Inhibition of Radical-Initiated Vinyl Acetate Polymerisation by Tobacco Smoke Fractions*

by M. A. Nisbet and Ms. S. Schmeller

Imperial Tobacco Products Limited, Montreal, Quebec, Canada

INTRODUCTION

In seeking a way to chemically describe tobacco smoke, considerable effort has been directed towards unravelling the chemical composition of this unusually complex mixture (1).

In parallel to the extensive analytical work, a second method of deriving a chemical profile of smoke has been evolving. It is based on investigation of the chemical properties of smoke: properties which may, in some cases, be due to a single compound but generally will be due to broad classes of compounds which, by virtue of similar functional groups, are capable of taking part in the same chemical reaction.

One of the earliest reports of work of this nature was concerned with measuring the reducing property of smoke by reacting it with Fehlings solution (2). Attempts were made to relate the reducing property, estimated in this fashion, with smoke quality. Since that time, alternative methods of measuring the reducing property of smoke have been developed in this laboratory (3) and by other workers (4, 5). Examples of other types of properties or activities which have been reported are:

> Alkylating activity (6),
> Copper binding activity (7),
> Sulphydryl binding (8),
> Photosensitising activity (9).

We have recently investigated the ability of smoke to intercept free radicals involved in chain processes. This property is determined by measuring the degree to which smoke inhibits benzoyl-peroxide-initiated vinyl acetate polymerisation. The experimental procedure and inhibitory activities of a number of different tobacco smokes have been reported (10).

The present paper deals with an examination of the inhibitory activities of smoke fractions. The results of the fractionation studies have been used to clarify the mechanism by which smoke exerts its inhibitory activity.

MATERIALS AND METHODS

Vinyl acetate, free of stabilisers, was obtained from Gulf Oil (Canada) Ltd. Benzoyl peroxide was crystal-

lised from chloroform prior to use. Glassware was scrupulously cleaned by a sequence of washings in methanolic potassium hydroxide, chromic acid, distilled water and finally methanol. The tubes were dried and stored in a dust-free, warm-air oven.

Cigarettes and cigars were smoked to the standard regime of one 35 ml puff of 2-second duration per minute to butt lengths of 30 and 50 mm respectively. Pipes were smoked at one 35 ml puff of 2-second duration at 10-second intervals. Condensates were collected by electrostatic precipitation.

Smoke fractionation was carried out by the procedure described by Stedman (11) and also by that of Grimmer (12) for subfractionation of the nitromethane solubles. Samples were made ready for testing by dissolving either the total condensate or appropriate fraction in vinyl acetate at a concentration of 5 mg per ml.

