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DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY

AND INDUSTRIAL PHARMACY

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B. Pharm. (Hons), M.P.S.N.

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY AND INDUSTRIAL PHARMACY IN THE FACULTY OF PHARMACEUTICAL SCIENCES AS PART OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHARMACY DEGREE OF THE UNIVERSITY OF NIGERIA, NSUKKA

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DECLARATION

We certify that Miss Stella Anagam Megwa carried out this research work in the Department of Pharmaceutical Technology and Industrial Pharmacy. The work presented herein is original and has not been previously reported any where else.

DR. O. K. UDEALA
SUPERVISOR

PROFESSOR H. C. MITAL
HEAD OF DEPARTMENT

TO THE CHILDREN YET UNBORN

C O N T E N T S

Appendix 1 - Calibration Curve Data

Appendix 2 - Drug Content of Suppositories

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Stella.

Nsukka
JULY, 1984.

ABSTRACT

A solid vegetable fat derived from the seeds of Irvingia gabonensis was investigated either in its pure form or in combination with Olive oil and nonionic surfactants as suppository base. The results obtained are compared with those obtained using the conventional cocoa butter base.

Chloroquine phosphate, aminophylline and aspirin suppositories were investigated. The choice of these drugs are of great significance. Both parenteral and oral administration of chloroquine phosphate may cause certain unpleasant side effects such as pruritis and vomiting. The gastro-intestinal symptoms can be avoided by rectal administration in suppository. When administered orally, aminophylline is unpredictably absorbed. Suppositories are an alternative to injections since the latter needs specially trained personnel for the administration of the dosage form. Obtaining adequate aminophylline release and its attaining adequate blood concentrations have always presented problems with the known suppository bases. The reasons are attributed to physical and chemical

instabilities of the drug and the suppository bases. Aspirin is most unstable in many of the fatty bases so far studied.

Results obtained in the present investigation show that dika fat can be formulated to yield release patterns comparable to those of cocoa butter. Apart from the colour change observed with aminophylline formulation, all three drugs are most stable in the dika fat blends during a 12 week storage period. The observed change in colour in no way hindered *in vitro* release of aminophylline from dika fat base.

The fact that the release of aminophylline does not decrease with storage is an important contribution in the evolution of an acceptable suppository base for aminophylline. The level of stability of aspirin in this new base recommends its use in the formulation of aspirin suppositories. The presentation of chloroquine phosphate in a suppository base is novel and may be preferred in paediatric application.

INTRODUCTION

GENERAL INTRODUCTION

A suppository is a medicated solid dosage form generally intended for use in the rectum, vagina and to a lesser extent the urethra (1, 2, 3, 4). Its therapeutic effect becomes evident when it melts at body temperature or dissolves in the aqueous secretions of the mucous membrane. Either of these mechanisma makes possible the release and contact of the medicament with rectal mucosa.

Therapeutic Uses of Suppositories.

Suppositories have been used since ancient times both for their local and systemic effects.

The use of suppositories to produce local effect is well documented. Local rectal medication may have one of the two contrasting therapeutic effects; the soothing effect of a local anaesthetic or an anti-infective such as the preparations used for hemorrhoidal treatment or the effect of the evacuant suppositories and the cleansing enemas. These are used to stimulate the defecation reflex and to empty the rectum and lower colon. Hippocrates used this form of treatment for the administration of cathartics in a vehicle of soap and honey (5, 6).

The mucous membrane of the rectum permits the absorption of many soluble drugs and thus can exert systemic effects (7, 8, 9). Aspirin suppositories and those containing isoprenaline have been used with complete success in children immediately following open heart surgery (6). Similarly, the pre-operative use of thiopentone sodium in the form of suppository has become more wide-spread (6).

The rectal route has several advantages over oral therapy, some of which may include (10):

(i) drugs destroyed or inactivated by acid or enzymatic activity of stomach or intestines need not be exposed to these destructive environments,

(ii) drugs such as indomethacin (11) which is irritating to the stomach may be given without causing such irritation,

(iii) drugs destroyed by portal circulation by-pass the liver after rectal absorption — more than half (50-70%) of rectally administered drugs were reported absorbed directly into the general circulation (12); the lymphatic circulation also helps divert absorbed drugs from the liver (13),

(iv) the route is convenient for administration of drugs to adults or pediatric patients who may be unable or unwilling to swallow medication, and,

(v) it is an effective route in the treatment of patients with vomiting episodes.

There have been divergent views on the worth of suppositories. Gilbaldi and Grundhofer (14) questioned the usefulness of aspirin suppository dosage forms for aspirin or salicylate therapy. That the acidity of aspirin aggravates gastric distress for ulcer patients is a known fact. The administration of aspirin through the rectal route can obviate this problem. An anonymous publication (15) also raised doubts as to the usefulness of suppositories. Howbeit, a drug such as theophylline is absorbed from the stomach in an unpredictable manner. There is therefore some advantage gained by the administration of this drug in a suppository. The emphasis in investigating suppositories has been on physical characteristics (16, 17), the influence of the base (18-20) and the <u>in vitro</u> release of the drug (21). There is scope for further investigation into the stability, release and absorption of drugs from various bases in this dosage form.

Suppository Bases.

A variety of substances has been used as suppository bases throughout the history of medicine. Their uses and applications were prompted by availability rather than scientific knowledge. A general classification of suppository base is possible on the basis of their physical properties.

The fatty or oleaginous bases are most often used. They are predominantly mixtures of triglycerides of fatty acids and they usually melt at body temperature. Most popular in this class is theobroma oil with a melting point range of 30-36^{o}C. Theobroma oil has a number of serious disadvantages that decrease its suitability for use as a suppository base. It is polymorphic. At certain melting temperatures and cooling rate, it can solidify to yield alpha, beta and gamma crystalline forms. Theobroma oil does not contract enough on cooling to loosen the suppositories from the mould. Sticking may occur particularly with worn moulds. Due to its low softening point, handling is quite difficult in tropical and sub-tropical countries. Soluble ingredients like chloral hydrate, volatile oils, phenol and creosote may lower its melting point to such an extent that the suppositories are too soft for use.

