A Rapid Colorimetric Method for the Assay of Xylocaine* and the Determination of some other Organic Bases, using Ammonium Reineckate

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The new local anaesthetic α -diethylamino-2,6-acetoxylidide, known as Xylocaine or Lidocaine, has acquired an ever increasing application since it was first synthesised by Löfgren^{1,2} and Lundqvist¹, and there is now a great need for a rapid and certain method for a quantitative estimation of the substance in pharmaceutical preparations. The existing procedure, involving solvent extraction of the Xylocaine and subsequent titration, is troublesome and time-consuming, and we therefore began as early as 1948 to experiment with an alternative colorimetric method.

Many organic bases are known to give precipitates with ammonium reineckate, $([\text{Cr}(\text{SCN})_4] \text{NH}_4)_2$, and the reaction has been utilised for quantitative estimations by Canbäck³, Steigner and Hippenmeyer⁴, Brandelin, Slifer and Pankratz⁵ and others. It has now been found that under controlled conditions Xylocaine also is readily and quantitatively precipitated by ammonium reineckate, and by colorimetric measurement of an acetone solution of the precipitate an accurate estimation of the base can be achieved.

PROCEDURE

A known volume (not more than 10 ml) of solution containing approximately 0.1 mg-equiv. (25 mg) of Xylocaine is acidified to pH 2 or a little lower with hydrochloric acid, and 5 ml of a saturated aqueous solution of ammonium reineckate is then *slowly* added dropwise with vigorous stirring at room temperature; the addition should extend over at least one minute. The mixture is then allowed to stand for a period; this should

* Regd. trade mark.

Fig. 1. Extinction-concentration curves (Brunius colorimeter filter S 53, 2 cm cell).

Reineckates of
 + procaine
 □ aniline
 ▽ LL 31
 × Xylocaine
 △ "Thiorgan"

be not less than 5–10 minutes, but may be prolonged to as much as 24 hours without adverse effect. The precipitate is collected by suction on a small filter and washed with water (two portions of 2 ml); when drained it is dissolved in acetone whilst still on the filter and the resulting solution transferred to a volumetric flask and diluted to 50 ml. The extinction value of the solution is then measured, either on a Brunius type colorimeter * using a S53 filter and a 20 mm cell or on an EEL type ** instrument with filter 624 and the appropriate round cell. The extinction value is directly proportional to the amount of Xylocaine present (see Fig. 1), and a previously prepared standard curve or a conversion factor gives the actual weight of base in the sample of solution taken.

If the concentration of Xylocaine in the solution to be examined is less than 3 mg/ml, the determination can be carried out on a larger volume than 10 ml such that the amount of base taken is again of the order of 25 mg, provided that the volume of ammonium reineckate reagent used is also increased by 5 ml for every additional 30 ml of the Xylocaine solution. In such cases the mixture should be allowed to stand for an hour or more before filtration of the precipitate.

The saturated ammonium reineckate solution should be freshly prepared each day; it is conveniently made by mechanically shaking 1.5 g of the salt with 100 ml of water at room temperature for 15 minutes and then filtering. The salt used should be of good quality it deteriorates slowly on prolonged storage and may then give rise to low results in the Xylocaine estimation (a sample of the salt which had been stored for ten years gave values 14 % lower than those given by a fresh sample).

The acetone employed should be free from ester impurities, which may cause extraneous coloration of the solution of precipitated reineckate; the water-content has little influence on the results of the estimation provided that it is below 10 %. Suitable tests for the purity of the solvent have been described by Merck (*Prüfung der chemischen Reagenzien auf Reinheit*, Darmstadt (1939) p. 1), and when these are satisfactorily met the extinction value of the acetone solution at a wavelength of 530 mμ remains unchanged for at least 5 hours at room temperature.

* A single-cell photoelectric colorimeter constructed by Professor Brunius, State Institute for Public Health, Stockholm, Sweden.