The tests were performed by placing 10.00 ml vinyl acetate and 20 mg of benzoyl peroxide in 20×150 mm glass tubes. From the stock solution of the material under test, 0.03 ml was added to the 10 ml of monomer-peroxide mixture.
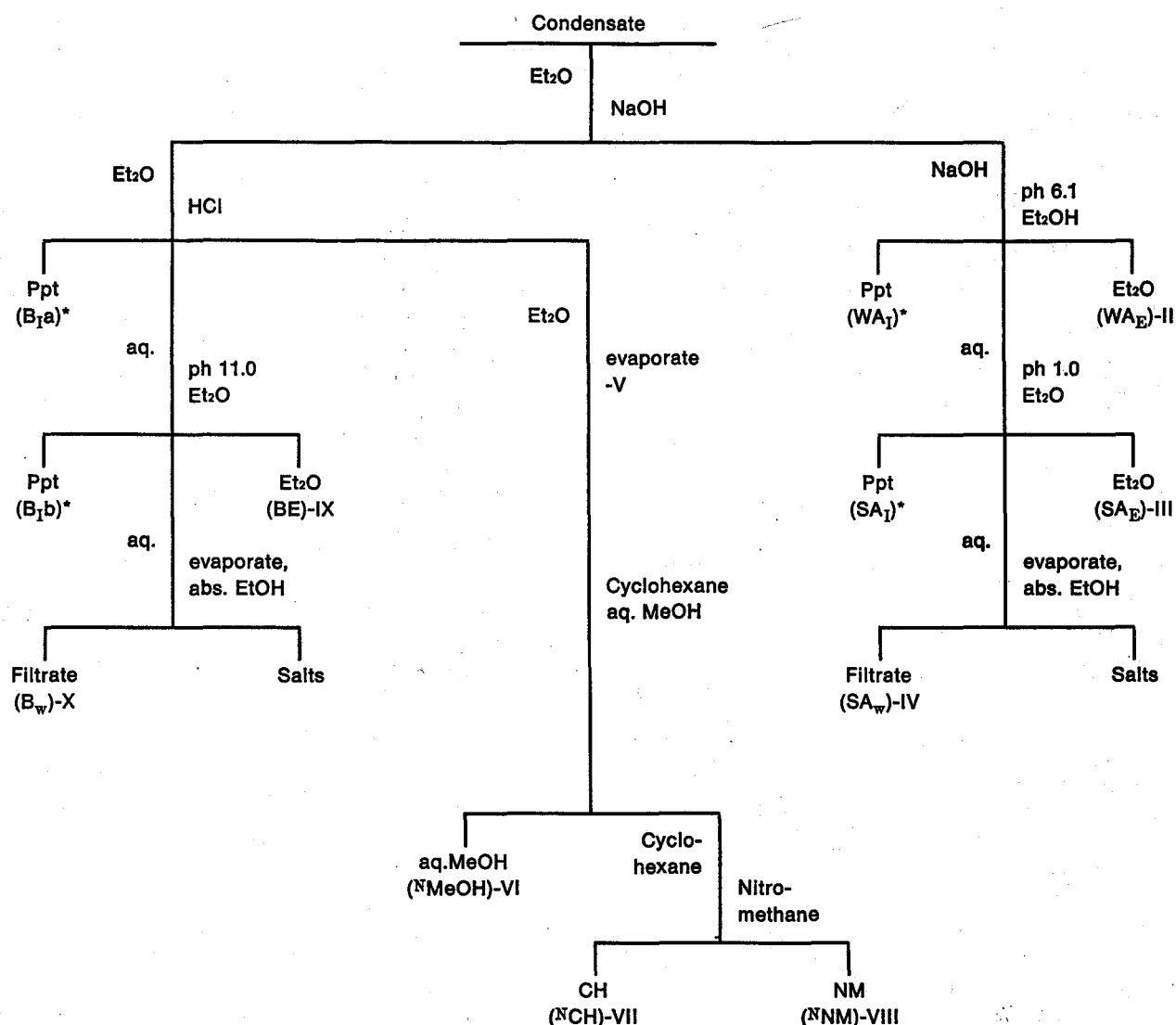
To the control nothing was added. The tubes were immersed in a constant temperature bath at $70 \pm 0.5°$ C and the time required to reach a spontaneous boil was recorded. For ease of reading, four tubes were immersed simultaneously, three test samples and one control. The presence of a control, with each test set, was designed to counter the effects of fluctuations in bath temperature.

The degree of inhibition was based on the difference in time taken to reach spontaneous boiling by the control and test mixtures. The inhibition factors were calculated as minutes delay in boiling per part per million of inhibitor and expressed as min. $ppm^{-1} \times 10^3$.

The diphenylpicrylhydrazyl [DPPH] test for the presence of free radicals was carried out by preparing stock solutions of the smoke fractions under investigation at a concentration of 5 mg/ml in a mixture of cyclohexane-ethanol (3:2). A solution of DPPH was made up in the same solvent system to give O. D. 0.82 at 525 nm in a 1 cm light path. To 2.9 ml DPPH solution, 0.1 ml of test solution was added and the O. D. was recorded at one minute intervals.

Figure 1.

Condensate
Et$_2$O
NaOH

Et$_2$O
HCl

NaOH
ph 6.1
Et$_2$OH

Ppt
(B$_I$a)*
aq.

Et$_2$O

Ppt
(WA$_I$)*
aq.

