law limits), % range of error (0.05 to 0.01 confidence limits) were calculated for all the methods and the results are summarized in Table 1. The values obtained for the determination of SLD in several pharmaceutical formulations (tablets) by the proposed and reported methods are compared in Table 2. Interference studies revealed that the common excipients and other additives usually present in the dosage form did not interfere in the proposed methods. In conclusion the proposed extractive spectrophotometric methods for the estimation of SLD are simple, sensitive, cheap, accurate and may found useful in the routine quality control analysis and quantitative determination of sildenafil from its pharmaceutical preparations.

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Antimicrobial Activity of Newly Synthesized Organic Complexes

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Halogenated organic complexes containing chromium as metal chelate, prepared by solvent extraction method were studied for antimicrobial activity. Solutions of the complexes were prepared with alcohol, acetone and chloroform and used in the study. Preliminary screening was done by disc diffusion method. Minimum inhibitory concentration and phenol co-efficient of the complexes was studied by tube dilution method. Of the 15 complexes studied (5 from each of Cl, Br and F), tetraphenyl phosphonium halochromate and tetraphenyl arsonium halochromate were found to be effective against 9 pathogenic bacterial strains, that include *Escherichia coli, Shigella sonnei, Shigella dysenteriae, Shigella flexnerae, Shigella boyedii, Salmonella typhimurium, Klebsiella sp., Pseudomonas aeruginosa and Vibrio cholerae.* The fluoro substituted complexes of chromium and arsenic were found to be most effective among the three halogenated organic complexes. The phenol co-efficient of the above two complexes was determined to be 1.60.

Laboratory-synthesized chemicals have long been used as antimicrobial agents, as antiseptics and disinfectants[1-3]. The usefulness of most of these agents became limited due to the development of resistance by various microorganisms[4-6]. Therefore, the present study has been taken up to investigate the antimicrobial efficiency of some newly synthesized organic complexes. The complexes studied contain cyclic components such as phenol, pyridine, quinoline and tetrazole to which halogens and metal ions have been attached as activity enhancing functional groups[7]. Minimum inhibitory concentrations (MIC), nature of toxicity and phenol co-efficient of the complexes are determined in this

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investigation.

Five classes of complexes (Table 1) were investigated for antimicrobial efficacy. Ten pathogenic bacteria that included *Escherichia coli, Shigella sonnei, Shigella dysenteriae, Shigella flexneri, Shigella boyedii, Salmonella typhimurium, Salmonella paratyphi, Klebsiella sp., Pseudomonas aeruginosa, Vibrio cholerae* and 2 pathogenic fungi, *Candida albicans* and *Cryptococcus neoformans* were used in the study. The bacterial and fungal strains were maintained in nutrient agar (NA) and Sabouraud dextrose agar (SDA) slants respectively in the laboratory. Nutrient broth (NB), NA, Sabouraud dextrose broth (SDB), SDA and Mueller Hinton agar (MHA) were procured from Hi-Media, Mumbai and prepared according to manufacturer's instruction.

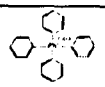
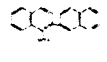
The complexes have been synthesized by solvent extraction method. Chromium trioxide was dissolved in aqueous haloacids to give the halochromate ions (CrO_3X; X=F/Cl/Br). These halochromate ions form two categories of ion association complexes, one with large cations such as 2,3,5-triphenyltetrazolium and tetraphenyl phosphonium ions to form $M^+CrO_3X^-$ type complexes, and the other with nitrogen containing organic bases like 2,2'-biquinoline and 2,2-bipyridyl and its analogs to form $BH^+CrO_3X^-$ type complexes. The method of synthesis of these halochromate complexes and their characterization by various physico-chemical methods has already been reported earlier[8,9].

For preliminary screening, the disc diffusion method (DDM) as described by Bauer *et al.* (1966)[10] was followed with some modification. Briefly, sterile Whatman No. 50 filter paper discs (1 cm dia) were placed over NA and SDA plates inoculated with broth culture of the test organism. Six discs were placed on a plate; 3 served as control to which 20 µl of the pure solvents (alcohol, acetone and chloroform) were added, and the other three discs were impregnated with 20 µl of the complex solutions separately. The above plates were incubated at 37° and 28±2° for 18-24/48 h for bacterial and fungal strains respectively. The plates were observed for zone of inhibition around the discs.

