Revista de Ciências Farmacêuticas Básica e Aplicada
*Journal of Basic and Applied Pharmaceutical Sciences*

**Rev Ciênc Farm Básica Apl.**, 2011;32(2):175-180
ISSN 1808-4532

# Preformulation studies of *Gymnema sylvestre* extract powder formulation for hard gelatin capsules

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Recebido 09/07/2010 / Aceito 24/03/2011

## ABSTRACT

***Gymnema sylvestre* extract (GSE) is a plant product widely used as an adjuvant in the treatment of diabetes mellitus and commercially available as a powder. Owing to its low flowability, the manufacturing of hard gelatin capsules containing GSE faces specific problems. The purpose of this study was to investigate the best excipient (starch, lactose or microcrystalline cellulose) for hard gelatin capsules containing GSE. The technological properties such us bulk density (ρβ); tapped density (ρ*t*); inter-particle porosity (Ie); Carr index (CI); Hausner ratio (HR); loss on drying (%LOD) and particle size distribution (%Pf) of the various GSE mixtures were investigated with the aim of identifying the best excipient. The need for lubricants (talc/magnesium stearate) was also assessed. GSE was characterized as a fine powder with more than 50% of its particles between 0.149mm to 0.250mm; furthermore, CI=25.6%; RH=1.3 and Ie = 25.6% and, as expected with such properties, it showed poor flowability. All the excipients investigated were able to change the technological properties of GSE and the powder mixture containing microcrystalline cellulose gave the best results.**

*Keywords: Gymnema sylvestre*, preformulation studies, technological properties, solid dosage forms, hard gelatin capsules.

## INTRODUCTION

*Gymnema sylvestre* (Asclepiadaceae) is a plant characteristic of the south of China, including the provinces of Guangdong, Guangxi and Fujian and tropical India, where it is used for gastrointestinal affections and as a diuretic, but mainly as an ancient ayurvedic treatment for diabetes ('sugar destroyer'). Its anti-diabetic action is due to the saponin fraction of the leaves, commonly known as "gymnemic acid", which has an anti-sweetening effect. This plant has been shown to be able to inhibit glucose absorption in the small intestine and suppress the elevated levels of glucose in the blood following the administration of sucrose. In addition, it has been suggested that this glucose regulation in the blood is achieved through an increase in serum insulin levels provided by the repair or regeneration of the pancreatic β-cells (Ye et al., 1999; Porchezhian; Dobriyal, 2003; Kanetkar et al., 2006; Zhu et al., 2008; Ahmed et al., 2010).

The biochemical mechanism of depletion of hepatic glycogen on ingestion of *G. sylvestre* leaf extract (GSE) together with glucose is not very clear at present. However, some studies suggest that this effect of GSE might be initiated in one of the following three ways, a) direct stimulation of glucose release by glycogenolysis in the liver, with rapid glucose utilization, b) secondary effect following increased peripheral uptake of glucose in the tissues, c) sum of the effects of the extra insulin released and the action of the drug on the liver cells (Chattopadhyay, 1998; Ye et al., 1999; Porchezhian; Dobriyal, 2003; Zhu et al., 2008). Recently, Ahmed and coworkers demonstrated anti-diabetic activity due to β-cell regeneration (Ahmed et al., 2010).

The GSE is taken orally as hard gelatin capsules. It is an established fact of pharmaceutical technology that the homogeneity of powder mixtures is fundamental to the pharmacological activity of solid dosage forms. Pharmaceutical powder mixtures for the production of solid dosage forms, such as hard gelatin capsules (Ansel et al., 2005), must exhibit certain mechanical characteristics. However, in physical tests, it was observed that commercially used GSE does not have suitable properties for hard gelatin capsule production.

The processing of many particulate materials to prepare solid pharmaceutical dosage forms involves unit

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operations such as fluidization, pneumatic conveying, packing and storage in bins, and feeding through hoppers. The behavior of powders is strongly influenced by particle properties and also by the design and operating conditions of these unit operations. The flowability of powders is the most important issue and it can strongly influence the efficiency of these operations in a compounding pharmacy.

