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Stable Antibiotic Sensitivity Disks

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Two methods of preparing sensitivity disks were compared for their effect on disk stability at 25 and 37 C. One method consisted of applying a solution of the antibiotic to blank disks by the conventional procedure; the second method consisted of applying the antibiotic to the disks as a suspension of crystals. Of the four β-lactam antibiotics that were studied, disks made with suspended crystals were substantially more stable than corresponding disks made by the conventional method. The increased stability is related to the greater chemical stability of the antibiotics in the crystalline versus the amorphous state.

Antibiotic sensitivity disks are generally stored at refrigerator temperature to prevent degradation of the antibiotic. Current Food and Drug Administration guidelines (1) permit the use of disks that are between 150 and 66% of labeled potency. Despite the breadth of this provision, informal surveys have indicated that clinical laboratories at times use disks that are less than 66% of labeled potency. This premature degradation may be the result of exposing packaged disks to higher temperatures at some time during their shelf-life. Unrecognized use of subpotent disks may yield incorrect conclusions about microbiological susceptibility or resistance.

The purpose of this paper is to call attention to a method for the preparation of sensitivity disks that are stable for long periods under ambient conditions. Although the experiments described were performed with only β-lactam antibiotics, the findings should apply as well to disks made with other degradable antibiotics.

MATERIALS AND METHODS

Cephalothin sodium was acquired as Keflin from Eli Lilly & Co.; microbiological potency, 945 μg/mg.

Penicillin G potassium was obtained from Eli Lilly & Co.; microbiological potency, 1,595 U/mg.

Cefazolin sodium was acquired as KEFZOL from Eli Lilly & Co.; microbiological potency, 963 μg/mg.

Cefamandole lithium was prepared from cefamandole sodium (see CMT in reference 3). Cefamandole sodium was dissolved in an aqueous solution of lithium acetate and methanol was added to effect crystallization; microbiological potency, 976 μg/mg.

Polyvinylpyrrolidone (PVP) was obtained as Povidone, N.F. All solvents were analytical reagent grade.

Sterile blank disks (6.5 mm) were acquired from BBL, Cockeysville, Md.

A metering syringe and holder, model PB-600, with a microliter syringe was obtained from Hamilton Co., Whittier, Calif.

A mill, type 63C, was acquired from Alpine A. G., Augsburg, Germany.

Preparation of "suspension" disks. The crystalline cephalothin sodium was ground by a single pass through the mill (200 mesh) at a setting of 50. For each experiment, 100 mg of this powder was suspended in 50 ml of liquid. Liquids used were chloroform and isopropanol containing small amounts (usually 1%) of PVP. A few drops of the stirred suspension were periodically examined under the microscope to check for complete dispersion of crystals. A metering microsyringe was filled with suspension and 20-μl portions were promptly applied to the center of one face of blank disks (40 μg of antibiotic per disk). The moist disks were spread on a stainless-steel wire cloth and dried in a moving-air oven at 60 C for 5 min.

Suspension disks containing the other antibiotics were made in a similar fashion. Any necessary potency adjustments were made by altering concentrations so that the applied volume remained at 20 μl.

Preparation of "solution" disks. Control disks were made by applying the respective antibiotics in a 1:1 methanol-water solution (pure water for penicillin G), using blank disks, a syringe, volumes, and drying conditions as above. For purposes of discussion, commercial disks are included in this category.

Physical comparison of suspension with solution disks. Cephalothin sodium disks of both kinds were made for optical examination as described above except that blank disks were punched from glass fiber paper (grade 934AH, Reeve Angel Co., Clifton, N.J.). These (dried) disks were soaked briefly in immersion oil to displace trapped air and examined through a microscope under plane-polarized light. Photomicrographs (Fig. 1, a and b) showed that the antibiotic on the suspension disk (Fig. 1a) was in the form of crystals, whereas no crystals could be seen on the solution disk (Fig. 1b).

Testing potency and stability. Initial and periodic potency determinations were performed by standard disk-plate methods (1). Twelve disks were assayed on each of two successive days and the results for each day were averaged.

