>
<td>2.35±0.31</td>
<td>218.92±13.43</td>
</tr>
<tr>
<td>12</td>
<td>22.25±1.49</td>
<td>30.26±1.56</td>
<td>10.54±1.33</td>
<td>2.37±0.12</td>
<td>245.22±12.47</td>
</tr>
<tr>
<td>13</td>
<td>23.65±2.36</td>
<td>30.25±1.88</td>
<td>10.65±0.47</td>
<td>1.80±0.08</td>
<td>227.43±19.96</td>
</tr>
<tr>
<td>14</td>
<td>23.50±3.24</td>
<td>30.33±1.24</td>
<td>9.60±0.47</td>
<td>2.40±0.33</td>
<td>231.36±23.12</td>
</tr>
</tbody>
</table>

Figure 1. Trace (Cox) graph for Compressibility index. A: Lactose monohydrate. B: Microcrystalline cellulose. C: Polyvinylpyrrolidone. Reference blend: A:38.25%; B: 30.50%; C: 5.75%

The ability to flow by the granules using the angle of repose and the flow rate (Figure 2A and 2B, respectively) were evaluated. The best flow ability takes place when the amount of PVP in the formulations was high. Lactose monohydrate had no significant effect on the variability of the flow properties. The lower angle of repose and the higher flow rate occurs when the amount of microcrystalline cellulose in the formulation is low (30.00%). For the angle of repose and the flow rate, the data were fitted to quadratic models (Table 4). In both cases, high values of $R^2$ and $Q^2$ and a minimum difference between them were observed. PRESS value in each case was low among the possible models obtained.
Statistics for fitted mathematical models obtained to each response on the granules formulations.

<table>
<thead>
<tr>
<th>Response</th>
<th>R²</th>
<th>Q²</th>
<th>PRESS</th>
<th>P (Anova)</th>
<th>p (Lack of fit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compressibility index</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>0.4624</td>
<td>0.2515</td>
<td>99.68</td>
<td>0.0131</td>
<td>0.0095</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.7200</td>
<td>0.5023</td>
<td>66.29</td>
<td>0.0429</td>
<td>0.7067</td>
</tr>
<tr>
<td>Special cubic</td>
<td>0.7000</td>
<td>0.3408</td>
<td>87.80</td>
<td>0.5160</td>
<td>0.0332</td>
</tr>
<tr>
<td>Angle of repose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>0.9597</td>
<td>0.9451</td>
<td>4.76</td>
<td>0.0001</td>
<td>0.0466</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.9883</td>
<td>0.9800</td>
<td>1.73</td>
<td>0.0041</td>
<td>0.5827</td>
</tr>
<tr>
<td>Special cubic</td>
<td>0.9872</td>
<td>0.8964</td>
<td>8.99</td>
<td>0.2953</td>
<td>0.0468</td>
</tr>
<tr>
<td>Flow rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>0.8757</td>
<td>0.8489</td>
<td>8.03</td>
<td>0.0001</td>
<td>0.1257</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.9706</td>
<td>0.9511</td>
<td>3.22</td>
<td>0.0028</td>
<td>0.2419</td>
</tr>
<tr>
<td>Special cubic</td>
<td>0.9794</td>
<td>N/A</td>
<td>N/A</td>
<td>0.7891</td>
<td>0.0738</td>
</tr>
</tbody>
</table>

In both cases, lack of fittest was not significant (p-value>0.05). Table 4 shows the values of the coefficients of each of the factors and the observed interactions between these factors, for both properties. PVP interacts with lactose monohydrate and microcrystalline cellulose. Both interactions improve the fluidity of the granules. The lower angle of repose and the higher flow rate were observed in runs 3 and 11. These runs had the same composition, lactosemonohydrate (36.50%); microcrystalline cellulose (30.00%) and 7.00% of PVP.