In this study, a preformulation study was carefully planned and carried out, with the aim of developing a powder mixture suitable for the preparation of hard gelatin capsules in such a pharmacy. Thus, three excipients, starch, lactose and microcrystalline cellulose, alone or combined with the lubricants talc and magnesium stearate, were tested in mixtures with GSE.

## MATERIAL AND METHODS

### Chemicals and Reagents

*Gymnema sylvestre* extract (GSE) was purchased from Pharma Nostra®, Brazil, magnesium stearate, starch, lactose and talc from Henrifarma®, Brazil, and microcrystalline cellulose from Merk®, Brazil.

### Powder mixture preparation

The three diluents (starch, lactose and microcrystalline cellulose) and two lubricants (talc and magnesium stearate) most commonly employed in the compounding pharmacies in Brazil (Batistuzzo et al., 2002) were chosen for the powder mixtures in this study. The amounts of the various components were based on the volume of powder required to fill completely a hard gelatin capsule (size 0; 0.68mL). Thus, the physical properties of the components were investigated to determine their densities. Next, the various powder mixtures were prepared in a mortar and pestle. The weight of GSE was fixed at 150mg per capsule and the other excipients were added in amounts calculated to fill the hard gelatin capsules (size 0; 0.68mL). After the effects of the three excipients had been tested, a lubricant was added (1%). The compositions of the different mixtures are shown in Table 1.

Table 1. Compositions of powder mixtures containing GSE.

| Formulation | Composition |
|---|---|
| 1 | GSE/lactose (GSEl) |
| 2 | GSE/microcrystalline cellulose (GSEc) |
| 3 | GSE/starch (GSEs) |
| 4 | GSE/ lactose/magnesium stearate (GSEls) |
| 5 | GSE/lactose/talc (GSElt) |
| 6 | GSE/microcrystalline cellulose/magnesium stearate (GSEcs) |
| 7 | GSE/ microcrystalline cellulose/ talc (GSEct) |
| 8 | GSE/starch/magnesium stearate (GSEss) |
| 9 | GSE/ starch/talc (GSEst) |

The preformulation studies were performed on GSE, starch, lactose, microcrystalline cellulose and the powder mixtures prepared for encapsulation. The properties determined were:

***Bulk density (ρb)***

As described in Section 2.9.15 of Eur.Ph. (European Pharmacopoeia, 2005), the volume of a known weight of powder was measured in a graduated glass cylinder.

***Tapped density (ρt)***

Again by the method described in Section 2.9.15 of Eur. Ph. (European Pharmacopoeia, 2005), this density was calculated from the final volume reached after 1250 taps from a settling apparatus on the measuring cylinder.

***Inter-particle porosity (Ie)***

The inter-particle porosity of the powder mixture was calculated by the following equation:

$$Ie = (\rho t - \rho b)/(\rho t \times \rho b) \quad \text{(Equation 1)}$$

***Carr index (CI%)***

This was calculated from ρb and ρt as:

$$CI = (\rho t - \rho b / \rho t) \times 100 \quad \text{(Equation 2)}$$

***Hausner ratio (HR)***

This was calculated from ρb and ρt as:

$$HR = \rho t / \rho b \quad \text{(Equation 3)}$$

***Angle of repose (α)***

The angle of repose is the angle of the conical pile formed when the product flows from a funnel with the following dimensions: funnel height 9.5 cm, upper diameter of spout 7.2 cm, internal diameter at the narrow bottom end of spout 1.8 cm. This funnel was placed on a support, 20cm above the surface of a table. The height of the cone formed and the radius (r) of its base were measured and the tangent of the angle of repose (α) was calculated by the equation:

$$\tan(\alpha) = h/r \quad \text{(Equation 4)}$$

α was then deduced from its tangent.

***Flowability (t")***

This was determined by the method described in Section 2.9.16-2 of Eur. Ph (4).

***Loss on drying (%LOD)***

The loss-on-drying test was carried out in accordance with the General method 2.2.32 in Eur. Ph. (European Pharmacopoeia, 2005). The powder was dried

in an oven at 105ºC + 2ºC, until a constant weight was obtained.

