Evaluation of Physicochemical Characteristics of Suspensions Containing Hydrochlorothiazide Developed for Pediatric Use

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SUMMARY. Hydrochlorothiazide (HCTZ) is a thiazide diuretic used in pediatric patients despite the lack of a liquid form commercially available. Pediatric suspensions containing 0.6% of CMC-Na (F1) or 0.6% of HPMC (F2) were developed and their physicochemical characteristics were analyzed. The in vivo activity of the F1 and F2 was carried out in rats. The formulation F1 showed zeta potential value of -22.6 mV ± 1.6, while for F2 the found value was -2.01 mV ± 2.3. The mean particle size found for F1 and F2 were 44.1 μm ± 2.3 and 16.3 μm ± 1.9, respectively. The F1 sediment was easily redispersed with soft agitation of 13.3 s. On the other hand, F2 with non-charged HPMC, was denser and more difficult to redisperse. Both formulations showed an increase in urinary volume and electrolytes excretion (Na+, K+, Cl–) in rats.

INTRODUCTION

Primary hypertension in children is related to several metabolic abnormalities that are associated to overweight, metabolic syndrome, and type 2 diabetes 1. Hydrochlorothiazide (HCTZ), chemically known as 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide, is a thiazide diuretic and antihypertensive drug, largely used in pediatric patients although there is not a liquid formulation commercially available 2.

Pharmaceutical suspensions are commonly used as a dosage form for drug administration, particularly to children who cannot ingest solid oral dosage forms such as tablets and capsules 3. When solid forms are prescribed to children, usually the tablet is crushed, and its content is administered with a palatable drink or mixed with solid food 4. Besides the possibility of interaction of the active pharmaceutical ingredient with the drink or food, the poor reproducibility of the tablet breaking could compromise the correct dose administration and the efficiency of the treatment 5,6.

Physicochemical stability is an important requirement for pharmaceutical suspensions. The irreversible aggregation of the particles must be avoided and, at most, the suspension should stay in a flocculated state thus making it possible to reversibly change it to a homogeneous dispersion before dispensation 7.

The development of an acceptable formulation requires the analysis of different suspending agents, like polymers, which play an important role in the mechanisms of flocculation of particles. As HCTZ is a hydrophobic drug, water soluble polymers can be used to expect that they adsorb on the particle surface. These polymers can be ionized to some extent or nonionized. The degree of protection provided is related to the extent of adsorption and the chain length. They have many functional groups and, because of the flexibility of the macromolecule in solution, some of these functional groups
could be adsorbed at a solid surface, arouse wide spectrum of actions - from flocculation of dispersions to their stabilization. Of these polymers, hydroxypropylmethylcellulose (HPMC) and carboxymethylcellulose sodium (CMC-Na) are often used for pharmaceutical suspensions to get uniform dispersion of the water-insoluble particles in water. Selecting appropriate water-soluble polymers and their concentrations is, however, difficult because the behavior of the suspensions changes with combination of the drug and polymer characteristics, polymer concentrations, coexisting electrolytes and other additives. In some cases, selection of inappropriate polymers and their concentrations may increase flocculation or caking of the suspension.

Therefore, measuring the zeta potential, sedimentation volume and redispersibility of suspensions are a good approach for predicting the flocculation of water-insoluble particles, and selection of appropriate water-soluble polymers. Furthermore, the rheological behavior of pharmaceutical suspensions is a key factor in assessing the overall performance characteristics of a product. Although the developmental effort to achieve acceptable flow properties can be substantial for a suspension, the consumer is quite aware of product efficacy and elegance. By this way, the product characteristics should be outlined and achieved early in the stage of development.

New formulations of HCTZ suspension have been developed using different polymers, and to assure their quality for further studies in vivo (protocol already approved CEPSH-UFSC: 259/06), a physicochemical characterization should be carried out to choose the best one. At the moment there is no published study for the analysis of HCTZ suspensions containing HPMC or CMC-Na as suspending agents. In this way, the purpose of this study was to evaluate the effects of polymers with different ionic characteristics on the physicochemical parameters of developed pharmaceutical suspensions containing HCTZ for pediatric use.

**MATERIALS AND METHODS**

**Reagents**

The HCTZ reference standard (99H1207) was obtained from Sigma-Aldrich (Steinheim, Germany). The HCTZ raw material was donated by LAFESC (Laboratório Industrial Farmacêutico de Santa Catarina, Florianópolis, Brazil). Hydroxypropylmethylcellulose (Methocel K4M® - HPMC) and Carboxymethylcellulose Sodium (Denvercel PH-2008A® - CMC-Na) were donated by Colorcon (São Paulo, Brazil) and Denver (São Paulo, Brazil), respectively. Ultra-pure water was provided by a Milli-Q® purification system (Millipore, Bedford, USA). All chemicals used were of pharmaceutical or special analytical grade.

