Acta Pharmaceutica Sciencia
51: 281- 288 (2009)

Effect of interacting variables on the mechanical and release properties of chloroquine phosphate suppositories

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Abstract

The individual and interaction effects of type or nature of suppository base (N), concentration of surfactant (C), and storage (S) on the mechanical and release properties of chloroquine phosphate suppositories have been studied using a 2^3 factorial experimental design. Suppositories (1g) containing 100mg chloroquine phosphate each with or without 2%w/v Tween 20 as adjuvant, were prepared in Suppocire® AS2 and Witepsol® H15 bases. The mechanical properties of the suppositories were determined using crushing strength and dissolution properties were assessed using dissolution times (t_{50} and t_{80} - time for 50% and 80% drug release), and rates. The concentration of surfactant had the highest individual effect on the release properties of the suppository formulations while storage had the lowest effects. Thus, the type of suppository base and concentration of surfactant used in suppository formulations need to be carefully chosen in order to obtain suppositories of desired mechanical and drug release properties.

Key words: Chloroquine, suppository base, surfactant, variables, release properties.

Introduction

Malaria remains the most common and most fatal infection in Africa (Sowunmi et al. 1997). Chloroquine phosphate is one of the first line drugs used in the treatment of malaria. The need to formulate chloroquine phosphate as suppositories has been emphasised because of the side effects associated with the other routes of administration (WHO 1990). The oral formulations have been associated with nausea and vomiting due to the bitter taste of chloroquine which has reduced their acceptance especially by children (Tjoeng et al. 1991). Furthermore, in the treatment of severe cerebral malaria, oral administration of the drug is ineffective while the parenteral forms have been associated with several reports of cardiotoxicity (Looareesuwan et al. 1986). Thus, efforts have been geared towards developing chloroquine as suppository formulations with the aim of providing a more practical alternative for the administration of the drug (Abdul–Gawad and El-Din 1989). Although some work have been done on the formulation of chloroquine suppositories (Anita-Obong et al. 1995, Onyeji et al. 1999), the individual and interacting effects of various formulation variables that could contribute to the optimum release profile for chloroquine suppositories have remained largely uninvestigated.

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Studies have shown that the physical and release properties of many suppositories depend considerably on the physicochemical properties of the drug, suppository base and formulation adjuvants (Zuber et al. 1998, Onyeji et al. 1999) and a lot of formulation work is normally required to optimise the properties of suppository preparations. Suppository bases are vehicles in which medicaments intended for rectal administration are incorporated and the types of bases employed in the formulation of suppository are important factor in the absorption process as they determine the pattern of drug release (Muller 1986). Witepsol® H15 and Suppocire® AS2 are synthetic hard fats prepared by hydrolysing and hydrogenating vegetable oil. The resulting fatty acids are then re-esterified by heating with glycerol. Controlled modification of the process yields a wide range of materials containing mixtures of mono-, di- and tri-glycerides of saturated fatty acids with chain lengths C_9 to C_{17}. Suppocire® AS2 has a melting range of 35.0-36.5°C and hydroxyl value of 15-25 while Witepsol® H15 has a melting range of 33.5-35.5 °C and hydroxyl value of <15. Both bases are versatile and suitable for all types of production equipment (Berko et al. 2002). Moreover, synthetic hard fats have good resistance to oxidation and different grades are available which can be chosen to suit particular climatic conditions. They are therefore likely to be suitable for the production of suppositories to be used and stored especially in the hot and humid topical climate.

Excipients such as diluents, adsorbents, surfactants, lubricants, antimicrobials, preservatives and colorants may also be added to suppository formulations when necessary to improve the properties of formulated suppositories (Adegboye and Itiola 2003). Surfactants such as Polyoxyethylenesorbitan fatty esters (Tween 20 or 80) and sodium salicylate are included in suppository formulations to enhance release and subsequent absorption of drug from the suppository formulations. Surfactants act by lowering the surface tension of the suppository base and thus increasing particle contact between drug and base (Herman 1995). The hydrophilic nature of the non ionic surfactant (Tween 20) has also been reported to aid this kind of particle contact thus further promoting the absorption enhancing capability of this group of chemical agents (Akala et al. 1991).

Thus in the present work, the relative individual and interaction effects of type or nature of base (N), concentration of surfactant (C), and storage on the mechanical and release properties of chloroquine phosphate suppositories have been studied using a 2^3 factorial experimental design (Woolfall 1964, Odeku and Itiola 2003a), which has already proved useful in the analysis of the quantitative individual and interaction effects of various formulation factors on metronidazole suppositories (Adegboye and Itiola 2003). The mechanical properties of the suppository which are indicator of the ability of the suppositories to withstand the rigours of handling involved in manufacture, transportation, dispensing and usage, were assess using the crushing strength (CS), and the release properties of the suppositories were assessed using the dissolution times (t_{50} and t_{80} - the time required for 50% and 80% of drug to be released) and dissolution rates, k_1 and k_2.

