Reactions of Acetaminophen in Pharmaceutical Dosage Forms: Its Proposed Acetylation by Acetylsalicylic Acid

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Abstract □ Acetylation of acetaminophen (paracetamol) by acetylsalicylic acid to *O,N*-diacetyl-*p*-aminophenol (DAPAP) was not evident in experimental pharmaceutical dosage forms. DAPAP, which was found to be present in small quantities in commercial grade acetaminophen, is unstable in pharmaceutical preparations. The destruction of DAPAP increases rapidly with the temperature and moisture content of the system. Solid DAPAP seems to exist in at least two forms, as shown by their IR spectra.

The observed color darkening of suspensions containing acetaminophen is mainly due to the oxidative degradation of the liberated *p*-aminophenol. The presence of codeine phosphate and caffeine seems to enhance the color deterioration of solid preparations containing acetaminophen kept at 45° and in a humid atmosphere. It is recommended that moisture be excluded, as much as possible, from such preparations.

Keyphrases □ Acetaminophen reactions—dosage forms □ Aspirin—acetaminophen acetylation □ Codeine PO_4, caffeine effect—acetaminophen stability □ Humidity effect—acetaminophen stability □ TLC—analysis □ IR spectrophotometry—identity □ UV spectrophotometry—identity

Previous publications have indicated that drugs containing amino- or phenolic-groups, such as phenylephrine (1) or codeine (2), as well as proteins (3) can be acetylated by acetylsalicylic acid in tablet formulations.

In 1967, it was reported (4) that acetaminophen (*N*-acetyl-*p*-aminophenol) can be acetylated by acetylsalicylic acid in tablet formulations, with the formation of *O,N*-diacetyl-*p*-aminophenol and salicylic acid. The same authors (4) proposed a linear relationship between the rate of formation of salicylic acid and the rate of formation of *O,N*-diacetyl-*p*-aminophenol and indicated the presence of *p*-aminophenol in some tablets, but they did not discuss its formation. However, a study of the kinetics of the formation of *p*-aminophenol by the hydrolysis of acetaminophen in aqueous solutions has been reported earlier (5).

Because there was a strong move in 1967 to have phenacetin removed from analgesic preparations being sold in Australia, many manufacturers replaced the phenacetin in their formulations by the same weight of acetaminophen. In this laboratory, however, it was found that in aspirin–acetaminophen preparations held at 45° under humid conditions or aspirin–acetaminophen suspensions held at 45°, *p*-aminophenol, together with its colored oxidation products, was being produced in a relatively short time.[1] In a number of these aspirin–acetaminophen preparations or suspensions the presence of *O,N*-diacetyl-*p*-aminophenol has also been established.

The formation of *p*-aminophenol in preparations containing acetaminophen is of some concern, therefore, and the presence of *O,N*-diacetyl-*p*-aminophenol attracted the author's attention, because any acetylation of acetaminophen (APAP) by acetylsalicylic acid (ASA) to the *O,N*-diacetyl-*p*-aminophenol (DAPAP) could, in addition to the direct hydrolysis of acetaminophen, be responsible for an alternative reaction path affecting the rate of the formation of *p*-aminophenol (PAP). However, the results of this paper show also that, contrary to what has been assumed earlier (4), acetylation of APAP by ASA was not evident under the conditions examined.

EXPERIMENTAL

Thin-layer Chromatography (TLC)—Analyses by TLC were carried out on 0.25 mm. Silica Gel GF_{254} (Merck reagent). Solvent systems (4) used in all cases were as follows: (*a*) chloroform–acetone–acetic acid (80:18:2), and (*b*) chloroform–ethanol–acetic acid (88:10:2).

TLC plates were usually eluted only once but, when necessary, a second elution (placing the plate in the solvent tank for a second time) improved the separation of spots. The various spots separated on the TLC plate could be easily seen against the green fluorescence of the plate by viewing in the UV light of short wavelength (254 mμ).

