SUMMARY

1. A series of hydroxypyrazines, with a phenylcarbinol moiety in the 3-position, was prepared. The prepared compounds were unobtainable by any other existing method.

2. A study of β-phenylserine indicated that the amide of this acid is more stable in the threo form than in the erythro form. The erythro form is preferentially obtained by reaction mechanism due to the excess pressure required in the reaction. The inversion during the formation of the amide from the ester is minimized by the use of the bulky carbobenzoxy group.

3. It was shown, by infrared analysis and low yield of methoxy compound from reaction with dimethylsulfate, that the compounds exist in predominantly the keto or pyrazone form. In addition, it was shown that sufficient crowding of the 2-position is present to prevent normal hydroxylic and ketonic reactions due to preventing sterically the formation of the necessary transition states required for these compounds.

4. The steric crowding has little effect on the formation of the methyl ethers because of the size and the linearity of the entering group.

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Influence of Excipients on the Analysis of Tablets and Capsules by Nonaqueous Titrimetry

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Investigation of the effect of twenty-seven tablet and capsule excipients on the titration of medicinal organic acids and bases has shown that a careful choice of solvents can limit interference to a very small percentage of the excipients. Certain of these excipients which are titratable when dissolved alone in common nonaqueous solvents do not always consume titrant when combined with strong organic acids and bases. Polyvinylpyrrolidone and stearic acids cause the most difficulty in the titration of medicinal organic bases and acids, respectively.

ALTHOUGH the scientific literature of the past decade is filled with reports on the application of nonaqueous titrimetry to the analysis of drugs and chemicals, only a relatively few workers, by comparison, have adapted this technique to the analysis of pharmaceuticals. This situation can be attributed in part to the extensive use made of glacial acetic acid as a solvent, both by numerous investigators in the field as well as by such authoritative compendia as the British and United States pharmacopoeias (1, 2). While the wide solubilizing properties of glacial acetic acid enhance its utility, it is this very factor that limits its application in the analyses of pharmaceuticals. A second contributing cause to the reluctance of some workers in the field to accept nonaqueous titrations as a practical means of assaying pharmaceuticals has been the lack of investigations into the effect of excipients on the quantitative determination of active components of pharmaceutical forms.

The purpose of this report is to assess the influence of a number of excipients and to investigate means of obviating interference by altering the solvent system. The effect of the presence of drugs on interfering excipients is also within the scope of this work.

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EXPERIMENTAL

Apparatus

A microburet graduated to 0.01 ml.; electromagnetic stirrers; a precision Shell titrometer fitted with Beckman glass electrode No. 1190–80, and Beckman sleeve-type calomel electrode No. 1170–71.

Reagents

Perchloric acid in dioxane 0.05 *N;* potassium hydroxide in anhydrous methanol 0.1 *N;* and 6% mercuric acetate in glacial acetic acid. Solvents: acetone, A.C.S.; acetonitrile A.C.S. redistilled at 81.6° prior to use; benzene A.C.S.; chlorobenzene A.C.S., redistilled at 131–132° prior to use; chloroform A.C.S.; dimethylformamide, practical, redistilled at 150° prior to use; ethylene glycol A.C.S.; technical *n*-hexane; isopropanol A.C.S.; anhydrous methanol A.C.S.; practical nitromethane redistilled at 101.2°; propylene glycol A.C.S.

Indicators

Bromocresol purple 0.5% in anhydrous methanol; crystal violet 0.5% in glacial acetic acid; methyl red 0.1% in glacial acetic acid; methyl red 0.5% in anhydrous methanol; methylene blue 0.1% and quinaldine red 0.2% in anhydrous methanol; thymol blue 0.5% in anhydrous methanol.