MIC of the complexes showing appreciable zones of inhibition against the test organism was determined by tube dilution method as described by Cruickshank *et al.* (1975)[11]. In a nut shell, a two fold serial dilution of the complex was followed with 1 ml of sterile NB/SDB in test tubes to provide varying concentrations, 0.15-1 mg/ml of the complexes. NB and SDB were used to test the MIC values of the complexes against the bacteria and fungi respectively. Ten microliters of the test organism was added to each tube and incubated at 37° and 28±2° for 24/48 h. The highest dilution of the complex completely inhibiting the test organism was considered as MIC value of the complexes respectively. MIC at chloroform solution of the complexes could not be determined due to its insolubility in water.

Further, microbicidal or microbiostatic activity of the complexes was determined by taking one loopful of the culture from MIC tubes and subculturing onto NA or SDA plates. The plates were incubated at appropriate temperatures. No growth after the incubation period indicated microbicidal nature while growth on sub culturing indicated microbiostatic

TABLE 1: MOLECULAR FORMULAE AND STRUCTURES OF THE COMPLEXES STUDIED.

	Complex*	Mol. formula	Structure
1.	Tetraphenyl phosphonium halochromate	$TPPCrO_3X$	
2.	2,3,5 triphenyl tetrazolium halochromate	$TPTCrO_3X$	
3.	2,2'-Diquinolinium halochromate	$BiqHCrO_3X$	
4.	2,2'-Bipyridyl halochromate	$BipyHCrO_3X$	
5.	Tetraphenyl arsonium halochromate	$TPAsCrO_3X$	

*Solution of the complexes were prepared by dissolving their powdered forms in alcohol (A), acetone (B) and chloroform (C). The amount taken was 1 g/l. For thorough mixing, the solutions were shaked continuously for 24 h in a mechanical shaker at room temperature.

nature of the complexes.

Further more, an experiment was designed to estimate the efficacy of the test complexes by comparing them with phenol taken as standard disinfectant as reported earlier[11]. *E. coli* was taken as standard organism for all the complexes. The phenol coefficient value of the complexes was calculated using the formula. Phenol co-efficient = highest dilution of test complex killing the test organism in 10 min/highest dilution of phenol killing the test organism in 10 min.

Results obtained from the DDM indicate that fluoro substituted tetraphenyl phosphonium fluoro chromate ($TPPCrO_3F$) and tetraphenyl arsonium fluoro chromate ($TPAsCrO_3F$) showed the highest zones of inhibition around discs (Table 2). This is followed by the bromo substituted and chloro substituted derivatives respectively. The other complexes exhibited very low degree of antimicrobial activity in the decreasing order of $TPTCrO_3X$>$BiqHCrO_3X$>$BiPyHCrO_3X$, in terms of zones of inhibition around the discs. All the bacterial strains except *Salmonella paratyphi* were inhibited by the above two complexes. But the fungal strains were not inhibited at concentrations as high as 100 µl/disc.

The MIC values of the complexes (only alcohol and acetone fractions) which showed appreciable zones of inhibition in DDM were determined, the results of which have been listed in Table 3. The MIC values of the complexes range between 0.25 to 0.50 mg/ml, in most of the cases.

From the nature of toxicity studies, it was observed that, the complexes are bactericidal in nature as no growth appeared on subculturing onto solid agar plates from the MIC dilution tubes.Phenol co-efficient of the $TPPCrO_3F$ and $TPAsCrO_3F$ were found to be 1.60 and $P \leq 0.5$ indicates the statistical significance of the phenol co-efficient value of the complexes.

In conclusion, out of the 15 complexes tested for antimicrobial activity, two complexes $TPPCrO_3F$ and $TPAsCrO_3F$ gave most promising results against the 9 common patho-

TABLE 2: ANTIMICROBIAL ACTIVITY OF PREPARED COMPLEXES.