The disadvantages inherent in theobroma oil, have prompted search for suitable substitutes. These substitutes are produced from a variety of materials which may be either synthetic or natural in origin. These include vegetable oils that are modified by esterification, hydrogenation and fractionation at different melting ranges. These newer bases are triglycerides of higher fatty acids with carbon chain length of C_{11} to C_{17}. They have higher melting points, lower iodine and acid values and are capable of emulsifying water. Caldwell (22) suggested the use of hydrogenated palm kernel oil and soya-bean oil. The use of hydrogenated coconut oil and beef fat fractions which compared favourably with theobroma oil and synthetic lauric acid glyceride have been reported (23). Fats from seeds of shorea specie (24) and mango (25) have also been investigated. Hartman et al (26) suggested the use of partially or completely hydrogenated cotton seed oil such as cotoflakes and cotomar together with hexadienal. Fauli and co-worker (27) used synthetic excipients. These excipients include hardened fatty alcohols, and fatty acid esters of glycerin, propylene glycol and fatty alcohols. In the present study, dika fat, an oily extract from Irvingia gobonensis seed,

with a melting point range of 38 to 41°C is being investigated. Some of its physical properties like low iodine and acid values, high saponification value and melting range qualify it for use especially in the tropics where environmental temperatures are quite high.

Water soluble or water miscible bases form a second class of suppository bases. Prominent in this group is the glycerinated gelatin base. It does not melt at body temperature but slowly dissolves in the secretion of the mucous membrane of the rectum. This base is not frequently used because it is not only liable to microbial decomposition but also has physiological action; it is used per se as evacuant suppository. It is doubtful whether it would dissolve completely in the small volume of fluid known to exist in the rectum (28). A quantitative release of drug is thus not assured.

The shortcomings of glycerinated gelatin base led to a search for other alternative water soluble bases. Early work on the water soluble and water miscible class was carried out on glycerol and glycol esters of stearic, lauric and palmitic acids (6). The polyethylene glycol polymers superceeded the glycerol base. They are

polymers of ethylene oxide and water prepared to give various chain lengths, molecular weights and physical states. In order to obtain a desired consistency, two or more polymers are combined. Polyethylene glycol suppository bases do not melt at body temperature but dissolve slowly in the rectal fluids. An area of great concern in the use of these bases is their good solvent properties. This may result in the retention of drug in the liquified base with subsequent reduction in therapeutic activity. They are incompatible with bismuth salts, tanins and phenol. Whitworth et al (29) reported that decomposition of aspirin in polyethylene glycol is due primarily to a transesterification reaction.

The third or miscellanous group are bases which are mixtures of water soluble and oleaginous materials. They are water dispersible and the non-ionic surface active agents belong to this group. Gross and Becker (30) recommend a high melting point suppository base consisting of polyoxethylene 30 stearate, water, white wax and Aerosol OT. It was found that bases containing 35 to 40% of emulsifying agents gave best release of the medicaments.

Aerosol OT must be used with caution. It has adsorption and viscosity increasing properties and may retain some of the drug incorporated into the base (31). This reaction depends on the chemical characteristics of the drug.

Dose Characteristics and Factors Affecting Rectal Absorption of Drugs.

Conflicting opinions exist concerning the amount of drug that should be administered rectally as compared to the oral dose. The suggested rectal dose generally covers ranges from one half to twice the oral dose. Parrott (9) reported that the rectal dose of aspirin and sodium salicylate is equivalent to the oral dose. Atropine, chloral hydrate, methylene blue, morphine and sodium salicylate were reported to be absorbed rectally more quickly than with oral administration (32). The dose requirement of sulfanilamide is said to be the same for tablet and a glycerinated gelatine base suppository (33). A good formulation thus requires a concurrent consideration of the suppository base and the amount of incorporated drug. Assuming constant physiological factors, the proper rectal dose would be dependent on the physiochemical properties of the drug and on the properties of the suppository (3).

Drugs administered rectally in suppositories are placed in intimate contact with the rectal mucosa which behaves as a normal lipoidal barrier (34). The absorption of drugs from the colon as described by Schanker (35) and the kinetics of rectal absorption as determined by Reigelman and Crowell (36) agree in principle. It might thus be said that the absorption of drugs from the colon and that from the rectum are quite similar. It is worth noting , however, that the area to which rectal retention enema (36) is applied is much greater than that of suppository. Physiological factors and physiochemical factors of the drug and the base (37) affect the rectal absorption of a drug administered in a suppository.

Physiological Factors. Wagner (38) pointed out that the amount of faecal matter in the rectum at the time of drug administration by the rectal route influences the rate and extent of absorption. The state of the ano-rectal membrane also plays a role in drug absorption. The membranous wall is covered with a relatively continuous mucous blanket, which can act as a mechanical barrier, for the free passage of drug through the pore space where absorption occurs.

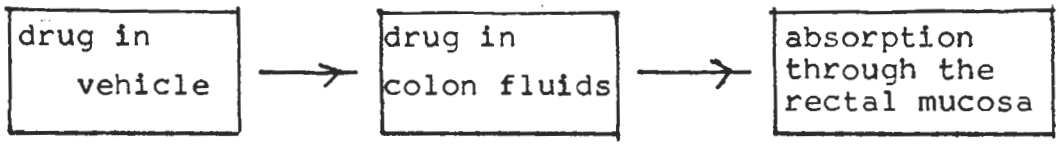
Reigelman and Crowell (36) demonstrated that one of the rate-limiting steps in drug absorption from the rectum is the diffusion of the drug to the site on the rectal mucosa at which absorption occurs. The diffusivity is influenced not only by the nature of the medicament, but also by the amount and chemical nature of the fluids and solids present in the rectum. A drug will obviously have greater chance of making contact with the absorbing surface of the rectum in the absence of faecal matter. The rectal absorption of a drug will be generally more rapid and efficient if an enema is given some time prior to the administration of the suppository. This will not obtain if the rectum and colon are not first cleansed with an enema prior to the administration of a suppository. Other conditions such as diarrhoea, colonic obstruction due to tumorous growths, and tissue dehydration can all influence the rate and degree of drug absorption from the rectal site.

The real anatomic difference between the rectum and colon, with perhaps a physiologic advantage, is that the rectum is supplied with the lower and the middle haemorrhoidal veins, which pass directly into the general circulation. The upper haemorrhoidal vein passes into the hepatic portal system. The amount of drug absorbed directly into the general circulation depends on where

the drug is released in the rectum. If the suppository remains in the lower part of the rectum, a higher proportion of the drug (12) will enter the inferior vena cava and general circulation. A lower proportion would enter if the suppository migrates up the gastro-intestinal tract. The drug administered via the rectal route therefore, does not necessarily or reproducibly by-pass the liver.