Excipients

Acacia, 1; beeswax, 2; calcium carbonate, 3; calcium phosphate, 4; calcium phosphate tribasic, 5; calcium sulfate, 6; carnauba wax, 7; cetyl alcohol, 8; gelatin, 9; lactose, 10; magnesium carbonate, 11; magnesium hydroxide, 12; magnesium stearate, 13; magnesium sulfate, 14; methylcellulose, 15; polyethylene glycol 4000, 16; polyvinyl pyrrolidone, 17; sodium alginate, 18; sodium benzoate, 19; sodium carboxymethylcellulose, 20; sodium silicate, 21; sodium sulfate, 22; starch, potato, 23; stearic acid, 24; sucrose, 25; talcum, 26; tragacanth, 27.

PROCEDURE

General.—An excess amount of excipient was placed in a 150-ml. beaker, 50 ml. of solvent added, and stirring was then carried out for 15 to 20 minutes. The undissolved excipient was removed by filtration through a fine sintered-glass funnel. The appropriate indicator was added, and the filtrate was then titrated with 0.05 *N* perchloric acid in dioxane in order to determine those excipients which consume this titrant. A second set of filtrates was titrated with 0.1 *N* potassium hydroxide in methanol in the presence of the indicator of choice, in order to determine which excipients behave as acids.

All titrations with potassium hydroxide in methanol were performed in a closed system to prevent contamination by atmosphere. A blank was carried out on each solvent system and in all cases was found to be within reasonable limits.

Special procedures.—In the phenol-chloroform-acetonitrile system, a stock solution of 25% phenol in chloroform was prepared. The excipients were stirred with 25 ml. of this solution for the prescribed time, filtration was carried out and 25 ml. of acetonitrile was added to facilitate the visual titration.

In the glycols-isopropanol systems (25:25), because these solvent systems were viscous, filtration was carried out through a sintered-glass funnel of medium porosity. Using *n*-hexane, excipients were dissolved in 30 ml. of hexane, filtration was carried out, and 20 ml. of acetone added to facilitate the titration.

Testing the Effect of Interfering Excipients in the Presence of Drugs.—A quantity of drug sufficient to give a 3 to 4 ml. titration was accurately weighed and dissolved in the solvent of choice by stirring electromagnetically for 15 minutes. The appropriate indicator was added and the titration performed with 0.05 *N* perchloric acid in dioxane for bases. In the presence of a halide salt, 2 ml. of mercuric acetate solution was added to the titration. Organic acids were titrated by 0.1 *N* potassium hydroxide in methanol. All assays were carried out in duplicate. In certain instances, the visual end points was verified potentiometrically.

A similar quantity of active ingredients was accurately weighed and dissolved in 50 ml. of the solvent of choice. Varying amounts of excipients were added. The solution was stirred for 15 minutes and then filtered through a sintered-glass funnel of medium porosity to remove undissolved excipients. Two ml. of mercuric acetate solution was used when necessary. The indicator of choice was added and the titration performed with the appropriate titrant. Blanks were subtracted.

For perchloric acid titrations, 0.1% methyl red in glacial acetic acid was used as the indicator in every instance except for isopropanol where 0.5% thymol blue in methanol was used.

When potassium hydroxide was the titrant, 0.5% bromothymol blue was the indicator of choice for the solvent anhydrous methanol. For all other solvents, 0.5% thymol blue in methanol was the most suitable indicator.

TABLE I.—EXCIPIENTS TITRATABLE WITH 0.05 *N* PERCHLORIC ACID

Solvent	Indicator	Interfering Excipients
Acetone	Methyl red (CH_3COOH)	17, 19, 23
Acetonitrile	Methyl red (CH_3COOH)	17, 19
Benzene	Crystal violet	16, 17
Chlorobenzene	Crystal violet	16, 17
Ethylene glycol	Methyl red (CH_3OH)	13, 19, 21
n-Hexane	Methyl red (CH_3COOH)	None
Isopropyl alcohol	Methyl red (CH_3COOH)	17, 19
Methanol	Thymol blue	12, 13, 14, 17, 18, 19, 20, 21, 22, 27
Nitromethane	Methylene blue & quinaldine red	16, 17, 18, 19, 20, 21, 22
Phenol-chloroform-acetonitrile	Methyl red (CH_3COOH)	15, 19
Propylene glycol	Thymol blue	12, 13, 14, 17, 18, 19, 21, 22

RESULTS AND DISCUSSION

Although suitable indicators were known for some of the solvent systems, it was necessary to find indicators for others. This was accomplished by checking the color changes against a potentiometer which was equipped with a glass-calomel electrode system.