** Evans Electroselenium Ltd., Harlow, Essex, England.

APPLICABILITY OF THE METHOD TO BASES OTHER THAN XYLOCAINE

Although Xylocaine reineckate is practically insoluble in water and in the ammonium reineckate reagent, the solubilities of the reineckates of other bases vary considerably (see Table 1); the method is therefore not of universal applicability and for the present must be tested in each individual case. The procedure described above has been found to give good results for the estimations of β -diethylaminoethyl 4-aminobenzoate (procaine), diethylamino acetomesidide ("LL31"), and 10- $[\beta$ -(dimethylamino)-propyl]-phenthiazine ("Thiorgan"). With aniline and diethylaminoethanol it was found possible to obtain quantitative results by using a modified procedure. A more concentrated solution of the base was employed, containing about 0.05 mg-equiv. per ml; 2 ml of this was precipitated at 0° with 5 ml of reagent, and the mixture allowed to stand at that temperature for a few hours before filtration. Finally, the precipitate was washed with ice-cold water (two portions of 2 ml). The reineckates of α -(3,4-dihydroxyphenyl)- β -methyl-aminoethanol (adrenaline), 1-phenyl-2-aminopropane (phenopromine), diethylamine, diethanolamine and guanidine all proved to be far too soluble to give quantitative results. (This

Table 1. Losses of some freshly precipitated reineckates in different washing procedures. Precipitates (corresponding to approx. 0.12 mg.-equiv. base) washed on filter, extinction values measured in acetone solution. Differences between values so obtained and theoretical values corresponding to amount of base taken given as % of latter.

Washing agent	Temp. °C	Reineckate of				
		Aniline	"Thiorgan"	"LL 31"	Xylocaine	Procaine
50 ml dist. water	20	48	10	8	8	25
	10	40	5	5	10	23
	3	21	2	6	8	17
2 × 2 ml dist. water	20	5	3	0	0	0
	3	1	0	0	0	0
	0	0	0	0	0	0
50 ml 0.15 % ammonium reineckate solution followed by 1 ml dist. water	20	63	0	0	0	17
	3	15	0	0	0	—

fact has rendered it possible to accurately estimate Xylocaine in pharmaceutical preparations such as "Umbradil Viskös" and "Isofen", in which diethanolamine and diethylamine are also present. Such solutions, however, must be specially treated before the estimation can be carried out).

It is clear that the applicability of the reineckate method is not governed merely by the strength or type of the base concerned. Thus, among the substances which give quantitative precipitates under the specified conditions, are five aromatic compounds having pK_a values between 9 and 4.5, but adrenaline, which has a pK_a value in this range (approx. 7), is not precipitated quantitatively and 1-phenyl-2-amino-propane (pK_a approx. 10) gives only a negligible precipitate. Among the aliphatic bases investigated only one, diethylaminoethanol (pK_a approx. 10), gives a quantitative result and that only under modified conditions.

Extinction values for the reineckates of all the bases which have been investigated are given in Table 2.

SOME GENERAL OBSERVATIONS ON THE METHOD

The reineckate method has been tested at first hand in rapid routine analyses of preparations which apart from Xylocaine itself contain only sodium chloride, methyl *p*-hydroxy-benzoate and small amounts of adrenaline (the latter does not interfere owing to its very small concentration and to the solubility of its reineckate). A statistical calculation of the accuracy of the method has been made from data obtained in analyses on twelve solutions of "Xylocaine-Exadrin 2 %", in which the Xylocaine content was also independently estimated by the older extraction method. The ratio of corresponding colorimetrically and titrimetrically determined values was 1 ± 0.03 , the average ratio being 1.003. The standard deviation for individual ratios $\left(\sqrt{\frac{\sum A^2}{n}}\right)$ was $\pm 2\%$; this figure includes the experimental errors of both methods. In the case of nine samples of "Umbradil Viskös" solutions "B" and "H", colorimetric determinations gave values of $100 \pm 2\%$. The standard deviation here was $\pm 1.6\%$, which represents variations not only in the estimations themselves but also in the pre-treatment of the solutions. A group of 120 arbitrarily chosen solutions of "Xylocaine-Exadrin 2 %" were analysed by eight different persons; the average, the maximum, and the minimum values obtained were respectively 20.2, 21.0 and 19.2 mg/ml, and the standard deviation of the individual values was $\pm 2\%$ (this includes possible inaccuracies in the preparation of the solutions).