Materials and Methods

The materials used were: Suppocire® AS2, (USP/NF) (GATTEFOSSE, Cedex, France), Witepsol® H15 (Brome and Schimmer Ltd., Hampshire, England), Tween® 20 (polyoxyethylene (20) sorbitan) (BDH Chemical Ltd Poole, England), Chloroquine phosphate powder (Bayer, Leverkusen, Germany),

Anhydrous disodium hydrogen orthophosphate (BDH Chemical Ltd., Poole, England), Potassium dihydrogen orthophosphate (BDH chemical Ltd., Poole, England.)

Preparation of chloroquine suppositories

Chloroquine suppositories (1g) were prepared by the fusion method (BP, 1994) using Suppocire® AS2 and Witepsol® H15 as suppository base. The chloroquine phosphate powder was passed through a 100μm mesh sieve before incorporation into the base. The suppositories were prepared in metal moulds with six cavities. The displacement values of the bases were determined and suppositories containing 100mg chloroquine phosphate with or without surfactant Tween 20, were prepared in batches. The suppositories were kept at room temperature for 24 h after removal from the mould to allow for uniform solidification and crystal transformation. They were then stored in the refrigerator at 4°C and some were stored at normal laboratory conditions (Average temperature of 25±2°C) to assess the effect of storage.

Evaluation of the mechanical and release properties of the suppository

The crushing strength of the suppositories was determined using a Monsanto hardness tester (Monsanto, Cambridge, UK). The force required to deform or break the suppository in Newton (N) was measured.

Content uniformity was determined using spectrophotometric method (USP, 1980). Chloroquine suppositories selected from each of the batches of the samples were melted in 100 ml with the dissolution medium (0.2M phosphate buffer pH 7.0). After dilution, the solution was assayed using a UV/Visible Spectrophotometer (Jenway 6305, RealLabware Unit 33, Watford Herts., UK) at a wavelength of 342nm. The drug content in each batch (20) of suppositories was found to be ≥ 95%.

Dissolution test for the chloroquine suppositories was carried out using the Hanson's easy lift dissolution test apparatus (Hanson Research, Northridge CA, USA). Each suppository was placed in a basket which was fitted to a spindle rotated at 100rpm. The basket was lowered into a flask containing 500 ml of 0.2 M phosphate buffer, pH 7.0 (USP 1980) maintained at constant temperature of 37±1°C. 5 ml samples were taken at different time intervals and replaced with 5 ml of fresh dissolution medium maintained at the same temperature. The amount of chloroquine released was determined spectrophotometrically at 342nm.

The integrated form of the equation of Noyes and Whitney (1897), which has found wide application in describing the release kinetics of drugs from tablet dosage forms (Kitazawa et al. 1975, Odeku and Itiola 2003b) used for the analysis of the suppository formulation, is given below:

$$\ln[C_s/(C_s-C)] = kt \qquad (1)$$

Where C_s is the concentration of the solute at saturation, C is its concentration at time t, and k is a dissolution rate constant. Adegboye and Itiola (2003) have found this equation applicable to the release kinetics of suppository formulations. Kitazawa plots of ln [Cs/(Cs-C)] versus t were constructed for all formulations (Kitazawa et al. 1975).

Data analysis

The factorial experimental design which involved the application of simple statistics was used to study the effect of the nature of suppository base (N), concentration of surfactant (C), and storage (S) on the mechanical and release properties of chloroquine suppository (Woolfall 1964, Odeku and Itiola 2003a). The basis of the experimental design was that each of the three variables was utilized at a "high" level (denoted by the subscript, H) and a "low" level (denoted by the subscript, L). The number of experiments in the design was $2^3 = 8$. Using the above nomenclature the various combinations between the variables used in the design were:

$N_LS_LC_L$, $N_LS_LC_H$, $N_LS_HC_L$, $N_LS_HC_H$

$N_HS_LC_L$, $N_HS_LC_H$, $N_HS_HC_L$, $N_HS_HC_H$

N_L = Nature of base (Suppocire® AS20)

N_H = Nature of base (Witepsol® H150)

C_L = concentration of the surfactant, (0%w/v)

C_H = Concentration of the surfactant (Tween 20- 2%w/v)

S_L = Storage time (0 months)

S_H = Storage time (six months)

By grouping the results into a number of sets, it was possible to assess the effects that each of the three variables had separately on the mechanical and release properties of the tablets and also to determine whether the variables were interacting or acting independently of each other.

