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Full Length Research Paper

Influence of fixed-oils in the dispersion of some water-insoluble antimicrobial compounds

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Ampicillin trihydrate, salicylic acid and griseofulvin were subjected to interphasal partitioning between an organic and aqueous phases formed from mixtures of sterile fixed-oils and distilled water. The fixed-oils used were groundnut oil, cotton-seed oil, vegetable oil and cod-liver oil. At each of the varying concentrations of the respective antimicrobial compounds, more molecules of each compound were found to have partitioned into organic (oily) phase than the aqueous phase. Based on physico-chemical and susceptibility studies report with *Staphylococcus aureus*, groundnut oil and cod-liver oil ranked better than cotton-seed oil and vegetable oil oils in their dispersion ability of the drugs. The results support the use of the local fixed-oils as suitable dispersion media in pharmaceutical oil-based preparations and susceptibility testing.

Key words: Interphasal partitioning, local fixed-oils, pharmaceutical oil-based preparations, suscepti-bility testing.

INTRODUCTION

Antimicrobial compounds and some other substances are often formulated to include solubilising or emulsifying agents as dispersants with a view to enhancing the solubility (Collet and Aulton, 1989) of the drugs in the body system. In *in vitro* tests, water-insoluble antibiotics are also often dissolved in certain organic solvents as diluents so that adequate molecular concentration may reach the target cells. For a drug to be effective on microbial cells, it must reach its target in sufficient concentration (Penn, 1977) essentially in solution form (Croshaw, 1983). The solubilisers or dispersants could be any of the cationic, anionic or non-ionic surface-active agents known as surfactants (Florence and Attwood, 1981; Aulton, 1988). The diluents include ethanol, acetone, solvent ether and dimethyl sulphoxide (DMSO) often with their own antimicrobial activity.

Metabolisable fixed-oils such as groundnut and soybean oils have been included in some pharmaceutical formulations, as dispersants. They include emulsions such as total parenteral nutrition (TPN), small-volume oily injections meant for depot effect and as a base in eye ointment (Allwood, 1983). However, the use of fixed-oils as dispersants in susceptibility testing is yet to be appreciated and embraced. Utilization of oils in this regard was reported by Adeleke (1991) following a favourable partitioning of some selected water-insoluble antibiotics into organic (oily) phase relative to aqueous phase. From the same study, a similar pattern of distribution for some microorganisms was reported (Osazuwa et al., 1999). Also, Adeleke and Agunbiade (1999) reported a higher antistaphylococcal activity for ampicilin trihydydrate when dispersed in a fixed oil than in water. It has, therefore, become necessary to involve more of local fixed-oils in interphasal separation of water-insoluble antibiotics between oil and water. This is with a view to making credible contribution towards the use of fixed-oils as dispersion media for such antibiotics during susceptibility testing.

MATERIALS AND METHODS

Sample collection

Cotton-seed (CSO), groundnut (GNO), vegetable (VO) and cod-liver (CLO) oils were obtained in local markets in Ibadan, Nigeria.

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Each oil was sterilized in 100 ml volume in partially opened MacCartney bottles at 160° C for 1 h, 30 min in an oven sterilizer (Gallenkamp). Ampicillin trihydrate (AT), griseofulvin (GR) and salicylic acid (SA) were also obtained locally. Each of them was used in four graded concentrations; 0.1, 0.05, 0.025 and 0.0125% prepared in serial dilutions.

Selection of wavelength

Taking into consideration the weights of active constituents and excipients in every drug, 0.1% (w/v) of each compound was prepared in sterile distilled water. With the water as blank, the absorbance peak of each solution was determined for the three drugs tested by scanning each solution through wavelength on UV-VS Spectrophotometer (SP8-400, PYE UNICAM).

Selection of blank

This was selected to nullify UV-absorbing contaminants from oil. Here, a mixture of 20 ml of each oil and distilled water was shaken up for 2 min in a 250 ml separatory funnel. It was then allowed to stay clamped on a retort stand for 30 min. The separated aqueous phase was then discharged into clean 25 ml universal bottle as oily water (o.w.) which served as blank in later determinations.

Distribution experiments

By 2-fold serial dilution, four aqueous concentrations of each antimicrobial compound tested were prepared; 0.1, 0.05, 0.025 and 0.0125% (w/v). Thereafter, 20 ml of each concentration as well as each of the fixed-oils was shaken up for 1-2 min as earlier stated followed by phase separation within 30 min. Using the oily water (o.w.) earlier obtained, the absorbance of each discharged aqueous phase was measured on a Spectrophotometer (SP8-400, PYE UNICAM) at the pre-determined wavelengths for the absorbance peak of each compound.

