TABLE 1: ANALYSIS OF TIZANIDINE HYDROCHLORIDE TABLETS.

Tablet Formulation	Label Claim (mg/tab)	Amount found (mg/tab)	% of label claim* ±standard deviation	Standard Error	% Recovery*
Sirdalud (Novartis)	2	1.98	99.0±0.71	0.006	99.9
Tizan (Sun Pharma)	2	2.02	101±0.27	0.002	99.5
Tizpa (Blue Cross)	2	2.04	102±0.65	0.006	99.3

*Mean of five determinations.

used for the routine analysis of tizanidine hydrochloride in bulk drug and formulations.

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REFERENCES

1. Budavari, S., Eds., In; The Merck Index, 12th Edn., Merck and Co., Inc., White House Station, NJ, 1996, 1618.
2. Parfitt, K., Eds., In; Martindale, The Complete Drug Reference, 32nd Edn., The Pharmaceutical Press, London, 1996, 1322.
3. Reddy, M.N., Murthy, T.K., and Sankar, D.G., **Eastern Pharmacist.**, 2001 ,44,121.

1,1'-Oxy Bis[(4-Methoxy Cinnamidopropyl Dimethyl Ammonio) Ethane] Dichloride, a New Photoprotective Agent for Personal Care

N. M. KOSHTI*, A. H. JAWALE, B. B. PARAB, S. D. NAIK, M. M. MOGHE, T. S. JADHAV AND S. S. NASHTE

Galaxy Research Centre, Galaxy Surfactants Ltd.,
C-49/2, TTC Ind'l Area, Pawne, Navi Mumbai–400 703.

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The synthesis and photoprotection efficacy of a new water-soluble photoprotective agent, 1,1'-oxy bis[(4-methoxy cinnamidopropyl dimethyl ammonio) ethane] dichloride is reported. This non-irritating hydrolytically stable UV-absorber is substantive to both skin and hair due to its cationic nature. It can be easily formulated into a variety of hair and skin care formulations.

Harmful effects of solar UV-radiation on skin and hair are well documented[1-4]. Over-exposure to UV rays is the most common cause of skin cancer. The problem is becoming more acute with continuous depletion of ozone layer. The damage to blond hair is significant too! Solar UV-radiation makes hair brittle, rough and difficult to comb. The human hair has been shown to lose the tensile strength as a result of cleavage of disulphide bond of hair keratin upon exposure to UV-radiation.

Today's consumer is very well aware of the need for photoprotection to both skin as well as hair. The conventional UV-absorbers such as derivatives of salicylic acid, benzophenones, benzotriazoles, cinnamic acid, amino benzoic acid are not substantive to skin and hair. Neverthe-

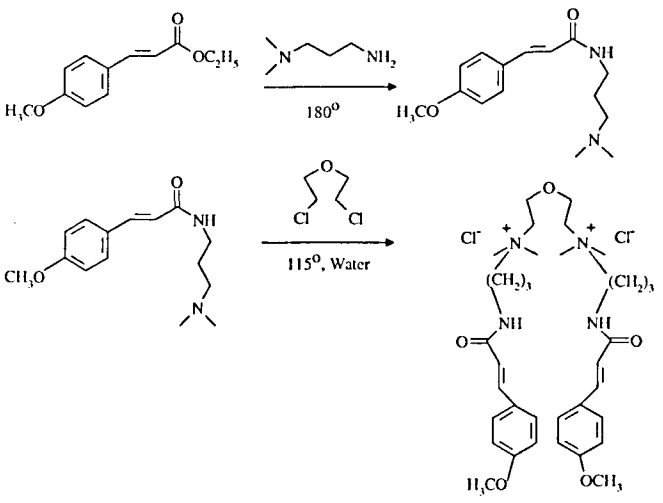
*For correspondence
E-mail: nirmal.koshti@galaxysurfactants.com

less, they can be rendered substantive by either anchoring UV-absorbing unit on to a polymeric backbone or by introducing UV-absorbing chromophore into cationic portion of the molecule. Both approaches have been successfully employed[5-7].

Here, we report synthesis and properties of new substantive *bis*-quaternary ammonium salt, 1,1¢-oxy bis[(4-methoxy cinnamidopropyl dimethyl ammonio) ethane] dichloride[8]. The excellent substantivity of this water-soluble UV-absorber is achieved through two quaternary ammonium centres.

