

Full Length Research Paper

Efficacious formulation of anti-malarial dry suspension for pediatric use

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This study was designed for concealing the bitter taste of an anti-malarial drug, artemether, by using various coating substances. Four granular formulations (formulations A, B, C and D) of artemether were prepared using different excipients and then were coated using methocil™ E5 and opadry® enteric solutions in a simple coating pan. In the initial 2 min, the cumulative percentage amount of drug release in phosphate buffer at pH 6.8 from pure artemether and formulation A, B, C and D was 59.4 ± 4.2 , 55.7 ± 3.5 , 48.2 ± 3.8 , 43.4 ± 4.1 and 30.8 ± 3.7 , respectively. Formulation D exhibited minimum bitterness as its taste masking efficacy (44.73 ± 4.98) was significantly ($P < 0.05$) greater than other formulations. It can be concluded from results that granules of formulation D prepared by sodium metabisulphite and methyl paraben sodium and coated by opadry® enteric had maximally reduced the bitterness of artemether with suitable dissolution behavior.

Key words: Artemether, dry suspension, *in vitro* test, taste analysis.

INTRODUCTION

Artemether is a derivative of artemisinin, which has exhibited greater hydrophilicity compared to that of artemisinin and possesses an efficient activity as a first-line anti-malarial drug with little toxicity (Chen et al., 2004). The customary Chinese herb *Artemisia annua* is a source of this drug (Dhingra et al., 2000). Being a drug with very bitter taste, it is needed to prepare an oral dosage form which can mask the unpleasant taste of artemether. Its adult dose is 1.6 mg/kg of body weight per dose given every 8 hourly. Otherwise, the identical total

dose may be administered once daily for three days (Ashley et al., 2005). They are widely available in the different pharmaceutical dosage forms including tablets, injections, suppositories and dry powders (Van der Meersch, 2005).

The oral route of drug delivery is not suitable especially in children who are not likely to accept the bitter or unpleasant tastes of medicines (Roy, 1994; Szejtli and Szente, 2005). There is a long list of oral drugs like artemether and sparfloxacin that have bitter taste (Shirai et al., 1993, 1994; Katsuragi et al., 1995, 1997; Yajima et al., 1999), which is responsible for non-compliance of the patients. Therefore, many physical methodologies like encapsulation and techniques for chemical modification have been introduced to mask the unpleasant and bitter

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Table 1. Formulation and process variables used in the preparation of artemether granules.

| Parameter | Group 1 | | Group 2 | |
|---------------------------|---------------|-----------------|---------------|-----------------|
| | Formulation A | Formulation B | Formulation C | Formulation D |
| Artemether (mg) | 35 | 35 | 35 | 35 |
| Starch (g) | 3.5 | 3.5 | - | - |
| PVP-K30 (g) | 4.5 | 4.5 | - | - |
| IPA (ml) | 10 | 10 | - | - |
| Carbopol 934P (g) | - | - | 25 | 25 |
| Sodium metabisulphite (g) | - | - | 60 | 60 |
| Methyl paraben sodium (g) | - | - | 80 | 80 |
| Distilled water (ml) | Qs (~10) | Qs (~10) | Qs (~15) | Qs (~15) |
| Coating solution | Methocil™ E5 | Opadry® enteric | Methocil™ E5 | Opadry® enteric |
| Drying time (min) | 45 | 45 | 50 | 50 |

taste of drugs (Cuna et al., 1997; Barra et al., 1999). However in general, liquid preparations are recommended for pediatric patients. Meanwhile, it has been determined that only the dissolved materials reduce taste feeling (Wilson et al., 1994). It was also observed that water-insoluble materials are tasteless or are too bitter to tolerate (Wilson et al., 1994).

One of the methods employed to achieve maximum taste masking characteristic is to prepare the drug suspension which creates a physical fence around the drug molecules (Shimano et al., 1995). Presently, the available dosage forms of artemether are tablets and capsules (Hakim, 2011). Thus, the current study was focused on preparing dry suspension of artemether using different coating materials to mask its bitter taste, followed by the study of its dissolution behavior at pH 6.8 of the oral cavity and pleasant taste perception test.

MATERIALS AND METHODS

Artemether was obtained as a gift sample from AMSON Vaccines & Pharma (Pvt.) Ltd. Islamabad, Pakistan. Methocel® E5 was procured from Dow Chemical Co., Midland. Opadry® was bought from Colorcon, West Point, PA. Carbopol 934P was purchased from B.F Goodrich, Cleveland, OH, USA. Polyvinylpyrrolidone (PVP-K30) and isopropyl alcohol (IPA) were procured from Fluka (Buchs, Switzerland). All these chemicals and reagents were of analytical grade and were used without any alteration.

