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DETERMINATION OF PENICILLINS AND CEPHALOSPORINS USING 3-BROMO-4,4-DIMETHYL-2-OXAZOLIDINONE

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SUMMARY

A quantitative assay procedure for penicillins and cephalosporins was developed using 3-bromo-4,4-dimethyl-2-oxazolidinone (NBDMO). The main advantage of NBDMO over N-bromosuccinimide (NBS) is its stability as a reagent solution, resulting in reliable and reproducible results. The new reagent should be useful in other bromometric type quantitative assays.

INTRODUCTION

The determination of penicillins and its formulations using an iodometric titration procedure has been known and practiced since 1946 (Alicino, 1946). The method is based on the fact that the intact penicillin molecule does not absorb iodine, while the alkaline hydrolysis product, sodium penicillinoate, does react with iodine. The accuracy of this volumetric method compares favorably with the microbiological cylinder-plate method and it is more convenient and rapid. The iodometric assay has been extended also to include synthetic penicillins, as well as cephalosporins (Alicino, 1961).

It was recently found that the iodometric assay has failed with some synthetic cephalosporins, presumably due to sensitivity of the side-chain under the assay conditions (Alicino, 1976). This observation prompted development of an alternative assay which could compliment the iodometric method.

A bromometric method has been reported for penicillin O where an alkali inactivation of the penicillin was no longer required (Weis et al., 1953). Similarly, the reaction of N-bromosuccinimide (NBS) with penicillin was also found not to require inactivation with alkali or penicillinase. However, use of this reagent in a direct assay procedure was complicated by the time dependence of its reaction with penicillin and the sensitivity of NBS stock solutions to light and air oxidation.

Recently, a new brominating agent, 3-bromo-4,4-dimethyl-2-oxazolidinone (NBDMO) was developed and was compared to NBS in its chemical behavior (Kaminski et al., 1976).

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NBDMO was found to be equivalent or better than NBS as a brominating agent in the reactions investigated and NBDMO appeared to exhibit advantages in terms of its stability and selective reactivity relative to NBS. These observations lead to the examination of NBDMO as an alternative to NBS in the assay of antibiotics.

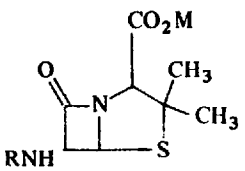
In the present work, the comparative assay of a number of penicillins and cephalosporins using both NBS and NBDMO is described. In addition, a comparison between the stability of stock solutions of NBS and NBDMO has also been examined.

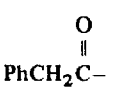
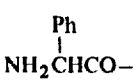
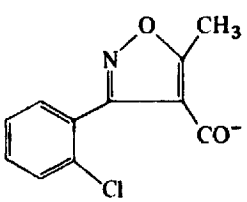
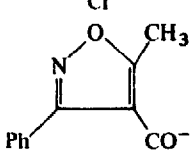
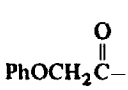
MATERIALS AND METHODS

N-Bromosuccinimide (NBS) was purchased from the Aldrich Chemical Company. The synthesis of 3-bromo-4,4-dimethyl-2-oxazolidinone (NBDMO) has been described previously (Kaminski et al., 1976). The NBDMO used in this study was recrystallized from water. The antibiotics used were obtained from Bristol and Eli Lilly Research Laboratories and are listed in Tables 1 and 2.

TABLE 1

PENICILLINS ASSAYED USING NBS AND NBDMO SOLUTIONS

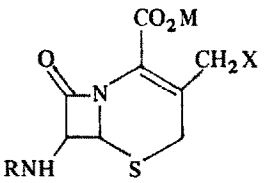


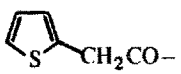
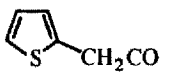
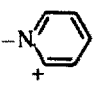
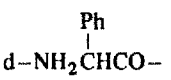
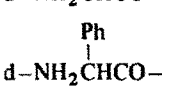
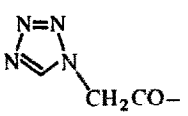
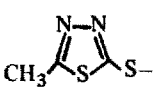
Compound	Nr.	R	M
Penicillin G potassium, $C_{16}H_{17}KN_2O_4S$, MW 372.48	1a [a] 1b 1c		K
Ampicillin, $C_{16}H_{19}N_3O_4S \cdot 3\,H_2O$, MW 403.46	2		H
Sodium cloxacillin, $C_{19}H_{17}ClN_3NaO_5S$, MW 457.89	3		Na
Sodium oxacillin, $C_{19}H_{18}N_3NaO_5S \cdot H_2O$, MW 441.45	4		Na
Penicillin V potassium $C_{16}H_{17}KN_2O_5S$, MW 388.48	5		K

[a] 1a, 1b and 1c were samples of penicillin G of different origin and potency.

