ORIGINAL PAPER

Nig. J. Pharm. Res. 2018, 14 (1) pp 53-59
ISSN 0189-8434 **Available online at http://www.nigjpharmres.com**

The Effects of Surfactant and Storage Conditions on the *In-Vitro* Release of Quinine Suppository Made from Witepsol Base

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: The need to develop quinine suppository formulation as an alternative to the intravenous parenteral dosage form in severe cases of malaria necessitates this study. The aim is to demonstrate the effect of surfactant, polysorbate (Tween® 20), and storage conditions on the *in-vitro* release rate of quinine suppositories made from Witepsol H15 base.

Methods: Different batches of quinine bisulphate suppositories were made from Witepsol HI5 base by fusion method incorporating Tween® 20, as a surfactant at 0, 0.5, 1.0, 2.0 and 4.0 % w/w concentrations. Some of these suppositories were stored at room temperature (30 ± 2 °C) while the rest were stored in the refrigerator (10 ± 2°C) for a period of 21 days and were then analyzed. The physicochemical properties of the suppositories were determined by several tests which include content uniformity, melting range test, hardness and in- vitro drug release rate.

Results: The melting ranged from 20 – 30 min for suppositories without surfactant while those with the surfactant were from 3 – 12 min. Suppositories with surfactant had the same trend in hardness. For preparation containing 4% w/w of surfactant, (3800 - 4000 g pressure at room temperature and 3800 – 2600 g pressure in a refrigerator), respectively for days 1 and 12. The active constituent for 0, 0.5, 1.0, 2.0 and 4.0% w/w concentrations of incorporated Tween 20® were 98.00, 82.30, 77.60, 81.90 and 76.73 % respectively while the in-vitro release on day 1 after 60 min were 78.32 ± 0.34, 75.69 ± 0.66, 84.34 ± 0.35, 90.50 ± 0.10 and 98.10 ± 0.30 %.

Conclusion: This study on day 1 reveals an enhanced effect of the surfactant on the *in-vitro* release of quinine bisulphate from the suppositories with the highest effect being at the concentration of 4.0 % w/w of Tween 20. Storage of the suppositories at room temperature and refrigeration had a surprising effect of increasing hardness and softening the suppositories, respectively with time.

Keywords: Antimalarial; Quinine; Suppositories; Surfactant; Witepsol

INTRODUCTION

Malaria is one of the most important diseases in Africa and it was estimated that about 214 million people were affected with the malaria parasite and an estimated 438,000 deaths mainly among children occurred worldwide in 2015 (WHO, 2015).

Over 3.2 billion people or almost half of the world's population are at risk from malaria. Over the last ten years the situation in most malaria endemic region has worsened because of increasing risk of transmission, and increasing international travels (CDC Malaria 2017). To further compound the problem, the anopheles vector has become increasingly resistant to insecticides and the parasite to current antimalarial drugs (Dai *et al.*, 2015).

Slow intravenous (IV) administration of quinine dihydrochloride is the most recommended treatment for severe malaria caused by chloroquine susceptible

or resistant *Plasmodium falciparum* (PrayGod, *et al.*, 2008). The parenteral route provides rapid onset of action but it is associated with risk of developing quinine-induced hyperinsulinaemia and hypoglycemia (Pasvol, 2005). Moreover, the parenteral route of administration required the availability of trained personnel, which is often lacking in some situations such as the rural areas. The use of oral route in treating the patients may not be feasible in some cases such as those who are unconscious, or unable to tolerate oral medication because of vomiting or pathological conditions of the alimentary tract. The practical solution to these problems is the use of the rectal route of administration. This route does not require any special technique. Consequently, studies have been carried out to investigate the possibility of formulating quinine into rectal dosage form using different bases (Barennes *et al*., 1995;Babalola, *et al.*, 2004; Soremekun, *et al.,* 2012). In a comparative bioavailability study by Babalola *et al.*, (2004) it was demonstrated that rectal and oral quinine formulations have a significant difference in their bio-availabilities with the implication that a given oral dose cannot be substituted by a similar rectal dose to provide the same anti- malarial effect. Thus, there is the need to further improve or modify quinine suppository formulations so as to enhance the rectal absorption of the drug.