***Particle size analysis***

The particle size analysis was performed by the traditional homogeneity test for pharmaceutical powders. A sample of 100g was subjected to vibration on a sieve stack for 15min at speed 10 (CISA vibrator). The meshes of sieves used were 0.297mm, 0.250mm, 0.210mm and 0.149mm. The mean particle size was determined from the weighted average of the size fractions obtained.

***Hard gelatin capsule preparation procedure***

Formulations 4, 5, 6, 7, 8 and 9, as shown in Table 1, were prepared with a manual capsule-filling apparatus. For all formulations, size 0 capsules were used (0.68mL).

***Quality control of hard gelatin capsules***

The quality control parameter used for the various formulations was the average weight and the procedure was that described in Eur. Ph. (European Pharmacopoeia, 2005). The variability was taken as the standard deviation (SD) and relative standard deviation (RSD%).

***Statistical analysis***

The results are expressed as mean ± SD. The RSD was calculated as 100 × (SD/mean). One-way analysis of variance (ANOVA) was employed to compare the experimental data. Dunnett's test was used for post-hoc multiple comparisons of group means and significance was accepted at p-values less than 0.05.

**RESULTS**

Uniformity of dosage is one of the most important problems faced in the preparation of hard gelatin capsules in the compounding pharmacy. The filling of hard capsules with plant extracts is not easy, owing to certain mechanical properties. Most of these extracts are fine powders with considerable moisture content, which interferes with the flowability of the material and, consequently, the filling step. In order to identify the best excipient for GSE, the physical properties of every isolated component of the powder mixtures were investigated. The particle size distribution for GSE is displayed in Figure 1.

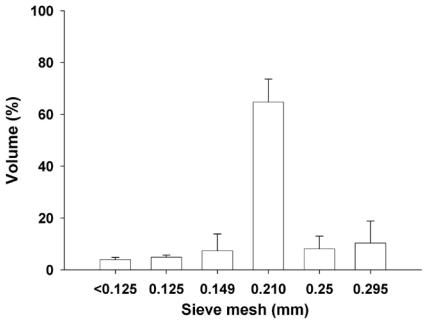


Figure 1. Particle size distribution for GSE.

The most prevalent particle size in GSE was around 0.210mm. The particle size distribution is an extremely important factor for the physical properties of powder mixtures. It influences both the compression and the flowability of the material. After the particle size analysis of GSE, the various excipients were mixed with the GSE and the particle size distribution of each mixture determined (Figure 2). The effects of the different excipients on GSE can be compared readily in Figure 3, where the cumulative distributions are plotted.

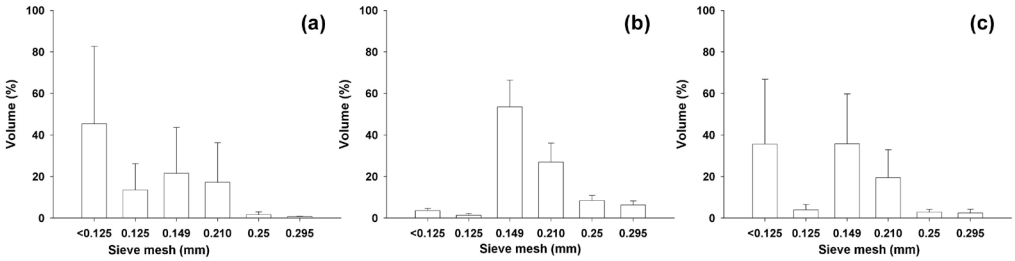


Figure 2. Particle size distribution for (a) GSEl; (b) GSEc and (d) GSEs.

The powder mixture that showed the most uniform particle size distribution was the one with microcrystalline cellulose, where more than 90% of the material had a particle size between 0.210 and 0.149mm. The mixtures with starch and lactose exhibited several particle sizes, with a considerable fraction around 0.125mm. This can be observed easily in Figure 3.

The particle size distribution should be assessed in conjunction with other indirect flowability parameters. The effect of the three excipients on the other physical properties was investigated and the experimental results are shown in the Table 2. GSE was chosen as control.