**Preparation of HCTZ suspensions**

In order to prepare a HCTZ suspension (2.50 mg mL⁻¹), the raw material was crushed in a mortar and pestle to get smaller particle sizes, and glycerin was added on the particles as a wetting agent. It was used polymeric solutions (CMC-Na - F1 and HPMC - F2), with sodium benzoate as a preservative, to prepare the suspensions. The citric acid was incorporated to the final formulation to correct the suspensions’ pH. The content of each formulation is described at Table 1.

<table>
<thead>
<tr>
<th></th>
<th>F1</th>
<th>F2</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCTZ</td>
<td>250 mg</td>
<td>250 mg</td>
</tr>
<tr>
<td>HPMC</td>
<td>–</td>
<td>0.6%</td>
</tr>
<tr>
<td>CMC-Na</td>
<td>0.6%</td>
<td>–</td>
</tr>
<tr>
<td>Glycerin</td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td>Sodium benzoate</td>
<td>0.1 %</td>
<td>0.1%</td>
</tr>
<tr>
<td>Citric acid</td>
<td>q.s. pH 3.3</td>
<td>q.s. pH 3.3</td>
</tr>
<tr>
<td>Water</td>
<td>q.s.p 100 mL</td>
<td>q.s.p 100 mL</td>
</tr>
</tbody>
</table>

Table 1. Content of each developed formulation (F1 and F2).

**Physicochemical Characterization of Suspensions**

**Determination of pH**

pH measurements were carried out using a WTW pH 3300i pH-meter with glass electrode.

**Zeta potential**

The zeta potential values were obtained from electrophoretic mobility measurements in equipment Zetasizer ZS (Malvern instruments, UK). The rate of particles movement under the influence of external electrical field with voltage of 150 V was measured with the help of Doppler’s laser anemometer.

**Particle size distribution in suspensions**

In order to obtain particles images, photomicrographs were carried out using a camera coupled to a biological microscope containing achromatic objectives of 4X, 10X, 40X and 100X.
Then, the determinations of diameter of 156 particles were done in software called “Size Meter”, and the particles size distribution was constructed.

**Sedimentation volume**

Each sample was shaken to ensure uniformity prior to the study, and then transferred to the measuring cylinder (100 mL capacity), where it was allowed to stand undisturbed for 24 h. Sedimentation height was measured after 2, 4, 6, 8 and 24 h. The sedimentation volume (SV) was calculated from the ratio of the ultimate height (Hu) of the sediment to the initial height (Ho) of the total suspension.

**Redispersibility**

The ease of redispersion was deduced from the time (s) required to resuspend the sediment through vertical inversion of each test tube containing the studied suspension. The vertical inversion was conducted by clamping the test tube vertically on a stand. The tube was then manually rotated, at a constant rate, up 180° and back down.

**Rheological behavior**

The rheological profile of each formulation was characterized using rotational viscometer (Haake VT 550) with sensor MV1 and cup MV. In order to provide a common and consistent shear history for samples, the suspensions were pre-sheared at a constant rate of 100 s⁻¹ for 2 min, and then followed by a 3 min rest period. Shear-dependent rheological characterization of the examined systems was evaluated through ascending and descending shear rate ramps from 0 to 300 s⁻¹ in 200 s, and from 300 to 0 s⁻¹ in 200 s, respectively. The apparent viscosity was measured at a shear rate of 300 s⁻¹, and was expressed in mPa.s. The flow curves were plotted between viscosity (η) and shear rate (γ).

The power law was used to describe the flow behavior of suspensions, according to equation [1]:

\[
\sigma = k (\gamma)^n
\]

where \(\sigma\) is the shear stress (Pa), \(\gamma\) is the shear rate (s⁻¹), \(k\) is a characteristic constant of the fluid, and \(n\) is the flow index (dimensionless). If \(n = 1\), the fluid is Newtonian, if \(n < 1\), the fluid is pseudoplastic, and, if \(n > 1\), the fluid is dilatant.

**Analysis of HCTZ in pharmaceutical suspension**

A stability-indicating HPLC method was used to quantify the HCTZ in pediatric suspensions. The method was previously validated according to ICH and was successfully applied for the quality control analysis of new pharmaceutical formulations of HCTZ for pediatric use.

