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Kinetics of Degradation and Stability Studies of Cephalexin Suspensions Marketed in Nigeria

Ofokansi, KC[1*], Uzor, PF[2], Nnaji, AM[1]

[1]Department of Pharmaceutics, University of Nigeria, Nsukka, 41001, Enugu State, Nigeria;
[2]Department of Pharmaceutical and Medicinal Chemistry, University of Nigeria, Nsukka, 41001, Enugu State, Nigeria.

ABSTRACT

An ideal pharmaceutical suspension should retain its physical, chemical and organoleptic properties within an acceptable time of storage. Cephalexin is considered by the World Health Organization (WHO) as one of the drugs with stability problems. The objective of this study, therefore, was to evaluate the degradation kinetics and stability of some commercially available cephalexin suspensions in Nigeria in order to ascertain the validity of shelf-life claims of the dry suspension and the reconstituted drug products. The study was carried out at exaggerated temperature conditions of 37 ^{o}C, 45 ^{o}C and 55 ^{o}C using four brands of cephalexin suspension coded as CEF A, CEF B, CEF C and CEF D. The degradation rate constants as well as the shelf-lives of the drug products at room temperature were evaluated from the Arrhenius equation. Results show that the degradation rate constant ranged from 0.10 to 0.14 year^{-1} while shelf-life ranged from 9 to 12 months for all suspensions studied. Degradation rates were found to follow first order while the shelf-lives for all the products were far below the label claims as stated by their manufacturers. The indication is that high tropical temperature affects the stability of cephalexin suspensions marketed in Nigeria and possibly leads to the degradation of the products long before the manufacturers' stated expiry dates. Results from degradation kinetics monitored at room temperature indicate that the studied drug maintained maximum stability at the ambient temperature of 25 ^{o}C and potency was retained for well over three weeks in a reconstituted state.

Keywords: Arrhenius equation;cephalexin suspension; stability; degradation kinetics.

INTRODUCTION

For over a decade, the World Health Organization (WHO) has been addressing the salient issues related to drug stability and storage, with particular attention being paid to the developing countries, most of which are located in the tropical climate zones where drug stability poses more serious problems, probably because of the more adverse climatic conditions [1].

In addition, there is increasing concern about the stability of pharmaceuticals marketed in Nigeria as a result of the poor storage conditions and the high level of substandard drugs in Nigerian market. It is known that temperature, humidity, light, etc., affect the stability of drugs and drug products. Thus, there is every likelihood, in many cases, that some drugs might have degraded beyond a level suitable for use long before the expiry date as claimed on the product label. The degraded product may not only lead to therapeutic failures as a result of the patient taking an under-dose of the drug but it could be deleterious to the health of the patient if the drug is degraded to toxic compounds. This necessitates the routine stability testing of drugs despite the label claims of such drug products.

According to WHO [2], the purpose of stability testing is to provide evidence on how the quality of an active substance or pharmaceutical product varies with time under the influence of a variety of environmental factors such as temperature, humidity, light etc. In addition, other factors known to affect the stability of the product include the chemical and physical properties of the active substance and the pharmaceutical excipients, the dosage form and its composition, the manufacturing

*Author for correspondence:*E-mail address: kcofokansi@yahoo.com, Tel: +234-81-31245821*

process, the nature of the container-closure system, and the properties of the packaging materials. As a result of stability testing, a re-test period for the active substance or a shelf life for the pharmaceutical product can be established, and storage conditions can be recommended.Stability studies are now an essential part of the activities of the pharmaceutical formulator as well as the hospital and clinical pharmacist, in order to ensure the safety and efficacy of pharmaceutical products. In the past, expiration dating was only necessary for drugs possessing deteriorative or unstable characteristics, but now all formulations are expected to bear such a date on their labels and, when necessary, their storage conditions, in order to ensure both patient safety and rational management of drug supplies [1].

It was common in the past to test the stability of drug products by subjecting it to the usual storage test but such method was time consuming and expensive. Accelerated stability technique devised during the product development stage would enable rapid prediction of the long term stability of a product within a short period of time [3].In most cases acceleration of chemical decomposition can be achieved by raising the temperature of the preparation; a procedure which was adopted in the present study. In addition, several techniques of quantitative analysis such as UV-Vis spectrophotometric analysis, HPLC [4-5], etc. could be used but the method adopted here was the microbiological method. This method often has the advantage of a wider margin of error, a great sensitivity and specificity for some antibiotics and it provides a standard method of resolving doubt with respect to possible loss of bioactivity of antibiotics [1].