Materials—Acetaminophen, phenacetin, and acetylsalicylic acid were commercial (pharmaceutical) grade reagents. Acetaminophen (Winthrop), which showed more than one spot by TLC examination, was recrystallized four times from methyl isobutyl ketone before the spot corresponding to DAPAP disappeared. Figure 1 shows the results of the TLC examination of the residues and of the final product obtained from the recrystallization of acetaminophen; also, it shows that when 2% DAPAP was added before or after purification, only one DAPAP spot was obtained by TLC. The spots which correspond to pure DAPAP have UV and IR spectra identical with those of DAPAP. The UV spectra in ethanol have $\lambda_{max.}$ at 246 mμ and log $\epsilon \cong 4.20$. Solid DAPAP seems to be polymorphic and the form obtained depends on the solvent from which it is recovered. Thus, when it was recrystallized from water, the IR spectrum of the solid showed two carbonyl frequencies at

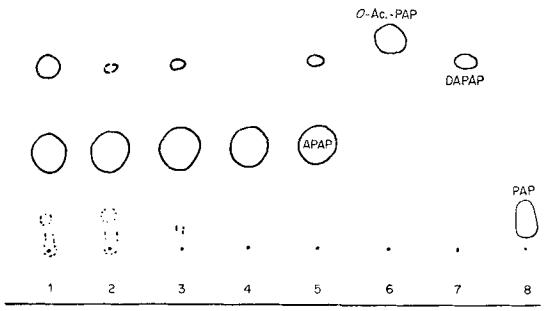

Figure 1—*Thin-layer chromatogram of APAP (Solvent* a) *Key:* 1, *residue from the filtrate of the first recrystallization of commercial APAP;* 2, *same as* 1 *but from second and third recrystallizations combined;* 3, *commercial APAP before recrystallization;* 4, *pure recrystallized APAP;* 5, *commercial APAP containing 2% DAPAP;* 6, *O-acetyl-p-aminophenol;* 7, *DAPAP; and* 8, *PAP.*

[1] Part of this work was presented at the I.U.P.A.C. 2nd International Congress on Pharmaceutical Chemistry, held at Münster/Westf. on July 22–26, 1968, in a paper on "Reactions of Acetylsalicylic Acid and Paracetamol in Dosage Forms," by B. G. Boggiano, E. Kalatzis, and F. E. Peters.

Table I—Uncompressed Mixtures of Powders

Mixture	APAP, g.	Phenacetin, g.	ASA, g.	SA, g.
1[a]	1.0	—	1.0	—
2[a]	1.0	—	—	1.0
3	—	1.0	1.0	—
4	—	1.0	—	1.0
5[b]	0.5	—	0.5	—
6[b]	0.5	—	—	0.5
7[b]	—	0.15	0.15	—

[a] Commercial (pharmaceutical) grade acetaminophen, which was shown by TLC to contain DAPAP. [b] Recrystallized acetaminophen, acetylsalicylic acid, and phenacetin.

1688 and 1738 cm.$^{-1}$ and an N—H frequency at 3364 cm.$^{-1}$. There were a shift and an increase in the complexity of these frequencies when the solid was recovered from other solvents. For example, the solid recovered from alcohol or benzene showed the two carbonyl frequencies at 1665 and 1752 cm.$^{-1}$ and what appeared as a number of N—H frequencies in the range of 3074–3292 cm.$^{-1}$. Furthermore, the solid recovered from acetone seemed to be a mixture of the two forms, because its spectrum contained all these frequencies. These changes, which may be due to the presence of the amide group, are reversible and have been observed with commercial DAPAP as well as with that recovered by TLC from commercial APAP. This problem is under investigation.

Phenacetin and acetylsalicylic acid were recrystallized three times from ethylacetate; they were shown by TLC to be pure (one spot). Salicylic acid (AnalaR), acetylsalicylic acid (May & Baker), codeine phosphate (May & Baker), and caffeine (D.H.A.) were used without further purification.

Commercial APAP containing 2% DAPAP was prepared as follows: 0.2 g. of DAPAP (Tokyo Kasei & Co.) together with 10 g. of APAP (commercial grade D.H.A. shown by TLC to contain no DAPAP) were dissolved in alcohol (20 ml.) and the clear solution obtained was then evaporated slowly to dryness under vacuum.

Preparations—*Suspensions*—Typical suspensions were prepared as follows: 10 g. acetylsalicylic acid, 10 g. acetaminophen, 200 ml. demineralized water, with or without 0.4 g. codeine phosphate or 1.0 g. caffeine, and with or without the following mixture: 4 g. compound powder of tragacanth BP and 20 ml. syrup of orange and 5 ml. concentrate chloroform–water (60% chloroform in 60% ethanol).