The pH of the rectal mucosa plays a significant role in drug absorption from the ano-rectal area. Schanker (35) reported that the pH of the colon lies between 6.8 and 7.0. Rectal fluids have virtually no buffering capacity, and as such, the dissolving drugs determine the existing pH according to their own properties of dissociation. Thus, weak acids and bases that remain undissociated are rapidly absorbed, whereas those drugs that are highly ionised are slowly absorbed. Weak organic electrolytes that are highly lipid-soluble were found to be absorbed more rapidly. For increased absorption, a drug may be presented in the suppository in the unionised form. Alternatively, the pH of the rectal fluids may be easily modified by a formulation component of the suppository which then maintains the pH at which the drug is best absorbed.

Physicochemical Factors of the Drug and the Base The sequence of events leading to drug absorption from the anorectal area can be diagramatically represented as follows(3):



For a drug to be available for absorption, it must be released from the suppository and distributed by the surrounding fluids to sites of absorption.

The lipid-water partition coefficient of a drug is an important consideration in the selection of the suppository base and in anticipating drug release from that base. Ansel (10) observed that a lipophilic drug that is distributed in a fatty suppository base in low concentration, has less tendency to escape to the surrounding aqueous fluid than would a hydrophillic substance, present in the fatty base to an extent approaching its saturation. This is in agreement with the findings of Allawala and Reigelman (39) who compared <u>in vivo</u> absorption of sodium salicylate and aspirin from cocoa butter base. A greater portion of sodium salicylate was absorbed. This was ascribed to the partition coefficient which favours partitioning of the water-soluble sodium salicylate

from the liquid oleaginous phase. In order to ensure a rapid and complete release from a suppository base, lipophilic drugs are best formulated in hydrophilic bases (31). Water soluble drugs are best incorporated in lipophilic bases (40). The transport of a drug from a suppository base to the rectal fluid is therefore a rate-limiting step in the path of the drug to its site of action. This is largely under the control of the partition coefficient of the drug.

If a drug of limited water solubility is placed in a suppository base, the particle size of the drug will influence its release to the rectal fluid since its dissolution rate will be proportional to its particle size. Parrott (9) studied the release of powdered aspirin and aspirin disks and found that the time for 50% of aspirin to dissolve from suppository containing the powdered form was 50 minutes. This is in contrast to 100 minutes obtained with the other formulation. Cooper and Gunn (41, 42) stated that dissolution rate of any drug or solid with limited water solubility is increased by an increase in surface area. Reduction in particle size is the only effective way of increasing surface area. Konning et al (43) attributed increased release of crystalline medicaments from ointments as particle size decreased to the fact that there is greater

dissolution in the base and hence increased availability of the drug at the ointment substrate interface. Particle size has an effect on the viscosity of suspensions (34). It is therefore preferable to avoid the incorporation of ultrafine crystals since melted excipient becomes too viscous. The above finding was confirmed by Jaminet (44). Formation of suspension or solution with low viscosity under the environmental rectal conditions is advantageous. The resultant fluid spreads into a film over a large surface area, thus increasing the diffusion rate of the drug from the suppository mass, and hence an increase in absorption occurs (28, 42).

The diffusion of a drug from a suppository base is a function of its concentration. Rate of diffusion of drug from the base matrix will increase as the concentration of drug in the base increases. There is, however, a limit to this phenomenon since the external surface of the suppository can get saturated with the drug and release into the receptor phase is slowed down. Thus, the release rate depends not only on the concentration, but also on the rate of distribution of the already released drug in the rectal fluid. This view is held by King (2) who stated that optimum concentration for a given drug largely depends on the transport mechanism utilised

in the absorption process. Also, Anschel and Lieberman (3) reported that if the luminal concentration of a drug is above a particular amount, the rate of absorption will not change with further increase in the drug.

Bevernage and Poldeman (45) reviewed the factors influencing drug release from suppositories. They emphasised the importance of the study of the melting behaviour of fatty suppositories. As the melting point approaches 37°C within 0.5°C (46, 47), there is a strong reduction in *in vitro* release of drugs from suppository bases. This decreased release is more pronounced with increasing water solubility. This may be expected since for drugs of low water solubility other steps, such as dissolution rate, may become rate limiting. A study (48) with various bases with melting points up to body temperature shows that the base with highest melting point gave a much slower release both *in vitro* and *in vivo* in rabbits and humans. Available data indicate that, the melting point of fatty suppositories should not exceed body temperature. There are reports of changes in the melting behaviour of aminophylline suppositories in various fatty bases (49, 50, 51). Cieszynski (52), Brower *et al* (53) and Coben et al (54) attributed the increase in the melting

point of these suppositories to the decomposition products of the fats. The decomposition product of the fats was isolated and identified as the amide reaction products of ethylenediamine with glyceryl esters of fatty acids (triglycerides of C_{12}-C_{18} fatty acids). The presence of the amide causes the hardening phenomenon which induces an increase in the melting point of fatty suppositories and hence their inability to release the medicaments.

The rheological properties of a suppository can also affect the medicament release. Malati et al (55) showed that drug release from suppository bases is inversely related to the consistency of the bases. They measured consistency in terms of viscosity index. Most consistency tests are based on the resistance of the suppository to the increasing weight stress applied at room temperature (56).

The presence of adjuvants affect the rate of drug release and hence absorption from suppository bases. It has been shown that increasing the ionic strength in a microenema containing gentamicin, significantly enhanced the rectal absorption of the drug (57). The enhancing effect due to high ionic strength appeared to be additive to the enhanced rectal absorption effected by sodium

salicylate, a proven absorption adjuvant (58, 59). One of the two factors mentioned in the ionic strength enhancing effect on absorption of gentamicin was sodium transport (57). There is also sodium transport in adjuvant assisted rectal drug absorption. Fix et al (60) confirmed the above possibility. This may generally be applied to other water soluble compounds whose rectal absorption is enhanced by salicylate-type adjuvants (61, 62). The mechanism(s) by which active sodium transport affects rectal absorption of water soluble compounds is unknown, although the direction and magnitude of fluid movement is significantly affected by the sodium gradient (60). Regardless of the exact mechanism(s) involved, it is clear that active sodium transport is intimately related to rectal absorption systems. The regulation of sodium transport may provide a means of altering the permeability of the rectal mucosal cell barrier in a predictable manner. It may also afford a means of controlling adjuvant enhanced absorption of water soluble compounds.