Table 2. Summary of results for reineckates investigated. pK_a values of base components are approximated to one place of decimals without consideration of the difference between thermodynamic and stoichiometric constants. Values in parentheses are calculated from original potentiometric titration results; other values are taken from Löfgren² or Landolt-Börnstein "Physikalisch-Chemische Tabellen". Extinction values measured according to standard procedure and calculated on the amount of base in the original solution.

Reineckate of	Remarks	pK_a	$E_{1\text{ cm}}^{1\text{ g mol/l}}$ 526 m μ	$E_{1\text{ cm}}^{1\text{ g equiv/l}}$ 526 m μ	$E_{1\text{ cm}}^{1\text{ g equiv/l}}$ 395 m μ
Ammonium	Salt recryst. 3 times from water. Extinction constant after second and third recrystallisations.	9.3	106.0	106.0	85.5
Aniline	Freshly distilled aniline precipitated in ether as hydrochloride and recryst. twice. Nitrogen content (Dumas) taken to correspond to % aniline.	4.6	107.5	107.5	88.5
"Thiorgan"	100 % pure	(7.8)	105.5	105.5	85.0
Diethylamino-acetomesidide ("LL 31")	100 % pure m.p. 46–48°	7.9	106.0	106.0	81.9
Xylocaine	100 % pure m.p. 68–70°.	7.9	106.3	106.3	85.3
Procaine	Quality prescribed by Swedish Pharmacopeia Ed. XI. Purity (determined by titration) 100 %.	8.9	210.0	105.0	85.0
Diethylamino-ethanol	Titration indicated 96 % purity. The preparate was probably not entirely homogeneous.	(10.1)	104	104	84.5
1-Phenyl-2-aminopropane	Quality prescribed by Swedish Pharmacopeia Ed. XI. Purity (determined by titration) 100 %.	10.0	91	No quantitative precipitation with ammonium-reineckate	
Adrenaline	,	(8.9)	89		

Diethylamine	Purity (determined by titration) 100 %	12.0	—	No precipitate
Diethanol-amine	»	7.9	—	
Guanidine	»	rather strong base	—	
Average over the first six estimations				106.0 $\pm$ 0.4 85.1 $\pm$ 0.9

In the case of the other bases to which the reineckate method is applicable no large series of analyses have been carried out; the distribution of the points on the standard curve in Fig. 1 suggests, however, that the accuracy of the estimation is of the same order as that for Xylocaine.

The sensitivity of the method is mainly determined by the minimum extinction value which can be accurately measured. On the Brunius colorimeter this is approximately 0.15, and when the standard procedure is followed and the colorimetric measurements are made in a 2 cm cell this corresponds to about 0.04 mg-equiv. of base. Smaller amounts of base may however be estimated by increasing the cell thickness and diminishing the amount of acetone used to dissolve the reineckate precipitate; thus, employing a 3 cm cell and only 10 ml of solvent instead of 50 ml, the minimum that can be estimated is approximately 0.005 mg-equiv. (1.2 mg Xylocaine). The sensitivity is limited not by the absorption of the reineckate precipitate in acetone solution but rather by the solubility of the precipitate in the aqueous media employed in the estimation.

Fig. 2. Extinction-wavelength curves (Beckman Spectrophotometer DU, 0.004 M solutions, 1 cm cell).

+ *Reineckate of procaine*

o *Reineckates of aniline, LL 31, Xylocaine, and "Thiorgan"*

Table 3. Values of $10^3 \cdot E_{1\text{ cm}}^{0.004 M}$ for some reineckates in acetone solution (determined on Beckman DU spectrophotometer).