Cephalexin is a first generation cephalosporin that has a wide spectrum of antibacterial activity [6]. It is indicated for the treatment of infections of the respiratory tract, skin and skin structure, bone and genitourinary tract infections and otitis media when caused by susceptible microorganisms and may also be used to prevent bacterial endocarditis. The World Health Organization included cephalexin among the list of 108 less stable drug substances requiring particular storage attention [7]. Several workers have studied the stability of cephalexin and other cephalosporins by exposure to different conditions such as light, air and pH [8-13]. The commonly available forms of cephalexin in Nigeria are the capsules and dry powder for reconstitution as suspension. According to manufacturers' instructions, the dry suspensions usually have expiry dates ranging from 2 to 3 years and when reconstituted, the suspensions are expected to be used within 7 -10 days, provided the drugs are stored in a cool, dry place (preferably in a refrigerator) and protected from light. An ideal pharmaceutical suspension should retain its physical, chemical and pharmacological properties within an acceptable limit throughout its shelf-life.

This study was designed to investigate the stability of cephalexin suspensions marketed in Nigeria using the microbiological method of assay to monitor their degradation under the influence of exaggerated temperature as the stress condition.

MATERIALS AND METHODS

Materials

Cephalexin pure sample was a gift from Ranbaxy Nig. Ltd. Four brands of commercially available cephalexin suspensions were purchased from local pharmacy shops in Nsukka, Enugu State, Nigeria. They were coded as CEF A, CEF B, CEF C and CEF D. All the reagents and chemicals were of analytical grade and were purchased from their manufacturers and used without further purification.

Methods

Selection of microorganisms

Some microorganisms known to be susceptible to cephalexin were used in order to select the organism that showed the highest susceptibility. The organisms used were *Escherichia coli, Staphyloccocusaureus*and*Klebsiella pneumonia.* The test was done using the agar diffusion method [14]. *Staphyloccocusaureus*was selected based on this test.

Maintenance, Activation and Standardization of the microbial culture

The stock culture of the microorganism, *S. aureus*, was maintained on nutrient agar slants at 4 ^{o}C. The stock culture was purified from freshly prepared subcultures and incubated at 37 ^{o}C for 24 h. Standard suspensions of the microorganism was made by transferring a colony from the subculture into 5 ml of distilled water and the volume was adjusted to obtain a cell population of approximately 1.0×10^6 CFU/ml. One loop-full (0.02 ml) of such suspension was used as inoculum in all the tests.

Preparation of various concentrations of cephalexin suspension

Each brand of cephalexin suspension was reconstituted with purified water, according to the manufacturer's instruction, to give a final

concentration of 25 mg/ml (stock). From the stock suspension, ten-fold dilutions were made successively to give concentrations of 2.5 mg/ml, 0.25 mg/ml, 0.025 mg/ml and 0.0025 mg/ml. The concentration of each drop was calculated to give: 0.5 mg, 0.05 mg, 0.005 mg, 0.0005 mg and 0.00005 mg respectively.

Evaluation of the stability of cephalexin suspension

The stability of the reconstituted suspension was evaluated using microbiological assay method. The seeded agar plates were divided into three zones using a permanent marker and holes were bored on each zone on the plate using a sterile cork borer of 8 mm diameter. Three 10-fold serial dilutions of cephalexin pure sample were done and one drop of each dilution was introduced into the labeled holes using a sterile dropper. The plates were prepared in triplicate and incubated at 37 ^{o}C for 24 h. The pure drug sample was stored at room temperature of 25 ^{o}C and samples were withdrawn and assayed at four day intervals for a period of 20 days. Similarly, the pure sample of the drug was maintained in a humidity oven at varying temperature conditions of 37 ^{o}C, 45 ^{o}C and 55 ^{o}C. Samples were withdrawn at intervals and assayed microbiologically. The procedure was repeated using the four brands of cephalexin. Inhibition zone diameter (IZD) produced on the plates was measured in each case.

Estimation of shelf-life

For this test, the drug products (dry granules) of the various brands of cephalexin were stored at elevated temperature conditions of 37 ^{o}C, 45 ^{o}C and 55 ^{o}C and samples were withdrawn and assayed as described above. IZD was measured at each temperature. A standard plot of IZD^2 versus log concentration was obtained using the pure sample of cephalexin. The concentration of undecomposed drugs at each temperature was extrapolated from the graph. The specific rate constant or degradation rate constant (k) of the samples at various temperatures and concentrations was obtained from the plot of log concentration against time. The logarithms of 'k' were plotted against the reciprocal of the absolute temperature according to the Arrhenius equation (Eqn.1) [1].

$k = Ae^{-E_a/RT}$.....Eqn.1,

where k = specific rate constant

T= absolute temperature

E_a= energy of activation

R= gas constant

A= frequency factor

The specific rate constant at room temperature of 25 ^{o}C (k_{25}), extrapolated from the Arrhenius plot, was used to calculate the shelf-life (t_{90}) which is the time for 10 % of the drug to decompose, or 90 % of the drug to remain undecomposed, at room temperature [15]. The shelf-life ($t_{0.9}$) and half-life ($t_{1/2}$) were calculated from Eqns. 2 and 3 respectively as shown below.