Surface-active agents are one of the most important groups of adjuvants in pharmaceutical preparations. Their dispersing and emulsion-forming properties and their often times non-irritating properties are

advantageous features (30, 63). The widespread use of surfactants as well as their unique physico-chemical properties has elicited considerable interest in the possible influence of these agents on drug absorption. Enhancement as well as inhibition of the absorption and pharmacologic activity of drugs has been observed in the presence of surfactants. Eckert and Muhlemann (46) suggested that the speed of drug absorption from a medicated suppository base is increased when the drug is emulsified or suspended in a vehicle rather than dissolved. Studies of the administration of slightly water-soluble sulfonamides in suppositories (64) have shown that these drugs are absorbed more rapidly into the blood from water-soluble bases than from fat-soluble bases. It was also demonstrated that sodium sulfonamide gave the highest blood concentration when administered in a suppository based on a non-ionic emulsifier. Whitworth et al (65) further illustrated the usefulness of non-ionic surfactants in suppository formulations. Plaxo et al (66) reported that surfactants with HLB values of 11 to 14 showed greatest increase on the rate of absorption. The rate of absorption of 2, 4, 6, - triiodophenol is retarded by surfactants (66). Hydrolysis and subsequent solubilisation of the free phenol was postulated

to explain this effect. Above the critical micelle concentrations, nonionic surfactants decreased the rectal absorption of sulfonamides (67). Surfactants act by interaction with biologic membranes and modification of membrane permeability, interaction with the drug and interaction with the dosage form. These effects may be operative at the same time, some tending to enhance drug absorption, others tending to retard it. The net effect is dependent on the relative magnitude of each (68). Ayres et al (69) reported that surfactants act by increased solubilisation of the drug because of surfactant-drug interaction and hence a proportional increase in dissolution rate. They may cause emulsification (68) at the interface between the molten fatty bases and the aqueous body fluids. This leads to an increase in the interfacial area across which partition can occur and therefore an increase in drug release and absorption. The surface tension lowering and peptizing action of surfactants have been suggested as a mode of surfactant activity. Their cleansing action on the rectal mucosa surface makes additional pore spaces available for drug absorption, thereby facilitating drug movement across the rectal membrane barrier (36). Surfactants should, however, be used with caution.

Micellar concentrations decrease absorption of lipid soluble drugs. This is because the drugs become entrapped in the surfactant micelles and are thus unavailable for absorption. Rate of release of drugs from suppositories containing non-ionic surfactants remains unpredictable and should be determined for each drug in each base. Investigation carried out on this suggests that, erroneous conclusions may be reached when only a small number of surfactants have been investigated.

Quality Control Of Suppositories.

There are available many test methods applied to ensure that each manufactured batch of suppositories consistently meets the standards established during the manufacture of early experimental lots (16, 70). Finished suppositories are routinely inspected for appearance and, after slicing lengthwise, for uniformity of mix. They are assayed for active ingredients to ensure that each contains the labelled amount. Other parameters include liquefaction time and fragility tests. In vitro drug release study has been found a useful guide in the evaluation of suppositories. This provides information on the drug release characteristic.

The B.P. 1980 (71) states that when twenty suppositories are weighed singly and averaged, no suppository deviates from the average weight by more than 7.5%. Setnikar and Pietra (72) gave suggestions for weight uniformity specifications. On the other hand, Holm et al (73) reported that there is a great inter-dose variation with regards to the active ingredient without this appearing as weight variation. They concluded that weight control is not a satisfactory method for inter-dose control.

Liquefaction time test also called softening test is a measure of the time necessary for rectal suppositories to be completely melted when exposed to a closely simulated natural conditions. A number of devices have been suggested for its determination (54, 74, 75). Setnikar and Fantelli (16, 76) reviewed the proposed methods and suggested an improved test procedure. It was observed that for this test to be of any significance, results obtained should be compared with clinical experience.

An area of great importance in the quality control of suppositories is their content uniformity. This enables the formulator know if dosage form will deliver the specified amount of drug. Most literature report that suppositories contain not less than 90% and not more than

110% of labelled amount of drug. Problems concerning content uniformity control include analytical methods and statistical procedures chosen. The assay methods must be accurate, reliable, and specific as well as sufficiently sensitive (77). Setnikar and Fontani (17) stated that method variability interfers with assessment of actual content variability. This variation may lead to rejection of complying samples and acceptance of non-complying samples. They suggested that the evaluation and restriction of content variability be based on coefficient of variation.

Dissolution Rate Studies and Correlation Between In Vitro and In Vivo Results.

The dissolution rate of a drug from its solid dosage form gives an insight to bioavailability (78). This is because dissolution is usually the rate-limiting process in the absorption of poorly soluble drugs. Wagner (79) gave reasons for dissolution test as the need to ensure that a pharmaceutical product is essentially uniform from lot to lot, and to predict rate of absorption in man and/or availability of the drug for absorption. Such predictions, he pointed out, require careful correlation of in vivo and in vitro results.

Although it is not easy to design a test that will accurately mimic the conditions in the rectum, a large number of methods are available for the determination of drug release pattern from suppository bases (80, 81, 82). The techniques that have been in use include microbiological method (83, 84), chemical method (43), radio-active isotope technique (47) and dialysis through a membrane and subsequent spectrophotometric analysis (85). For this work, a modification of Cox (86) method is used. This method, apart from being devoid of the introduction of a rate-limiting step, as with dialysis, presents a constant area for spreading of the molten suppository. Roseman et al (87) summarised other available techniques. It is worthy of note that so far none of these techniques has proved totally satisfactory in correlating in vitro release with in vivo bioavailability. The technique chosen influences the dissolution rate profile obtained. This observation is confirmed by the report of Vidras et al (11). These test procedures, though based on unidentical working principles, tend toward attaining most of the mechanical and physico-chemical conditions operating during rectal absorption as outlined by Setnikar and Fantelli (16) and Ayres et al (69).

One basic problem in testing for drug release from a suppository is that the suppository softens, deforms,

melts or disintegrates during the test, exposing a variable interfacial area to the dissolution medium. Since the release rate is dependent on interfacial area, the variability of this factor leads to poor test reproducibility. A number of approaches have been used in a bid to solve this problem. Both membranes and relatively small bags have been used in restricting the area exposed to the dissolution fluid. This meant an introduction of an additional physical process, i.e. membrane transport, which complicates matters and may mask the real release characteristics for certain drug-suppository base combinations.