Slit no.	Wave-length $m\mu$	Reineckate of							
		Procaine	Xylocaine	"LL 31"	"Thiorgan"	Aniline	Ammonium		
							*	**	***
0.2	500	690	347	346	349	360	348	350	349
»	510	778	391	391	393	403	389	391	390
»	520	831	417	418	416	425	415	422	417
»	526	840	425	424	422	430	418	426	424
»	530	832	425	422	420	428	415	422	418
»	540	733	397	397	394	400	388	393	390
»	560	552	289	292	290	289	274	281	275

* Recrystallised once.

** , twice.

*** , three times.

The color given in acetone solution by reineckates of organic bases is, as has been pointed out by Canbäck³, due to the presence of the reineckate group. In equimolar solutions, therefore, the intensity of the color is independent of the actual base concerned. We found that the quantity $E_{1\text{ cm}}^{1\text{ g-equiv/l}}$ has a value of 85 ± 1 at $395\text{ m}\mu$ and 106.1 ± 0.2 at $526\text{ m}\mu$. This fact has been verified for 0.004 M solutions of ammonium, aniline, diethylaminoacetomesidide, diethylaminoethanol and 10- $[\beta\text{-(dimethylamino)-propyl}]$ -phenothiazine reineckates, all of which give the same curve, namely the lower one in Fig. 2 (the readings were taken on a Beckman DU spectrophotometer with a 1 cm cell). The degree of coincidence is also clearly seen from the extinction values in the wavelength region of $500\text{--}560\text{ m}\mu$, given in Table 3. This generalisation is, of course, only true if the base combines with a single reineckate residue. Procaine, for example, combines with two such residues, one at the primary and one at the tertiary nitrogen atom, and the resulting extinction value is therefore twice as great as that given by an equivalent amount of any of the other bases investigated. It should however be noted that the basicity of a substance with respect to reineckate is not always the same as when determined by titrimetric methods; thus procaine although it forms a di-reineckate, behaves only as a monobasic compound on titration.

This latter fact renders it possible to estimate individually the components of a binary mixture of bases, one of which exhibits identical, the other different

Table 4. Analyses of solutions containing mixtures of Xylocaine and procaine hydrochlorides.
Values given as % of total base-hydrochloride present.

Amount taken		Amount found		
% Xylocaine HCl	% Procaine HCl	% Xylocaine HCl	% Procaine HCl	% Total
11.3	88.7	13.8	87.2	101.0
46.7	53.3	46.7	52.4	99.1
91.2	8.8	89.6	9.8	99.4

equivalent weights in the two cases, by carrying out both colorimetric and titrimetric determinations. The accuracy is however poor if one of the bases is present in large excess compared with the other. In Table 4 are included the results of three such estimations on mixtures of Xylocaine and procaine (in order to increase the accuracy in these cases, a Beckman DU spectrophotometer was employed). Similarly it is possible to follow the decomposition of procaine into diethylaminoethanol and *p*-aminobenzoic acid, since the basicities of these compounds with respect to reineckate are two, one and zero respectively. In cases where the equivalent weight of a base cannot be directly determined titrimetrically owing to the presence of another base, the reineckate method may provide an alternative if the accompanying base does not give an insoluble reineckate. Thus Xylocaine may be determined in the presence of diethylamine, and procaine may be estimated in the presence of caffeine provided the pH is adjusted fairly exactly to 2 before precipitation. Procaine in procaine penicillin can also easily be determined with reineckate.

SUMMARY

A rapid method for the estimation of Xylocaine by means of ammonium reineckate is described. It is based on spectrophotometric measurement of the colour produced in acetone solution by the reineckate ion. In routine analysis the standard deviation of the results of individual estimations does not exceed $\pm 2\%$ and under favourable conditions it can be reduced to $\pm 1\%$. The method is also applicable to many other organic bases and is under certain conditions superior to the ordinary titrimetric procedure. By combining the latter with the colorimetric method it is possible to estimate separately the constituents of some binary mixtures of bases.

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