in the now lower values can be attributed to the closer packing of the planar pyridinium ion. It does not seem plausible that the lower saturation adsorption area of DPC in the presence of salt is due to its greater desolvation at the surface since KCl would be expected to desolvate DTAC and DEAC to a greater extent.

A comparison of CMC values of the various compounds in Table III indicate that the addition of salt affects the CMC of DPC to a greater extent than DTAC or DEAC. This can be explained on the same basis as the saturation adsorption phenomenon, where the addition of salts partially eliminates the repulsive effects of the ions and accentuates steric differences.

Thus, it is apparent that the small differences in surface activity due to the polar group may be evaluated by the techniques presented. These techniques, extended to a wider variety of polar groups, including nonionics and anionics, should prove useful to those interested in evaluating the effects of head groups at various interfaces. Future studies will discuss the effect of the polar group at various oil–water and biological interfaces.

SUMMARY

The adsorption of three quaternary ammonium salts, having the same chain length and counterion but differing in their polar group, has been measured at the air–water interface.

Differences in adsorption in the ideal region due to the polar groups have been evaluated thermodynamically and related to the entropy of adsorption. The possible role of ion hydration has been discussed.

Application of two-dimensional equations of state and the Gibbs adsorption isotherm have allowed for the determination of coareas, A_0, which are in good agreement with molecular models for the various compounds.

In regions of high surface concentration, the effect of the steric and electronic nature of the polar group has been evaluated by means of saturation adsorption, coarea, and CMC data.

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Drug Standards

Polarographic Determination of Chloramphenicol Preparations

By A. FRANCIS SUMMA

A polarographic method for the determination of chloramphenicol in commercial preparations is presented. The method has the necessary accuracy and precision for use in routine control analysis.

THE U.S.P. XVI assay (1) for chloramphenicol preparations is quite time consuming because it is based on a microbiological method of analysis. Chloramphenicol palmitate and its preparations, however, are assayed by an ultraviolet spectrophotometric procedure. The spectrophotometric procedure also is used for chloramphenicol preparations as an alternate method. In the analysis of dosage forms, chloramphenicol should be isolated from the excipients or vehicles prior to ultraviolet absorption quantitation, since these materials can lead to erroneously high results. The solubility characteristics of chloramphenicol, however, make isolation by liquid–liquid extraction difficult. Polarographic analysis should be less susceptible to interference from

Received September 25, 1964, from the Special Investigations Branch, Division of Pharmaceutical Chemistry, Bureau of Scientific Research, Food and Drug Administration, U. S. Department of Health, Education, and Welfare, Washington, D. C.
Accepted for publication November 11, 1964.

these excipient materials. However, chloramphenicol palmitate and its preparations can not be analyzed polarographically due to its solubility characteristics.

Both the ultraviolet method and the polarographic procedure are nonspecific because 1-*p*-nitrophenyl-2-amino-1,3-propandiol, a hydrolysis product of chloramphenicol, will interfere. It produces a well-defined wave which corresponds closely in half-wave potential to that obtained for chloramphenicol, and also absorbs in the ultraviolet region. In a report on the polarographic estimation of chloramphenicol, Hess (2) states that the hydrolysis product is not a normal decomposition product of chloramphenicol.

If the present assay for chloramphenicol U.S.P. is retained and all batches of chloramphenicol are analyzed microbiologically, the dosage forms then could be determined by a chemical method. With this in mind, the polarographic procedure would appear to be the method of choice for the routine analysis of chloramphenicol dosage forms.

METHOD

Apparatus

Sargent model XXI recording polarograph, H-type cell, with a saturated calomel electrode was employed.

Reagents

Isopropyl alcohol, A.C.S. reagent grade, 0.2 *M* potassium biphthalate solution, 0.2 *N* sodium hydroxide solution, and methylene blue solution, 0.1% in alcohol, were used.

Preparation of Samples

Chloramphenicol Capsules.—Transfer, as completely as possible, the contents of not less than 20 chloramphenicol capsules to a small tared dish and weigh. Mix the powder and transfer an accurately weighed portion, equivalent to about 10 mg. of chloramphenicol, to a 100-ml. volumetric flask.