In vitro drug dissolution patterns have been used to predict in vivo drug availability. Cooper (88) has stated that with proper experimental design, a dissolution rate test may predict in vivo behaviour. Predictive conversion of in vitro drug dissolution data into in vivo drug response versus time profiles is illustrated with plasma levels of warfarin (89). Ayres et al (69) working with benzocaine found out that small differences detectable in vitro were not seen in vivo in rats, although substantial in vitro differences were correlated well with in vivo results. Vidras and co-workers' (11) attempt at correlating in vivo and

in vitro release of indomethacin from various bases proved unsatisfactory. They however obtained a good correlation during the first 45 minutes of experiment in a sequential order correlation. Kerckhoffs and Huizinga (90) established that in vitro drug release from cocoa butter base was not the same as the in vivo performance. Kin (2) observed that in vitro studies on aspirin and calcium acetylsalicylate varied but their in vivo results were the same. Neuwald and Kunze (91) concluded that absorption of drugs from suppositories can only be elucidated by in vivo experiments in humans. Neuwald and Ackad (92) stated that prevailing theories on rectal absorption of drugs in man require revision.

It can thus be deduced from fore-going discussion that there are differences of opinion as to the extrapolation of in vitro drug dissolution results to the in vivo performance of such a drug. In vitro solution rates can not be indicative of the total in vivo absorptive process. Similarly, one cannot assume that because a drug is absorbed by the rectum of an experimental animal, that quantitatively similar absorption will occur in man. While in vitro release pattern could be taken as an in-line quality control process, the ultimate test should be an in vivo test.

MATERIALS AND METHODS

Materials. n-Hexane (Merck) was used as supplied by manufacturers. The dry seeds of Irvingia gabonensis were locally purchased.

Extraction of Dika Fat.

The seed coats were scraped off and the contyledons coarsely powdered. The seed meal so obtained was soxhlet extracted using n-hexane. The extraction was effected for a period of 24 h. The solvent was removed using a rotary-vacuum evaporator. The solid concentrate was further air dried at room temperature. The yellow solid dika fat was obtained. The fat was bottled and stored at 3.5-4.5^{o}C throughout the period of investigation.

Suppository Bases.

The following chemicals were used as supplied by the manufacturers: cocoa butter (Nigerian Cocoa Ind. Ltd.); Dika fat, obtained as described earlier, olive oil (George Lockhart & Co., England); Tween 60 and Span 85(Fluka)

The bases investigated are as shown in Table I. Twelve blends were prepared by mixing different ratios of dika fat and olive oil on one hand; and dika fat, olive oil, Tween 60 and Span 85 on the other hand. The mixtures were melted on

Table 1: The Composition of Various Bases Produced with Dika Fat and Other Additives.

Blend	Composition % w/w		Melting Point Range °C
Base A	Dika fat	50	34.6-35.0
	Olive oil	50	
Base B_1	Dika fat	60	36.0-36.7
	Olive oil	40	
Base B	Base B_1	60	
	Tween 60	30	37.6-38.4
	Span 85	10	
Base C	Cocoa Butter	100	33.5-34.7

water bath at about 50°C and blended by stirring till cold. The blends were stored at 4°C for at least 24 h. before further investigations were performed on them. Based on their melting points and consistency, two of these blends were used in formulation as suppository bases.

The physico-chemical properties of these bases were studied. These included their capillary melting temperatures, iodine numbers, saponification and acid values. The methods used are as specified in the British standard of analysis of fats and oils (93).

Capillary Melting Temperature (CMT). This is the temperature at which a column of fat, of specified length, rises in an open capillary tube under the specified conditions of the test. The fat was melted at a temperature not higher than 10°C above the point of its complete fusion and mixed thoroughly by adequate shaking. Four uniformly thin-walled capillary glass tubes, open at both ends, of average internal and external diameters 1.2 and 1.6mm respectively and of length 50 to 60 mm were inserted into the fat so that columns of about 10 mm long arose in them. The tubes were quickly transferred and left in an ice chamber at 0°C for 24 h. The prepared tubes were attached to a thermometer of suitable range, subdivided at every 0.2°C, with rubber band. The column of fat coincided

with the thermometer bulb. The thermometer was then suspended by means of a clamp in the middle of a beaker containing 350 ml of boiled water maintained at 15°C. The lower end of the fat column was 30 mm below the water surface. Heat was applied at the rate of 1°C per minute while the water was agitated by means of a mechanical stirrer. The temperature at which the fat begins to rise in the tubes is the melting point.

Saponification Value. This is the number of milligrams of potassium bydroxide required to neutralise the fatty acids resulting from the complete hydrolysis of 1g of the fat.

Materials. These included potassium hydroxide pellets (Merck); 95% ethanol (Merck); phenolphthalein solution; olive and castor oils (George Lockhart & Co.) all of B.P. standards.

Method The titrant was 0.5 N HCl while the medium was ethanolic potassium hydroxide. Phenolphthalein was the indicator. Blank determinations were concurrently carried out by the same procedure. Saponification value S, was calculated by the following expression:

$$S = \frac{56.1\ (b-a)\ T}{M}$$

Where a = volume of HCl used for test portion (ml)

b = volume of HCl used for blank solution (ml)

M = mass of test portion (g)

T = normality of the HCl.

Three determinations were carried out for each test portion and the values for olive and castor oils were also determined.

Acid Values. The number of milligrams of potassium hydroxide required to neutralise the free fatty acids in 1g of the fat is the acid value. A 50 ml volume of 95% ethanol was heated to above 70°C and neutralised with 0.1 N potassium hydroxide using phenolphthalein as indicator. The neutralised ethanol was added to a suitable weight of test portion and titrated with potassium hydroxide solution.

$$\text{Acid value} = \frac{56.1\ VP}{M}$$

Where V = volume of potassium hydroxide solution used

P = normality of potassium hydroxide solution

M = mass of test portion.

The acid values of olive and castor oils were determined.

Iodine Number. This is the amount of halogen expressed as iodine absorbed by 100g of test portion under the conditions of the test. The iodine monochloride method was used.

Materials. Iodine Monochloride solution (Wijs reagent)

Iodine Trichloride	(Fluka)
Iodine	(Fluka)
Carbontetrachloride	(Merck)
Glacial acetic acid	(Merck)

Potassium iodide and sodium thiosulphate (Merck)

An 8g quantity of iodine trichloride was dissolved in about 200 ml acetic acid. Iodine, 9 g in weight was dissolved in 300 ml carbontetrachloride. Both solutions were then mixed and the volume made up to one litre with more acetic acid.