Et$_2$O
(WA$_E$)-II

ph 11.0
Et$_2$O

evaporate
-V

ph 1.0
Et$_2$O

Ppt
(B$_I$b)*
aq.

Et$_2$O
(BE)-IX

Ppt
(SA$_I$)*
aq.

Et$_2$O
(SA$_E$)-III

evaporate,
abs. EtOH

Cyclohexane
aq. MeOH

evaporate,
abs. EtOH

Filtrate
(B$_w$)-X

Salts

Filtrate
(SA$_w$)-IV

Salts

aq.MeOH
(^{N}MeOH)-VI

Cyclo-
hexane
Nitro-
methane

CH
(^{N}CH)-VII

NM
(^{N}NM)-VIII

RESULTS AND DISCUSSION

We have previously reported that both vapour phase and total particulate matter (TPM) affect the rate of polymerisation of vinyl acetate (10). In the case of vapour, isoprene, which has an inhibition factor of 788 min. ppm^{-1}×10^8, and other conjugated systems were responsible for most of the inhibitory activity.

The experiments involving TPM showed that smokes from different tobaccos, or smoking vehicles, could be ranked on the basis of their ability to inhibit polymerisation.

Table 1 demonstrates the ranking which was obtained. The values range from 115 min. ppm^{-1}×10^8 for flue-cured cigarette smoke to 30 min. ppm^{-1}×10^8 for air-cured pipe tobacco smoked in a pipe. No information was obtained at this point about the mechanism by which TPM causes inhibition or the classes of compounds involved. To resolve the question, TPM has been fractionated and the activities of the various fractions have been tested.

The main fractionation was carried out as described by *Stedman* (11) (Fig. 1). Fractions marked with asterisks were not tested because they could not be dissolved in sufficient concentration in a solvent compatible with the reaction mixture.

Table 1.   Inhibition of polymerisation of vinyl acetate by different tobacco smoke condensates.

Tobacco	Form	Inhibition factor (min. ppm^{-1} × 10^8)
Flue-cured	Cut	115
Flue-cured	Granulated	114
Air-cured	Granulated	87
Stem	Cut	98
Stem	Granulated	95
Perique	Granulated	82
Reconstituted	Cut	71
Reconstituted	Granulated	66
Pipe		33
Cigar		90

Each value represents a mean of 24 results.

Table 2 shows the percentage by weight at each stage and the inhibitory activity. The highest specific activities, 122 and 135 min. $ppm^{-1} \times 10^3$, are found in the methanol-soluble neutral fraction and the nitromethane fraction respectively. Somewhat lower values in the region of 83 to 62 min. $ppm^{-1} \times 10^3$ occur in the weak and strong ether-soluble acids, total neutrals and the cyclohexane-soluble neutrals. Considerably lower activities are found for the water-soluble strong acid fraction and in both basic fractions.

The last column of figures in this table gives an estimate of the contribution of the various fractions to the inhibitory activity of the TPM. The figures are derived by multiplying the percentage weight of each fraction by its inhibition factor. It can be seen that the total neutral fraction accounts for a large amount of the activity. Of the neutral subfractions, the cyclohexane solubles, because of their greater percentage weight, provide the biggest contribution.

The acid fractions comprise 41 % of the weight of the TPM and so, despite their relatively low inhibition factors, are responsible for a considerable amount of the inhibitory activity of the TPM.

The fact that the nitromethane fraction did not show greater activity was somewhat surprising. Both *Georgieff*'s studies (13) and our own work (10) indicated that polyaromatic hydrocarbons have relatively high inhibition factors. To clarify this problem the nitromethane fraction was subfractionated after the method described by *Grimmer* (12) (Fig. 2).

The subfractions were tested for their inhibitory

Table 2. Inhibition factors of flue-cured tobacco smoke fractions.