Similar suspensions containing phenacetin instead of acetaminophen were also prepared. All suspensions were kept at room temperature (about 25°) or at 45° for 14 weeks.

Table II—Uncompressed Mixtures of Powders

Mixture	APAP, g.	ASA, g.	SA, g.	Codeine Phosphate, g.	Caffeine, g.
1[a]	1.0	1.0	—	—	—
2	1.0	—	1.0	—	—
3	1.0	1.0	—	0.043	—
4	1.0	—	1.0	0.041	—
5	0.75	1.23	—	—	—
6	0.75	—	1.23	—	—
7	0.75	1.23	—	—	0.15
8	0.75	—	1.23	—	0.15
9	2.0	—	—	—	—
I[c]	1.0	1.0	—	—	—
II	1.0	—	1.0	—	—
III	1.0	1.0	—	0.042	—
IV	1.0	—	1.0	0.041	—
V	0.75	1.23	—	—	—
VI	0.75	—	1.23	—	—
VII	0.75	1.23	—	—	0.15
VIII	0.75	—	1.23	—	0.15
IX	2.0	—	—	—	—

[a] Mixtures 1–9 contain APAP (commercial grade) which was shown by TLC to be free from DAPAP. [b] Mixtures I–IX contain APAP (commercial grade as in Mixtures 1–8) to which 2% DAPAP was added.

Powders—Uncompressed mixtures of powders were prepared by grinding the weighed quantities of the appropriate materials and mixing thoroughly. No excipients were included in these mixtures, as is seen in Tables I–III.

Each uncompressed mixture was divided into six equal parts which were kept at room temperature (about 25°) and at 45° under the following conditions: (*a*) in sealed vials (dry mixtures), (*b*) in a desiccator over water (humid mixtures), and (*c*) in sealed vials in which 1–2 ml. of water was added (moist mixtures).

Samples (25–50 mg.) were taken out at regular intervals of time, dissolved in alcohol (about 2 ml.), and examined by TLC. Satisfactory results were obtained when many applications (about eight each of about 5 μl.) were used for each sample, thus ensuring that the minor components, if present, would appear as recognizable spots on the thin-layer chromatograms. The limits of detection lie between 0.2 and 0.4 mcg. of DAPAP spots on the TLC plate by the method described.

Tablets—The tablets examined were commercially manufactured products.

RESULTS AND DISCUSSION

Suspensions—Suspensions containing phenacetin and held at 45° for 14 weeks showed no color change and, as expected, neither DAPAP nor PAP could be detected by TLC.

Suspensions containing acetaminophen (commercial grade, which was shown to contain DAPAP) rapidly turned brown and deposited a black precipitate. Spots indicating the presence of both PAP and DAPAP were observed. While the amount of PAP and its colored oxidation products increased with time, the amount of DAPAP decreased and eventually could not be detected.

The level of PAP in the aqueous phase of these suspensions rose to about 10^{-2} *M*, as determined spectrophotometrically by using 4-nitrobenzaldehyde and removing the colored oxidation products (6).

Similar, but much slower, changes were observed in suspensions kept at room temperature. For example, at the end of 14 weeks the level of PAP was less than 10^{-4} *M* and 24 weeks later these suspensions, although discolored, were not as dark as the suspensions kept at 45° for 14 weeks.

Powders—The results obtained after examining the powder mixtures containing recrystallized APAP (Table I) indicate that no DAPAP is formed in the dry mixtures when kept at room temperature for 6 months. Also, no DAPAP is formed in all the other mixtures held at room temperature or at 45° for 2 months.

On the other hand, humid and moist mixtures containing commercial acetaminophen (Table I) and kept at room temperature indicated that the original DAPAP remained almost completely unaffected for 2 months. Similar results were obtained with the dry mixtures kept at room temperature for 6 months and at 45° for 2 months. However, the DAPAP present in the humid and moist mixtures held at 45°, for 6 and 2 weeks, respectively, decreased steadily and at the end it could not be detected, presumably because of hydrolysis to APAP. This view is supported by the fact that no additional spots were revealed by TLC (*O*-acetyl-*p*-aminophenol appears as a different spot than that of APAP, as shown in Fig. 1).

Uncompressed powder mixtures based on two-tablet formulations of the Australian Pharmaceutical Formulary were also prepared. No excipients were added to these mixtures as seen in Table II.