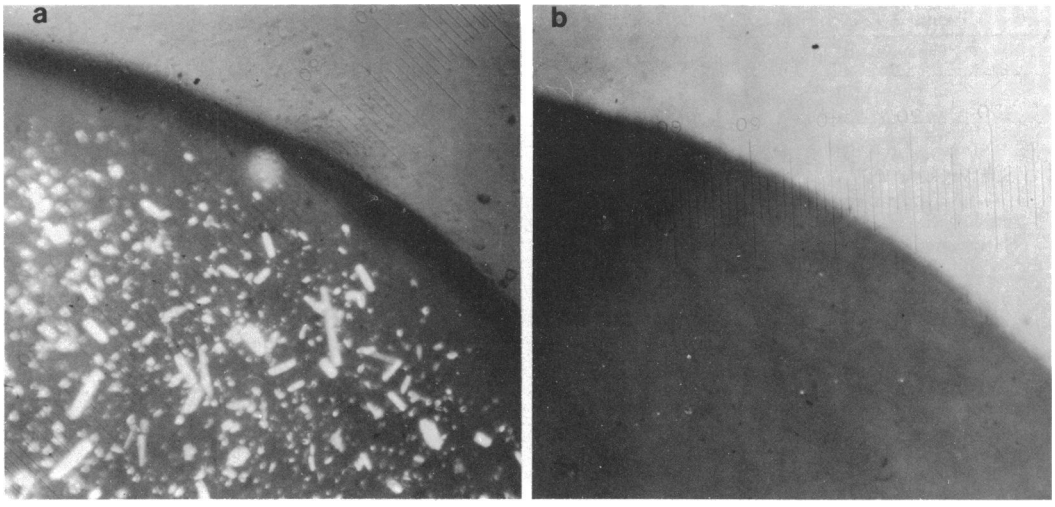

FIG. 1. *Comparison of suspension and solution disks. (a) Cephalothin sodium (40 μg) suspended in 20 μl of 1% PVP solution in isopropanol, applied to a glass fiber disk, and dried. Mounted in immersion oil. (Crossed Nicols, original ×50). (b) Cephalothin sodium (40 μg) dissolved in 20 μl of 1:1 methanol-water, applied to glass a fiber disk, and dried. Same photographic conditions as (a).*

For quick checking of antibiotic content and uniformity within a lot, individual cephalothin sodium disks were extracted by shaking with 3 ml of water. The absorbance of the resulting solution at 235 nm, read on a Cary model 14 spectrophotometer, was compared with a standard curve for cephalothin.

To test the microbiological availability of the crystalline antibiotic, cephalothin sodium suspension disks were placed on agar test plates, six disks with application side up and six disks with application side down, and assayed by the disk plate method.

Testing the adhesive effect of PVP. Suspension disks were made with 40 μg of cephalothin sodium and varying amounts of PVP (0 to 500 μg), which served as an adhesive to prevent mechanical loss of crystals during handling. Five disks of a given lot were mechanically shaken in a 15-ml capped bottle for 1 min with a stroke of 10 cm, 300 oscillations per min. The group of disks was then extracted with water, and the extract was analyzed spectrophotometrically, as described above.

To test the possible effect of PVP on potency stability, solution disks were made containing 40 μg of cephalothin sodium and 100 μg of PVP for comparison with solution disks containing only the antibiotic.

Storage. Dried disks were stored in glass vials resembling typical commercial disk vials, i.e., with polyethylene stoppers and a reservoir at the bottom filled with silica gel. The storage chambers were maintained at: (i) 25 C with 40% relative humidity, (ii) 37 C with 30% relative humidity, and (iii) 50 C with 20% relative humidity.

RESULTS

Stability studies. The stability of the various β-lactam antibiotic suspension disks and solution disks was measured. As indicated in Table 1, the suspension disks were more stable than the solution disks in every case. The data obtained with cephalothin sodium disks indicated that the suspension disks were only slightly less potent after 12 months at 25 C and 37 C than initially. In contrast, the solution disks of maximum initial potency fell below the lower Food and Drug Administration limit (66% of nominal 30 μg = 19.8 μg) within approximately 5 months at 25 C and 1 month at 37 C. Figure 2 gives a graphic comparison of the stability of cephalothin sodium suspension and solution disks at 37 C.

Similar results were obtained in analogous comparisons of penicillin G potassium disks, cefazolin sodium disks, and cefamandole lithium disks, each prepared by both methods.

General suitability of suspension disks: availability of crystalline cephalothin sodium. The microbiological test results were the same (mean of six zone diameters = 19.18 versus 19.05 mm, respectively) whether the antibiotic was diffused directly into the agar from the disk face that contained most of the crystals (application face-down) or was diffused first through the entire disk before reaching the agar (application face-up).

PVP and mechanical stability. Table 2 compares the effect of extremely vigorous shaking on loss of potency from solution disks and from suspension disks containing increasing amounts of water-soluble adhesive (PVP). As expected, the active compound, in the absence of adhesive, was held less firmly in suspension

TABLE 1. *Potency of antibiotic sensitivity disks prepared by solution and suspension methods*

Type of disk	Microbiological potency (μg/disk)[a,b]					
	Initial	1 mo	2 mo	3 mo	6 mo	12 mo
			T = 25 C			
Suspension	37.7			37.2	38.3	35.5
Cephalothin sodium[c]	36.7	46.5	31.0	40.9		
Cephalothin sodium[d]						
Solution	41.6	33.4	15.2	21.6	<15	
Cephalothin sodium[e]	45.5	36.8	33.6	21.5	22.0	<15
Cephalothin sodium[f]						
			T = 37 C			
Suspension	43.9	39.7	42.7	38.9	41.1	33.3
Cephalothin sodium[c]	36.7	49.0	31.7	38.6	37.5	
Cephalothin sodium[d]	32.9	28.8	34.3	34.8	30.2	27.8
Cefazolin sodium[c]	30.8	29.0	29.2	37.3	30.5	29.3
Cefamandole lithium[c]	13.8	12.6	12.8	11.7	9.4	8.3
Penicillin G potassium[c]						
Solution	43.4	15.0	<15			
Cephalothin sodium[e]	40.7	24.6	19.8	15.6	<10	
Cephalothin sodium[f]	40.5	18.5	16.4	<15		
Cefazolin sodium[e]	37.1	20.9	17.1	15.0	<10	
Cefamandole lithium[e]	10.4	<5				
Penicillin G potassium[g]	37.6	<15				
Cephalothin sodium[h]						