	Fraction	% Weight	Inhibition factor (IF)	IF × % weight
I	Total condensate	100	103	
II	Weak acids (ether-soluble)	2.3	83	191
III	Strong acids (ether-soluble)	2.5	71	177
IV	Strong acids (water-soluble)	36.0	12	432
V	Total neutrals (ether-soluble)	16.7	78	1.303
VI	Neutrals (methanol-soluble)	2.6	122	317
VII	Neutrals (cyclohexane-soluble)	12.2	62	756
VIII	Neutrals (nitromethane-soluble)	1.9	135	256
IX	Bases (ether-soluble)	4.0	28	112
X	Bases (water-soluble)	2.4	13	31

activity and the results are displayed in Table 3. The NM fraction V, which corresponds to the nitromethane fraction in the *Stedman* scheme, had in this case a value of 126 min./$ppm \times 10^3$. Fractionation by column chromatography did not lead to a concentration of activity in either of the fractions. The first eluate off the column had an inhibition factor of 180 min. $ppm^{-1} \times 10^3$, balanced by an activity of 50 min. $ppm^{-1} \times 10^3$ in the residual eluate.

Since the first subfraction represents a concentrate of the smoke polyaromatics, it must be assumed that the polycyclic hydrocarbons found in smoke are, on average, only moderately efficient radical interceptors.

To relate the information, which we have derived, about the activities of the main fractions to the mechanism of inhibition of polymerisation by TPM, consideration should be given to what is known about inhibition of chain processes.

Disruption of a radical-initiated chain reaction occurs when the radicals involved in the chain react with a substance to produce another radical which is too

Figure 2.

Table 3. Inhibition factors of neutral subfractions.

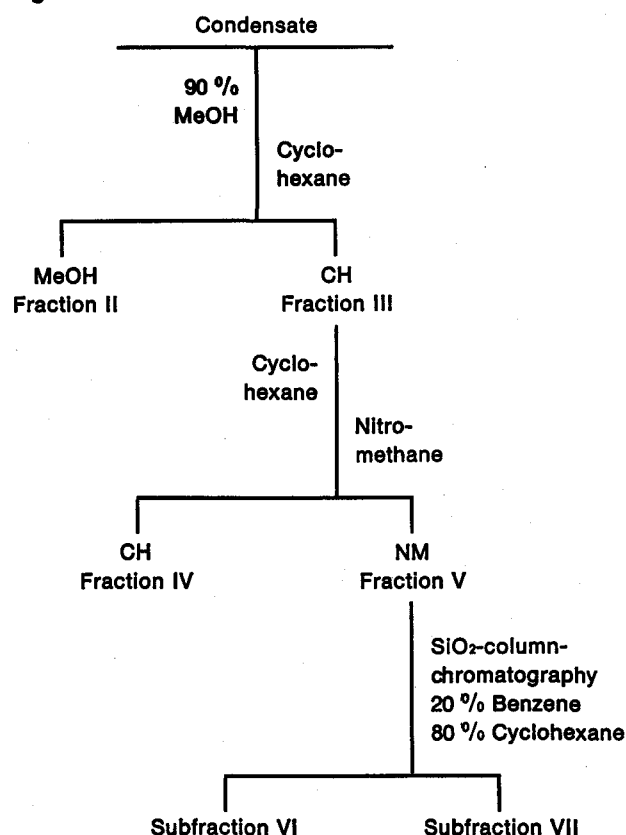| Fraction	Yield (%)	Inhibition factor (min. ppm—1 × 10³)
Total condensate	100	
MeOH II	71.36	80
Cyclohexane III	26.40	68
Cyclohexane IV	16.42	42
Nitromethane V	6.82	126
Subfraction VI	2.92	180
Subfraction VII	0.59	50

Table 4. Mechanisms of inhibition of radical-initiated polymerisation of vinyl acetate.

Inhibition mechanism	Reactive species	Examples in smoke
Addition/substitution	Double bonds	Unsaturated hydrocarbons
	Aromatic nuclei	Aromatic and poly-aromatic compounds
Atom transfer	Active hydrogen	Phenols Amines Reducing agents
Inhibition by radicals	Radicals	Resonance-stabilised smoke radicals

Table 5. Suggested inhibition mechanisms of TPM fractions.