Preparation of artemether granules

For solubility study, a surplus quantity of pure artemether was added to water for the preparation of test samples, shaken at 100 rpm and 37°C for 120 h using an orbital shaker (Eras, Pakistan) and then centrifuged for 10 min. The drug samples were withdrawn using a syringe attached with a 0.40 µm syringe filter, diluted and instantly analyzed using validated high performance liquid chromatography (HPLC) method (Gradient SPD 10A, Shimadzu,

Japan; method validation results are not presented) at 209 nm for drug analysis (Sharma et al., 2012). This experiment was repeated in triplicate.

For granulation, low-shear mixer (laboratory-scale, Asraw, Pakistan) was employed to fabricate the wet granules of artemether (formulations A, B, C and D in Table 1). Each batch size consisted of 50 g. During the preparation, powders were mixed using impeller rotated at 100 rpm for 10 min. For wetting the whole mixture, granulation liquid was mixed using impeller rotated at 400 rpm for 10 min. The formulated granules were dried at 60°C (at reduced pressure) with periodic shaking using impeller at a speed of 100 rpm for 10 s after every 100 s and angling the bowl to enhance the exposed surface area of granules for the maximum evaporation of solvents (Sharma et al., 2012). After allowing the granules to cool down at room temperature as thin layers in a tray, sieving was done to fractionate on the basis of granule size. In order to coat the formulated granules (Table 1), two coating solutions namely Methocil™ E5 (prepared by thorough mixing the solution of methocil™ E5 in 250 g of IPA with the solution of titanium dioxide and talcum in 50 ml of methylene chloride) and opadry® enteric (prepared by mixing opadry® enteric in methylene chloride and IPA) (Sharma et al., 2012) were employed using conventional coating pans.

Characterization of granules

Size measurement

Sieve analysis technique using vibrating shaker (Octagon Digital, Endecotts, London, UK) was employed to determine the granule size distribution at medium vibration level for 15 min and three standard sieves (Scientific Instruments, Milan, Italy) in the range 250 - 2000 µm. The collected fractions of granules were stored in desiccators at 30 ± 2°C (Sharma et al., 2012). This experiment was repeated in triplicate.

Artemether content determination

Granules (50 mg) were dissolved in phosphate buffer pH 6.8 (100 ml) and the contents of artemether in each formulation were determined using HPLC (Sharma et al., 2012). This experiment was repeated in triplicate.

Table 2. Excipients used to formulate finished dry suspension.

| No. | Ingredients | Quantity (g) |
|--------------|----------------------------|---------------------------------|
| 1 | Artemether coated granules | 0.400 |
| 2 | Sugar fine | 23.445 |
| 3 | Aerosil | 0.090 |
| 4 | Xanthan gum | 0.180 |
| 5 | Tit. Dioxide | 0.050 |
| 6 | Orange flavor | 0.050 |
| 7 | NaCl | 0.225 |
| 8 | Citric acid | 0.56 (q.s. to adjust pH at 3-5) |
| Total weight | | 25 g / Pack |

Loss-on-drying (LOD)

The LOD Box was placed at 105°C in oven for 30 min, cooled in desiccator and then weighed the empty LOD Box (W1) using analytical balance. One gram of sample was taken in LOD Box and weighed (W2). The LOD Box then put in the oven at 60°C for 1 h, cooled in desiccators and weighed (W3). The percentage was found out according to following formula:

$$LOD (\%) = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

In vitro dissolution tests

In vitro dissolution tests for artemether release were carried out in the following conditions: USP 24 paddle method (Pharmatest, Steinhein, Germany), paddle speed 60 rpm, phosphate buffer pH 6.8 (900 ml), temperature of medium $37 \pm 1^\circ\text{C}$, 35 mg of artemether in each sample, samples collection at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 10 h, sample analysis via HPLC Gradient SPD 10A (Shmadzu, Japan) at 209 nm, and experimentation in triplicate.