Table 1. Optical Density (OD) of 195 – 290 nm UV light absorbing substances in fixed oils.

Fixed oil	Wavelengths (nm) / OD (A)			
	195	**240**	**250**	**290**
GNO	2.67*	0.983	1.54	1.34
VO	2.645	1.895*	1.617	1.728*
CSO	0.890	1.036	0.944	0.855
CLO	2.130	1.700	1.820*	1.550

*Highest interference at the wavelength among the oils. GNO, groundnut oil; VO, vegetable oil; CSO, cottonseed oil; CLO, cod-liver oil.

RESULTS

Scanning the 0.1% aqueous concentration of every drug tested through various wavelengths produced the peaks of absorbance as 290 nm (griseofulvin, GR), 240 nm (salicylic acid, SA) and 250 nm (ampicillin trihydrate, AT). The oily water (o.w.) from each oil-water mixture gave ODs that showed the presence of UV-absorbing substances (Table 1). With oily water as blank, the ODs obtained for the aqueous phase from every fixed oil-aqueous drug mixture generally showed greater partitioning of the drug molecules in the oily phase (Table 2). The magnitude of partitioning varied with the drugs and oils as reflected in the following rankings in increasing order of dispersion ability:

For AT: GNO > CLO > CSO >VO
For SA: VO > CLO > GNO >CSO
For GR: CLO > GNO >CSO >VO

Partition coefficient (P) was calculated for every drug concentration brought into contact with a particular oil, after phase separation using the Nernst equation (Vogel, 1961):

$$P = \frac{C_o}{C_a}$$

Where C_o and C_a are the concentrations of drug in the organic (oily) phase and aqueous phase, respectively. C_a was determined using OD of the aqueous phase as OD_2 and OD of each drug in distilled water as OD_1 as follows:

OD_1 of n value contains a%

Therefore OD_2 of y value will contain

$$\frac{ay}{100n}$$

The partition coefficients (P) thus obtained reflected preferential dispersion of the drugs in fixed oils relative to water (Table 3). Susceptibility patterns of growth of *Staphylococcus aureus* in drugs dispersed in the different oil samples showed that with the same sample size, inhibition was noticed more in groundnut oil and vegetable oil than in the other oils (Table 4). However, better results were obtained with the paraffin used as standard while ampicillin trihydrate performed better than griseofulvin in any of the oils in terms of susceptibility studies.

DISCUSSION

In absorptiometry, a solution normally absorbs light of definite wavelength to the extent of the concentration of the molecules in solution (Vogel, 1961; Diamond and Denman, 1973). This study upholds the relationship in the different measurements of absorbance. One of such

Table 2. OD (A) ofaqueous concentration of drugs in relation to OD sepersted aqueous phase from oil-aqueous drug system.

Drug	OD (A) of aqueous drug concentration			
	0.1%	**0.05%**	**0.025%**	**0.0125%**
Aqueous drug concentration				
Aq.AT	3.913	3.702	3.225	3.214
Aq.SA	3.862	3.640	3.436	3.038
Aq.GR	2.800	1.600	0.900	0.390
Separated aqueous phase (oil-aq.drug)				
CSO-Aq.AT	2.424	2.162	2.078	2.0125
GNO-Aq.AT	2.093	1.860	1.682	1.650
PVO-Aq.AT	2.853	2.014	0.858	0.272
CLO-Aq.AT	2.853	2.014	0.909	0.612
CSO-Aq.SA	3.538	3.204	2.752	1.837
GNO-Aq.SA	2.947	2.540	2.206	1.329
PVO-Aq.SA	1.846	1.777	1.620	0.470
CLO-Aq.SA	2.226	1.920	1.773	0.840
CSO-Aq.GR	0.840	0.400	0.370	0.240
GNO-Aq.GR	0.550	0.470	0.430	0.210
PVO-Aq.GR	1.550	1.200	0.600	0.200
CLO-Aq.GR	0.870	0.800	0.420	0.050

GNO, groundnut oil; VO, vegetable oil; CSO, cottonseed oil; CLO, cod-liver oil; GR, griseofulvin; SA, salicylic acid; AT, ampicillin trihydrate; Aq, aqueous.

Table 3. Mean partition coefficient (P) of the drugs and grading of the oils for drug dispersion capacity.

Drug	Mean partition coefficient (P) x 10^2			
	CSO	GNO	VO	CLO
AT	1.61	2.27	1.27	1.75
SA	1.29	1.53	3.10	2.40
GR	2.03	2.02	1.53	3.1

GNO, groundnut oil; VO, vegetable oil; CSO, cottonseed oil; CLO, cod-liver oil; GR, griseofulvin; SA, salicylic acid; AT, ampicillin trihydrate.