The composition of suppository bases is an important factor in the absorption process by determining the pattern of drug release. Also, drug absorption from suppositories can be modulated by incorporation of absorption enhancers (surfactants, salicylates, enamines and fatty acid derivatives) (Onyeji, *et al*., 1999; Prasanna, *et al*., 2012). The main consideration for their selection in formulation is safety, efficacy and compatibility with other ingredients of the formulation (De Boer, *et al*., 1990). Previous studies on quinine suppository (Soremekun *et al*., 2012), used Cocoa butter and Fattibase™ as suppository base and varying concentrations of Polysorbate 80 as the surfactant. Cocoa butter, Fattibase™ and Witepsol are all classified as oleaginous or fatty bases but while cocoa butter has the disadvantage of polymorphism, rancidity on storage, stickiness to the molds and lowered melting point when soluble ingredients are incorporated thereby causing leakage from the body, Fattibase™ is a single entity synthetic base that has only one melting range. Witepsol has its solidifying point unaffected by overheating and they have different series with different melting range which could be suited for different climatic regions. The use of polysorbate 20 (Tween 20) as absorption enhancer was considered over Tween 80 and Brij 35 because of the superior performance of polysorbate 20 obtained in the release profile of chloroquine suppository as demonstrated by Onyeji and co-workers (Onyeji *et al.*, 1999).

This present work is a product of the continued effort to optimize and formulate a quinine suppository (Babalola *et al*., 2004) that is stable, cheap and an efficient alternative to both oral and parenteral route of its administration using Witepsol H15 (a synthetic fatty base) and Polysorbate 20 in varying concentrations as a base.

MATERIALS AND METHODS

Quinine bisulphate powder was obtained from Courting and Warmer, Sussex, U.K. Witepsol H15 was procured from Penn Pharmaceutical Ltd. (Tredegar, South Wales, U.K.) and Tween® 20 from British Drug House (U.K.). All other chemicals and reagents used were of analytical grade .

Preparation of quinine bisulphate suppositories and analysis of samples

The quinine bisulphate suppositories were prepared by fusion method. The appropriate amount of the quinine bisulphate, the surfactant (Tween® 20) and the base (Witepsol H15) were calculated, using the displacement values, and weighed on a metal analytical balance. The witepsol and surfactant were heated to melt in a stainless dish over a water bath. The drug (300 mg) was incorporated into the melted Witepsol by levigation with a spatula. After a thorough mixing, the drug witepsol – surfactant mass was poured to overfill five metal molds, each having 6 cavities. After setting by allowing to solidify at room temperature, the excess was trimmed off with a razor blade. Finally, they were all carefully removed from the moulds into paper cardboard boxes and labeled accordingly. Five batches of the formulations were prepared using metal molds with six cavities. Some of the suppositories were stored in the locker at room temperature (30 $\pm$ 2 $^{\circ}$C) while the rest were stored in the refrigerator (10 $\pm$ 2° C) until when needed. Displacement value was based on the following equation:

$$F = \frac{D}{(A - C)} \qquad \text{Eqn 1}$$

Where:

F is the displacement value, D is the weight of active ingredient in the suppositories, A is the weight of suppositories using the base alone and C is the

amount of base present in the medicated suppositories.