Characterization of the tablets

Table 6 shows the results for D-optimalmixture design for tablets preparation. The models adjusted for each response for tablets are shown in Table 7. In the same way, Table 8 shows the selected fitted model for each property with the corresponding statistic. The hardness showed a linear behavior related to the excipients proportions (p< 0.05). The test of lack of fit was not significant. There was not found any statistical significance for all other considered model (Table 7). The adjusted model showed excellent values of R² and Q². This model was selected because, in spite of that, the R² and Q² are lower than the other models; the difference between them was minimal (0.0253). This fact implies that the fraction of variability explained for linear model is very close to the fraction that can be predicted by itself. The last is expressed as a minor PRESS value of this model . Experimental runs 3 and 11 showed higher hardness (Table 6). PVP showed greater influence on the tablet hardness, than the rest of the excipients (Figure 3A; Table 8). With increasing the content of PVP in the formulation, the tablet hardness increase. The highest hardness was found in the formulation containing greater amount of PVP, the lowest percentage of microcrystalline cellulose and around the average of the addition of lactose.
Figure 2. Trace (Cox) graph for angle of repose (A) and flow rate (B). A: Latose monohydrate. B: Microcrystalline cellulose. C: Polyvinylpyrrolidone. Reference blend: A:38.25%; B: 30.50%; C: 5.75%.

Table 6. Experimental matrix obtained in the D-optimal mixture design for tablet formulation with the standard deviation (n=3).

<table>
<thead>
<tr>
<th>Run</th>
<th>Hardness(kg/f)</th>
<th>Disintegration time (min)</th>
<th>Friability(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.76±0.26</td>
<td>13.50±1.14</td>
<td>1.30±0.04</td>
</tr>
<tr>
<td>2</td>
<td>5.76±0.35</td>
<td>12.50±1.10</td>
<td>1.29±0.07</td>
</tr>
<tr>
<td>3</td>
<td>5.96±0.28</td>
<td>12.00±0.51</td>
<td>0.62±0.10</td>
</tr>
<tr>
<td>4</td>
<td>5.56±0.05</td>
<td>15.00±0.26</td>
<td>1.37±0.08</td>
</tr>
<tr>
<td>5</td>
<td>5.81±0.12</td>
<td>12.50±1.3</td>
<td>0.90±0.08</td>
</tr>
<tr>
<td>6</td>
<td>5.18±0.14</td>
<td>19.00±2.28</td>
<td>1.40±0.20</td>
</tr>
<tr>
<td>7</td>
<td>5.81±0.21</td>
<td>12.50±0.31</td>
<td>0.84±0.11</td>
</tr>
<tr>
<td>8</td>
<td>5.45±0.27</td>
<td>16.00±1.39</td>
<td>1.39±0.23</td>
</tr>
<tr>
<td>9</td>
<td>5.86±0.04</td>
<td>12.00±0.56</td>
<td>0.67±0.14</td>
</tr>
<tr>
<td>10</td>
<td>5.25±0.33</td>
<td>19.00±3.26</td>
<td>1.39±0.08</td>
</tr>
<tr>
<td>11</td>
<td>6.07±0.13</td>
<td>10.00±0.55</td>
<td>0.64±0.10</td>
</tr>
<tr>
<td>12</td>
<td>5.61±0.17</td>
<td>14.00±0.27</td>
<td>1.33±0.07</td>
</tr>
<tr>
<td>13</td>
<td>5.61±0.17</td>
<td>14.00±0.29</td>
<td>1.32±0.00</td>
</tr>
<tr>
<td>14</td>
<td>5.56±0.31</td>
<td>15.00±0.41</td>
<td>1.35±0.09</td>
</tr>
</tbody>
</table>

monohydrate (Figure 3B).