Table 4. Susceptibility test of *S. aureus* in two antibiotics dispersed in the different oil samples.

Drug	Oil type / zone of inhibition (mm)				
	Groundnut oil	**Vegetable oil**	**Cottonseed oil**	**Cod-liver oil**	**Paraffin**
Ampicillin trihydrate	0.28*	0.22	0..26	0.20	0.32
Griseofulvin	0.22	0.18	0.22	0.20	0.28

*Each value is the mean of duplicate determinations.

measurements showed the presence in fixed-oils of UV light absorbing substances which had to be nullified before the actual measurements could be made of the drug molecules retrievable in the aqueous phase.

The favourable distribution of the drug molecules into the oily organic phase relates directly to the calculated varying high partition coefficients (P) for the drugs. These indicate high lipid solubility (Florence and Attwood, 1981) from a favourable hydrophobic interaction between the oils and water-insoluble drugs. Similar lipid-lipid interaction was reported by Rosenberg et al. (1980, 1982) in the adherence of microbial cells to certain hydrocarbons, while Brown (1975) reported a favourable uptake of polymyxin B by bacterial cells due to the same hydrophobic interaction. Consequently, this study has led credence to the use of oils of plant and animal origin as dispersion media for water-insoluble drugs in the preparation of some oil-based injections, total parenteral nutrition and also as media of interaction between bacterial cells and such drugs during susceptibility testing. Interestingly, Adeleke (1991) recorded a higher antibacterial activity for oil-dispersed ampicillin trihydrate than the water- dispersed drug. Similar report was also made against clinical isolates of *S. aureus* (Adeleke and Agunbiade, 1999). Furthermore, the organisms seem to follow the same sequence as observed for the dispersion of the drugs in the different oils, a result similar to that obtained by Adeleke and Agunbiade (1999).

In this study, cod-liver oil and groundnut oil ranked better than cotton seed oil and vegetable oil as media of dispersion. This work has added to the number of metabolisable fixed-oils suitable in pharmaceutical preparations and susceptibility testing. It is noteworthy also that any spectrophotometric measurements involving fixed-oils should take note of their UV light absorbing interfering substances as recorded in this study.

REFERENCES

Adeleke OE (1991). Fixed-oils as microbial dispersion and susceptibility testing media. An M.Sc. Thesis in the Department of Pharmaceutical Microbiol. University of Benin, Benin City, Nigr. p.172.

Adeleke OE, Agunbiade RA (1999). A fixed non-mineral oil in broth dilution and agar –diffusion for evaluating antibacterial activity of ampicillin trihydrate against sensitive strains of *Staphylococcus aureus* Afr. Biomed. Res. 2: 69-73.

Aulton ME (1988). The Science of dosage form design Churchill Livingstone. pp. 56-57.

Brown MRW (1975) . The role of cell envelope in resistance, In: Brown MRW. (ed.) Resistance of *Pseudomonas aeruginosa* 1st edn. John Wiley and Sons London New York Sydney. Toronto. pp. 71-79.

Collet DM, Aulton ME (1989). Solution of medicament of low water solubility. Pharmaceutical Practice Churchill Livingstone pp. 47-109.

Crowshaw B (1983). Evaluation of non-antibiotic antimicrobial agents In: Hugo WB, Russel AD (eds.) Pharmaceutical Microbiology 3rd edn. Blackwell Scientific Publications Oxford London. pp. 237-257.

Diamond PS ,Deman RF (1973). Laboratory techniques in Chemistry and Biochemistry 2nd edn. Buttersworth and Co. London Boston pp. 222-238.

Florence AT, Attwood D (1981). Physico-chemical Principles of Pharmacy. The Macmillan Press Ltd. London.

Osazuwa EO, Ahonkai I, Adeleke, OE (1999). Disribution of microorganisms between water and fixed-oils. W. Afr. J. Pharm. 13(1):?

Penn, RH. (1977). Pharmacology. Bailure Tindall. Rosenberg M, Gutnick D, Rosenberg, E (1980). Adherence of bacteria to hydrocarbons: a simple method for measuring cell surface hydrophobicity. FEBS Microbiol. Lett. 9: 29-33.

Rosenberg M, Romen S, Rosenberg E (1982). Cell-surface hydrophobicity of smooth and rough *Proteus mirabilis* strains determined by adherence to hydrocarbons. FEMS Microbiol. Lett. 13: 167-169.

Vogel AI (1961). A textbook of Quantitative inorganic analysis ELBS and Longman pp. 880-904.