For the analysis of quinine bisulphate samples, a calibration curve data were generated using an aqueous buffer solution of pH 8 to give a concentration range of 1×10^{-4} to 1×10^{-3} % (w/v), and absorbance was measured at 206.5 nm using a Cecil model CE 2021 UV/Vis spectrophotometer (Cecil®, England)

Assay of the quinine suppositories

A modification of the British Pharmacopoiea, (2012) non-aqueous titration method for assay of quinine sulphate tablet was used. First the titrant which is 0.1M perchloric acid was prepared by diluting 2.05 mL of 70 %w/w of perchloric acid with little quantity glacial acetic acid and 8 mL of acetic anhydride and allowed to stand for 24 h. The acetous perchloric acid was standardized using a primary standard which is a 0.25 g of pure oven-dried potassium hydrogen phthalate which is dissolved in acetic acid and refluxed in a condenser till the salt dissolves. 2 drops of crystal violet solution was used as the indicator. The equation of reaction

$$C_6H_4(COOK)(COOH) + HClO_4 \longrightarrow C_6H_4(COOH)(COOH + KClO_4$$

$$0.02042g \text{ of } C_6H_4(COOK)(COOH) = 1ml\ 0.1M\ HClO_4$$

For the assay of quinine suppositories, one suppository per batch was dissolved in 30 mL glacial acetic acid then 2 drops of indicator was added. The mixture was titrated with standardized 0.1M perchloric acid. The assay was complete with blank determination to make for necessary corrections in volume. The amount of quinine in each suppository was calculated using the milliequivalent relationship and the equation of reaction between quinine bisulphate and perchloric acid:

$$C_{20}H_{24}N_2O_2\ H_2SO_4 + 2HClO_4 \longrightarrow$$
$$C_{20}H_{24}N_2O_2\ .H_2SO_4 + 2ClO_4$$

Determination of suppository hardness

The hardness of the suppositories was determined using a suppository hardness tester (Erweka®, Germany). A suppository was placed on the lower bar of the apparatus at the starting time, after ensuring that the suppository's tapered end fitted into the indentation on the upper surface. At intervals of one minute, additional weight of 200 g each was placed on the rod until the suppository was crushed.

Melting range test

This was done using a modified method in which a tablet disintegration apparatus (Copley Construction Ltd, Kent, and U.K.) was used. One suppository was introduced into each tube of the assembly and suspended in the beaker containing water maintained at a temperature of 37°C ± 1 °C. The apparatus was operated such that the wire mesh bearing the suppository was stationed at about 90 mm vertically below the water surface in the beaker. The time it took the entire suppository to completely dissolve in the water was taken as the melting range. Duplicate determinations were carried out and the average time determined.

Determination of pH of suppositories

1% w/v of quinine bisulphate suppository from each of the batches was made by dissolving 0.5 g of the suppository in 50 mL of the distilled water in a beaker. This was heated to complete the dispersion and cooled. The pH was measured with a digital pH meter (Delta 340, Germany).

Dissolution studies

The rotating basket dissolution apparatus recommended in B.P (2012) was used. For each determination, one suppository was placed into a stainless steel wire mesh basket. The basket was then attached to the clamp at the lower end of the rotating shaft. The shaft was lowered into the dissolution vessel containing 600 mL of the dissolution medium phosphate buffer (pH 8) maintained at a temperature of 37 ±0.1 ^{0}C. The basket was rotated at a speed of 100 ± 1 rpm for 1 h. At time intervals of 5, 10, 20, 30, 40, 50 and 60 min during the dissolution process; 5mls of sample was withdrawn from the dissolution medium, filtered through a 0.45µm Millipore filter, and assayed for its drug content after appropriate dilutions and following the procedure described above. Fresh phosphate buffer (5 mL) maintained at the same temperature was added to the medium to maintain a sink condition. A minimum of triplicate release determinations were made for each suppository preparation. The amount of drugs released at each time interval was determined by calculation using the calibration curve equation and dilution factor employed.

Analysis of data

The following plots were made: Higuchi's square root and Peppa's kinetic models to determine the mechanism of drug release from the suppositories. The model that produced the highest correlation and consistent result among the various suppository preparations was used for evaluation of the drug release profiles. The extent of drug release was assessed from the total amount of drug present in the dissolution medium at the expiration of 60 min experiment. Analysis of variance and Bonferroni post test was conducted using Graphpad prism 5.0 to

compare the formulations and the various treatment means done in the analysis.

