Stability of Cough Linctus (Streptol) Formulated from Named Medicinal Plant Extracts

Maurice Iwu,[a] Christopher Okunji,[a] Michel Tchimene,[a] Ngozi Anele,[a] Kennedy Chah,[b] Uduma Osonwa,[c] Paul Achile Akpa,[c] and Godswill Chukwunweike Onunkwo*,[c]

[a] *International Centre for Ethno Medicine and Drug Discovery (InterCEDD); Nsukka, Enugu State, Nigeria;* [b] *Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Nigeria; and* [c] *Faculty of Pharmaceutical Sciences, University of Nigeria; Nsukka, Enugu State, Nigeria.*

Extracts of named medicinal herbs (*Garcinia kola*, *Zingiber oificinale*, *Aframonum melequeta* and *Ocimum viride*) were formulated into an antitussive preparation to alleviate cough. Some physical properties of the cough syrup formulation evaluated were: specific gravity, pH, viscosity, content uniformity, and shelf life. The specific gravity and viscosity of the formulations were stable on storage, with glycerin-based formulations having higher values. The pH of the formulation varied from 4.2 to 5.3 and was also stable on storage. Glycerin-based formulations had lower pH values. The total flavonoids content of Streptol was calculated based on GB1 and found to be 46 mg. The estimated shelf life of the Streptol cough syrup was 4.5 years.

Key words   Streptol; content uniformity; specific gravity; pH; shelf life

Since the dawn of civilization, humans have had recourse to medicinal plants as a means of providing relief for their illnesses.[1] The treatment of cough has traditionally been effected with linctuses. Most linctus contains ingredients with sedative, expectorant, and antimicrobial actions.[2] The vehicle usually utilized is syrup, which serves as a soothing material for the sore mucous membrane of the throat. Syrup B.P. is commonly employed as the flavoured vehicle for most linctus. This substance contains high concentrations of sucrose and provides a preservative effect owing to its high osmotic pressure.[3] However, linctuses containing water or aqueous solutions support microbial growth and may require the inclusion of a preservative.

Many medicinal herbs have been reported to possess antimicrobial, counter irritant, sedative, anti-inflammatory, and expectorant properties and could be employed in antitussive preparations to alleviate cough.[4] For instance, *Garcinia kola* has been reported to have anti-inflammatory, antimicrobial, and antiviral properties.[5] This herb is used in traditional medicine to cure chest colds and relieve cough. The seeds are used in the treatment of bronchitis and throat infections. Powerful antioxidative agents based on garcinoic acid isolated from *G. kola* have been shown to be responsible for these effects. The essential oil obtained from the seeds of *Aframonum melequeta* and the benzenoids components (paradol, gingerol, and shagaol) have been shown to possess antimicrobial and antifungal activities.[6] Roots of *Zingiber officinale* and leaves of *Ocimum viride* have been reported to possess antimicrobial activities.[7]

Formulation of a modern pharmaceutical liquid dosage form using medicinal herbs could have the following advantages: dosage precision, ease of dispensing, and an enhanced stability. These qualities are vital since herbal medicines have been widely criticized for lack of standardization.

This paper discussed the formulation of a cough linctus (containing extracts of *Garcinia kola* Heckel (family: Guttiferae), Ginger *Zingiber officinale* Roscoe (family: Zingiberaceae), *Aframonum melequeta* K. Schum (family: Zingiberaceae) and *Ocimum viride*—(family: Labiatae) and determi-

nations of some of its physical properties such as specific gravity, pH, viscosity, and content uniformity and shelf life.

Experimental

Materials   Sorbitol (Merck, Germany) and ethanol (95%) (M&B, England). Ethanolic extracts of the named medicinal plants were obtained in our laboratory.

Methods   The composition of the herbal extracts in 100 ml of syrup formulation is shown in Table 1. Ethanol (95%), simple syrup, sorbitol and glycerin were used to prepare the Streptol cough linctus formulations. Three cough linctus preparations were made; the compositions of the formulation ingredients in each preparation are as follows;

(a) Glycerin+ethanol (95%)
(b) Simple syrup+sorbitol+ethanol (95%)
(c) Simple syrup+glycerin+sorbitol+ethanol (95%)

Each of the preparations contained 10% ethanol to solubilize the ethanolic herbal extracts. Sorbitol was added in the formulations containing simple syrup to prevent crystallization of the sugar. Granulated sugar (66.7% (w/w)) and sorbitol powder (70% (w/w)) were used to prepare the simple syrup and sorbitol solutions, respectively.