Method. An appropriate weight of test portion was placed into an iodine flask. A 15 ml portion of carbon tetrachloride and 25 ml iodine monochloride were added, the flask shaken and kept in the dark for 30 minutes. A 20 ml aliquot of 10% w/w potassium iodide was then

added. The liberated iodine was titrated with a 0.1 N sodium thiosulphate solution. Blank determinations were carried out under the same conditions. The iodine number was computed using the following expression.

$$\text{Iodine number} = \frac{12.69\ N\ (V-V_1)}{M}$$

Where M = mass of test portion (g)

V = volume of sodium thiosulphate used for blank titration

V_1 = volume of sodium thiosulphate used for test portion

N = normality of sodium thiosulphate

Iodine numbers of olive and castor oils were also determined.

Calibration Curves

Materials. These include chloroquine phosphate (Serva); aminophylline (Sigma) and aspirin (Merck).

Method All stock solutions were prepared with 0.1 N HCl. Stock solutions containing 3, 5 and 6 mg % of aspirin, chloroquine phosphate and aminophylline were respectively prepared and serially diluted with 0.1 N HCl. With 0.1 N HCl as reference sample and using quartz

cuvettes of 10 mm path length, the prepared solutions were scanned on the SP 8-400 UV/VIS Spectrophotometer (Pye Unicam). The wavelength for maximum absorbance was noted for each drug. This was 229, 257 and 272 nm for Aspirin, chloroquine phosphate and aminophylline respectively. Figs 1a, b and c show the plot of absorbance Vs concentration. The linearity of the plots and the fact that they passed through the origin indicated that the systems obeyed Lambert-Beer's law. This analytical method was thus considered suitable for monitoring the release of drugs into the dissolution medium.

Formulation of Suppositories.

Test suppositories contained 100 mg of the particular drug. Eight steel suppository moulds of nominal weight, 1 g were used. A mixture of the drug and an appropriate amount of base was melted in a water bath at the lowest possible temperature and mixed well. The amount of base to be incorporated was obtained by adopting the double casting procedure (2). In this method, the amount of drug for the specified number of suppositories was weighed and mixed with a quantity of base that was not sufficient to fill the moulds. This mixture was melted and poured into the moulds. The empty moulds were filled with the molten

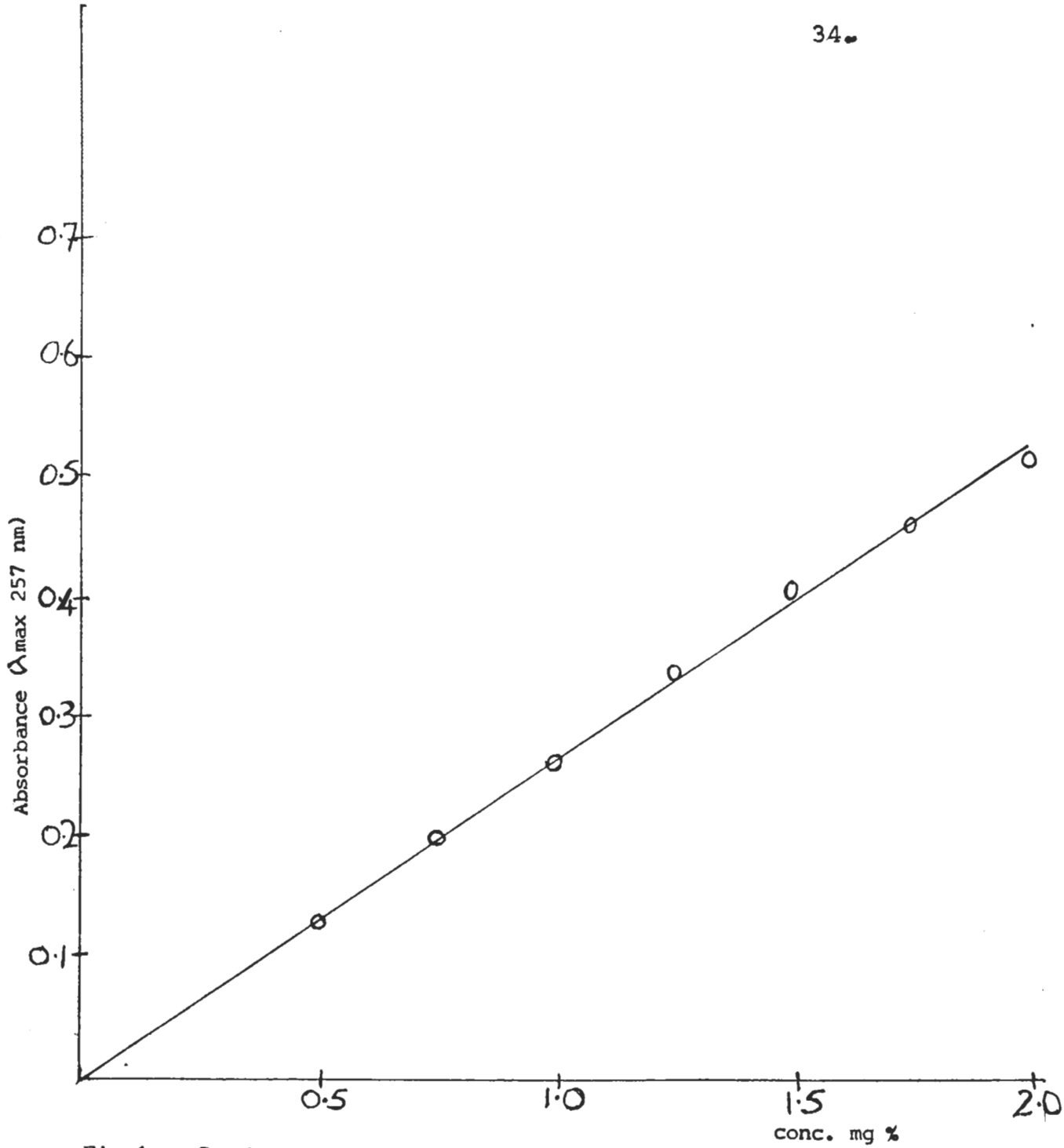


Fig 1 a. Graph of Absorbance Versus Concentration for Chloroquine Phosphate.