The effects of increasing N, from its "low" level to its "high" level on the various parameters were found by summing all the results (CS or t_{50} or t_{80} or t_1 or k_1 or k_2) of samples containing "high" level of N and subtracting the sum of the results of samples containing "low" levels of N. That is:

$$¼[(N_HS_LC_L+N_HS_HC_H+N_HS_HC_L+N_HS_LC_H) - (N_LS_HC_H+N_LS_LC_L+N_LS_HC_L+N_LS_LC_H)$$

The amount by which the result departs from zero (irrespective of whether positive or negative) was a quantitative measure of the effect of nature of base on crushing strength (CS) or release properties (t_{50} or t_{80} or t_1 or k_1 or k_2). Similar expressions were used to evaluate values for C and S.

To determine whether there was any interaction between two variables, the (CS) or release properties (t_{50} or t_{80} or t_1 or k_1 or k_2) results of the combinations in which they appear together at either "high" or "low" levels were summed and the sum of other combinations subtracted from this to obtain the interaction coefficient. For example, for N and C:

$$¼[(N_HS_HC_H+N_HS_LC_H+N_LS_LC_L+N_LS_HC_L)–(N_HS_LC_L+N_HS_HC_L+N_LS_HC_H+N_LS_LC_H)]$$

A zero result indicates no interaction. A significant departure from zero indicates that the two variables interact with each other. The magnitude of the extent to which the values departs from zero is an indication of the level of interaction. Similar expressions were used to determine the interaction if any between N and S and C and S.

Statistical Analysis

Statistical analysis to compare the individual and interaction effects of the formulation variables on the crushing strength and drug release properties of chloroquine suppository was done with the Kruskal-Wallis test, a non-parametric multiple comparison test, using the computer software Graphpad Prism® 4 (GraphPad Software Inc., San Diego, USA). Individual differences between the formulations were performed using the Dunn's multiple comparison tests. At 95 % confidence interval, *p* values less than or equal to 0.05 were considered significant.

Results and Discussion

The amount of chloroquine phosphate released from the suppositories was plotted against time and representative plots for suppositories with and without the surfactant is presented in Figure1.

The values of t_{50} and t_{80} (i.e. time required for 50% and 80 % of chloroquine phosphate to be released respectively) were calculated. Typical plots of Ln [Cs/ (Cs-C) versus t (Kitazawa et al. 1975) for the suppositories are shown in Figure 2.

In all cases, two straight regression lines of slopes k_1 and k_2 were obtained. The time at which the lines intersect, which represent the time that the release rate of the drug from the suppository changes from k_1 to k_2, is denoted by t_1. The release kinetics of the suppositories prepared from the two bases was biphasic with release rates k_1 and k_2 (Kitazawa et al. 1975, Odeku and Itiola 2003b). Values of k_2 were greater than k_1 indicating that the release rate became faster after t_1 for suppositories. The biphasic release rate character of the suppositories may be as a result of the release of chloroquine being limited initially by the available surface area of the suppository and probably controlled by spreading, while the second (rapid release)

phase corresponds to the increasing drug release from a melting suppository mass (Moolenaar et al. 1995).

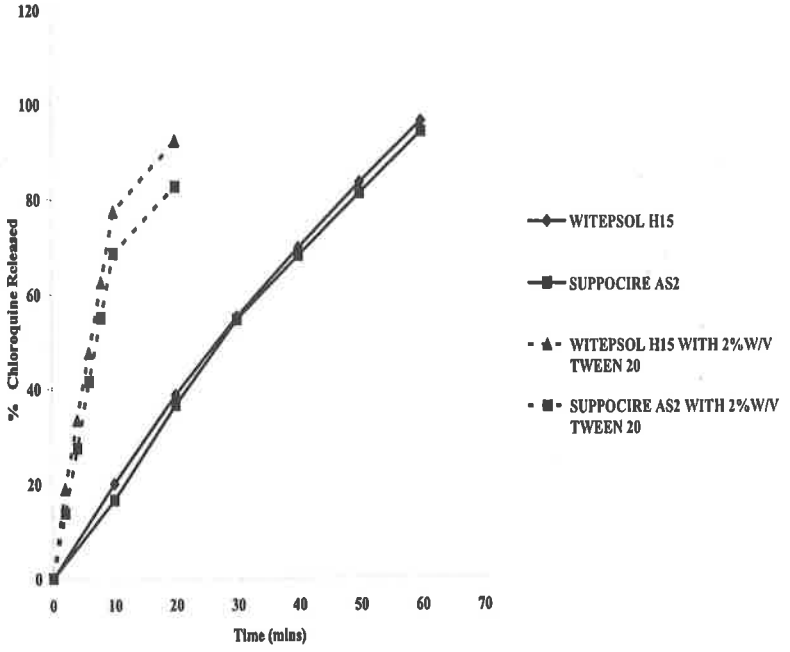

Figure 1. Dissolution profiles of chloroquine phosphate suppositories.