	Initial	1 mo	2 mo	4 mo	6 mo	12 mo
			T = 50 C			
Suspension	37.5	35.1		31.6	38.8	28.8
Cephalothin sodium[c]						
Solution	40.9	<15				
Cephalothin sodium[f]						

[a] Units per disk for penicillin.
[b] All values are means of results obtained with 24 disks. Standard deviations are <5.5%.
[c] One percent PVP-isopropanol.
[d] A 0.5% amount of PVP-chloroform.
[e] A 1:1 methanol-water ratio.
[f] Commercial disks.
[g] Water.
[h] A 0.5% amount of PVP-1:1 methanol-water ratio.

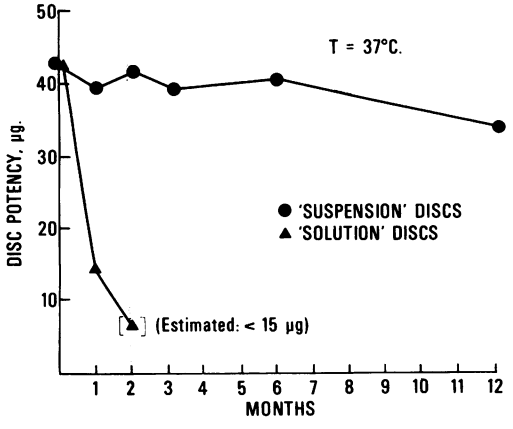

FIG. 2. *Potency of cephalothin sodium suspension and solution disks at 37 C.*

disks than in solution disks. Adding PVP, however, had an appreciable adhesive effect, and suspension disks made with 200 μg of PVP (1% PVP in the suspending vehicle) approached the solution disks in mechanical stability, as measured by this test.

The possible role of PVP as a potency-stabilizing factor was ruled out by observing that solution disks degraded no less rapidly at 37 C when PVP was added (see Table 1, last entry).

DISCUSSION

In contrast to the relatively rapid degradation of antibiotics in disks that require refrigeration, the same antibiotics in ampoules lose less than 10% of their potency during 2 years on the pharmacy shelf. Clearly, the two forms of the antibiotic are fundamentally different. One

TABLE 2. *Effect of PVP on retention of potency of suspension disks when vigorously shaken*

Disk	Disk content (μg)	Loss due to shaking (%); avg of 5 disks
Cephalothin sodium applied as suspension, plus:	40	
PVP	0	78
PVP	100	15
PVP	200	5
PVP	500	3
Cephalothin sodium applied as solution	40	3

obvious difference is in the amount of exposed surface. In the manufacture of sensitivity disks (1), a sheet of paper, wetted with antibiotic solution, is quickly dried to minimize decomposition. During the evaporation, a small amount of the antibiotic is thus deposited on a relatively large surface of cellulose in a thin amorphous film. The result is extensive exposure of the antibiotic molecules to detrimental environmental factors such as oxygen or moisture. In contrast, a crystalline compound has only a small fraction of its molecules at a surface, i.e., a crystal face, where attack by environmental factors most readily takes place. A second important difference relates to the greater thermodynamic stability of many compounds in their crystalline (ordered) versus their amorphous (disordered) state. Thus, Mathews and co-workers (2) reported that the heat stability of pure amorphous potassium penicillin G was remarkably improved when the same preparation was crystallized. With compounds susceptible to degradation, a disk manufacturing process that assures complete crystallinity of the antibiotic on the disk therefore should have a distinct advantage over one that leaves the antibiotic in an amorphous state.

In the present study the validity of this argument was tested using the following guidelines to make disks containing crystalline antibiotic. (i) The active compound must be applied to the disk paper as a suspension of crystals; (ii) the suspending vehicle must be volatile; (iii) the finished disk must be mechanically stable; and (iv) it must meet Food and Drug Administration requirements of performance.

In making suspension disks containing compounds that exhibit polymorphism, there is a risk of losing desired crystallinity while the compound is in contact with the suspending fluid or while the disk is being dried. In such cases it is advisable to choose the suspending vehicle and the drying conditions only after an appropriate physical study, e.g., by microscopy, X-ray diffraction, or infrared spectroscopy, has confirmed their suitability. The uniform application of the crystals to paper, whether by dipping, rolling, spreading, or spraying, is dependent on having a suspension whose physical stability is consistent with the operation. Obvious factors that are to be considered in this regard are size of the crystals, effect of additives (such as PVP), and specific gravity of the fluid vis-a-vis that of the crystals. With care, these factors can generally be favorably manipulated without adverse effects on the antibiotic.

ACKNOWLEDGMENT

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