**Instruments and analytical conditions**

The liquid chromatography system consisted of a Shimadzu LC-10A system (Kyoto, Japan). The injection volume was 20 µL and the detector was set at 254 nm. The experiments were carried out on a reversed-phase Phenomenex (Torrance, USA) Luna C18 column (250 mm x 4.6 mm I.D., with a particle size of 5 µm and pore size of 100 Å), maintained at 40 ± 1 °C. A security guard holder (4.0 mm x 3.0 mm I.D.) was used to protect the analytical column. The mobile phase consisted of 0.1 M sodium phosphate buffer pH 3.0 and acetonitrile (70:30 v/v), and was eluted isocratically at a flow rate of 1.3 mL min⁻¹.

**Preparation of Solutions**

Before the measurements, each suspension vial was stirred on a magnetic stir plate during 60 s to assure homogeneity at the time to take the aliquot. An aliquot of 0.3 mL was pipetted from the suspension and quantitatively transferred to a 25 mL volumetric flask containing 2 mL of methanol, and stirred in an ultrasonic bath for 10 min. The volume was completed with the mobile phase (30 µg mL⁻¹), and the resulted solution was filtered in a 0.45 µm nylon membrane. Besides, a work standard solution (30 µg mL⁻¹) was prepared with the HCTZ reference standard.

**Drug Analysis**

For quantification of HCTZ in the dosage form, six batches of each formulation containing 2.50 mg mL⁻¹ of HCTZ were analyzed as described previously. An aliquot of the sample solution was injected in the HPLC for the analysis and the amount of drug in the suspension calculated against the reference standard.

**Evaluation of diuretic action in rat Animals**

Adult female Wistar rats weighing 200-220 g obtained from our local animal facility were housed under standard environmental conditions (22 ± 1 °C, 55 ± 5% humidity and 12 h/12 h light/dark cycle). The animals were allowed free access to tap water and standard laboratory rat food. The care and handling of rats were in accordance with the internationally accepted
standard guidelines for use of animals, and the protocol was approved by our institutional committee on animal care (P00053/CEUA and 23080.015492/2006-94/CEUA/UFSC).

**Diuretic action**

The animals were given an oral loading of normal saline (5% bw) before the experiment. Subsequently, they were randomized in six groups with four animals per group. Group 1 (negative control) was given distilled water. Group 2 (positive control) was given HCTZ dissolved in distilled water. Groups 4 and 6 (test groups) were given formulations F1 and F2, respectively. Groups 3 and 5 (negative controls) were given CMC-Na 0.6 % and HPMC 0.6 % solutions. The formulations containing HCTZ and the negative control solutions were administered orally at doses of 10 mg kg⁻¹ bw and 10 ml kg⁻¹ bw, respectively. Each animal was then placed in an individual metabolic cage, without water access. The excreted urine was collected after 8 h, and the volume measured in a graduated cylinder. The ratio of urinary excretion in the treated group to urinary excretion in the control group was used as a measure of the diuretic action for the given dose of the drug ¹⁷,¹⁸ [Eq. 2].

Diuretic action = UEₜₐₑₜ / UEₜₑₙₑᵣ [Eq. 2]

where UEₜₐₑₜ = urinary excretion of treated group and UEₜₑₙₑᵣ = urinary excretion of control group.

The content of urinary electrolytes, which included sodium (Na⁺), potassium (K⁺) and chloride (Cl⁻), was determined by using ion selective electrodes (Electrolyte Analyzer system E4A, Beckman).

Variance analysis was used to compare the results obtained in the different groups. A difference was considered significant when \( P \leq 0.05 \).

**RESULTS AND DISCUSSION**

**Determination of pH**

HCTZ undergoes hydrolysis to yield formaldehyde and 4-aminoo-6-chloro-m-benzene disulfonamide in an equilibrium process. The reaction is reversible, and the equilibrium constant is independent of pH from 1.5 to 8.2. In this pH range the equilibrium favors the HCTZ. In very alkaline solutions complete hydrolysis can occur ¹⁹. In this way, the suspensions’ pH was adjusted to 3.3, where HCTZ exhibits acceptable stability.

**Zeta potential**

The formulation F1 showed value of zeta potential of -22.6 mV ± 1.6, while for F2 the found value was -2.01 mV ± 2.3. The different zeta potential values found for F1 and F2 are related to the fact that HCTZ is an acid with pKₐ of 8.75 and 9.88. Therefore, at pH < 6 it is an uncharged molecule ²⁰,²¹. So, the high negative zeta potential in F1 resulted from the surface charge of the anionic polymer (CMC-Na) adsorbed on the drug particle, producing bridging flocculation. This sort of flocculation occurs when polymer chains simultaneously adsorb on the surfaces of different particles. Polymer bridging represents long range forces and occurs in systems with a low surface coverage by the adsorbed polymer ²². CMC-Na can flocculate a suspension by a combination of steric effects and electrostatic repulsion, which depend on the equilibrium between attraction and repulsion forces ²³.