None of the powder mixtures flowed through the funnel used, so flowability was investigated indirectly through other properties. All three excipients clearly modified the compaction properties of the GSE. The mixtures with cellulose and lactose had significantly lower moisture contents ($P<0.05$) than GSE.

After the effects of all the excipients on the flow-related properties of the GSE had been observed, combinations with flow agents (1% magnesium stearate or talc) were tested by preparing hard capsules from the different powder formulations containing talc (t) or magnesium stearate (s) and subjecting them to a quality

control test (Table 3). In this experiment, GSE was not used as a control, since the amount of GSE (150mg) was insufficient to fill completely the hard capsule chosen (size 0; 0.680mL)

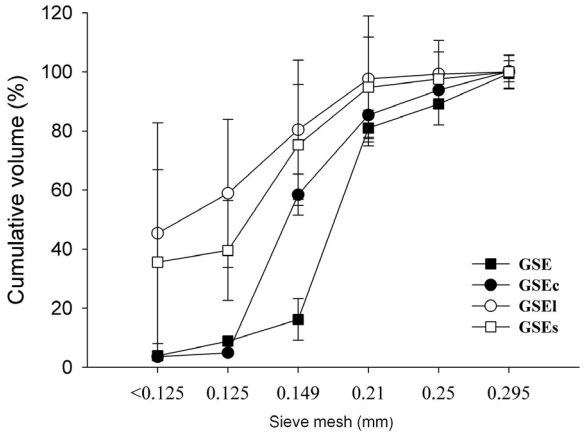


Figure 3. Cumulative particle size distribution for different powder mixtures.

Table2. Technological properties of GSE and the two-powder mixtures.

| Property | GSE | GSEl | GSEc | GSEs |
|---|---|---|---|---|
| Bulk density (ρb) | 0.25 ± 0.001 | 0.34 ± 0.010* | 0.32 ± 0.002* | 0.32 ± 0.005* |
| Tapped density (ρt) | 0.33 ±0.001 | 0.45 ± 0.060* | 0.44 ± 0.006* | 0.43 ± 0.005* |
| Inter-particle porosity(*Ie*) | 25.6 ± 0.001 | 25.7 ± 0.83 | 27.7 ± 0.46* | 26.13 ± 1.2 |
| Carr index (*CI%*) | 25.60 ± 0.01 | 25.80 ± 0.83 | 26.91 ± 1.05 | 24.96 ± 0.97 |
| Loss on drying (*%LOD*) | 7.1 ±0.36 | 3.9 ± 0.10* | 6.7 ± 0,15* | 8.7 ± 0.47 |
| Hausner ratio (*HR*) | 1.34 ±0.0002 | 1.35 ± 0.015* | 1.37 ± 0.020 | 1.33 ± 0.020 |
| Angle of repose (α) | nf | nf | nf | nf |

*Statistically different versus GSE (P<0.05); nf: failed to flow from funnel.

Table3. Average weight and variability of each formulation (n=3)

| Formulation | Identification | Average weight | RSD% |
|---|---|---|---|
| 4 | GSEls | 285.96 ± 10.77* | 3.76 |
| 5 | GSElt | 284.9 ± 9.62* | 3.38 |
| 6 | GSEcs | 264.38 ± 10.53 | 3.98 |
| 7 | GSEct | 267.08 ± 9.15 | 3.43 |
| 8 | GSEss | 281.02 ± 11.26* | 4.01 |
| 9 | GSEst | 275.88 ± 14.41 | 5.22 |

*Statistically different from GSEct (P<0.05)

Between the mixtures containing microcrystalline cellulose (GSEcs and GSEct), the one containing talc as flow agent (GSEct) showed a smaller relative standard deviation in quality (RSD= 3.43%). This sample was thus chosen as the statistical control, in the comparative analysis of the different mixtures. No statistical differences were found between GSEcs and GSEct. The mixtures that showed the highest values for RSD were those containing starch as diluent.