Chloramphenicol Otic Solution.—Transfer an aliquot, equivalent to about 10 mg. of chloramphenicol, to a 100-ml. volumetric flask.

Chloramphenicol Ophthalmic Solution and Chloramphenicol Sodium Succinate for Injection.—Reconstitute the sample as directed on the label and pipet an aliquot equivalent to about 10 mg. of chloramphenicol into a 100-ml. volumetric flask.

Chloramphenicol for Aqueous Suspension.—Reconstitute the sample as directed on the label. Dilute quantitatively and stepwise to obtain a solution having a concentration of about 1 mg. of chloramphenicol per milliliter. Pipet 10 ml. of the solution into a 100-ml. volumetric flask.

Procedure

Add 5 ml. of isopropyl alcohol to the sample solution and agitate for a few minutes. Add 25 ml. of 0.2 *M* potassium biphthalate solution, 2 ml. of 0.2 *N* sodium hydroxide solution, and 0.2 ml. of 0.1% methylene blue solution. Mix and dilute to volume with distilled water. Transfer a portion of this solution to a polarographic cell immersed in a water bath regulated at 24.5° to 25.5° and deaerate by bubbling purified nitrogen through the solution for 10 min. Insert the dropping mercury electrode of a suitable polarograph and record the polarogram from −0.10 to −0.90 v., using a S.C.E. as the reference electrode. Determine the height of the diffusion current at −0.78 v. Calculate the quantity, in milligrams, of $C_{11}H_{12}Cl_2N_2O_5$ in each milliliter of sample solution taken by the formula $0.1C[(id)_u/(id)_s]$, in which $(id)_u$ is the observed diffusion current of the unknown solution, and $(id)_s$ is that determined similarly on a solution of U.S.P. chloramphenicol reference standard, the concentration of which is *C* mcg. in each milliliter (about 100 mcg. in each milliliter).

Chloramphenicol Ophthalmic Ointment, Chloramphenicol Ointment, and Chloramphenicol, Paromomycin, Hydrocortisone Ointment.—Weigh a quantity of ointment, equivalent to about 10 mg. of chloramphenicol, transfer to a separator, add 10 ml. of solvent hexane, and mix by shaking vigorously. Extract successively with 15- and 10-ml. portions of 0.2 *M* potassium biphthalate solution and finally with 15 ml. of distilled water, filtering each extract through a pledget of cotton, previously wetted with water, into a 100-ml. volumetric flask. Add 5 ml. of isopropyl alcohol, 2 ml. of 0.2 *N* sodium hydroxide solution, and 0.2 ml. of 0.1% methylene blue solution and make to volume with distilled water Proceed as directed under *Procedure*, beginning with "Transfer a portion of this solution to a polarographic cell. . . ." Calculate the quantity, in milligrams, of $C_{11}H_{12}Cl_2N_2O_5$ in the portion of the ointment taken by the formula $0.1C[(id)_u/(id)_s]$, in which the terms are as defined therein.

Chloramphenicol Cream.—Weigh a portion of the cream equivalent to about 10 mg. of chloramphenicol, transfer to a separator, add 25 ml. of distilled water, and mix by shaking vigorously. Extract with three 25-ml. portions of chloroform–ethyl acetate (2:1), filtering each extract into a 150-ml. beaker, through a pledget of cotton previously wetted with the chloroform–ethyl acetate mixture. If persistent emulsions occur, add anhydrous sodium sulfate to the separator to break the emulsion prior to filtration. Evaporate the extracts to dryness on a steam bath. Dissolve the residue in 5 ml. of isopropyl alcohol and 25 ml. of 0.2 *M* potassium biphthalate solution and transfer to a 100-ml. volumetric flask with the aid of distilled water. Add 2 ml. of 0.2 *N* sodium hydroxide solution and 0.2 ml. of 0.1% methylene blue solution and dilute to volume with distilled water. Filter the solution and proceed as directed above, beginning with "Transfer a portion of this solution to a polarographic cell. . . ." Calculate the quantity, in milligrams, of $C_{11}H_{12}Cl_2N_2O_5$ in the portion of the cream taken by the formula $0.1C[(id)_u/(id)_s]$, in which the terms are as defined therein.