Determination of Specific Gravity   Specific gravity was determined by finding the ratio of the mass or weight of the Streptol linctus that is equal to a measured quantity of water. This is expressed in the following mathematical relationship:

$$\text{specific gravity} = \frac{W_s}{W_w} \tag{1}$$

where W_s=weight of Streptol and W_w=weight of an equal volume of water.

Determination of pH   The pH of the Streptol formulations was determined using a digital pH meter (Model 7065, Electronic Machines Ltd., U.K.).

Determination of Viscosity   Determination of viscosity was carried out using a glass capillary viscometer. The procedure was based on measuring the rate of flow of the test liquid through the orifice of the viscometer[8] according to Poiseuille's law expressed as follows:

$$\eta\eta = \frac{\pi r^4 t P}{8L} \tag{2}$$

Table 1.   Composition of the Herbal Extracts in 100 ml of Syrup Formulation

Bitter Kola (*Garcinia kola*)–Seed–80 mg
Ginger (*Zingiber officinale*)–Fresh rhizome–10 mg
Grain of Paradise (*Aframomium melequeta*)–Seed–5 mg
Wild Basil (*Ocimum viride*)–Leaf–5 mg

Where, $r=$ the radius of the capillary tube, t is the time of flow, P is the pressure difference across the ends of the tube, L is the length of the capillary tube, and V is the volume of liquid; p_1 and p_2 are the densities of the dispersed phase and the dispersion medium respectively and $\eta=$Newtonian viscosity of the dispersion medium in poises.

Since the radius and length of the capillary tube as well as the volume of liquid flowing through the tube are constants for a given viscometer, then:

$$\eta=KtP \tag{3}$$

where $K=\pi r^4/8LV$.

The pressure difference, P, is dependent upon the density, p, of the liquid (in this case the Streptol formulation), the acceleration due to gravity, g, and the difference in heights of the two menisci in the two arms of the viscometer. Given that the values of g and the levels of the liquids are constants, these may be reflected in the following relationships relating the viscosity of an unknown and a standard liquid.

$$\eta_1=K^1t_1p_1 \tag{4}$$

$$\eta_2=K^2t_2p_2 \tag{5}$$

Thus when the flow times for two liquids are compared using the same viscometer, division of Eq. 4 by Eq. 5 gives

$$\frac{\eta_1}{\eta_2}=\frac{t_1p_1}{t_2p_2} \tag{6}$$

Determination of Content Uniformity The technique of chemical standardization was adopted in which the percent composition of active ingredients was calculated with reference to an authenticated sample.[9—11] These determinations were carried out by checking the absorbance of graded concentrations of Streptol syrup at the wavelength of 291 nm established as the wavelength of maximum absorption for the marker compound GB-1. The active drug in Streptol was calculated based on GBI, since *Garcinia kola* seed extract constitutes about 80% of the active drug. GBI is a standard flavonoid compound isolated from *Garcinia kola* seed. A pure sample of GBI was used to construct a Beer's plot.

Shelf Life Determination Shelf life determination was done based on standard methods.[9,12] Three relative humidity ovens were set at 30 °C, 50 °C, and 70 °C. The atmosphere inside each oven was maintained at 75% RH using saturated NaCl solution. The bottles containing the syrups were loosely kept open in the ovens. Samples were withdrawn at fixed time intervals and assayed. The experiment was carried out for 4 weeks.

The percentage of active drug remaining was calculated based on GBI. A plot of log percentage of active drug remaining (Log 100-Q) was made against time (d). The degradation rate constants at the various temperatures were determined. Log of the degradation rate constants (Log k) was plotted against the reciprocals of the corresponding temperatures (converted to Kelvins) (K) ($1/T \cdot 1000$), to obtain the degradation rate constant (k) at 25 °C. The shelf life (time for the active drug to degrade by 10%) was calculated from the equation $0.1052/k$. The shelf life at 30 °C was also calculated.