$t_{0.9} = {0.105}/{k_{25}}$.....Eqn.2;

$t_{1/2} = {0.693}/{k_{25}}$.....Eqn.3.

Statistical analysis

The data obtained were analysed by SPSS version 16 and recorded as mean ± standard deviation (SD, n = 3). Statistically significant difference between brands of cephalexin was evaluated using the Students' t-test and one way analysis of variance (ANOVA; Fischer LSD post-hoc test). Significant differences between means were considered at $p < 0.05$.

RESULTS AND DISCUSSION

A linear relationship was found to exist between log concentration and the IZD^2 of the cephalexin pure sample with regression coefficient (r^2) of 0.9993 (Figure 1). From the relationship, the concentration of the different samples producing the IZDs was determined.

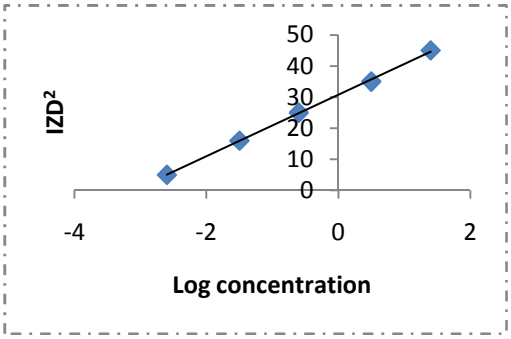

Figure 1: Standard plot of cephalexin pure sample

The degradation rate constant data of the various brands of cephalexin suspension at various temperatures, obtained from a plot of logarithm of concentration against time, are presented in Table 1. The pattern of degradation of CEF A brand of cephalexin under the influence of temperature is illustrated graphically in Figure 2; other brands showed similar pattern. The regression coefficient values (r^2) ranged from 0.847 to 0.996 indicating the linearity of such relationship. This linearity is a further indication that the degradation of cephalexin

suspensions followed a first-order reaction. In a related study, the degradation kinetics and mechanism of a cephalosporin derivative, cefadroxil, in aqueous solution were investigated. It was shown that at constant pH and temperature, the degradation of cefadroxil followed first-order kinetics [16]. Other workers have also shown that the degradation of cephalexin and cefazolin followed pseudo-first order kinetics [17].

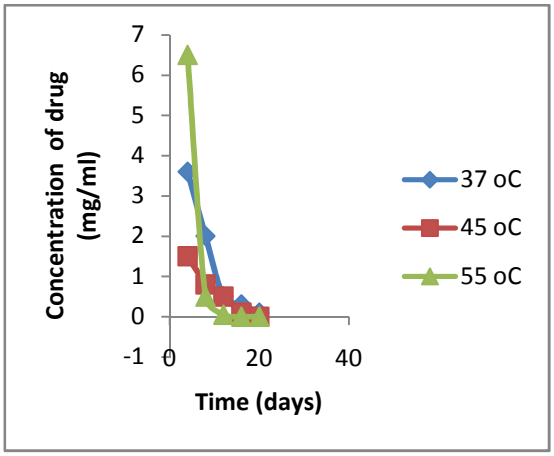

Figure 2: Effect of temperature on the degradation kinetics of CEF A brand of cephalexin suspension

Table 1 shows a linear relationship between temperature and degradation rate constant of the various brands of cephalexin. As shown in the Table, the respective degradation rate constants of CEF A-D increased from 0.180 to 0.392 year^{-1}, 0.115 to 0.921 year^{-1}, 0.161 to 0.370 year^{-1} and 0.201 to 0.350 year^{-1} respectively as temperature increased from 37 ^{o}C to 55 ^{o}C. These changes signify that higher temperatures produced higher reaction rate or degradation of the drug. Degradation of drugs is an indication of reactions taking place and reaction rates have been observed to depend on such factors as temperature, concentration of the reacting species, light, solvent, pressure, pH, presence of catalyst as well as the nature of the reaction [19-20]. Also the speed of many reactions increases about two to three times with each 10 ^{o}C rise in temperature [1]. The colour changes of the products at elevated temperatures after 6 h are shown in Table 2. The changes in colour suggest degradation of the products at high temperatures. Common degradation mechanisms for drugs include hydrolysis, oxidation, photodegradation, thermolytic degradation, isomerization and racemization. Several hydrolytic degradation products of cephalosporins have been reported as cephalosporanic acid, desacetylcephalosporin, desacetylcephalosporin lactone [18]. The degradation of these drug products at elevated temperature might have occurred through one of hydrolysis, oxidation and thermolytic degradation or a combination of these mechanisms.