DISCUSSION

In the polarographic procedure for chloramphenicol reported by Hess (2), large amounts of thymol were

TABLE I.—POLAROGRAPHIC, SPECTROPHOTOMETRIC, AND MICROBIOLOGICAL DETERMINATION OF CHLORAMPHENICOL PREPARATIONS[a]

Product	Microbiological (*M*)	Polarographic (*P*)	Spectrophotometric (*S*)	% $P/M \times 100$	% $P/S \times 100$
Otic	5.82 mg.	5.9 mg.		101.4	
	5.82 mg.	5.9 mg.		101.4	
	5.60 mg.	5.8 mg.		103.6	
	6.79 mg.	6.6 mg.		97.2	
	6.53 mg.	6.4 mg.		98.0	
Ophth. oint.	10.4 mg.	10.6 mg.		101.9	
	10.9 mg.	11.1 mg.		101.8	
	10.7 mg.	10.7 mg.		100.0	
Cream	10.2 mg.	10.2 mg.		100.0	
	10.4 mg.	10.3 mg.		99.0	
Soln.	0.617 Gm.	0.607 Gm.		98.4	
	0.633 Gm.	0.628 Gm.		99.2	
Sod. succinate inj.	395 mg.	394.7 mg.		99.9	
Oint.	10.4 mg.	11.0 mg.	11.0 mg.	105.8	100.0
	11.0 mg.	11.0 mg.	11.0 mg.	100.0	100.0
	9.9 mg.	10.1 mg.	9.2 mg.	102.0	109.8
	10.1 mg.	10.5 mg.	9.6 mg.	104.0	109.4
Ophth. aqueous		26.4 mg.	26.7 mg.		99.0
Aq. susp.		1.18 Gm.	1.16 Gm.		101.7
		1.19 Gm.	1.21 Gm.		98.4
		1.32 Gm.	1.35 Gm.		97.8
		1.27 Gm.	1.27 Gm.		100.0
		1.18 Gm.	1.21 Gm.		97.5
		259.6 mg.	261 mg.		99.5
		263.4 mg.	255 mg.		103.3
Capsules		240.1 mg.	244 mg.		98.4
		246.9 mg.	251 mg.		98.4
		249.6 mg.	254 mg.		98.3

[a] Each result in the above table represents an average of at least three determinations on different lot numbers of the various preparations by each method.

used to supress maxima. The curves so obtained became deformed, and measurements were not reproducible. The polarographic behavior of chloramphenicol was re-examined more carefully by Knobloch and Svatek (3) and compared with the behavior of related compounds.

The polarographic procedure reported here is essentially that of Hess (2), modified for application to dosage forms. Methylene blue was found to be a suitable maxima suppressor for solutions from 40 to 500 mcg. of chloramphenicol per milliliter, in which range the wave height is also proportional to concentration.

The polarographic data reported in Table I represent the averages of six separate determinations on each dosage form (deviation ±1%). The results are compared with results obtained by either the microbiological or the spectrophotometric method.

Except for chloramphenicol ointment and cream, sample preparation is quite simple: a portion of the dosage form is diluted with buffer solution and polarographed.

The extraction procedure used in the U.S.P. XVI assay method (4) for chloramphenicol ointment was followed to separate chloramphenicol from the ointment base, except that the polarographic buffer solution was used for extraction instead of pH 6.0 phosphate buffer specified in the official method. Because the chloramphenicol cream is an oil-in-water emulsion type of ointment base, the modified extraction used for the ointment could not be followed. By using the procedure which Levine and Fischbach (5) used to separate chloramphenicol from blood and urine, it was possible to extract completely the chloramphenicol prior to polarographic analysis.

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