The synthesis of this *bis*-quaternary ammonium compound was accomplished in two steps as shown in the scheme. Ethyl *p*-methoxy cinnamate was reacted with a bifunctional amine, *N,N*-dimethyl propyl diamine to get the corresponding *p*-methoxy cinnamidopropyl dimethyl amine. The tertiary nitrogen of the latter was then alkylated using *bis*(2-chloroethyl) ether. Both high yielding steps of this reaction sequence were conveniently carried out under pressure using stoichiometric quantities of reactants. The quaternisation was performed in aqueous medium and the progress of the quaternisation was easily followed by estimating liberated chloride.

The NMR spectra were recorded at Regional Sophisticated Instrumentation Centre, Indian Institute of Technology, Mumbai, India. Skin and mucous membrane irritation and cumulative dermal irritation analyses were performed by National Toxicology Centre, Pune. Phototoxicity tests were performed by Vimta Labs Ltd., Hyderabad and mutagenicity study was done by Institute of Toxicological Studies, Pune.



Scheme 1

The sythesis of the title compound was achieved in two steps. *p*-Methoxy cinnamidopropyldimethylamine was sythesised from ethyl *p*-methoxy cinnamate and *N,N*-dimethylpropylamine and follows. Ethyl *p*-methoxy cinnamate (206 g, 1.0 mol), *N,N*-dimethylpropylamine (102 g, 1.0 mol) and sodium methoxide (2.0 g) were charged in a pressure reactor. The air inside the reactor was flushed out by purging of nitrogen. The reaction mixture was then stirred at 180° (this generated pressure of 5 kg/cm²) for 36 h. The progress of reaction was monitored by disappearance of ethyl *p*-methoxy cinnamate on chromatography (TLC and HPLC). The TLC was performed on aluminium coated silica gel plates (Merck - 60-F-254) and viewed with a UV lamp at 254 nm. HPLC was performed using reversed phase technique on a C-18 bonded (octadecyl silane) column and 60 % aqueous methanol as mobile phase (1.0 ml/min) and detection at 280 nm. The crude pale yellow solid thus obtained was crystallised in methanol to give the amidoamine as off-white needles (226 g, 86 %) with m.p. 80°. Molar extinction coefficient, ϵ, in methanol was found to be 26,600 at 290 nm. Elemental analysis: Calculated for $C_{15}H_{22}O_2N_2$, C, 68.70 %; H, 8.40 %. Found: C, 69.00 %; H, 8.80 %. IR in dichloromethane showed carbonyl stretching of amide at 1660 cm^{-1} and NH stretching at 3300 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 1.73 (p, 2H, J = 6.6 Hz), 2.26 (s, 6H), 2.42 (t, 2H, J = 6.6 Hz), 3.45 (q, 2H, J = 6.0 Hz), 3.81 (s, 3H), 6.27 (d, 1H, J = 15.6 Hz), 6.86 (d, 2H, J = 8.7 Hz), 7.43 (d, 2H, J = 8.7 Hz), 7.53 (d, 1H, J = 15.6 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ 26.64, 39.15, 45.05, 55.27, 58.28, 114.22, 119.27, 127.80, 129.23, 139.70, 160.74 and 166.47.

p-Methoxy cinnamidopropyl dimethyl amine thus obtained was quaternised by *bis*-(2-dichloroethyl)ether. *p*-Methoxy cinnamidopropyldimethylamine (263 g, 1.0 mol) and *bis*-(2-dichloroethyl)ether (71.5 g, 0.5 mol) and deionised water (334.5 ml) were charged in a pressure reactor. The air inside was flushed out by purging nitrogen. The reaction mixture was then stirred at 115° (pressure 3 kg/cm²) for 36 h. The progress of the reaction was followed by analysing the chloride content. The reaction yielded 669 g of product as light amber colored liquid with slight cinnamom like odor, pH 7.5, chloride content of 5.2 % and solids 50 %. Removal of water afforded off-white, sticky, hygroscopic solid. Elemental analysis: Calculated for $C_{34}H_{52}O_5N_4Cl_2$, C, 61.16 %; H, 7.79 %. Found: C, 60.71 %; H, 7.43 %. The molar extinction coefficient, ϵ in aqueous solution at 292 nm λmax was found to be 42,729. ^1H NMR (300 MHz, D_2O): δ 1.98 (m, 4H), 3.11 (s, 12H), 3.29 (t, 4H, J = 6.3 Hz), 3.39 (m, 4H), 3.60 (improperly resolved triplet, 4H), 3.71 (s, 6H), 3.94 (improperly

resolved triplet, 4H), 6.27 (d, 2H, J = 15.6 Hz), 6.84 (d, 4H, J = 8.7 Hz), 7.29 (d, 2H, J = 15.9 Hz), 7.38 (d, 4H, J = 9.0 Hz). Hereafter, the bis-quaternary ammonium salt, 1,1'-oxy bis[(4-methoxy cinnamidopropyl dimethyl ammonio) ethane] dichloride will be referred to as BisQuat.

