cultured in a nitrogen-deficient medium—conditions under which the organisms grow very poorly but produce a higher percentage (but not absolute amount) of lipid (2). Yields were so low in this medium that neither niacin nor B_6 activity could be satisfactorily determined with the procedures employed.

SUMMARY

In *C. vulgaris* and *C. pyrenoidosa* there is a continuous decrease in the concentration of niacin and of vitamin B_6 (mmcg./mg. dry weight of cells) during the second and third weeks of a 3-week culture period. However, despite the decrease in *concentration* of the two vitamins in the cells, there is a substantial increase in the *absolute amount* present in the cultures because of the large increase in total mass of cells during the same period.

C. pyrenoidosa excels *C. vulgaris* as a source of B_6 activity whether results are expressed in terms of concentration in the cells or of total yield from the harvested cells, although the superiority of the former species diminishes with increasing age of the cultures. The two species are approximately equivalent with respect to niacin content.

The niacin/B_6 ratio remains constant in both species during the culture period, but the ratio maintained by *C. vulgaris* is higher than that maintained by *C. pyrenoidosa*. Similarly, *C. vulgaris* contains more protein relative to B_6 than does *C. pyrenoidosa*.

As a source of B_6 and niacin, both species of *Chlorella* compare favorably with dietary vegetable sources.

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Scope of Acetonitrile as a Solvent in the Nonaqueous Titration of Organic Medicinals

By C. A. MAINVILLE* and L. G. CHATTEN

Acetonitrile has been shown to be a useful solvent for the assay of a variety of salts of organic bases. Its scope as a solvent must be investigated for each individual compound, since solubility cannot be predicted on the basis of chemical similarity. This solvent has also been demonstrated to be particularly valuable in the assay of tablets and capsules because so few excipients cause interference in it.

ACETONITRILE has been shown to be a satisfactory solvent medium for the titration of a limited number of organic compounds (1–3). However, its use as a primary solvent for salts of medicinals had been previously applied on a small scale by Mizukami and Hirai (4). In other investigations the role of acetonitrile in the titration of organic medicinal agents has been restricted to that of a stabilizing solvent (5–7).

From the results of a recent publication (8), which dealt with the titratability of tablet excipients in a variety of organic solvents, it was decided to select those solvents in which minimal interference by excipients occurred and to determine the scope of each as a titration medium for the analysis of medicinals and their pharmaceutical forms. Acetonitrile was one of those solvents chosen for this investigation.

Received June 3, 1963, from the Faculty of Pharmacy, University of Alberta, Edmonton, Alberta, Canada, and the Food and Drug Directorate, Ottawa, Ontario, Canada.
Accepted for publication July 26, 1963.
Presented to the Scientific Section, A.PH.A., Miami Beach meeting, May 1963.
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The authors are indebted to the following drug manufacturers for the generous supplies of crystalline materials: Abbott Laboratories, Ltd., Montreal, for methamphetamine HCl and pramoxine HCl; Ayerst McKenna and Harrison, Montreal, for isothipendyl HCl; Burroughs-Wellcome, Montreal, for methoxamine base and chlorcyclizine HCl; Ciba and Co., Montreal, for tripelennamine HCl; Eli Lilly and Co., Toronto, for piperocaine HCl; W. S. Merrell Co., Weston, Ontario, for diperodone HCl and doxylamine succinate; Parke Davis and Co., Brockville, Ontario, for diphenhydramine HCl; Poulenc, Ltd., Montreal, for the hydrochlorides of chlorpromazine, promethazine, and prochlorperazine; Sandoz Pharmaceuticals, Montreal, for thioridazine HCl; E. R. Squibb & Sons, Ltd., Montreal, for triflupromazine HCl; The Upjohn Co., Don Mills, Ontario, for methoxyphenamine HCl and pyrathiazine HCl; John Wyeth and Brother, Windsor, for promazine HCl.

EXPERIMENTAL

Apparatus

An A. C. titrometer, Precision Scientific Co., equipped with a glass-calomel electrode combination, 5-ml. microburets graduated to 0.01 ml., and electromagnetic stirrers were employed.