TABLE 2

CEPHALOSPORINS ASSAYED USING NBS AND NBDMO SOLUTIONS



Compound	Nr.	R	X	M
Cephalothin sodium $C_{16}H_{15}N_2NaO_6S_2$, MW 418.44	6		CH_3CO_2-	Na
Cephaloridine $C_{19}H_{17}N_3O_4S_2$, MW 415.50	7			–
Cephaloglycin $C_{18}H_{19}N_3O_6S$, MW 405.42	8		CH_3CO_2-	H
Cephalexin $C_{16}H_{17}N_3O_4S$, MW 347.39	9		H	H
Cefazolin sodium $C_{14}H_{13}N_8NaO_4S_3$, MW 476.52	10			Na

(1) N-Brominating agent solutions

N-Bromosuccinimide (NBS), 1.78 g (0.01 mol) and 3-bromo-4,4-dimethyl-2-oxazolidinone (NBDMO), 1.94 g (0.01 mol), were each dissolved in 5 ml dimethylformamide (DMF). The dimethylformamide solutions were each diluted to 1 liter using distilled water. The resulting solutions were each transferred into amber bottles. The concentrations of the NBS and NBDMO solutions were each determined by iodometric titration using standardized sodium thiosulfate (9.93×10^{-3} N). The concentration of the NBS and NBDMO solutions were 1.85×10^{-2} N and 2.05×10^{-2} N, respectively.

(2) Antibiotic assay

A 0.5 M sodium acetate, pH 5.4 buffer solution was prepared by dissolving 41 g (0.5 mol) sodium acetate in 1 liter of distilled water containing 2 ml concentrated sulfuric acid.

General procedure. A 10–15 mg sample of each penicillin and cephalosporin to be analyzed was accurately weighed and dissolved in 20 ml of the sodium acetate buffer. To each antibiotic solution was added 15 ml of the N-brominating agent solution. At predetermined time intervals after the addition of the N-brominating agent (0.5, 1, 2, ... 5 h), the samples were analyzed by iodometric titration relative to a blank sample of the N-brominating agent using standardized sodium thiosulfate. The results of these studies have been described in Tables 3 and 4 and Fig. 1.

TABLE 3

TIME DEPENDENCE OF THE REACTION OF NBS AND NBDMO WITH PENICILLIN G (1a)

Time (h)	NBS assay		NBDMO assay	
	mg/mEq [a]	mmol NBS / mmol Pen. G	mg/mEq	mmol NBDMO / mmol Pen. G
0.5	70.06	2.66	69.21	2.69
1	64.93	2.88	62.91	2.96
2	61.13	3.06	58.43	3.18
3	59.02	3.18	56.12	3.31
4	59.10	3.19	53.67	3.47
5	57.55	3.29	52.25	3.56

[a] mg compound/mEq of sodium thiosulfate

(3) Stability of the N-brominating agent solutions

The NBS and NBDMO solutions in amber bottles were stored at ambient temperature. The concentration of the NBS and NBDMO solutions were determined daily by iodometric titration using standardized sodium thiosulfate. A semi-logarithmic plot of the volume of titrant against time for both the NBS and NBDMO solutions is shown in Fig. 2. No significant change in the concentration of the NBDMO solution over the time interval examined was observed. The change in the concentration of the NBS solution appears to exhibit first-order kinetic behavior, $k_1 = 4.8 \times 10^{-2}$ day^{-1}, $t_{1/2} = 14.5$ days, $r_c = 0.997$.

TABLE 4

DETERMINATIONS OF PENICILLINS AND CEPHALOSPORINS [a]

Compound	NBS assay		NBDMO assay		
	mg/mEq	mmol NBS / mmol antibiotic	mg/mEq	mmol NBDMO / mmol antibiotic	NBDMO equivalents / NBS equivalents
1a	61.13	3.06	57.66	3.22	1.05
1b	61.59	3.04	57.16	3.25	1.08
1c	61.07	3.07	58.00	3.20	1.05
2	58.38	3.47	62.61	3.21	0.93
3	86.60	2.66	82.15	2.78	1.05
4	87.69	2.54	80.78	2.72	1.09
5	49.12	3.97	46.47	4.17	1.06
6	42.97	4.87	41.68	5.01	1.03
7	41.14	5.05	40.11	5.17	1.03
8	44.20	4.59	44.84	4.51	0.99
9	28.15	6.27	27.61	6.28	1.02
10	38.30	6.26	34.71	6.05	0.97

[a] Samples were analyzed 2 h after mixing.