Organisms	Diameter of zone of inhibition (mm)																	
	Complexes																	
	$TPPCrO_3Cl$			$TPPCrO_3Br$			$TPPCrO_3F$			$TPASCrO_3Cl$			$TPAsCrO_3Br$			$TPAsCrO_3F$		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
E.coli	13	10	11	16	16	18	19	22	14	13	9	11	15	13	13	21	18	12
Shigella sonnei	15	15	13	17	15	18	16	21	15	15	15	13	12	15	13	23	21	16
S. dysenteriae	11	10	13	-	-	17	10	9	11	11	10	-	11	8	12	13	10	11
S.flexnerae	13	9	10	-	16	10	11	14	14	13	9	13	8	11	9	12	12	14
S. boyedii	11	-	-	8	-	-	21	25	23	11	8	8	11	10	11	20	16	21
Salmonella paratyphi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Klebsiella sp.	10	8	-	-	-	-	10	8	10	10	8	-	-	-	-	-	-	8
S. typhimurium	-	-	-	-	-	-	24	27	27	-	-	-	8	-	10	23	18	25
P. aeruginosa	12	10	9	-	-	-	13	13	10	12	10	10	10	8	-	16	8	10
Vibrio cholerae	19	15	-	-	-	-	25	28	27	13	12	8	13	-	-	23	19	21
*Candida albicans**	-	-	-	-	-	-	9	8	19	-	-	-	-	-	-	10	-	9
*C. neoformans**	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	-	-

Complexes were dissolved in different solvents. 100 µl of the solution was applied per disc. Zones represented are mean and values from three experiments. A, B and C represents the solution of the complexes in organic solvents like alcohol, acetone and chloroform respectively. Dash represents that no inhibition was observed.

TABLE 3: MIC VALUES OF THE COMPLEXES.

Organisms	$TPPCrO_3Cl$		$TPPCrO_3Br$		$TPPCrO_3F$		$TPPCrO_3Cl$		$TPPCrO_3Br$		$TPPCrO_3F$	
	A	B	A	B	A	B	A	B	A	B	A	B
E. coli	0.50	0.50	0.25	0.25	0.25	0.25	0.50	0.50	0.50	0.50	0.25	0.25
Shigella sonnei	0.50	0.50	0.25	0.50	0.25	0.25	0.50	0.50	0.50	0.50	0.25	0.25
S. dysenteriae	0.50	0.50	-	-	-	-	0.50	-	-	0.50	0.50	0.50
S. flexnerae	0.50	0.50	-	0.25	0.50	0.50	0.50	0.50	-	0.50	0.50	0.50
S. boyedii	0.50	-	-	-	0.25	0.25	0.50	0.50	0.50	0.50	0.25	0.25
S. paratyphi	-	-	-	-	-	-	-	-	-	-	-	-
Klebsiella sp.	0.50	-	-	-	0.50	0.50	0.50	-	-	-	-	-
S. typhimurium	-	-	-	-	0.25	0.25	-	-	-	-	0.25	0.25
P. aeruginosa	0.50	0.50	-	-	0.50	0.50	0.50	0.50	0.50	-	0.25	0.50
Vibrio cholerae	0.20	0.25	-	-	0.25	0.25	0.50	0.50	-	-	0.25	0.25

Dash represents no inhibition of the organisms at 1 mg/ml concentration of the complexes. MIC values are in mg/ml.

genic bacteria tested. The antimicrobial activity may be due to the presence of a halo group, such as fluorine (F) and elements like chromium in these complexes. Haque and Russel (1976)[12] reported similar effects of chromium as antimicrobial against a number of gram -ve bacteria. Further, Diver (1989)[4] reported the bactericidal activity due to inhibition of DNA synthesis inside the cell by quinolone and ketone compounds. Since the complexes used in this study contain a biquinolone base, it would be interesting to study whether these complexes have any interaction with DNA. However, efforts are on for separation of the active principles and to study the mechanism of action of these newly synthesised complexes.

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