Fraction	Addition/ substitution	Atom transfer	Radical coupling
Total condensate	+++	+++	+++
Weak acids (ether-soluble)	+	+++	+++
Strong acids (ether-soluble)	+	++	+
Total neutrals	++	++	++
Neutrals (methanol-soluble)	+	++	++
Neutrals (cyclohexane-soluble)	++	++	+
Neutrals (nitromethane-soluble)	+++	+	+
Bases (ether-soluble)	+	+	+

stable to enter the kinetic chain or is reactive along a different pathway.

There are three mechanisms by which interception of radicals can take place. These are summarised in Table 4.

Table 5 shows the kinds of activity expected in the most active fractions. It must be pointed out that we do not have direct proof for the occurrence of addition/substitution or atom transfer mechanisms. However, our knowledge of the composition of smoke fractions and our information of the classes of compounds likely to take part in these mechanisms enables us to suggest the type of mechanism likely to occur.
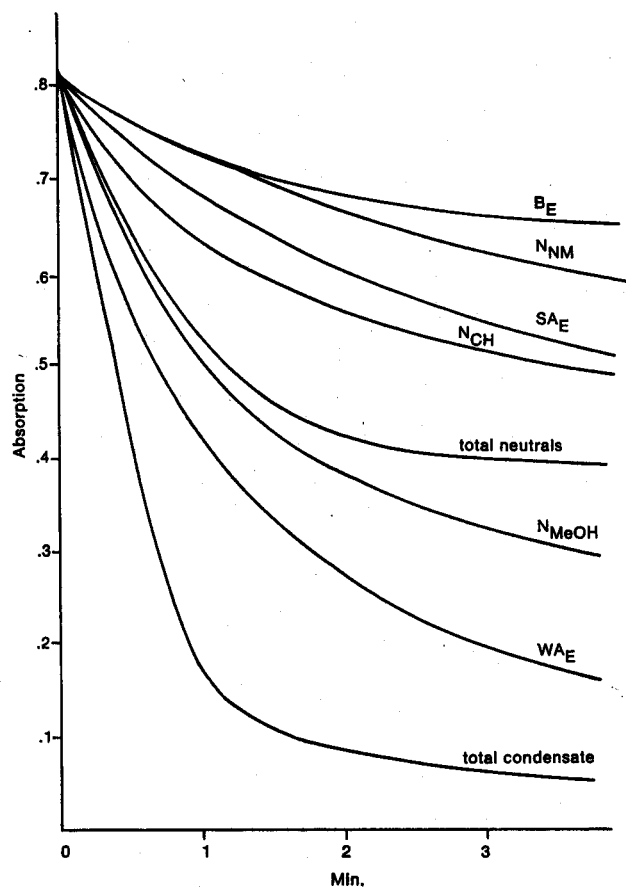
Figure 3. Decolorisation of DPPH by smoke fractions.

Evidence of radical coupling was obtained by reacting the fractions with diphenylpicrylhydrazyl [DPPH], a stable free radical, and observing loss of colour, as the unpaired electron of DPPH combines with that from another radical to form a covalent bond. DPPH has been used as a radical trap in kinetic studies (14) and also as a means of measuring the freshness of smoke condensates (15).

The rates of decolorisation of DPPH by various smoke fractions are shown in Fig. 3. The results suggest that resonance-stabilised radicals are widely distributed throughout the fractions. However, the assumption is being made that decolorisation of DPPH is due only to radical coupling. We have not eliminated the possibility that other reactions could be causing decomposition of the reagent.

It is apparent that most of the fractions exert their inhibitory action through all three mechanisms. But it was not expected that free-radical coupling would be so widely distributed. The final part of our work was directed toward examining the inhibitory activity of the main TPM fractions of smoke from different sources. Table 6 shows the profiles which were obtained.

No precise conclusions can be reached from examination of the activities. A general conclusion can be made, that the activity patterns of the fractions from the different condensates give profiles which are specific for a given type of smoke.