On the other hand, F2 showed zeta potential almost nonexistent, because of HPMC is a non-ionic polymer, without electric charges. The particle surface is covered with polymer so densely that there is no place for the bridging between the particles. The steric repulsion takes place, where a mechanical barrier keeps the solid particles at such distances where attractive forces become insignificant. For such a sterically system, interparticle repulsion can be considered to arise from a swelling pressure at the point of sufficiently close approach ²³,²⁴.

**Particle size distribution**

HCTZ raw material was crushed in a mortar and pestle to get smaller particle sizes, and the mean value obtained before its dispersion in polymeric solutions was 15.9 µm ± 3.1. On the other hand, when particles are dispersed in an aqueous medium they are bound to influence the state of dispersion or flocculation and therefore, the particle size. The mean particle sizes found for F1 and F2 were 44.1 µm ± 2.3 and 16.3 µm ± 1.9, respectively, and the graphs of distribution of particle sizes can be visualized in Figure 1.

The higher particle size found for F1 in comparison to F2 reflects the flocculation of particles in F1, with formation of aggregates, while in the F2 the measured size is from an individual particle (Figure 2).

**Sedimentation volume (SV) and redispersibility**

Since adequate and uniform dosage is a prerequisite for any pharmaceutical suspension, the necessity of controlling the particle sedimenta-
tion is obvious. According to Table 2, F1 showed higher SV ratio than F2, because of the negatively charged CMC-Na could flocculate the particles, forming a loosely linked polymer network system within the suspension. Therefore, the F1 sediment was easily redispersed with soft agitation of 13.3 s. On the other hand, F2 with non-charged HPMC, was denser and more difficult to redisperse than F1, requiring 61.9 s to re-

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Sedimentation Volume (SV) a</th>
<th>F1 b</th>
<th>F2 b</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.95 ± 0.2</td>
<td>1.0 ± 0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.85 ± 0.3</td>
<td>0.95 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.65 ± 0.8</td>
<td>0.80 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>0.25 ± 0.8</td>
<td>0.65 ± 1.0</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>0.11 ± 1.8</td>
<td>0.001 ± 0</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Results of sedimentation volume and redispersibility for F1 and F2. a SV = ratio of the ultimate height (Hu) of the sediment to the initial height (Ho) of the total suspension. b each value represents the mean ± Relative Standard Deviation (R.S.D.) of three replicates.

suspend the sediment with vigorous shaking. The supernatant liquid was cloudy, and the settled particles arranged themselves into a hard-packed cake, that results in a product lacking in dose consistency because of failure to obtain and maintain a good degree of dispersion.

Because settling velocity is proportional to the second power of the particle radius, it is apparent that flocculates (F1) will settle more rapidly than properly dispersed particles (F2). A deflocculated system (F2), that is, one in which the dispersed particles are discrete and in which there is little or no association, exhibits slow sedimentation of particles over time, as can be visualized in Table 2.

Rheological behavior

The typical flow curves obtained are shown in Figure 3. It can be seen that the apparent viscosity decrease with shear rate, and the variation is exponential, i.e., the suspensions exhibit shear-thinning or pseudoplastic behavior over entire range of shear rate studied. The apparent viscosity (300 s⁻¹ and 25 °C) found were 322 mPa.s ± 2.5 and 230.3 mPa.s ± 0.57 for F1 and F2, respectively.
The shear-thinning flow of suspensions is explained by the progressive breakdown of flocculated structures in shear fields 11,25,26. From this point, a narrow shear rate range with constant viscosity can be observed, followed by an increase in viscosity, which is due to particle orientation by the flow of the disperser phase: this orientation favors inter-particle interactions, resulting in new clustering, as already been characterized by others authors in some particular systems 27.

There are many different models to describe the rheology of non-Newtonian systems 21. The degree of pseudoplasticity can be measured by the flow behavior index \( n \), which decreases when pseudoplasticity increases. The power law model was fitted to the experimental flow curves and it was observed that the values of fluidity index \( n \) were < 1 for both formulations, indicating a pseudoplastic behavior. \( F_1 \) showed \( n = 0.4529 \) and \( F_2 \) \( n = 0.7443 \). Since that \( n \) value near to 1 indicates less pseudoplasticity, we can verify that \( F_1 \) is more pseudoplastic than \( F_2 \), which is in accordance with Figure 3, where can be visualized that the \( F_2 \) flow curve is almost linear.