Table III—Uncompressed Mixtures of Powders

Mixture	APAP, g.	ASA, g.	SA, g.	Codeine Phosphate, g.	PAP, g.
1[a]	0.5	0.5	—	—	—
2[a]	0.5	0.5	—	0.05	—
3[b]	0.5	0.5	—	—	—
4[b]	0.5	0.5	—	0.10	—
5	—	1.0	—	—	1.0
6	—	—	1.0	—	1.0
7	—	—	—	—	2.0

[a] Commercial (pharmaceutical) grade acetaminophen, which was shown by TLC to contain DAPAP. [b] Recrystallized acetaminophen and acetylsalicylic acid.

The formulations are: (*a*) acetylsalicylic acid, 250 mg.; acetaminophen, 250 mg.; and codeine phosphate, 8 mg.; and (*b*) acetylsalicylic acid, 225 mg.; acetaminophen, 150 mg.; and caffeine, 30 mg.

The APAP used was a commercial grade, but TLC examination showed that it contained no DAPAP. Accordingly, DAPAP was added and thus two series of powder mixtures were prepared: one with APAP free from DAPAP and the other with APAP containing 2% DAPAP (Table II).

All the mixtures were exposed to the same conditions as indicated above. The results have shown that again no DAPAP was formed under any of the conditions examined and that the DAPAP added had a fate similar to that of the DAPAP present in the previous powder mixtures (Table I). These findings hold true for all the mixtures including those containing codeine phosphate or caffeine. Increased quantities of codeine phosphate added in a number of uncompressed powder mixtures (Table III) gave similar results.

No appreciable decrease in the DAPAP was observed in the dry mixtures held at room temperature and at 45° for 2 months (Fig. 2*a*). The DAPAP present in the humid mixtures which were held at 45° for 2 months decreased steadily to traces and disappeared completely when codeine phosphate was present. This acceleration in the destruction of DAPAP is possibly due to the liberation of phosphoric acid in the presence of moisture.

Only a slow decrease in the DAPAP present in the moist mixture held at room temperature was observed and DAPAP was still present after 2 months. On the other hand, the decrease in the DAPAP in the moist mixtures kept at 45° was rapid and no DAPAP could be seen after about 2 weeks (Figure 2*b*).

These observations seem to be consistent whether ASA or SA is mixed with APAP. Of course, ASA hydrolyzes to SA and acetic acid, and this can be easily seen by TLC, because ASA appears as a green spot in the UV light (254 mμ) against the fluorescent background of the plate while SA appears as a blue spot. Adequate separation of the ASA from SA can be effected in Solvent *b* referred to in the *Experimental* section.

Powder mixtures containing phenacetin (Table I) have shown no recognizable changes except that, as in the case of those containing acetaminophen, humid and moist mixtures develop SA rapidly at 45° and more slowly at room temperature.

Because the results of the present work have shown that DAPAP is not stable and that its destruction, like the hydrolysis of ASA, increases with temperature and moisture content of the systems under consideration, the work reported by the earlier authors (4) has been reexamined. Figure 3 shows a plot of the number of moles of free SA against the number of moles of DAPAP based on their data (4).

Such a plot, however, does not seem to support satisfactorily the suggestion made by the above authors (4) that there is a relationship between the rate of formation of SA and the rate of formation of DAPAP. For example, in the case of their results with Product C,

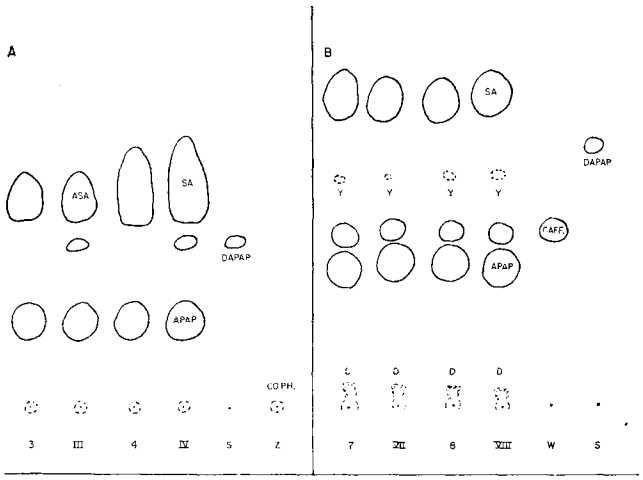

Figure 2—*Thin-layer chromatogram of uncompressed powder mixtures. (Solvent* a) *Key: for* 3 *to* IV *and* 7 *to* VIII, *see Table II;* S, *DAPAP;* Z, *codeine phosphate;* W, *caffeine;* Y, *yellow spots (seen in ordinary light); and* D, *rather dark brown spots. Chromatogram* A *was eluted once and Chromatogram* B *was eluted twice. (Dry* A *and moist* B *mixtures at 45°).*