Reagents

Acetone A.C.S., acetonitrile A.C.S., chloroform A.C.S., glacial acetic acid A.C.S., anhydrous

TABLE I.—TITRATABILITY OF DRUGS IN ACETONITRILE

Drug	Mol. Wt.	% Purity[a]	% Purity in Acetonitrile[b]	ΔE, (Mv.)
Local Anaesthetics				
Diperodon HCl	433.93	99.1 (*a*)	99.9	
		99.4	99.3	
			100.5[c]	[49]
Piperocaine HCl	297.83	100.6 (*a*)	100.5	
		100.2	100.1	
			100.7[c]	[49]
Pramoxine HCl	329.88	101.1 (*a*)	100.9	
		100.7	100.8	
			101.0[c]	[42]
Procaine HCl	272.77	100.0 (*a*)	100.6	
		99.5	100.2	
			100.4[c]	[15]
Sympathomimetics				
Ephedrine HCl	201.70	100.4 (*b*)	Insoluble	
		100.4		
Methamphetamine HCl	185.69	99.7 (*b*)	100.6	
		100.4	100.4	
			100.2[c]	[46]
Methoxamine (base)	211.24	99.8 (*b*)	98.6	
		99.1	98.2	
			99.5[c]	[120]
Methoxyphenamine HCl	215.72	100.1 (*b*)	100.1	
		99.9	99.9	
			99.8[c]	[48]
Antihistamines				
Chlorcyclizine HCl	337.30	99.2 (*e*)	99.4	
		99.5	99.4	
			100.1[c]	[11]
Diphenhydramine HCl	291.8	100.5 (*b*)	100.3	
		100.2	100.0	
			100.3[c]	[49]
Doxylamine succinate	388.45	98.9 (*c*)	98.8	
		99.6	98.8	
			98.6[c]	[44]
Isothipendyl HCl	321.88	100.7 (*d*)	100.3	
		100.8	99.8	
			100.3[c]	[37]
Tripelennamine HCl	291.83	100.3 (*b*)	100.4[c]	[40]
		100.4	100.2[c]	[40]
			100.5[c]	[40[
Phenothiazines				
Chlorpromazine HCl	355.53	100.4 (*f*)	100.2	
		100.2	100.0	
			100.8[c]	[24]
Mepazine HCl	364.92	100.6 (*f*)	100.1	
		100.9	100.4	
			100.4[c]	[41]
Prochlorperazine dimaleate	303.04	99.9 (*c*)	Insoluble	
		99.3		
Promazine HCl	320.88	99.8 (*f*)	100.1	
		99.2	100.5	
			101.6[c]	[33]
Promethazine HCl	320.88	99.9	99.7	
		99.7	99.7	
			100.6[c]	[46]
Pyrathiazine HCl	332.89	100.7	99.7	
		100.5	99.5	
			100.1[c]	[32]
Thiopropazate HCl	518.94	100.1 (*b*)	Insoluble	
		99.4		
Thioridazine HCl	407.11	100.0 (*f*)	100.5	
		100.9	100.4	
			100.9[c]	[18]
Trifluprom azine HCl	388.90	100.7 (*f*)	99.4	
		100.4	99.4	
			100.4[c]	[40]

[a] (*a*), (*b*), (*c*), (*d*), (*e*), and (*f*) indicate the procedure employed to establish purity of the drug. [b] *V* was 0.01 ml. [c] Determined potentiometrically.

methanol A.C.S., 6% mercuric acetate in glacial acetic acid, and 0.1 *N* perchloric acid in dioxane. The following indicators were utilized: (*a*) 0.5% crystal violet in glacial acetic acid, (*b*) 0.1% methyl red in glacial acetic acid, (*c*) 0.1% methyl red in anhydrous methanol, (*d*) 0.25% methyl red in phenol-chloroform, and (*e*) 0.5% thymol blue in anhydrous methanol.