RESULTS

Preparation of Quinine bisulphate

The displacement value of quinine bisulphate in the Witepsol H15 base was obtained to be 1.54. This value was used to calculate the amount of the base required for each formulation (batches A- E).

Assay of the Quinine suppositories

Table 1 shows the composition, percent drug content and pH of the formulations (batch A- E). The quinine content of formulations ranged from 76.73 - 82.30 % for batches B - E which contains from 0.5 to 4.0 % of surfactant (Tween 20). Batch A which contains 0.0 % of Tween had the drug content within the (B.P, 2012) acceptable limit (95.0 -105.0 %). The pH of the formulations (A –E) was shown to be within 3.37 and 3.60.

TABLE 1. Composition, quinine content and pH of the prepared suppositories

Batch	%w/w Tween 20	Quinine bisulphate (mg)	% Quinine Content	pH
A	0.0	200	98.00	3.37
B	0.5	200	82.30	3.38
C	1.0	200	77.60	3.40
D	2.0	200	81.90	3.45
E	4.0	200	76.73	3.60

Hardness test result

The effect of surfactant, storage condition such as room temperature (RT) and refrigerator (RG) and time on the hardness of suppositories are shown on Figures1 A and 1B. There was an increase in hardness (3300 - 3900) and (3300 – 3400) g pressure respectively after storage in RT and RG for batch A with increase in time. Batches B – E showed a close range in hardness (3800 – 3900) g pressure for suppositories stored in RT and (3850 – 2800) g pressure for that stored in a RG with time. There was a decrease in hardness with increase in content of Tween 20 and the range is almost constant with increasing storage time. This trend is observed in all the batches containing surfactant.

Melting range test

Figures1 C and 1D are the graphical representation of the effect of surfactant, storage condition and time on the melting range of the different batches of suppositories. Batch A (0.0% surfactant) showed an increase in melting range (19 - 30 min) for storage in RT
and (19- 35 min) in RG while batches B - E showed a reduction in melting range with increase in surfactant (6.5 - 3.0 min) for day 1 and an increase in melting range with storage time.

Dissolution studies

The in-vitro drug release profiles from different batches (A-E) were shown in figure1 E. The release profiles for batches B and C were lower than that for batch A (0 % Tween20). At 15 min their release profiles showed quinine content of 71.83, 55.83, and 65.47% for batch A, B, C respectively. The dissolution increased with increase in surfactant concentration and started from batch D (2.0 %w/w) up to batch E (4.0 %w/w). The quinine content of the formulations were 76.91 - 90.50 % and 86.04 – 98.19 % for batches D and E respectively for 15 – 60 min. The dissolution data for batches A – E were fitted to various drug release kinetic models. The model that gives maximum 'R' value is considered as the best fit model for the release data.

DISCUSSION

The effect of polysorbate 20, as a surfactant, on the *in-vitro* release rate of quinine bisulphate from suppositories made from Witepsol H15 has been demonstrated. Graded concentrations (0.0, 0.5. 1.0, 2.0 and 4.0 %) w/w of Polysorbate 20 were used to formulate the suppositories. The choice of this surfactant was guided by the positive effect it had on the *in-vitro* release rate of chloroquine from polyethylene glycol (PEG) –based suppository (Onyeji *et al*., 1999). Witepsol H15 was used as the suppository base in this study because of its compatibility with quinine and Polysorbate 20 (Pharmaceutical Codex, 1994)