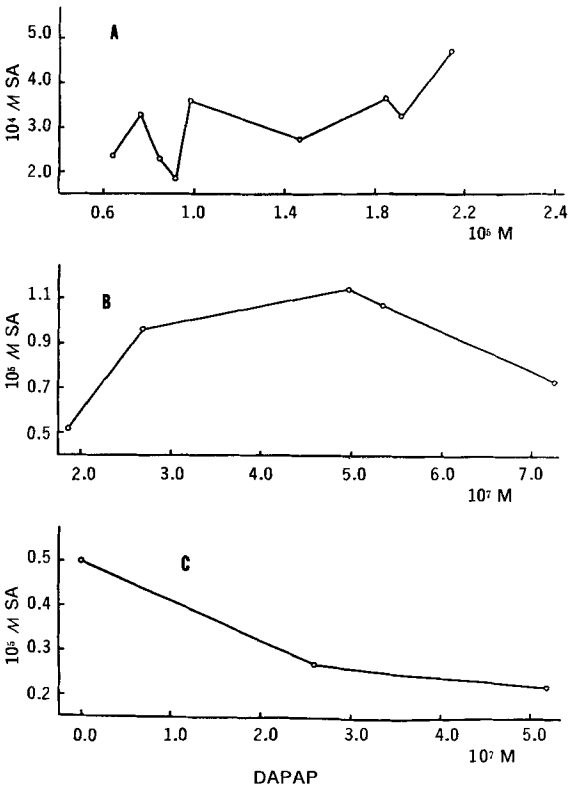

Figure 3—*Plot of the number of moles of free SA against number of moles of DAPAP in the samples of products* A, B, *and* C, *from the data of* Reference 4.

a fivefold increase in the number of moles of DAPAP corresponds to an approximate twofold decrease in that of the free SA.

These results rather suggest a random distribution of DAPAP in the various products. Furthermore, their (4) proposed linear relationship between the amount of DAPAP and that of SA formed with time in tablets from the same batch is not in agreement with the results of the present work, which indicates that the DAPAP is unstable especially under accelerated storage conditions. Since the storage conditions for the different products were probably not identical, the amount of DAPAP determined at a certain time would be expected to vary according to the history of the products concerned and the amount, if any, of DAPAP initially present. Moreover, all the figures quoted by the above authors (4) indicate that the number of moles of SA formed as a result of the proposed acetylation is far greater than that of DAPAP. For example, their (4) proposed linear relationship states that about 6 moles of SA are formed as a direct result of the formation of 1 mole of DAPAP. These results, however, are not in agreement with the proposed acetylation of APAP by ASA when it is considered that the formation of 1 mole of DAPAP is expected to be accompanied by the formation of 1 mole of SA.

An approximate estimation (semiquantitative TLC examination) of the DAPAP in commercial APAP (Table I) indicated that the level of DAPAP is within the range of 1.1–1.3%.

It is interesting to note that when APAP, with or without 2% DAPAP (Table II), was subjected to the same treatment as the previous mixtures, the destruction of DAPAP present in the moist sample held at 45° was complete within 6 weeks. Moreover, no color deterioration was observed.

Tablets—*p*-Aminophenol was detected (6) in very small amounts (less than 0.005%) in tablets containing acetaminophen, but was not detected in tablets containing phenacetin. Tablets containing acetaminophen (codeine phosphate or caffeine present) held at 45° in a humid atmosphere discolored or developed a mottled appearance after about 4 days.

In view of these discolorations, an examination of the color changes taking place in the powder mixtures discussed above in the *Powders* section of *Results and Discussion* is of interest.