Procedures

Determination of Drug Purity.—An accurately weighed quantity of the crystalline salt was dissolved in 50 ml. of solvent by stirring electromagnetically for 15 minutes. A suitable indicator was added, and the solution was titrated with 0.1 *N* perchloric acid in dioxane. A blank was determined on the solvent. All assays were performed in duplicate by one of the following techniques. (*a*) Dissolve sample in 25 ml. of phenol-chloroform (5:25) and 25 ml. of acetonitrile; add 2 ml. of mercuric acetate solution and 2 drops of methyl red in phenol-chloroform. (*b*) Dissolve sample in 50 ml. of glacial acetic acid; add 2 ml. of mercuric acetate solution and 1 drop of crystal violet in glacial acetic acid (9). (*c*) The same as (*b*), except mercuric acetate solution was not added. (*d*) Dissolve sample in chloroform-acetonitrile (1:1), add 2 ml. of mercuric acetate solution and 2 drops of methyl red in glacial acetic acid (7). (*e*) Dissolve sample in 50 ml. of chloroform-glacial acetic acid (1:1); add 1 drop of crystal violet in glacial acetic acid (7). (*f*) Dissolve sample in 50 ml. of acetone; add 2 ml. of mercuric acetate solution and 2 drops of methyl red in glacial acetic acid (10).

Methods of Assay of Crystalline Salts in Acetonitrile.—A sample of the salt was accurately weighed and dissolved in 50 ml. of acetonitrile by stirring electromagnetically for 15 minutes. A suitable indicator was used, and the solution was titrated with 0.1 *N* perchloric acid in dioxane.

All assays were performed in duplicate by this technique; the results were compared with that of a potentiometric titration. A blank was determined on the solvent system.

(*a*) Dissolve sample in 50 ml. of acetonitrile; add 2 ml. of mercuric acetate solution and 3 to 4 drops of methyl red indicator in glacial acetic acid. Titrate to red end point. (*b*) Dissolve sample in 50 ml. of acetonitrile; add 1 drop of crystal violet indicator in glacial acetic acid. Titrate to blue end point.

Method of Assay of Pharmaceuticals Using Acetonitrile as Solvent.—Weigh and powder 20 tablets or empty the contents of 20 capsules. Accurately weigh a sample of the powder which is equivalent to 50 mg. of the active ingredient. Add 50 ml. of acetonitrile and stir electromagnetically for 30 minutes; suction filter through a sintered-glass funnel of medium porosity, and wash beaker and funnel with 10 ml. acetonitrile. Add 4 drops of 0.5% methyl red indicator in glacial acetic acid and 2 ml. of 6% mercuric acetate solution if salt is a halide. The filtrate is titrated to a deep red with 0.1 *N* perchloric acid in dioxane.

RESULTS AND DISCUSSION

Because a number of chemically different compounds were investigated, it was necessary to use a variety of established nonaqueous procedures to determine the purity of each substance. The duplicate results of such assays appear in Table I, together with the appropriate letter which indicates the method employed.

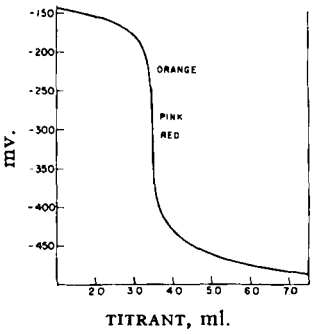

Fig. 1.—Titration of diphenhydramine HCl in acetonitrile.

Where solubility was adequate, triplicate determinations were performed on each drug in acetonitrile, and the results are reported in Table I. To check the validity of the visual end points, one of the triplicate assays was performed potentiometrically on the Precision-Shell titrometer, and the result appears in *footnote c* of Table I. An excellent titration curve was obtained in each instance; to demonstrate the magnitude of potential change at the end point, the ΔE is included in Table I for each drug. The ΔV, in the vicinity of the end point, was 0.01 ml. Figure 1 illustrates the titration curve for diphenhydramine hydrochloride in acetonitrile and is typical of the behavior of the stronger bases in this solvent.