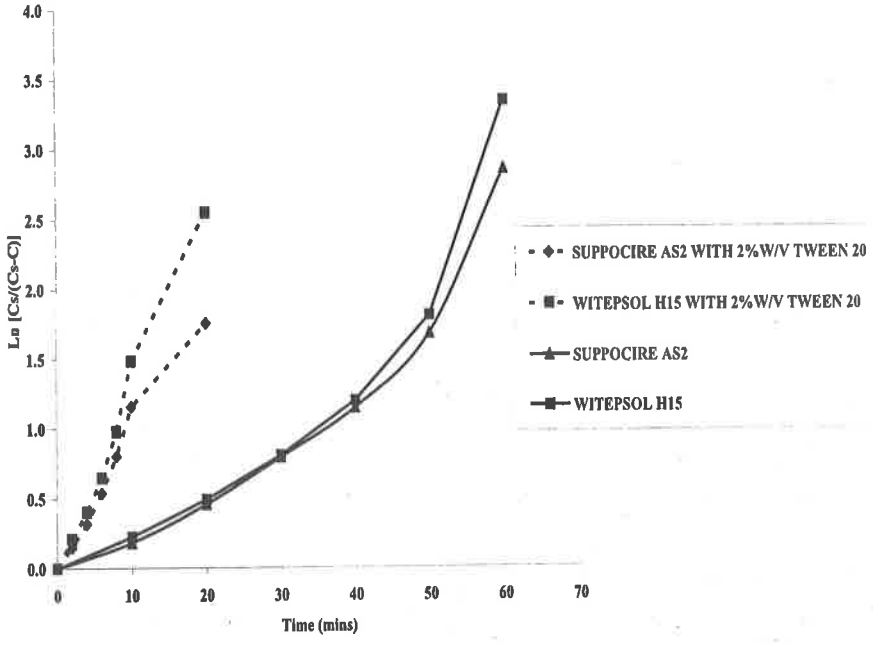

Figure 2. Representative plot of Ln [Cs/ (Cs-C)] against time (mins) chloroquine phosphate suppositories.

The values of CS, t_{50}, t_{80}, t_1, k_1 and k_2 used for the factorial experiment are presented in Table 1.

Table 1. The crushing strength (N) and dissolution times (mins) of chloroquine suppositories for factorial experimental design.

Combination codes	Crushing strength (N)	t_{50} (mins)	t_{80} (mins)	t_1 (mins)	k_1	k_2
$N_L S_L C_L$	14.71	26.00	48.00	44.00	0.03	**0.04**
$N_L S_L C_H$	17.17	7.50	17.50	10.00	0.01	**0.09**
$N_L S_H C_L$	17.66	29.00	52.00	47.00	0.03	**0.04**
$N_L S_H C_H$	19.61	8.00	19.00	14.00	0.08	**0.09**
$N_H S_L C_L$	21.09	23.00	46.00	38.00	0.03	**0.05**
$N_H S_L C_H$	29.42	6.50	11.50	8.00	0.12	**0.13**
$N_H S_H C_L$	24.52	25.00	50.00	40.00	0.03	**0.04**
$N_H S_H C_H$	31.88	7.00	14.00	12.00	0.10	**0.11**

These values were used to calculate the individual and interaction coefficients using relevant expressions and their values are presented in Table 2.

Table 2. Individual effects of Nature of suppository base (N), Concentration of surfactant (C), storage time (S) on Crushing force (N) and dissolution times (mins) of chloroquine suppository.

Variables	CS	t_{50}	t_{80}	t_1	k_1	k_2
Independent Coefficient						
N	9.44	-2.25	-3.75	-4.25	0.03	**0.02**
C	5.03	-18.50	-33.50	-31.25	0.05	**0.06**
S	2.82	1.50	3.00	3.25	0.01	**-0.01**
Interaction Coefficient						
N-C	2.82	0.75	-1.75	2.25	0.03	**0.01**
N-S	0.13	-0.25	0.25	-0.25	-0.02	**-0.01**
C-S	**0.37**	**-1.00**	**-1.00**	**0.75**	**-0.01**	**-0.00**

The individual and interaction coefficient values provide a clear indication of the quantitative effects of the three variables studied on the crushing strength and release properties of chloroquine suppositories. In comparing the formulation, the ranking of the independent coefficient values on crushing strength (CS) was N>C>S, on t_{50}, t_{80}, t_1 was C>>>N>S and on k_1 and k_2 was C>N>S. The negative value indicates that the values of the parameter decreased.