Pleasant taste perception test

Rather than the solid samples, pleasant taste perception (PTP) test was carried on the solution of granules for the evaluation to minimize the extensive changeability of drug concentration in the mouth due to various salivation states among volunteers. At the Gustatory Evaluation Laboratory of the Institute of Biotechnology, Bahauddin Zakariya University, Multan-Pakistan, PTP test was carried out by six young human volunteers as presented previously (Robson et al., 1999). To determine the perception and bitterness recognition threshold, seven standard solutions of pure artemether in distilled water at different concentration levels (0.00, 0.01, 0.025, 0.05, 0.1, 0.2% and 0.4% (w/v) were prepared. The volunteers were then asked to taste 0.1% solution (5 ml) by retaining it in mouth for 5 seconds followed by requesting them to tick any of the following option: (i) "I feel a bitter taste", (ii) "I do not feel any difference between 0.00% and 0.05% solutions", and (iii) "I feel something but I cannot identify the taste". For the volunteers who replied "I do not feel any difference between 0.00% and 0.05% solutions", they were asked for tasting 0.1% solution, while the volunteers who replied "I feel a bitter taste", they were asked to taste 0.025% solution. As a

result, the perception threshold at a level of 0.05% (w/v) with a range of 0.025 - 0.08% (w/v), as well as bitterness recognition threshold at a level of 0.08% (w/v) with a range of 0.06 - 0.2% (w/v) was set. An expert team evaluated some solutions for taste using various concentration levels of artemether to achieve homogeneous sensation of bitterness intensity among the human volunteers. Then the volunteers were communicated the bitterness scores in arrange of 0-100 for these solutions.

To test the prepared granules for taste, 250 mg of each granule sample was dispersed in 100 ml of water resulting in a 0.21% (w/v) solution of artemether, which was more concentrated as compared to bitterness recognition threshold. For assigning bitterness score, volunteers were then asked for tasting each sample (5 ml) as formerly illustrated.

Formulation of dry suspension

Keeping in view the behavior of finished coated granules and the raw materials, the finished dry suspension (each pack of 25 g powder) was formulated as follows:

Strength of the Suspension: 160 mg artemether / 80 ml
 Targeted pH of Diluted Product: 3 - 5
 Assay on granules on anhydrous basis: 40%

All the ingredients (Table 2) were mixed well in a small mixing bag for 15 min. This powder was then carefully shifted to small graduated cylinder. Then deionized water was added till the final volume of the suspension becomes 80 ml. The volume of water used was noted. The suspension was then shifted to a polyvinyl chloride (PVC) bottle and capped, then shaken very well and the final volume of the suspension was noted in the bottle by putting a mark (same volume of the water was added to the granules to bring the suspension on the required concentration). Initially the taste of diluted suspension was checked and it was observed that the taste was very good, and the pH was 3.9. Subsequently, it was then stored in the refrigerator and checked after seven days. All the physical parameters were satisfactory.

Statistical analysis

The significance of difference among parameters of various granular formulations was analyzed using the unpaired student's t-test and a value of $P < 0.05$ was considered significantly different.

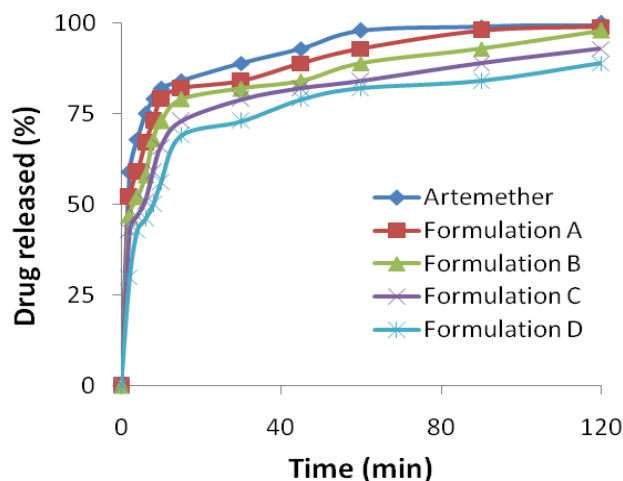


Figure 1. *In vitro* dissolution curves of artemether dry suspensions.

RESULTS AND DISCUSSION

The solubility of artemether in water was low (0.14 ± 0.02 mg/ml), which is in accordance with that of previous reports (Sharma et al., 2012; Chukwu et al., 1991; Al-Omran et al., 2002; Hashimoto et al., 2002). The contents of artemether in the groups 1 and 2 formulations were non-significantly ($p > 0.05$) different from each other (they were in the range of 89.2 ± 0.1 - $92.1 \pm 0.2\%$ (w/w). Moreover, all the formulations were developed for producing good quality and uniform shape granules. The granules of formulation A exhibited smaller size principally, which could be attributed to the incorporation of PVP (Al-Omran et al., 2002). However, the granules of formulation B were comparatively larger in size than those of formulation A. The cumulative frequency for group 1 formulations was > 37 , 30 and 18% for granules with a size range of < 250 , 250 - 500 and > 1500 μM , respectively. The cumulative frequency for group 2 formulations was 20 , 44 and 36% for granules with a size range of < 250 , 250 - 500 and > 1500 μM , respectively. On the whole, group 2 formulations produced significantly ($p < 0.05$) larger granules as compared to that of group 1 formulations. The percentage yield of granules from group 1 formulations ($96.6 \pm 0.2\%$, w/w) was non-significantly ($p > 0.05$) greater than that of group 2 formulations ($97.1 \pm 0.5\%$, w/w), indicating little loss of materials through the process of granulation.