Results and Discussion

Results of the determinations of specific gravity are shown in Table 2. It can be observed that the formulations based on glycerin showed the highest specific gravity, which tends to vary between 1.2540 and 1.2537. This may be attributed to the high viscosity of glycerin. Formulations based on simple syrup and glycerin exhibited intermediate specific gravities whereas those composed of simple syrup showed the lowest values of specific gravity. These results suggest that the optimum vehicle for Streptol formulation may be a combination of simple syrup and glycerin since the specific gravity is adequate to guarantee free flow of the formulation through orifices.

Specific gravity can be derived from the ratio of the mass of a substance to the mass of an equal volume of another substance taken as a standard (normally water in the case of liquids and solids[13]). The measurement is dimensionless and only serves as an indicator of the density of the sample under

Table 2. Specific Gravity of Streptol Cough Linctus Formulated with Various Excipients after 6 Months of Storage

Time (months)	Simple syrup	Simple syrup + glycerin	Glycerin
1	1.1954 (7.79)[a]	1.2276 (9.81)	1.2543 (3.65)
2	1.1952 (3.37)	1.2276 (1.15)	1.2541 (4.10)
3	1.1952 (5.91)	1.2274 (1.63)	1.2540 (1.41)
4	1.1950 (1.40)	1.2272 (1.41)	1.2540 (5.34)
5	1.1948 (1.89)	1.2271 (2.91)	1.2538 (8.98)
6	1.1948 (5.35)	1.2271 (4.76)	1.2537 (2.31)

a) Values in bracket represent S.D. $\times 10^{-4}$.

Table 3. Viscosity of Streptol Cough Linctus Formulated with Various Excipients after 6 Months of Storage

Time (months)	Glycerin	Simple syrup	Simple syrup + glycerin
1	87.42 (3.65)[a]	49.55 (8.21)	62.50 (4.76)
2	87.30 (8.17)	49.38 (2.31)	62.35 (5.47)
3	87.10 (4.08)	49.15 (7.35)	62.22 (5.94)
4	86.95 (4.76)	49.00 (3.46)	62.10 (4.62)
5	86.85 (4.97)	48.89 (4.08)	61.95 (6.53)
6	86.70 (4.89)	48.70 (6.58)	61.85 (5.77)

a) Values in bracket represent S.D. $\times 10^{-2}$.

Table 4. pH of Streptol Cough Linctus Formulated with Various Excipients after 6 Months of Storage

Time (months)	Glycerin	Simple syrup	Simple syrup + glycerin
1	4.35 (3.65)[a]	5.31 (6.73)	4.50 (5.77)
2	4.35 (2.94)	5.30 (4.76)	4.52 (5.17)
3	4.36 (6.53)	5.29 (3.51)	4.55 (5.22)
4	4.37 (5.38)	5.28 (2.94)	4.56 (5.48)
5	4.38 (4.62)	5.28 (6.22)	4.57 (3.46)
6	4.38 (6.22)	5.27 (4.62)	4.59 (5.20)

a) Values in bracket represent S.D. $\times 10^{-2}$.

examination.

Results of the determination of the viscosity of the various formulations of Streptol are shown in Table 3. There was minimal lowering of the viscosity of the preparations over the period of storage in all the cases studied. Preparations containing glycerin alone exhibited the highest viscosity values ranging from 87.42—86.70 at the end of the 6 month storage period. Preparations containing simple syrup showed the lowest viscosity values (between 49.55—48.70) whereas the samples formulated with a combination of glycerin and simple syrup had viscosity values ranging between 62.50—61.85. From these findings, it might be said that the viscosity of the Streptol formulation is stable on storage.

The viscosity of a fluid is a reflection of its innate resistance to flow or movement under an applied stress. The viscosity of a liquid preparation may ultimately influence the availability of drug from its formulations. Other significant applications of the viscosity of fluids lie in the area of mixing, passage through orifices, packaging in bottles, and passages through hypodermic needles as well as the physical stability of disperse system.[3]

The results obtained from the pH study (Table 4) of the formulations indicate that the formulations made with glyc-

erin, simple syrup, and combinations of glycerin and simple syrup all had pH values between 4.3—5.3, which makes the medium slightly acidic, implying maybe that medicaments that might remain in solution at these pH values could be used in this formulation.