Disintegration time is a measure of how well the tablets are broken up when they are in contact with the body fluids. For uncoated tablets a time not more than 15 min is recommended (USP, 2012). As is shown in Table 7, the best-adjusted model for this property was quadratic. The statistical significance of the Anova test (p<0.05), the higher values of R² and Q², and the lower PRESS compared with the other considered models, demonstrated that the quadratic model is the better predictor of the disintegration time variability. As the quantity of PVP augment on formulation, a lower disintegration time was reached (Figure 4A). Lactose monohydrate showed a similar behavior (Figure 4A). As literature report, lactose monohydrate produces soft tablets that disintegrate very well (Lieberman et al., 1981). It was observed that there was a statistical significance for interactions between lactose monohydrate-PVP and microcrystalline cellulose-PVP (p<0.05).

Both interactions tend to reduce the disintegration time. The lowest disintegration time was reached with the proportions lactose monohydrate 36.50%, microcrystalline cellulose 30.00% and PVP 7% (Figure 4B). Figure 5A presents a trace graph showing the influence of the excipients on the friability of the tablets. Lactose monohydrate showed little influence on this property. When the percentage of microcrystalline
Table 7. Statistics for all possible model obtained to each response on tablets formulation.

<table>
<thead>
<tr>
<th>Property</th>
<th>Adj-$R^2$</th>
<th>$Q^2$</th>
<th>PRESS</th>
<th>$P_{(\text{Anova})}$</th>
<th>$P_{(\text{Lack of fit})}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hardness</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>0.9090</td>
<td>0.8837</td>
<td>0.09</td>
<td>0.0001</td>
<td>0.0919</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.9367</td>
<td>0.8959</td>
<td>0.10</td>
<td>0.1242</td>
<td>0.1890</td>
</tr>
<tr>
<td>Special cubic</td>
<td>0.9280</td>
<td>0.5754</td>
<td>0.36</td>
<td>0.8725</td>
<td>0.0970</td>
</tr>
<tr>
<td><strong>Disintegration time</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>0.8330</td>
<td>0.7643</td>
<td>20.49</td>
<td>0.0001</td>
<td>0.1572</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.9441</td>
<td>0.8792</td>
<td>10.50</td>
<td>0.0077</td>
<td>0.7437</td>
</tr>
<tr>
<td>Special cubic</td>
<td>0.9398</td>
<td>0.8362</td>
<td>14.24</td>
<td>0.5318</td>
<td>0.6778</td>
</tr>
<tr>
<td><strong>Friability</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linear</td>
<td>0.8330</td>
<td>0.7643</td>
<td>0.20</td>
<td>0.0001</td>
<td>0.0020</td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.9441</td>
<td>0.8792</td>
<td>0.03</td>
<td>0.0001</td>
<td>0.1918</td>
</tr>
<tr>
<td>Special cubic</td>
<td>0.9398</td>
<td>0.8362</td>
<td>0.05</td>
<td>0.0882</td>
<td>0.3013</td>
</tr>
</tbody>
</table>

Adj-$R^2$, adjusted $R$-squared; $Q^2$, predicted $R$-squared; PRESS, Predicted Residual Sum of Squares. ($\alpha=0.05$).

Figure 3. Trace (Cox) graph (A) and contour (B) for tablet hardness. A, lactose monohydrate; B, microcrystalline cellulose; C, polyvinylpyrrolidone. Reference blend: A: 38.25%; B: 30.50%; C: 5.75%.

cellulose increased in the formulation, tablet friability was increased accordingly, this probably was due to a decrease in tablet hardness. Conversely, when the percentage of PVP was increased, the hardness increased and lower friability values were observed (Figure 5A and 5B).

To describe the behavior of tablets, friability a quadratic model was selected (Table 7). This model showed the highest values of $R^2$ and $Q^2$ and the lowest value of PRESS. On the other hand, Anova test were significant ($p<0.05$) and the lack of fittest was not significant ($p>0.05$). Table 8 shows the positive influence of PVP and microcrystalline cellulose for tables’ friability (negative).