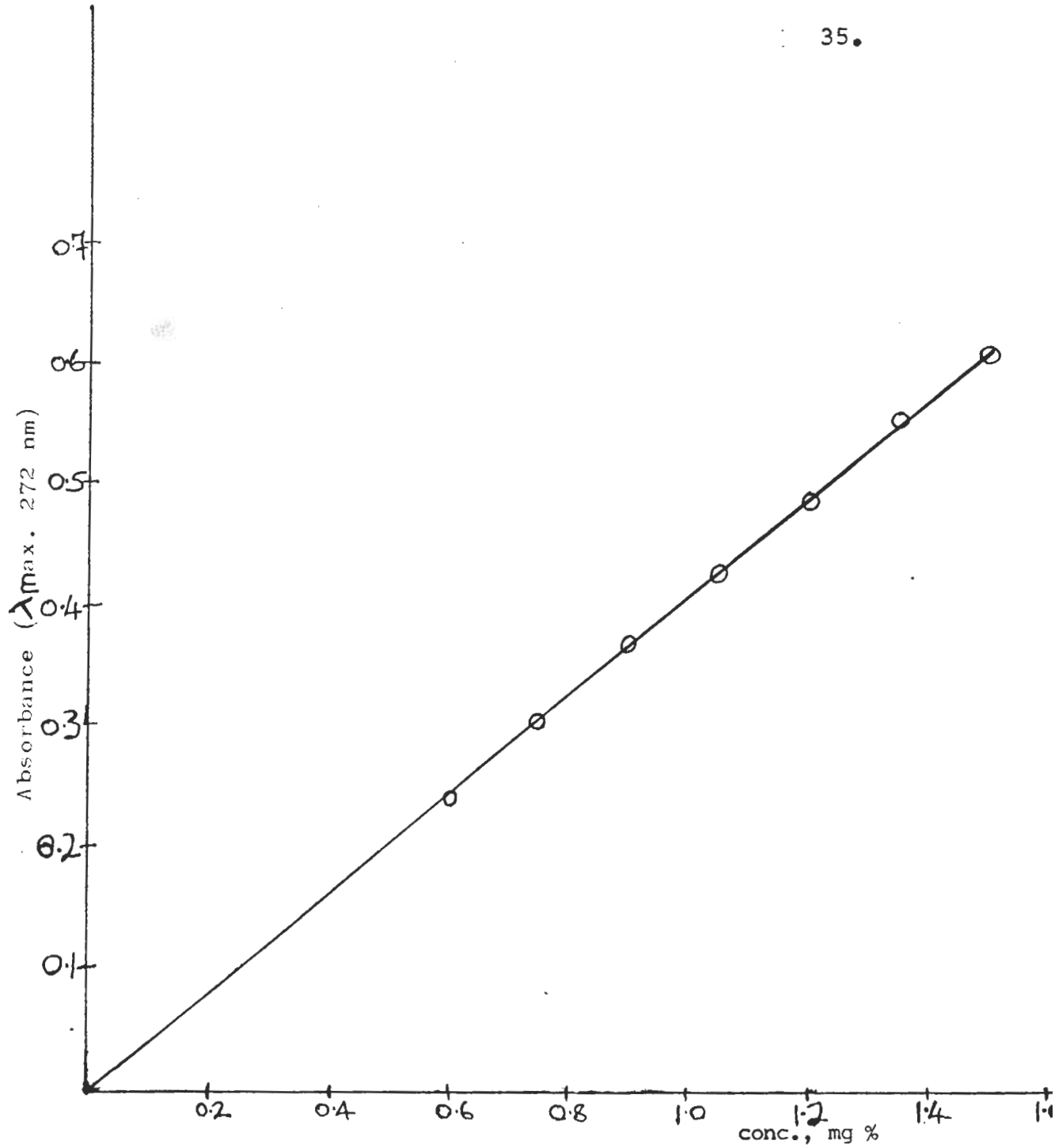


Fig 1 b. Graph of Absorbance Versus Concentration for Aminophylline

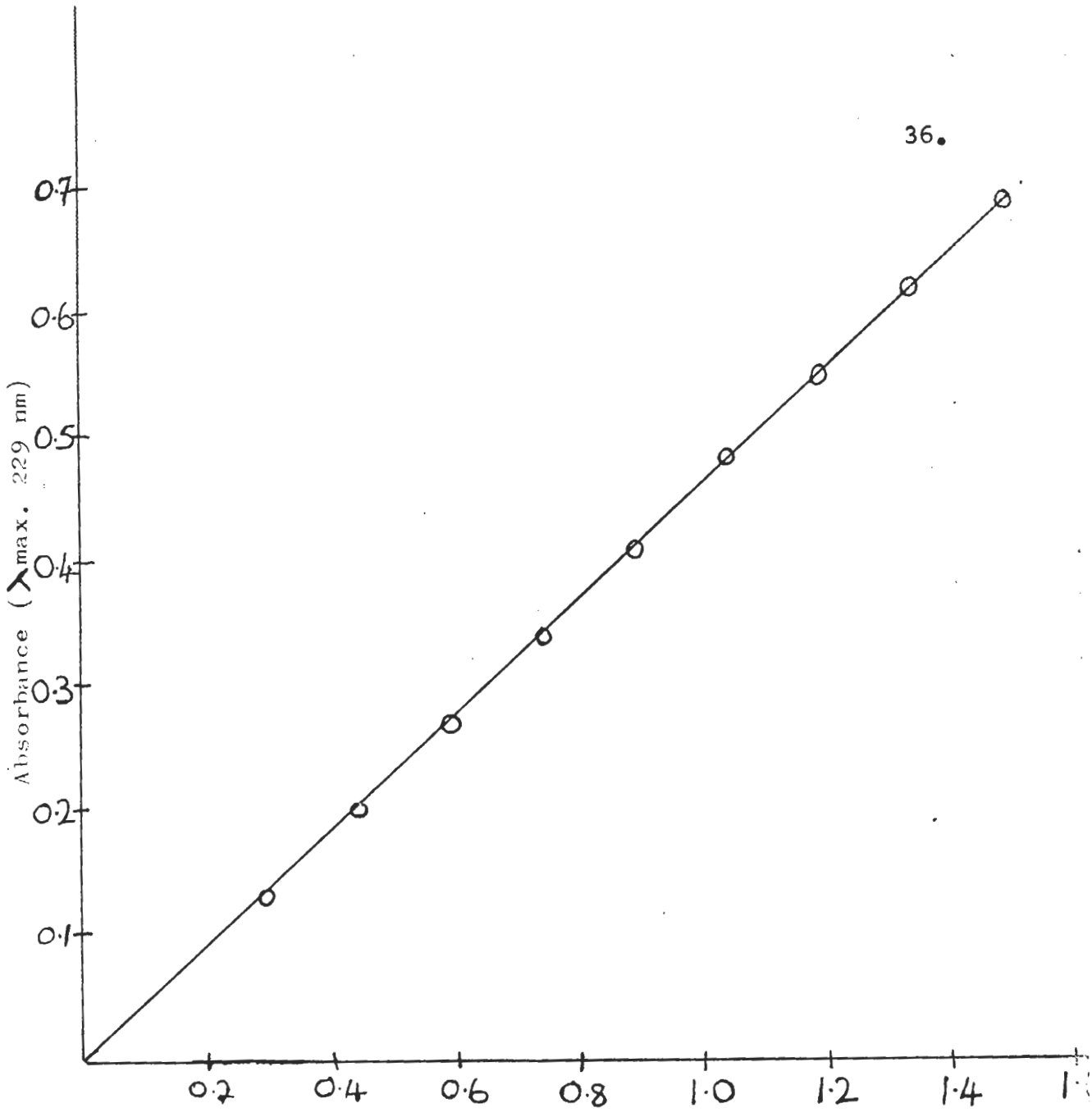


Fig. 1C. Graph of Absorbance Versus Concentration for Aspirin.

plain base. The suppositories were then allowed to set at 4°C. They were then brought out of the moulds, pulled together, remelted, remoulded in the steel moulds and cooled at 4°C. When theobroma oil was used, the moulds were lightly lubricated with liquid paraffin.

Drug Content Analysis.

From a mould of six suppositories, the second, fourth and sixth suppository were analysed for drug content. Each of these suppositories was melted in 500 ml of 0.1 N HCl in a water bath. A sample of 5 ml was diluted to 100 ml of 0.1 N HCl. It was found that the presence of surfactants in Base B interferred with the absorbance measurement of those drugs formulated with this base. This interference was eliminated by treating 10 ml sample withdrawn from the stock solution with 5 g of magnesium sulphate. The reaction mixture was shaken, allowed to cool and filtered. A 5 ml aliquote of the filtrate was then diluted to 100 ml of 0.1 N HCl. The electrolyte cracked whatever emulsion was formed by dehydrating the surfactants in the solution. The surfactants were thus salted out and were subsequently removed from solution by filtration. The absorbance was determined spectrophotometrically at the appropriate wave length using

0.1 N HCl as reference sample. By interpolation, the concentration of drug was calculated from the calibration curve prepared earlier.

The remaining suppositories were wrapped with aluminium foil and stored in brown glass bottles at room temperature (27-31°C). Some of the suppositories were stored at an average temperature of 4°C for one day to 12 weeks. Two hours after moulding, the suppositories were transferred to the appropriate storage temperature.

Dissolution Rate and Stability Studies.

Apparatus. Dissolution-Tester (Erweka Type DT-D; Model 50288).

Method. Drug release from the suppository bases was studied in a modified Cox (86) apparatus. The suppository was suspended in a wire mesh basket covered with a rubber bung. This was lowered into the dissolution chamber of the dissolution rate apparatus containing 900 ml of 0.1 N HCl maintained at a temperature of 37 ± 0.5°C. The paddle was adjusted to revolve at an average speed of 100 rpm. Samples of 10 ml were withdrawn from the dissolution medium at pre-determined time intervals. These were made made up to 100 ml with 0.1 N HCl in volumetric flasks. When

Base B was used, the withdrawn samples were treated with 7 g of magnesium sulphate, allowed to cool and filtered. The filtrate was then made up to 100 ml The volume of dissolution medium withdrawn in each case was replaced with 0.1 N HCl kept at 37 $\pm$ 0.5^{o}C. The absorbance was spectrometrically determined at the appropriate wavelength, using 0.1 N HCl as blank. From the calibration curve, the concentration and hence the percentage drug released at each time interval were calculated. Each release study was carried out in duplicate. The initial release study was undertaken 24 h. after moulding the suppositories. Subsequent studies were carried out after 1, 2, 4, 8 and 12 weeks storage under the appropriate storage conditions.