Table 1: Degradation kinetic parameters of the various brands of cephalexin suspension at various temperatures

Cephalexin Brand	Temp (^{o}C)	k (year^{-1})	r^2
CEF A	35	0.180	0.847
	45	0.253	0.864
	55	0.392	0.978
CEF B	35	0.115	0.972
	45	0.230	0.987
	55	0.921	0.991
CEF C	35	0.161	0.993
	45	0.300	0.954
	55	0.370	0.964
CEF D	35	0.201	0.996
	45	0.311	0.992
	55	0.350	0.957

Table 2: Colour change of the various brands of cephalexin suspension at elevated temperature

Cephalexin brand	Original Colour	Colour change
CEF A	Orange	Brown
CEF B	Pink	Reddish brown
CEF C	Orange	Dirty-brown
CEF D	Yellow	Dark brown

Thedegradation rate constants at room temperature (k_{25}) for the various brands of cephalexin suspension were obtained from the plot of logarithm of degradation rate constants (log k) against the reciprocal of absolute temperature (1/T), according to the Arrhenius equation (Figure 3).

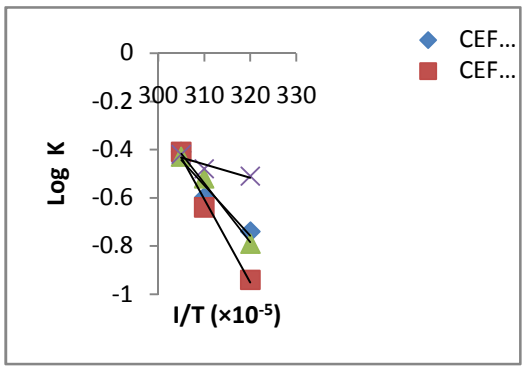

Figure 3: Arrhenius plot of the various brands of cephalexin suspension

The values of k_{25}, the calculated half-life and shelf-life are presented alongside the manufacturer's claim of the shelf-lives of the various brands of the drug in Table 3. It is evident from Table 3 that CEF B brand had the lowest degradation rate constant at room temperature (0.10 $year^{-1}$) while CEF C had the highest value (0.14 $year^{-1}$). The corresponding values of the half-life and shelf- life of these two brands were 6.93 $year^{-1}$ and 12.0 months respectively for CEF B and 4.95 $year^{-1}$ and 9.0 months respectively for CEF C. Furthermore, comparing the shelf-lives with the manufacture's claim for the dry granules showed that each of the brands had shelf-life far lower than the one stated on the product label. In each case, the shelf-life was statistically different (p>0.05) from the manufacturer's claim. These observations suggest that the drug in each of the brands is most likely to have degraded long before the expiry date set by the manufacturer. These are possibly due to poor storage conditions of these products (exposure to high temperature, humidity and light for quite a long time) during transportation and in warehouses and shops; erratic power supply compounds this situation as refrigerators and air conditioners need power to operate. Other factors include poor packaging and manufacturing processes. Also the general limitations of Arrhenius equation as a true predictor of the shelf life of a product must be recognized despite its merits [1]. Nevertheless, these findings have brought to light the necessity of strict adherence to the instructions on the proper storage of drugs and drug products. For instance, the storage condition stated in the British Pharmaceutical Codex is that the drug suspension should be stored in airtight containers, protected from light and at temperature not exceeding 30 ^{o}C [21]. Furthermore, data (not shown) indicate that the drug in a reconstituted suspension maintained maximum stability and potency at the ambient temperature of 25 ^{o}C. Storage of drugs at the proper conditions as recommended would guarantee the maintenance of quality, safety and efficacy throughout the shelf-life of the product.

Table 3: Stability parameters of the various brands of cephalexin suspension and their manufacturer's stability claims

Cephalexin Brand	k_{25} ($year^{-1}$)*	$t_{1/2}$ (year)*	Shelf-life* ($t_{0.9}$) (month)	Manufacturer's claim of shelf-life	
				Dry granules (month)	Reconstituted suspension (day)
CEF A	0.11	6.30	11.40	37	10
CEF B	0.10	6.93	12.00	24	7
CEF C	0.14	4.95	9.00	36	7
CEF D	0.13	5.33	9.72	37	10

*Parameters refer to the stability of dry granules and data are the mean of three determinations

CONCLUSIONS

The results of the present study have accentuated the long-held knowledge that high temperature increases the degradation rate of most drugs and more importantly for drugs marketed in Nigeria. The suspensions studied recorded shelf-live values far below the label claims as stated by their manufacturers. It is recommended that drug products should be protected from light and stored in a cool and dry place with strict adherence to the instructions on the storage temperature. Use of time -expired drug products and products that are nearing their expiry dates should be discouraged due to doubts about their storage conditions since the time of their manufacture.

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