## DISCUSSION

GSE exhibited particle sizes distributed mainly between 0.125mm and 0.295mm, with a peak at 0.210mm. This small particle size explains the low flowability of commercial GSE. The greater contact surface of smaller particles leads to a stronger electrostatic interaction among the particles (cohesion) and between them and the surface of the container (adhesion), which hinders its flow. The addition of excipients to the powder mixtures decreases these particle surface interaction phenomena, changing the physical properties of the bulk (Geldart, et al., 2006, Kim et al., 2007; Emery, 2009).

The particle size distribution is a very important technological property for pharmaceutical powders and for the processing of any solid dosage form, mainly because of its relationship with the flowability of the particulate material (Santomaso et al., 2003; Kim et al., 2005). In Figure 2 it can be seen that all the powder mixtures showed a most frequent particle size between 0.125mm and 0.210) mm. However, the most uniform particle size distribution was seen in the powder mixture with microcrystalline cellulose (GSEc), as can be verified in Figure 3.

Some parameters can supply indirect information about the flowability of a powder. As observed in Table 2, the tapped and bulk densities of all the powder mixtures were significantly higher (P<0.05) than those found for GSE. The results obtained for GSEc were the most suitable for a powder intended for preparation of a pharmaceutical solid dosage form such as hard gelatin capsules in a local pharmacy, especially with respect to the inter-particle-porosity values (Ie). The porosity may be used to characterize the packing geometry of a powder mixture, and it is directly related to bulk density (ρb), which is always smaller than tapped density (ρb), owing to the pores and empty spaces among the particles (Aulton, 2005; Hering et al., 2007).

Higher values of ρb normally signify greater hindrance to flow, due to the many points of contact among the particles, giving rise to the denser packing. On the other hand, higher porosity indicates less dense packing for the powder mixtures, which explains the poor flowability of the GSE relative to various powder mixtures. The addition of excipients increased *Ie*. The mixture with the highest value of *Ie* was GSEc (27.7 ± 0.46), which is thus expected to show better flowability than GSE and the other powder mixtures, GSEl (25.7 ± 0.83) and GSEs (26.13 ± 1.2).

All the powder mixtures tested were incapable of flowing through the funnel. Considering the values of *CI* identified for all the samples (24.96-26.91), which were not significantly different (P<0.05), it is possible to explain the poor flowability observed. Particle mixtures that act as free-flowing material have *CI* values less than 20% to 21% (De Saavedra; Cuadra, 2001; Aulton, 2005; Perez et al., 2006; Hering et al., 2007), well below those observed here.

From the technological data obtained on GSE and the various powder mixtures, it was possible to observe that the addition of the excipients improved only the porosity (Ie). However, to assess the flowability, the particle size must be considered too. Moreover, the results of the particle size analysis (Figure 2) showed that GSEc had a more uniform distribution. The high percentage of

fine powder identified in the mixtures with lactose (>45%) and starch (>35%) indicates that these excipients were not suitable diluents for GSE in mixtures intended for hard gelatin capsules filled in the compounding pharmacy. The particle size distribution is very important for flowability and the segregation of the excess fine powder from the mixture is an undesirable possibility that may occur during the preparation (Brittain et al., 1995; De Saavedra; Cuadra, 2001; Liss et al., 2004; Aulton, 2005; Perez et al., 2006; Hering et al., 2007).

The low flowability of all the samples may be explained by the small particle size identified, which increases the cohesion among the particles, due to electrostatic interaction and, consequently, hinders the flow (Brittain et al., 1995; Chiou et al., 1970). On adding lactose or starch to GSE, the proportion of fine powder (<0.125mm) increased to more than 45% and 35% for GSEl and GSEs, respectively (Figure 3). On the other hand, with the addition of cellulose, the percentage of particles smaller than 0.149mm decreased from about 8.4% (GSE) to 4.9% (GSEc). Moreover, the particle size distribution of the GSEc was more uniform, with more than 90% of the particulate material between 0.149mm and 0.295mm, confirming microcrystalline cellulose as the best diluent for GSE routinely prepared in the compounding pharmacy.

Granulation, carried out with the aim of increasing both particle size and the uniformity of particle size distribution, is a possibility that may be tried, but this procedure would require stability studies to ensure the efficacy of this new form. Moreover, the longer time needed to prepare the hard gelatin capsules should be taken into consideration in the routine of the pharmacy.