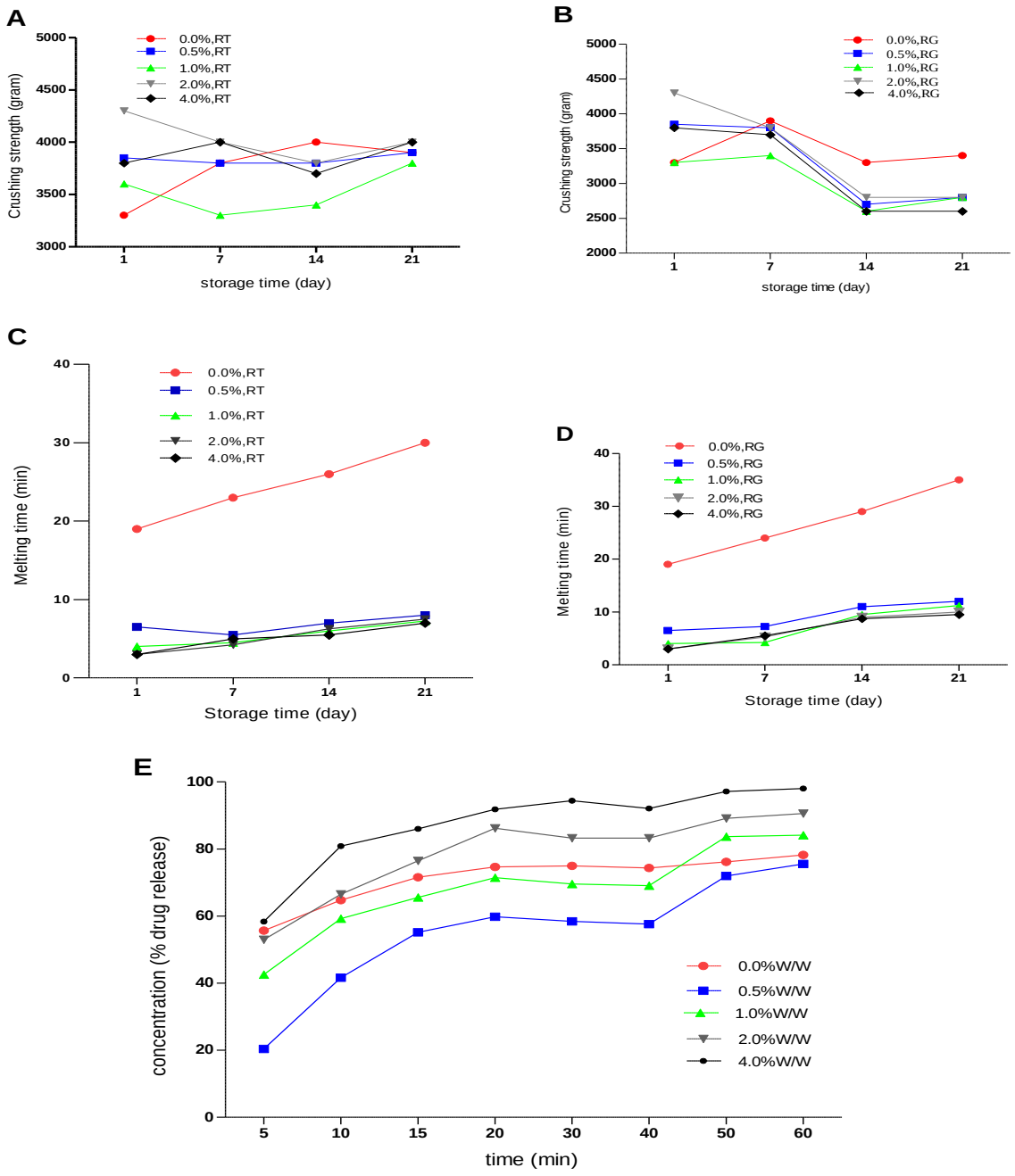

Figure 1. Effect of storage (temperature and time) on hardness and melting time (A) and (C) respectively for quinine suppositories stored at room temperature and for samples stored at refrigeration temperature (B) and (D) are the hardness and melting time respectively. In vitro dissolution profile (E) of quinine suppositories. All suppositories were made with Witepsol H15 base and varying %w/w of surfactant.

Only the batch without the surfactant falls within the official (B.P 2012) limit (95 – 105 %). The decrease and irregularity in the percentage yield of quinine bisulphate in the suppositories with increase in the surfactant concentration is attributed to the sedimentation problem observed in their preparations.