Since pH refers to the hydronium ion concentration of a solution, it is pharmaceutically significant in the determination of drug solubility, stability, activity, and absorption.[14] Modern medicaments are often salts of weak acids and bases. Some of these salts are usually soluble in water while most of the unionized acids and bases are practically insoluble. If a solution of a salt of a weakly basic drug is made alkaline, the free base may be precipitated, while precipitation of free acid may occur if a solution of a weakly acidic drug is acidified. The likelihood of precipitation occurring or not depends on the solubility of the unionized acid or base, the pH of the solution, and the pK_a of the acid or base.

Since *Garcinia kola* extract constitutes about 80% of the active principles in the formulation, its total flavonoid content was calculated based on GB-1[15] and was found to be 46 mg/100 ml.

The shelf-life of Streptol cough syrup was calculated using accelerated temperature stability studies, employing Arrhenius equation, which shows a quantitative relationship between the specific reaction constant (K) and temperature (T).[16] The equation can be used to determine the stability of a formulation, predict shelf life, and establish optimal storage conditions.

Arrhenius showed that the variation with temperature of the rate constant of chemical reactions could be expressed by the equation:

$$k = se^{-E_a/RT} \tag{7}$$

where k is the rate constant, s is the frequency factor, E_a is the activation energy, R is the gas constant, and T is the absolute temperature. In the logarithmic form, the Arrhenius equation becomes:

$$\mathrm{Log}\, k = \mathrm{Log}\, s - E_a/2.303RT \tag{8}$$

If the equation is valid, a straight line is obtained on plotting log k against the reciprocal of the absolute temperature.

The first order plots (Fig. 1) were linear and the degradation constants at the various temperatures were calculated from this plot. The plot of log K against $1/T$ also yielded a straight line (Fig. 2) and was used to obtain K at room temperature. The value of K_{25} was calculated to be $6.2356 \cdot 10^{-5}$ giving a shelf life of about 4.5 years, which is the time for a drug to decompose to 90% of its original concentration at 25 °C. The shelf life at 30 °C was also found to be approximately 3 years.

In a related research study, capillary electrophoresis (CE) was used in the quantitative analysis of *Garcinia kola* biflavonoids in Hepa vital tea and Hang over tonic formulations. The use of the CE provides a sensitive, simple, and quantitative method that simultaneously quantifies the 4 major biologically active biflavonoids of *G. kola* (GB1, GB2, and GB1, glycoside and kola flavonone).[17]

Conclusion

The results obtained in this study demonstrate that stable cough linctus could be formulated with active principles
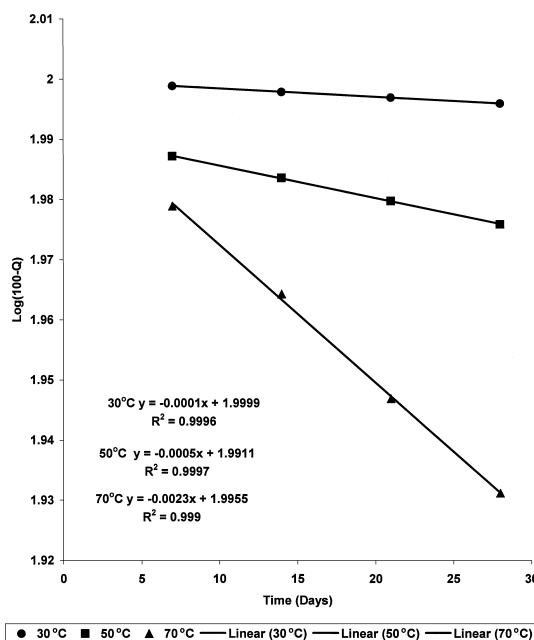