Twenty-seven excipients which occur most commonly in tablets and capsules were titrated in the manner outlined under Experimental, and those which consumed the amount of titrant in excess of the blank are reported in Tables I and II. Titration of these same excipients in chloroform and glacial acetic acid have been reported in a previous publication (3).

Table I shows that acetone, acetonitrile, benzene, chlorobenzene, *n*-hexane, isopropanol, and phenol-chloroform-acetonitrile are highly satisfactory solvents for the titration of medicinal compounds in tablets and capsules by perchloric acid, as very few excipients consume titrant in these solvents. Interference by polyvinylpyrrolidone and sodium benzoate seems to be widespread. It was found that in *n*-hexane no excipient consumed titrant. The utility of this solvent will be investigated and results published in a later report.

Table II shows that acetone, acetonitrile, benzene, chlorobenzene, and *n*-hexane are highly desirable solvents for the titration of acidic medicinal agents which are found in pharmaceuticals. Beeswax, polyvinylpyrrolidone, and stearic acid, however, are the common interfering agents.

From the results reported here, it is apparent that a careful choice of solvent system can markedly reduce and may even virtually eliminate interference by excipients in the nonaqueous titration of tablets and capsules.

The recoveries of the organic bases and acids are given in Tables III and IV, respectively. It can be seen from these tables that interfering excipients do not always cause overestimation when in the presence of some drugs and in certain solvents. It must be remembered that excipients were present in an amount far in excess of their normal occurrence in tablets. This produced, in certain instances, end points which were difficult to detect because they were seriously obscured by the formation of precipitates.

TABLE II.—EXCIPIENTS TITRATABLE WITH 0.1*N* POTASSIUM HYDROXIDE

Solvent	Indicator	Interfering Excipients
Acetone	Thymol blue	2, 19, 24
Acetonitrile	Thymol blue	17, 24
Benzene	Thymol blue	2, 24
Chlorobenzene	Thymol blue	2, 24
Chloroform	Thymol blue	2, 13, 17, 24
Dimethylformamide	Thymol blue	2, 7, 13, 14, 17, 19, 24
Ethylene glycol	Methyl red (CH_3OH)	24
Hexane	Thymol blue	2, 24
Isopropyl alcohol	Thymol blue	2, 17, 24
Methanol	Bromocresol purple	17, 24
Propylene glycol	Bromocresol purple	17, 18, 24

TABLE III.—TITRATION OF DRUGS AND EXCIPIENTS WITH PERCHLORIC ACID IN DIOXANE

Solvent	Active Ingredient	Excipient	Recovery, %
Acetone	Diphenhydramine HCl	...	100.5[a]
	Diphenhydramine HCl	Polyvinylpyrrolidone	109.7
	Diphenhydramine HCl	Sodium benzoate	100.8
	Diphenhydramine HCl	Starch	99.3
	Naphazoline	...	98.6[a]
	Naphazoline	Polyvinylpyrrolidone	109.2
	Naphazoline	Sodium benzoate	99.4
	Naphazoline	Starch	97.8
Acetonitrile	Diphenhydramine HCl	...	100.4
	Diphenhydramine HCl	Polyvinylpyrrolidone	99.7
	Diphenhydramine HCl	Sodium benzoate	102.7
	Naphazoline	...	98.6
	Naphazoline	Polyvinylpyrrolidone	99.2
	Naphazoline	Sodium benzoate	99.4
Benzene	Naphazoline	...	99.1
	Naphazoline	PEG 4000	98.6
	Naphazoline	Polyvinylpyrrolidone	97.6
	Methoxamine	...	99.3
	Methoxamine	PEG 4000	99.4
	Methoxamine	Polyvinylpyrrolidone	98.0
Isopropyl alcohol	Diphenhydramine HCl	...	98.9
	Diphenhydramine HCl	Polyvinylpyrrolidone	100.8
	Diphenhydramine HCl	Sodium benzoate	209.0
	Methoxamine	...	98.2
	Methoxamine	Polyvinylpyrrolidone	99.3
	Methoxamine	Sodium benzoate	145.6
Phenol-chloroform-acetonitrile	Diphenhydramine HCl	...	100.2
	Diphenhydramine HCl	Methylcellulose	103.4
	Diphenhydramine HCl	Sodium benzoate	193.6
	Ephedrine HCl	...	99.5
	Ephedrine HCl	Methylcellulose	100.8
	Ephedrine HCl	Sodium benzoate	206.5