Examination of the data in Table I shows that there is satisfactory agreement between the assay results obtained by standard or previously reported methods and the proposed procedure employing acetonitrile as primary solvent. Ephedrine was included in Table I because of its chemical similarity to methamphetamine. Although the latter compound is readily soluble, the former is not. For a similar reason, prochlorperazine and thiopropazate are included with those phenothiazine derivatives which are soluble. Such evidence indicates that chemical similarity is insufficient to predict solubility in this solvent. Each compound must be checked for solubility on an individual basis.

In addition to those compounds which are reported, the solubility of several sodium salts of barbiturates, some alkaloidal salts, and sodium *p*-aminosalicylate were investigated in acetonitrile. All had a solubility which was too low for practical value; hence no data are reported for them.

Several pharmaceutical preparations were assayed by the described procedure, and comparative analysis were obtained either by the official method (9) or by the control technique exercised by the manufacturer of the product. In the majority of instances, satisfactory agreement occurred as shown in Table II. The pharmacopeial method could not be applied to Abbott's chlorcyclizine hydrochloride because the orange color of the tablets appeared to interfere with the result and caused serious overestimation. No method of comparison was available from the manufacturer for the promazine tablets.

For the analyses of commercial preparations, a 30-minute stirring time is generally recommended. In

TABLE II.—ASSAY OF PHARMACEUTICALS IN ACETONITRILE

Drug	Mfr.	Official or Mfg. % Recovery	% Recovery, in Acetonitrile
Chlorcyclizine HCl U.S.P.	Burroughs Wellcome	97.9	97.8
		99.0	98.3
			98.4
Chlorcyclizine HCl U.S.P.	Abbott	...	103.0
			103.4
			101.3
Chlorpromazine HCl U.S.P.	Poulenc	98.8	98.1
		97.5	97.4
			97.8
Diphenhydramine HCl U.S.P.	Parke Davis	105.1	101.9
		106.2	101.5
			102.5
Doxylamine succinate U.S.P.	Merrell	98.0	97.3
		98.2	97.4
			97.3
Isothipendyl HCl	Ayerst	102.5	98.5
		103.2	99.0
			98.2
Methoxyphenamine HCl	Upjohn	97.3	100.3
		98.2	99.8
			99.6
Promazine HCl	Empire	No method	105.6
			105.6
			104.7
Promethazine HCl U.S.P.	Poulenc	99.8	88.0
		98.8	88.0
			87.2
Trifluprom azine HCl	Squibb	99.9	97.1
		100.0	97.1
			96.8
Thioridazine HCl	Sandoz	102.5	97.8
		102.5	97.3
			97.2
Tripelennamine HCl U.S.P.	Ciba	96.6	88.5
		97.6	87.1
			88.0

certain instances, it was necessary to increase this to 1 hour to extract the maximum amount of active constituent. This was particularly true of isothipendyl HCl tablets and trifluprom azine HCl capsules, where the ratio of drug was small compared to that of the excipients. For these products, a 1-hour stirring time is recommended. The results obtained for tripelennamine tablets and those for promethazine were low. No significant difference was obtained between a 30-minute stirring time and 1 hour. Since the crystalline salt of tripelennamine is quite soluble in acetonitrile, no satisfactory explanation can be offered for the values which are markedly below those obtained by the official procedure. For promethazine, it is obvious that the proportion of active constituent to that of tablet excipients is too small to permit complete extraction.

The present report should not be construed as representing the complete scope of acetonitrile as a solvent for the salts of organic medicinals. It does, however, give an indication of the utility of the solvent. Undoubtedly further investigation might reveal many more organic salts that could be titrated in acetonitrile.

A previous report (8) revealed that both isopropanol and *n*-hexane might be useful solvents for pharmaceutical analysis because of the small number of excipients that are titratable in them. Preliminary investigation shows that both of these solvents could also be useful in extending the applicability of nonaqueous titrimetry to the analysis of pharmaceuticals. Work is being continued in this direction.

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