Among the diluents under study, microcrystalline cellulose afforded the powder mixture with the most suitable properties. Another important excipient in the design of hard capsules is the flow agent. This pharmaceutical adjuvant is used to facilitate the filling operation of hard capsules. The Brazilian Regulatory Agency (ANVISA) lays down some important quality control procedures for solid dosage forms prepared in compounding pharmacies (Brasil, 2007; 2008). Mean weight is the most important control quality parameter, which not only detects problems with the dosage of the drug, but can also indicate poor flowability of the powdered material. By the average weight test (Table 3), it was possible to observe that there was no significant difference ($P<0.05$) between the samples prepared with talc and those prepared with magnesium stearate, for any of the diluents tested (starch, microcrystalline cellulose and lactose). These results indicate that no difference in weight was identified between the two flow agents used. However, the mixtures prepared with talc showed lower RSDs, implying higher precision.

The mixtures prepared with starch showed unfavorable results in the average weight test. GSEl and GSEc showed smaller RSD values than GSEs and the precision achieved for GSEl and GSEc may be considered adequate (RSD<4%), according to the limits recommended in USP 28. Thus, from the properties discussed earlier and the results of the average weight test, the most suitable diluent for GSE was microcrystalline cellulose. Its association with 1% talc as lubricant led to a smaller variability in quality than with 1% magnesium stearate. However, no significant difference was identified between the results obtained with these flow agents. The experimental data showed that microcrystalline cellulose was better than lactose or starch as a diluent and that, together with 1% lubricant, it gave a GSE powder mixture ready for encapsulation in hard gelatin capsules, according to the parameters established for solid dosage forms (Brasil, 2008).

This study demonstrates the importance of preformulation studies in the development of powder mixtures for filling hard gelatin capsules with plant-based products in routine practice in a compounding pharmacy. A carefully conducted study can lead to an improved formulation and avoid quality problems. The excipients tested were capable of modifying the technological properties of GSE and, among the various diluents investigated, microcrystalline cellulose proved most suitable, especially when associated with a lubricant. Furthermore, according to the values of the investigated parameters, it was possible to establish a formulation for ready encapsulation of GSE in hard gelatin capsules prepared in compounding pharmacies.

## ACKNOWLEDGEMENTS

We are thankful to the Pharmaceutical Technology Laboratory at the School of Pharmaceutical Sciences of UNESP-Araraquara-SP for providing the equipment necessary for this study.

## RESUMO

*Estudos de pré-formulação de cápsulas gelatinosas duras contendo formulação de extrato de Gymnema sylvestre em pó*

**O extrato seco de *Gymnema sylvestre* (EGS) é um produto fitoterápico amplamente utilizado como adjuvante no tratamento da diabetes, sendo comercializado na forma de pó. O objetivo do presente estudo foi investigar a influência da adição de adjuvantes (amido, lactose ou celulose microcristalina) à formulação para a preparação de cápsulas gelatinosas duras contendo o EGS. As propriedades tecnológicas como densidade aparente (Da), densidade aparente de compactação (Dc), porosidade interparticulas (Ie), Índice de Carr (IC), Fator de Hausner (FH), perda por dessecação (PD%) e análise do tamanho de partículas (%Pf) das diferentes misturas preparadas foram investigadas com o objetivo de escolher o melhor excipiente. A necessidade de agentes lubrificantes (talco/estearato de magnésio) também foi avaliada. O EGS foi caracterizado como um pó fino, com mais de 50% do material particulado compreendido entre 0,149-0,250mm; IC=25,6%; FH=1,3 and Ie = 25,6%, o que justifica seu fluxo pobre. Todos os excipientes testados foram capazes de modificar as propriedades tecnológicas do EGS, sendo a mistura de pós que apresentou melhores resultados aquela obtida com a adição de celulose microcristalina.**
*Palavras-chave: Gymnema sylvestre*, estudos de pré-formulação, caracterização de sistemas particulados, formas farmacêuticas sólidas.

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