In conclusion, our work on the inhibition of vinyl acetate polymerisation by smoke and smoke fractions indicates that smoke is a relatively efficient free-radical interceptor and that all three mechanisms of interception, addition reactions, atom transfer and radical coupling, play a role.

Smokes from different sources can be distinguished by their efficiencies as inhibitors. Additionally, when different types of smoke are fractionated, the activities of the fractions provide a profile which is distinctive for each type of smoke.

Table 6. Inhibition factors of smoke fractions from different sources.

Fraction	Source Flue-cured	Air-cured	Recon-stituted sheet	Stem	Perique	Pipe	Cigar
I Total condensate	101	87	75	98	70	37	94
II Weak acids (ether-soluble)	94	84	103	127	80	89	157
III Strong acids (ether-soluble)	82	53	90	99	63	137	153
VI Neutrals (methanol-soluble)	122	128	69	103	123	108	109
VII Neutrals (cyclohexane-soluble)	62	63	54	64	57	69	79
VIII Neutrals (nitromethane-soluble)	135	149	158	167	117	89	148

It is unlikely that this property of smoke has much biological significance. Relatively innocuous materials such as anthracene can be highly efficient radical interceptors, while compounds which are biologically active, formaldehyde and acrolein for example, are poor interceptors. The results of our study tend to run contrary to the theory that either free radicals present in smoke or promotion of free radicals from smoke components, may cause biological damage to the smoker. Smoke is more likely to act as a scavenger rather than a source of free radicals.

SUMMARY

Both the vapour and particulate phases of tobacco smoke have been shown to retard benzoyl-peroxide-initiated polymerisation of vinyl acetate by interception of the radicals involved in the polymerisation process.

The extent of inhibition of polymerisation by test compounds is estimated by measuring time taken for a mixture of monomer and benzoyl peroxide, immersed in a water-bath at $70°$ C, to reach a spontaneous boil and comparing it with the time required for a similar mixture with added retarder to reach boiling point. Units are expressed as minutes of inhibition per part per million of inhibitor $\times 10^3$.

Inhibition by the vapour phase can be attributed to conjugated unsaturated compounds, chiefly isoprene which has an inhibition factor of 788 min. $ppm^{-1} \times 10^3$ respectively.

To trace the groups of compounds responsible for the inhibitory activity of the particulate phase, the particulate matter was fractionated by the method of *Stedman* et al. The fractions containing the highest activities were ether-soluble weak acids, methanol-soluble neutrals and nitromethane-soluble neutrals with inhibition factors of 83, 122 and 135 min. $ppm^{-1} \times 10^3$ respectively.

Since the nitromethane fraction contains most of the aromatic hydrocarbons produced on smoking and some polyaromatics are known to have high inhibition

factors, it was subfractionated after the method of *Grimmer* but no subfraction with activity appreciably higher than the crude nitromethane fraction was obtained.

The distribution of activity in the major fractions of the particulate phase of the smoke from a number of different tobacco types and smoking vehicles has been examined.

These results and the mechanism by which smoke inhibits polymerisation are discussed.

ZUSAMMENFASSUNG

Es wurde gezeigt, daß sowohl die Dampfphase als auch die Partikelphase des Tabakrauches die durch Benzoyl-peroxid ausgelöste Polymerisation von Vinylacetat durch Abfangen der am Polymerisationsprozeß teilnehmenden Radikale verzögert.

Das Ausmaß der Polymerisationsverzögerung durch Testverbindungen wird ermittelt, indem die Zeit bestimmt wird, die eine in einem Wasserbad von $70°$ C erwärmte Mischung von Monomer und Benzoylperoxid bis zum spontanen Sieden braucht. Dieser Zeitraum wird mit demjenigen verglichen, den eine ähnliche Mischung mit einem zugesetzten Reaktionsverzögerer bis zum Erreichen des Siedepunktes braucht. Als Maßeinheit dient die Dauer der Hemmwirkung (in Minuten) pro Inhibitor (in ppm) $\times 10^3$.