Figure 1 depicts the *in vitro* dissolution behavior of the developed granules in comparison of pure artemether. In the initial 2 min, the cumulative percentage amount of drug released from pure artemether and formulation A, B, C and D was: 59.4 ± 4.2 , 55.7 ± 3.5 , 48.2 ± 3.8 , $43.4 \pm$

4.1 , and 30.8 ± 3.7 , respectively. These results were in accordance with the previous results (Sharma et al., 2012). Furthermore, the results observed from the PTP test represented that the taste-masking approach depends upon the technique of granulation as well as the selection of formulation and coating substances. The bitter taste intensity scores for formulation A, B, C, and D were 83.31 ± 3.26 ($p > 0.05$), 76.27 ± 5.21 ($p > 0.05$), 70.47 ± 4.45 ($p > 0.05$), and 44.73 ± 4.98 ($p > 0.05$), correspondingly as compared to that of pure drug. The PTP results verified the excellent taste masking efficiency of formulation D granules as compared to other formulations. These results are in accordance with the previous results (Sharma et al., 2012; Ameye et al., 2002; Ansari et al., 2009; Beatrice et al., 2004). LOD of granules were determined 3.76 ± 0.21 , 3.45 ± 0.11 , 3.25 ± 0.02 and 4.01 ± 0.01 , respectively and results were in the standard range (4.0%).

After getting reproducible results from the coated granules of DHA, formulation of finished dry suspension was carried out. Dry suspension is usually a stable dosage form but very technical, since any wrong choice of the excipients may lead to a number of problems in the product development when reconstituted with water for use. After seven days (the maximum time that the patient can store the suspension in refrigerator. The product was discarded after seven days), the assay of the dry suspension was done [107.63% (limit 90 - 110%)] and pH was 4.25 (limit 3 - 5) and all physical parameters like color (off white suspension), texture (thick suspension), taste (pleasant) and smell (fresh orange smell) were determined.

In an earlier work, a pediatric dry suspension had been developed with Artemether as active compound with concentration of 180 mg/60 ml after reconstitution in water (Gabriels, 2004), whereas in our formulated dry suspension (Table 2) artemether as active compound having 160 mg/80 ml is comparable with similar characteristics that offers several advantages: (i) maintenance of the chemical stability of the drug until (and after) reconstitution, (ii) reduction in transportation cost and possibility to adapt the dosage for different age groups. Once the particles of dry suspension have been wetted, they must be distributed uniformly throughout the liquid medium (Atemnkeng, 2007). The preparation contains macromolecules as suspending agents with thixotropic behavior. Other solid excipients include taste and coloring agents (sugar fine, orange flavor and titanium dioxide). In addition, a fair amount of aerosil was added to protect the active ingredient against moisture (LOD was 3 - 4%). Hence, this makes the preparation to contain a high amount of solid excipient powder making the analysis of the active ingredient and preservatives complex. The advantages of high-precision dosing,

manufacturing efficiency and patient compliance make solid dosage form like dry suspension, the most popular (Patel, 2006).

Conclusion

Thus far, the trials for the formulation of granules using different agents, as well as the trials for coating of these granules using different coating materials have been explored. The dry suspension was formulated and then evaluated by physical and chemical means. The concentration of the suspending agents, xanthan gum, was optimized to obtain a permanently physical stability of the suspension after reconstitution. This characteristic was required due to the low dosage of the drug in the suspension, namely 2 mg/ml. Viscosity was taken as response in the design. The most suitable amounts of the suspending agents, xanthan gum, in the suspension was 0.72% for artemether. An initial stability study showed sufficient physical and chemical stability of the active artemether, in the reconstituted suspension during the whole period of administration (seven days). The suspension as well as the taste and the coloration were optimized. Via taste tests with children and students, sugar fine and orange flavor were selected to mask the bitter taste of artemether.

It can be concluded from the results that granules of formulation D prepared by sodium metabisulphite and methyl paraben sodium and coated by opadry® enteric had maximally reduced the bitterness of artemether with suitable dissolution behavior. The formulation presented seemed to be a good base for preparing low-dose suspension of the active compounds in the range of 1 to 5 mg/ml, in which the aesthetic aspect of the suspension was optimized. This study has, therefore, opened a new approached for masking the taste of bitter drugs.

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