**Drug analysis**

In the HPLC analysis the HCTZ retention time was about 3.4 min, and typical chromatograms of \( F_1 \) and \( F_2 \) were obtained by the previously validated method. The quantitative assay of six batches of HCTZ in suspensions for pediatric use was 100.1% ± 0.32 for \( F_1 \) and 100.7% ± 0.31 for \( F_2 \).

**Evaluation of diuretic action in rat**

HCTZ reaches its maximum effect at a dose of 10 mg kg\(^{-1}\), coinciding with scientific literature. It reveals a remarkable excretor effect so much in water as in electrolytes, typical in saluretic diuretics 28.

The urinary volume obtained from different groups after eight hours of experiment is showed in Table 3. It is observed an increase in urinary volume near to 50% in treated groups (2, 4 and 6) in comparison with respective control groups (1, 3 and 5), which confirms the diuretic action of the HCTZ formulations (P<0.05). The diuretic action calculated was of 1.87 to group 4 (treated with \( F_1 \)) and 1.70 to group 6 (treated with \( F_2 \)) that is in accordance with previous results which indicated that \( F_1 \) was easier to redisperse than \( F_2 \), and therefore, leads to a higher precision in dosage.

Concerning electrolyte excretion values (Na\(^{+}\), K\(^{+}\) and Cl\(^{-}\)) the treated groups (2, 4 and 6) showed a significant increase in excretion of all electrolytes compared to respective control groups (Table 3).

**CONCLUSION**

In the present study, the relationship between polymers with different ionic characteristics and the physicochemical stability of a pediatric suspension has been reported. \( F_1 \) and \( F_2 \) had pseudoplastic and non-Newtonian behavior, which is in accordance to literature for the water-soluble polymers employed. The quantitative assay of \( F_1 \) and \( F_2 \) was in an acceptable range. It was concluded from the results of zeta potential, particle size, sedimentation volume and redispersibility that an adequate HCTZ suspension could be obtained by adding the polymer CMC-Na (\( F_1 \)), which due to the negative charges, leads a flocculated suspension, forming a loosely linked polymer network system that is easy to redisperse with soft agitation. Moreover, \( F_1 \) showed the best diuretic effect in rats, with increase of urine volume and of electrolytes excretion. The \( F_2 \), because of uncharged HPMC, showed caking formation, that result in a product lacking in dose consistency because of failure to obtain and maintain a good degree of dispersion.

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<table>
<thead>
<tr>
<th>Groups</th>
<th>Urinary Volume (ml/8h) a</th>
<th>Diuretic action</th>
<th>Na(^{+}) (mEq total) a</th>
<th>K(^{+}) (mEq total) a</th>
<th>Cl(^{-}) (mEq total) a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.63 ± 0.75</td>
<td>–</td>
<td>0.99 ± 0.12</td>
<td>0.68 ± 0.05</td>
<td>4.70 ± 0.54</td>
</tr>
<tr>
<td>2</td>
<td>11.75 ± 0.64*</td>
<td>1.77</td>
<td>2.25 ± 0.27*</td>
<td>0.94 ± 0.13*</td>
<td>8.38 ± 0.62*</td>
</tr>
<tr>
<td>3</td>
<td>5.88 ± 0.75</td>
<td>–</td>
<td>0.82 ± 0.13</td>
<td>0.44 ± 0.05</td>
<td>4.17 ± 0.56</td>
</tr>
<tr>
<td>4</td>
<td>11.0 ± 1.47*</td>
<td>1.87</td>
<td>2.18 ± 0.32*</td>
<td>0.90 ± 0.13*</td>
<td>8.40 ± 0.99*</td>
</tr>
<tr>
<td>5</td>
<td>6.88 ± 0.85</td>
<td>–</td>
<td>1.18 ± 0.18</td>
<td>0.56 ± 0.08</td>
<td>4.87 ± 0.59</td>
</tr>
<tr>
<td>6</td>
<td>11.75 ± 0.96*</td>
<td>1.70</td>
<td>2.07 ± 0.12*</td>
<td>0.87 ± 0.06*</td>
<td>8.08 ± 0.57*</td>
</tr>
</tbody>
</table>

Table 3. Results of urinary volume, diuretic action and electrolyte excretion for the different HCTZ preparations. a Each value represents the mean ± Relative Standard Deviation (R.S.D.) of four rats. * P < 0.05.
REFERENCES