None of the mixtures containing phenacetin (Table I) and held at room temperature showed any color deterioration, while at 45°

Table IV—Summary of Main Observations

	DAPAP[a]			Color Deterioration[a]		ASA Hydrolysis, APAP and Phenacetin Mixture
	Formation		Hydrolysis, Complete			
	APAP Mixture, DAPAP Absent	Phenacetin Mixture	APAP Mixture, DAPAP Present	APAP Mixture	Phenacetin Mixture	
At room temperature, about 25°						
Suspensions	No	No	No	Darkening, 6 months	No	Greatly increased
Mixtures						
dry	No, 6 months	No, 6 months	No, 6 months	No	No	Negligible
humid	No	No	No	No	No	Slight
moist	No	No	No	Slight	No	Greatly increased
Tablets humid	—	No	No	Negligible, more than 2 months	No	Slight, more than 2 months
At 45°						
Suspensions	No	No	Yes	Excessive darkening	Slight	Complete
Mixtures						
dry	No	No	No	No	No	Very slight
humid	No	No	Yes	Noticeable mainly in the presence of codeine phosphate or caffeine	No	Greatly increased
moist	No	No	Yes, 2 weeks	Excessive darkening of all mixtures	Slight	Almost complete
Tablets humid	—	No	No	Rather dark and mottled appearance	Slight	Greatly increased

[a] Observations carried out for a period of 2 months, unless otherwise stated.

only the moist ones showed a very slight deterioration. These results are different in many respects from those obtained from mixtures containing APAP.

At room temperature all APAP mixtures showed no color deterioration except for the moist mixtures containing caffeine, which turned light yellow.

At 45° the dry mixtures showed no color deterioration. Of the humid mixtures, those containing codeine phosphate or caffeine became slightly red initially with subsequent darkening of the color. This deterioration was more pronounced in mixtures containing codeine phosphate and SA (Table II). On the other hand, all the moist mixtures turned dark brown and eventually black due mainly to the oxidative degradation of *p*-aminophenol produced from the hydrolysis of APAP, as was observed in the case of the suspensions already discussed.

For comparative purposes, therefore, mixtures containing *p*-aminophenol instead of APAP (Table III) were prepared and examined in the same way as was done above. Color deterioration of these mixtures took place within a day or two, even at room temperature. They were examined periodically by TLC and compared with the moist acetaminophen mixtures, because the darkening of the color was similar in both these groups. The results showed that the darkening of the color of the samples of both groups was due to yellowish, dark brownish, and to some extent reddish products (Fig. 2*b*). They also showed that PAP can be acetylated by ASA with the formation of APAP and that its oxidation was very pronounced in the presence of SA. However, no DAPAP could be observed under any conditions.

Deterioration in the color of these mixtures greatly increases with temperature and moisture content, and sealed samples kept at room temperature did not show as many dark spots by TLC. It is worth noting, however, that acetylation of PAP to APAP by ASA takes place readily at room temperature also in the dry, humid, or moist mixtures.

In view of the above findings, APAP (1 g.), ASA (1 g.), and SA (1 g.), each containing codeine phosphate (40 mg.) or each containing caffeine (150 mg.), were placed at 45° in a humid atmosphere (above water in a desiccator) for 12 weeks. The color of the mixtures containing APAP deteriorated faster than those containing ASA or SA. Even codeine phosphate or caffeine kept at 45° and in humid atmosphere on their own showed a color deterioration. On the other hand, when these mixtures were heated at 45° for the same period of time in sealed vials, *i.e.*, in a rather dry atmosphere, no color deterioration could be observed.

It is, therefore, suggested that color deterioration in APAP tablets, kept under accelerated storage conditions, may be due partly to interactions between the codeine phosphate or caffeine, if present, with the other components or breakdown products in the tablets, and partly to the oxidative degradation of *p*-aminophenol formed from APAP.

SUMMARY AND CONCLUSION

A summary of the main observations is presented in Table IV.

From the results it can be seen that acetylation of acetaminophen to *O*,*N*-diacetyl-*p*-aminophenol by acetylsalicylic acid is not evident under the conditions examined and that the stability of the pharmaceutical preparations containing acetaminophen decreases rapidly with increase in their moisture content and temperature. Furthermore, when the moisture of these preparations is kept to the minimum possible level (without any special drying of the ingredients), their stability is greatly improved. It is, therefore, recommended that such preparations be kept in a dry atmosphere in order to increase their stability during storage.

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