Table 8. Optimization of tamarind tablet formulation.

Tablets optimization

For the optimization of this oral dosage form, a numerical method based on desirability function was used. This method takes into account various criteria for different response in only one mathematical equation. The relative important of the response are expressed as a scale from 1 to 5, with 5 being the most important. The conditions to optimize the tamarind tablet formulation were; minimized is integration time (importance of 5); maximize hardness (importance of 4) and minimize tablets friability (importance of 4). The higher importance of disintegration time is obvious.

Figure 6 shows the ramp graph of the optimized tablets.
Figure 4. Trace (Cox) graph (A) and contour (B) for disintegration time. A, lactose monohydrate; B, microcrystalline cellulose; C, polyvinylpyrrolidone. Reference blend: A:38.25%; B: 30.50%; C: 5.75%.

Figure 5. Trace (Cox) graphs (A) and of contour (B) for tablet friability. A, lactose monohydrate; B, microcrystalline cellulose; C, polyvinylpyrrolidone. Reference blend: A:38.25%; B: 30.50%; C: 5.75%.

Table 8. Adjusted mathematical model selected for each response on tablets formulation, expressed as real components, with the coefficients statistical probability.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Hardness</th>
<th>Disintegration time</th>
<th>Friability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>p-value</td>
<td>Coefficient</td>
</tr>
<tr>
<td>LM</td>
<td>0.088</td>
<td>&lt;0.0001</td>
<td>-0.096</td>
</tr>
<tr>
<td>MCC</td>
<td>0.041</td>
<td>&lt;0.0001</td>
<td>1.174</td>
</tr>
<tr>
<td>PVP</td>
<td>0.233</td>
<td>&lt;0.0001</td>
<td>34.779</td>
</tr>
<tr>
<td>LM*PVP</td>
<td>-</td>
<td>-</td>
<td>-0.504</td>
</tr>
<tr>
<td>MCC*PVP</td>
<td>-</td>
<td>-</td>
<td>-0.644</td>
</tr>
</tbody>
</table>

LM: Lactose monohydrate, MCC: Microcrystalline cellulose, PVP: polyvinylpyrrolidone. (α=0.05)

The proportions that guarantees the minimum disintegration time with an adequate hardness
Table 9. Observed and predicted properties used for the optimization process (n = 5).

<table>
<thead>
<tr>
<th>Properties</th>
<th>Predicted</th>
<th>Observed</th>
<th>t-test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disintegration time (min)</td>
<td>11.04</td>
<td>9.50±1.00</td>
<td>-3.8891</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hardness (kg/F)</td>
<td>6.04</td>
<td>6.15±0.45</td>
<td>-0.7778</td>
<td>0.4376</td>
</tr>
<tr>
<td>Friability (%)</td>
<td>0.63</td>
<td>0.67±0.08</td>
<td>0.3987</td>
<td>0.6905</td>
</tr>
</tbody>
</table>

Figure 6. Ramp graph for the tablet optimal formulation. Red points indicate the amount of the ingredient optimized by the software. The slope indicates the direction where the response is improved.

The D-optimal mixture design allowed the obtaining of optimal proportions of the excipients for the tablets formulation. PVP was the excipient that showed more influence on the tablets properties. The obtained tablets showed good technological properties. The proportions of the optimal mixture; expressed as percentage was lactose monohydrate 36.54%; microcrystalline cellulose 30.00% and PVP 6.96%.

Abbreviations

MCC, microcrystalline cellulose; Ar, angle of repose; LM, lactose monohydrate; Fr, flow rate; PVP, polyvinylpyrrolidone; RH, residual humidity; CSD, colloidal silicon dioxide; PS, particle size; CCNa, sodium croscarmellose; MS, magnesium stearate; TSE, tamarind soft extract; CI, compressibility index; Dt, tapped density; Db, bulk density;

Conflicts of interest

The authors declare that they have no conflicts of interest.

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