The effect of C indicates that presence of surfactant had the highest individual effect on the release properties of the suppositories. The suppository formulations containing the surfactant, Tween 20, at a concentration of 2% w/w showed significantly ($p>0.001$) higher CS, lower dissolution times and faster dissolution rates than those containing no surfactant. The crushing strength which is the amount of force required to deform or break a suppository is a measure of the strength and consistency of the suppository. This indicates that suppositories containing the surfactant possess more strength and consistency than those containing no surfactant at all. This shows that the presence of surfactant plays an important role in the dissolution profile of chloroquine suppositories. The non-ionic surfactant, Tween 20, is known to increase water incursion into fatty bases, which reduces their interfacial tension and results in increased contact between the drug particle and base (Adegboye and Itiola 2003) and thus promoting drug release from the suppository (Abdul–Gawad and El – Din, 1989). The hydrophilic nature of Tween 20 has also been reported to aid the formation of stronger bonds which will lead to an increase in crushing strength with the resultant increase in the ability of the suppositories to withstand the rigors of handling involved in manufacture, transportation, dispensing and usage

of suppositories (Odeku and Itiola 2003b). This result is in agreement with those of previous worker (Onyeji et al. 1999).

The nature of base (N) on the other hand had the highest effect on the crushing force. The effect of N on CS was positive, indicating that changing the type of suppository base from Suppocire® AS2 to Witepsol® H15 led to an increase in crushing strength. This suggests that suppositories containing Witepsol® H15 as base were stronger than those from Suppocire® AS2. This may be due to the chemical composition such as the glyceride content and the structure of the bases as well as their hydroxyl value. Witepsol® H15 has a lower hydroxyl value than Suppocire® AS2 indicating that more particle- particle interaction will occur between the drug and base. The effect N on the dissolution times (t_{50} and t_{80}) was negative indicating that suppositories made with Witepsol® H15 had lower dissolution times than Suppocire® AS2, while the positive effects on dissolution rates indicated a reduction in the dissolution rates k_1 and k_2. This information is of importance where a high blood level of the drug is required for quick pharmacologic effect (Adegboye and Itiola 2003).

The storage time (S) generally had the lowest individual effect on the CS and release properties of the suppositories. The effect of storage on the properties of the suppositories was positive, indicating that the crushing strength and dissolution times of the suppositories increase with storage. This could be attributed to the fact that suppositories tend to become harder over time owing to intermolecular attractions. However, there were no significant difference ($p>0.05$) in the CS and release properties of the suppositories when freshly prepared and after storage at 25±2°C for a period of six months. Thus, the suppositories were able to retain their integrity on storage.

The interaction coefficient values indicate the effects of the variables in combination on the CS and release properties of the suppositories (Table 2). The values of the interaction coefficient indicate that the variables were interacting with each other to varying degree. The ranking of the interaction effects on crushing strength, t_{80} and t_1 was N-C >C-S > N-S, on t_{50} was C-S > N-C > N-S, on k_1 was N-C> N-S > C-S and on k_2 N-C = N-S > C-S. The interaction effect indicates that the concentration of surfactant (C) interacted with the two other variables, N and S, to influence the mechanical and drug release properties of suppositories. Thus, the type of base used in the preparation of chloroquine suppositories as well as the inclusion of the surfactant plays an important role in determining the crushing strength and release properties of chloroquine suppositories.

Conclusion

The results obtained suggest that changing the suppository base from Suppocire® AS2 to Witepsol® H15 led to an increase in the crushing strength and dissolution rates but decrease in the dissolution times. The inclusion of a surfactant into the suppository formulation led to an increase in the CS but decrease in the dissolution times. The interaction between nature of base (N) and concentration of surfactant (C) had the highest effects on the mechanical and drug release properties of suppositories. Thus, the nature of the base and concentration of surfactant used in suppository formulations need to be carefully chosen during formulation in order to obtain suppositories of desired mechanical and drug release properties.

Acknowledgments

We gratefully acknowledge the GATTEFOSSE, France, for the generous gift of Suppocire®.

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Received: 22.08.2008
Accepted: 15.04.2009