The effect of storage time (day 1 to 21) and conditions (room temperature and refrigerator) on the suppository hardness values is as shown in Fig.1A and 1B. The increase with time in hardness of the suppositories stored at room temperature and decrease for those kept in the refrigerator can be attributed to crystallization of the suppository base. The degree of crystallization was reduced by storage in the cold environment (Coben & Lordi, 1980).

The day 1 hardness (3300 g pressure) differs from the value (4500 g pressure) obtained in a previous similar work (Ermis & Tarlmci, 1995). The variation could be from the difference in the formulation process used. The observed increase in melting range with increase in storage time of all the suppositories (stored at room temperature and refrigerator) Fig. 1C and 1D is a consequence of their increasing hardness (Coben & Lordi, 1980). The reduction in the melting time with increase in the surfactants concentration is attributed to their increased melting power on the formulation. The suppositories stored in the refrigerator melted at a longer time than those kept at room temperature because the test was performed immediately after removal from the refrigerator. The disintegration time of all the suppositories from days 1 to 14 satisfied the B.P (2012) specification which gives allowance of not more than 30 min for fat - based suppositories. It can be observed that there was initial decrease in the day 1 release of quinine bisulphate from the suppositories with increase in concentration of Polysorbate 20 This could be due to the viscous barrier formed by the dispersion of the surfactant against the drug at concentrations less than the critical micelle concentration (CMC). At higher concentrations greater than the CMC (2.0 and 4.0 %), micelles rather than the dispersion of the surfactant are responsible for its solubilizing power. This accounts for the observed increase in the drug release rate at the higher concentration (Urban *et al*., 1991). The decrease in the release rate of quinine bisulphate from the suppositories with time is consequent to the increasing disintegration time due to prolonged storage.

The result of the Higuchi's and Peppa's plots of the drug release from the suppositories implies that the release of quinine bisulphate is anomalous; it is not diffusion – controlled (Table 2).

Table 2. Higuchi's and Peppa's Values obtained from plots of Quinine bisulphate release from the suppositories with Polysorbate 20[Tween® 20] (Day 1)

Batch/Concentration	0.0% w/w (N/S)	0.5%w/w Tween 20	1.0%w/w [Tween 20]	2.0% w/w [Tween 20]	4.0% w/w [Tween 20]
(a) Higuchi's					
Slope	12.30	26.11	15.64	18.77	16.48
Intercept	148.42	9.42	80.23	98.86	117.85
Correlation coefficient	0.9701	0.9891	0.8711	0.9811	0.9412
(b)Peppa's					
Slope(n)	0.12	0.40	0.24	0.20	0.16
Intercept	2.15	1.53	1.88	2.01	2.67
Correlation coefficient	0.9213	0.8898	0.9362	10.9255	0.8009

This implies that, the rate at which the drug molecule diffuses through the suppository base does not control the rate of its release. This is because quinine bisulphate is very soluble in water and therefore does not form a homogenous dispersion in the fatty suppository base matrix. Consequently the amount of drug per unit particle of the suppository dispersion into the dissolution medium is not expected to vary directly with time (Onyeji *et al.*, 1999)

CONCLUSION

The inclusion of an enhancer (Polysorbate 20) improved the release of quinine from the suppositories formulated using Witepsol H15 base, the 4.0 % Polysorbate 20 concentrations gave the highest release of 98.19% (294.57mg) of quinine drug. The drug release from the suppositories which decreased with storage time may have arisen as a result of an increased hardness of the suppositories on storage. The *in vitro* release of quinine (in bisulphate form) from the suppository formulation

was expectedly high due to the high solubility of the quinine salts in aqueous medium. The anomalous drug release observed in these formulations when considered *in vivo* may lead to unpredictable systemic drug release patterns which would be undesirable. Similarly sedimentation of the bisulphate salt is amongst the challenges which must be overcome in the study.

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Conflict of Interest: None declared

Received: 19 November, 2017

Accepted: 30 June, 2018