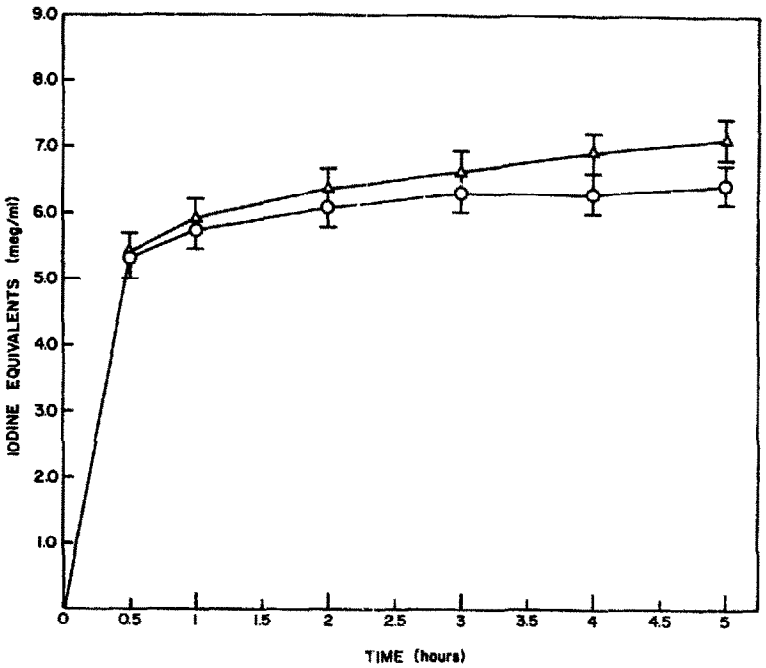


Fig. 1. Time-dependent characteristics of the reaction of NBS (○) and NBDMO (△) with penicillin G (1a).

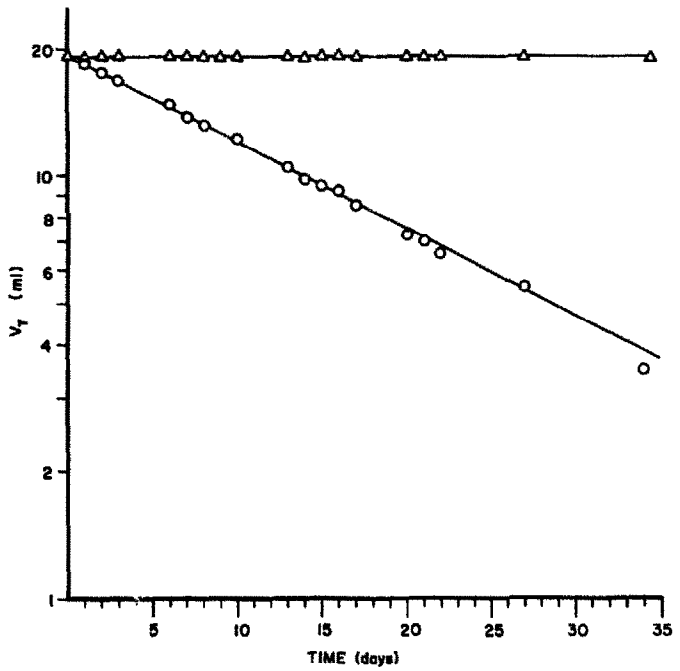


Fig. 2. Stability analysis of the NBS (○) and NBDMO (△) stock solutions. V_T is the volume of sodium thiosulfate solution (9.93×10^{-3} N) used for 10 ml of the reagent stock solutions.

RESULTS AND DISCUSSION

The penicillins of various structures and origins (Table 1) and the cephalosporins (Table 2) were assayed both by the NBS and NBDMO methods. A typical time vs reagent consumption sequence is given in Table 3 and the corresponding iodine equivalents are illustrated in Fig. 1. It can be seen that the two methods give essentially the same results, not only for penicillin G, but for all the various penicillins and cephalosporins studied, as indicated by Table 4. The NBDMO/NBS equivalents ratio is close to unity throughout the samples analyzed. The analysis of the NBS and/or NBDMO equivalents consumed for each antibiotic molecule indicates that while in the majority of the cases, integer number of equivalents were consumed in 2 h reaction time (such as compounds 1, 5, 6, 7, 10), in some cases the molequivalents are just fractional (~3.5 for 2; ~2.7 for 4, etc.). This underlines the importance of the time factor, but also the reliability of the reagents.

Comparative analysis of the two reagent solutions as shown in Fig. 2 indicate a dramatic difference in their stability. While the NBDMO solution did not show any sign of decomposition, the NBS solution underwent a very fast and steady decomposition with a half-life of about 14 days at room tempeature.

In conclusion, we are recommending the use of a bromometric assay based on 3-bromo-4,4-dimethyl-2-oxazolidinone (NBDMO) for the quantitative analysis of penicillins and cephalosporins. The new reagent should be useful in other bromometric type quantitative assays.

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