[a] Potentiometric determination.

TABLE IV.—TITRATION OF DRUGS AND EXCIPIENTS WITH POTASSIUM HYDROXIDE IN METHANOL

Solvent	Active Ingredient	Excipient	Recovery, %
Acetone	Acetylsalicylic acid	...	100.1
	Acetylsalicylic acid	Beeswax	101.1
	Acetylsalicylic acid	Sodium benzoate	99.8
	Phenobarbital	...	99.3
	Phenobarbital	Beeswax	100.8
	Phenobarbital	Sodium benzoate	99.8
Acetonitrile	Acetylsalicylic acid	...	99.8
	Acetylsalicylic acid	Cetyl alcohol	100.1
	Acetylsalicylic acid	Polyvinylpyrrolidone	100.2
	Phenobarbital	...	99.6
	Phenobarbital	Cetyl alcohol	99.7
	Phenobarbital	Polyvinylpyrrolidone	97.8
Chloroform	Acetylsalicylic acid	...	100.3
	Acetylsalicylic acid	Beeswax	105.2
	Acetylsalicylic acid	Magnesium stearate	111.1
	Acetylsalicylic acid	Polyvinylpyrrolidone	100.6
	Phenobarbital	...	99.7
	Phenobarbital	Beeswax	104.9
	Phenobarbital	Magnesium stearate	99.9
	Phenobarbital	Polyvinylpyrrolidone	100.8
Isopropyl alcohol	Acetylsalicylic acid	...	99.7
	Acetylsalicylic acid	Beeswax	100.2
	Acetylsalicylic acid	Polyvinylpyrrolidone	100.2
	Phenobarbital	...	99.8
	Phenobarbital	Beeswax	100.6
	Phenobarbital	Polyvinylpyrrolidone	100.4
Methanol	Acetylsalicylic acid	...	99.3
	Acetylsalicylic acid	Polyvinylpyrrolidone	99.9
	Benzoic acid	...	100.1
	Benzoic acid	Polyvinylpyrrolidone	100.8

As predicted, stearic acid readily consumed titrant in the presence of other organic acids and hence created a gross error in every solvent system. Beeswax, polyvinylpyrrolidone, and sodium benzoate were also major interfering agents but did not always consume titrant.

CONCLUSION

It has long been the feeling by some critics that the effect which excipients might exert on the quantitative analysis of tablets and capsules by nonaqueous titrimetry is probably so great as to limit its usefulness to the assay of the basic materials alone, or at best, to the products of those manufacturers where the control laboratory might be able to exert its influence in the formulation. The findings reported herein show that the careful choice of solvent systems can greatly minimize or even eliminate the possibility of interference by excipients.

It has been shown also that the nonaqueous titration of organic medicinal acids and bases suffers from very limited interference by tablet excipients, even when the excipients themselves are titratable. Sodium benzoate and polyvinylpyrrolidone appear to cause the major concern in the titration of bases. Only stearic acid causes serious and consistent interference in the titration of acids. This could be overcome by pharmaceutical manufacturers using magnesium stearate as a lubricant.

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