BisQuat was found to be non-irritant to skin and mucous membrane. Mucous membrane irritation potential was determined by conducting Draize test using 2.0 % aqueous solution. In fact, it was found to be totally non-irritant even in cumulative dermal irritation test wherein a cream containing 4.0 % of BisQuat was applied to shaved skin of rabbit for 21 d. Reverse mutation assay in *Salmonella typhimurium* was performed to test mutagenic potential. The bacterial cells were exposed at doses 5 to 100 μg/plate with and without metabolic system. There was no evidence of mutagenicity at any dose level in any strains of *S. typhimurium* (TA 97a, TA 98, TA 100, TA 102 and TA 1537). Since this being an UV-absorbing molecule, the phototoxicity tests were conducted to know light induced dermal irritation and light induced delayed contact hyper sensitiviy in albino guinea pigs (NIH – H strain) using UV-radiation and 8-methoxypsoralen as positive control. It exhibited no evidence of photo-irritation and photo-allergy in experimental guinea pigs. All the protocols adopted here for toxicity studies were approved by Institutional Animals Ethics Committee.

Substantivity of BisQuat to hair was measured using a procedure described by Vanemon[9]. Hair strands (5.0 g) were washed with 10 % sodium lauryl sulphate solution and rinsed with plain water. The hair tresses were treated with shampoo containing 2.0 % BisQuat. After the treatment the tresses were washed thoroughly with copious amount of water. The adsorbed quaternary was extracted from the hair surface by immersing them in isopropanol at 65° for 30 min. A known volume of this isopropanol/BisQuat mixture was analysed by UV-spectroscopy. The substantivity of BisQuat to hair was found to be 40 mg/100 g of hair (average of 10 measurements).

Substantivity to skin was measured through a bathing bar preparation. A soap containing 2.0 % BisQuat was applied on the inner side of forearm (hairless or shaved) on specified area (36 cm^2). It was then washed off by holding forearm under tap water. The deposited sunscreen was re-extracted with cotton swabs soaked in absolute ethanol. The volume of alcohol extract was made up and analysed spectrophotometrically. The substantivity of BisQuat to skin was found to be 6.0 μg/cm^2 of skin (average of 10 measurements). The substantivity of BisQuat to hair was found to be atleast three times higher when compared with conventional water-soluble UV-B absorber diethanol ammonium *p*-methoxy cinnamate, CAS No. 56265-46-4. Similarly, the substantivity of BisQuat to skin through soap was found to be two times of that of diethanol ammonium *p*-methoxy cinnamate.

Photoprotection efficacy of BisQuat on skin was evaluated using Mexameter that measures melanin content of human skin. (This instrument is manufactured by Courage Khazaka electronic GmbH, Mathias-Bruggen Strasse 91, B-50829, Koln, Germany). Marked area (36 cm^2) of inside of forearm (hairless or shaven) was washed with transparent bathing bar containing 2.0 % of BisQuat and rinsed off with copious amount of water. The treated and untreated portions of skin were then exposed to intense sun rays of mid-noon for 30 min. The melanin content (average of 10 measurements) was then measured using Mexameter and the reduction in melanin generation was taken as measure of efficacy of photoprotection to skin.

The melanin content of the untreated skin before exposure was 452 Mexameter units whereas for the skin treated with soap containing 2.0 % BisQuat, it was 453. After exposure to solar radiation for about 30 min, the melanin content for the untreated increased to 470 whereas for the treated skin went to 459. The melanin content readings were average of 20 measurements. Thus, the net increase in melanin content for the untreated skin was 18 Mexameter units as against 6 for the treated skin. Thus, it is evident that photoprotection through bathing bar containing 2.0% of BisQuat is about three times in 30 min exposure.