Stability studies were carried out by assaying two suppositories for their absolute drug content at each sampling time. In addition, the spectrum of the aqueous extract of freshly prepared suppository was obtained. This was compared with the spectrum similarly obtained at each sampling time.

RESULTS AND DISCUSSION

When subjected to exhaustive soxhlet extraction, the powdered seeds of Irvingia gabonensis yield between 60-65% fat. This high yield compensates for its fairly high cost of production. This underlines the potential commercial importance of dika fat.

Blends of Dika fat.

Dika fat **per se** has a firly high melting point range of 38-41^{o}C and as such, would not melt at body temperature. The production of blends characterised by melting temperatures close to body temperature thus becomes imperative. The blends in which drugs are incorporated should melt at or below body temperature. Therefore liquid oils such as olive oil are necessary additive for the lowering of the melting point. Surfactants find an entirely different function as solubilising agents. The surfactants employed in this work were Tween 60 and Span 85. The blend would thus be expected to release the drugs after dissolving in the dissolution medium. The choice of nonionic surfactants was based on thier relative lack of toxicity, stability and compatibility with excipients incorporated in the suppository base (30). Twelve blends were formulated

and evaluated in terms of their consistency and melting behaviours. Out of these, only two blends gave both suitable melting temperature ranges and workable consistency. In Table I (page 27) the proportion of additives and dika fat required to give various blends was presented. Most of the bases investigated were found to exhibit a high degree of hardening after one week storage at 4°C. Other blends gave an ointment base consistency at room temperature. The performance of Base B_1 which was made up of 60% dika fat and 40% olive oil was greatly improved by the addition of surfactants. After 120 minutes, Base B_1 gave only 37% and 30% release of chloroquine phosphate and aminophylline respectively. This is surprising considering that this base has a melting point range of 36-36.7°C. Smith et al (94) in their search for a reference partitioning system for drug design work, found that water-saturated olive oil attains very high emulsification properties. The olive oil forms a film round the drug particles thus making the drug unavailable to the aqueous phase. This film is broken by the added surfactants and hence the better performance observed with Base B. The high degree of water solubility and dispersibility imparted by the surfactant to the base further accounts for the improved