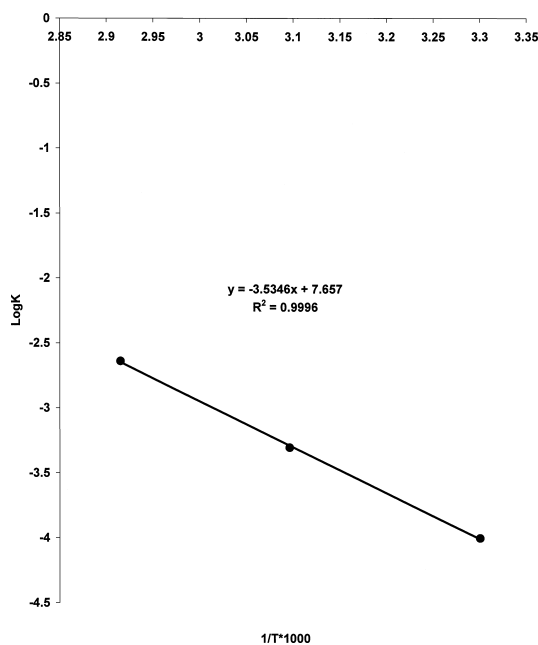Fig. 1. First Order Plots of Streptol Syrup Stored at Different Temperatures (°C)

Fig. 2. Plots of Degradation Constants of Streptol Syrup on Storage at Different Temperatures

from the named herbal extracts. The product could be assayed spectrophotometrically using GB1 as a marker compound and its shelf life determined.

References
1) Schuster B. G., Jackson J. E., Obijiofor C. N., Okunji C. O., Milhous W., Losos E., Ayafor J. F., Iwu M. M., *Pharmaceutical Biol.*, **37**, 84—99 (1999).
2) "Dispensing for Pharmaceutical Students," 12th ed., ed. by Carter S. J., Churchill Livingstone, Edinburgh, 1987, p. 78.
3) Aulton M. E., "Pharmaceutics, The Science of Dosage Form Design," 1st ed., Churchill Livingstone, Edinburgh, 1988, p. 254.
4) Ampofo O., "African Medicinal Plants," ed. by Sofowora E. A., University of Ife Press, Ile Ife, 1999, p. 67.

5) Iwu M. M., Igboko O. A., Onwuchekwa U. A., Okunji C. O., *J. Ethnopharmacol.*, **21**, 127—138 (1987).

6) Iwu M. M., Duncan A. D., Ononiwu J., Okunji C. O., "Herbal Medicinal Products used for HIV/AIDS," BDCP Press Silva Spring, 2003, p. 53.

7) Iwu M. M., Duncan A., Okunji C. O., "Perspectives on New Crops and New Uses," ed. by Janik J., ASHS Press, Alexandria, 1999, pp. 457—462.

8) Schott H., "Remington's Pharmaceutical Sciences," 16th ed., ed. by Osol A., Mack Publishing Company, 1980, pp. 323—339.

9) Onunkwo G. C., Akah P. A., Udeala O. K., *Phytother. Res.*, **10**, 418—420 (1996).

10) Onunkwo G. C., Egeonu H. C., Adikwu M. U., Ojile J. E., Olowosulu A. K., *Chem. Pharm. Bull.*, **52**, 649—653 (2004).

11) Onunkwo G. C., *West Afr. J. Pharm.*, **19**, 45—52 (2006).

12) Onunkwo G. C., Akah P. A., Udeala O. K., *Acta Pharm.*, **48**, 63—69 (1998).

13) Martin A. N., Swarbrick J., Cammarata A., "Physical Pharmacy," 2nd ed., Henry Kimpton Publishers, London, 1969, pp. 499—501.

14) Ravin L. D., "Remington's Pharmaceutical Sciences," 16th ed., ed. by Osol A., Mack Publishing Company, 1980, pp. 1364—1980.

15) Terashima K., Takaya Y., Niwa M., *Bioorg. Med. Chem.*, **10**, 1619—1625 (2002).

16) Methods of Stability Predictions Presented at the Manufacturing Controls Seminar, ed. by Carstensen J. T., Proprietary Association /FDA, Cherry Hill, New Jersey, 1971.

17) Okunji C. O., Wave T. A., Hicks R. P., Iwu M. M., Stanchy D. J., *Planta Med.*, **68**, 440—444 (2002).