Thus, BisQuat was prepared as 50 % aqueous solution. It showed very high UV-absorbing properties with ϵ of 42,729 at 292 nm. It was found to be very stable molecule both photolytically as well as hydrolytically. The *bis*-quaternary salt showed very safe toxicity profile. It was found to be non-irritant to skin and mucous membrane. It was found to be non-phototoxic and non-mutagenic in reversed mutation assay performed on six strains of *Salmonella typhimurium*. It exhibited very high levels of substantivity to hair (40 mg/100 g of hair) and skin (6.0 μg/cm^2 of skin) through rinse-off preparations like shampoo and soap containing 2.0% of the *bis*-quaternary compound. This high substantivity resulted in excellent photoprotection of skin as demonstrated by the measurement of melanin content after a short exposure of skin to sunlight. It was found to be compatible with commonly used cosmetic ingredients. Due to its very good water-solubility it can be formulated in non-greasy skin care preparations. Its high substantivity makes it suitable for com-

positions aimed at protecting skin from solar radiation in vigorous activity like swimming as well as for wash-off cosmetic preparations like soaps, shampoos, face washes etc.

REFERENCES

1. Rieger, M.M., **Cosmetics & Toiletries**, 1987, 102 , 91.
2. Taylor, L., **Skin Cancer Foundation Journal**, 1986, 4, 90.
3. Beyak, R., Kass, G.S. and Meyer, C.F., **J. Soc. Cosmet. Chem.**, 1971, 22, 667.
4. Hoting, E., Zimmermann, M. and Hocker, H., **J. Soc. Cosmet. Chem.**, 1995, 46, 85.
5. Gallagher, K.F. and Pereira, A.G., **US Patent No. US 5,601,811**, 1995.
6. Chaudhari, R.K., Alexander, A. and Gripp, A.A., **US Patent No. US 5,427,773**, 1995.
7. Sovak, M., Terry, R.C., Douglass, III J.G. and Bakir, F., **US Patent Nos. US 5,487,885**, 1996 and **US 5,741,924**, 1998.
8. Koshti, N.M., Jawale, A.H., Parab, B.B., Naik, S.D., Moghe, M.M., Jadhav, T.S. and Nashte, S.S., **US Patent No. US 6,426,435**, 2002.
9. Vanemon, P., **Cosmetics & Toiletries**, 1998, 113, 77.

Spectrophotometric Determination of Ornidazole and Norfloxacin in Tablets

U. N. KALE, K. R. NAIDU, AND M. S. SHINGARE*

Department of Chemistry, Dr. B. A. M. University, Aurangabad-431 004.

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Two simple spectrophotometric methods for the determination of ornidazole and norfloxacin in pharmaceutical preparations have been developed. First method is based on simultaneous equations. In the second method, derivative spectroscopy is used to eliminate spectral interference. Both drugs obey Beer's law in the concentration range employed for the analysis. The results of analysis have been validated statistically and by recovery studies.

Norfloxacin, 1-ethyl-6-fluoro-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid is used as antibacterial[1]. Its methods of analysis are given in USP[2], IP[3] and BP[4]. Further literature survey revealed some more methods for its estimation from pharmaceutical preparations and includes spectrophotometry[5-9], HPLC[10] and HPTLC[11]. Ornidazole, 1-(3-chloro-2-hydroxypropyl)-2-methyl-5-nitroimidazole is used as an antiinfective[12]. Literature survey describes spectrophotometry[13-16] and pH metery[17] methods for its determination from pharmaceutical preparations. There is no single method for simultaneous determination of ornidazole and norfloxacin from pharmaceutical preparations.

A PC-based JASCO V-560 UV/Vis spectrophotometer with 10 mm matched quartz cuvettes was used for the experimental purpose. Sodium hydroxide of analytical reagent grade and double distilled water were used. Ornidazole and norfloxacin were obtained as gift sample from M/s Aristo Pharmaceuticals (P) Ltd., Bhopal. A combination of both these drugs, ornidazole (500 mg) and norfloxacin (400 mg) in each tablet, is marketed by Mankind Pharma (Noragyl-OZ).

Standard stock solutions of ornidazole, 100 μg/ml and norfloxacin, 100 mg/ml were prepared separately in 0.1 N sodium hydroxide solution. Each stock solution was suitably diluted to different concentrations and linearity was studied. Linear relationships were observed in the range 2–20 μg/ml for ornidazole and 1–10 μg/ml for norfloxacin. Sample stock solution was prepared by crushing 20 tablets to fine powder. Powder equivalent to 10 mg of ornidazole and 8 mg of norfloxacin was dissolved in 50 ml of 0.1 N sodium

*For correspondence