Die Hemmwirkung der Dampfphase kann auf ungesättigte konjugierte Verbindungen, vor allem auf Isopren zurückgeführt werden, das einen Verzögerungsfaktor von 788 min. $ppm^{-1} \times 10^3$ hat.

Um die Verbindungsgruppen zu ermitteln, auf denen die Hemmwirkung der Partikelphase beruht, wurde diese unter Anwendung der Methode von *Stedman* et al. fraktioniert. Die Fraktionen mit der stärksten Aktivität waren ätherlösliche schwache Säuren, methanollösliche Neutralstoffe sowie nitromethanlösliche Neutralverbindungen mit Hemmfaktoren von 83, 122 bzw. 135 min. $ppm^{-1} \times 10^3$.

Da die Nitromethanfraktion die meisten der beim Rau-

chen entstehenden aromatischen Kohlenwasserstoffe enthält und einige Polyaromaten bekanntermaßen einen hohen Hemmfaktor haben, wurde diese Fraktion wiederum in Unterfraktionen nach der Methode von *Grimmer* aufgeteilt mit dem Ergebnis, daß keine der Unterfraktionen eine nennenswert höhere Aktivität aufwies als die rohe Nitromethanfraktion.

Bei einer Reihe von verschiedenen Tabak- und Abraucharten wurde die Verteilung der Aktivität zwischen den hauptsächlichen Fraktionen der Partikelphase untersucht.

Die Ergebnisse dieser Untersuchungen und die Mechanismen, durch die der Rauch die Polymerisation hemmt, werden diskutiert.

RESUME

On a démontré que la phase gazeuse et la phase particulaire de la fumée de tabac retardent la polymérisation de l'acétate de vinyle, polymérisation provoquée par le peroxyde de benzoyle, et ceci en interceptant les radicaux impliqués dans ce procédé.

On évalue le degré d'inhibition de polymérisation de composants test, en mesurant le temps nécessaire afin qu'un mélange de monomère et de peroxyde de benzoyle, plongé dans un bain-marie à $70°$ C, arrive spontanément à ébullition, et en comparant ce temps à celui requis afin qu'un mélange semblable additionné d'un retardateur atteigne le point d'ébullition. On exprime l'unité en minutes d'inhibition par partie par million d'inhibiteur $\times 10^3$.

On peut attribuer l'inhibition de la phase gazeuse à des composants insaturés conjugués, principalement l'isoprène qui possède un facteur d'inhibition de 788 min. $ppm^{-1} \times 10^3$.

Afin de déceler les groupes de composants provoquant l'activité inhibitrice de la phase particulaire, on a fractionné la matière particulaire par la méthode de *Stedman* et al. Les fractions contenant les activités les plus élevées sont les acides faibles solubles dans l'éther, les fractions neutres solubles dans le méthanol ainsi que les fractions neutres solubles dans le nitrométhane, qui ont respectivement des facteurs d'inhibition de 83, 122 et 135 min. $ppm^{-1} \times 10^3$.

Sachant que la fraction nitrométhane contient la plus grande partie des hydrocarbures produits en fumant et qu'on sait que certains polyaromatiques ont un haut degré d'inhibition, on a subfractionné selon la méthode de *Grimmer*, mais on n'a pas obtenu de subfraction contenant une activité sensiblement supérieure à la fraction nitrométhane brut.

On a examiné la distribution de l'activité dans les fractions principales de la phase particulaire de la fumée pour un nombre de types de tabac différents ainsi que de procédés de fumer.

On a discuté les résultats ainsi que le mécanisme d'inhibition de polymérisation par la fumée.

REFERENCES

1. Stedman, R. L.: Chem. Rev. 68 (1968) 153.
2. Shmuk, A. A.: Izvest. Akad. Nauk., U.S.S.R., Biological Division, 955, 1939.
3. Bilimoria, M. H., and M. A. Nisbet: Beitr. Tabakforsch. 6 (1971) 27.
4. Benedict, R. C., L. Lakritz and E. D. Strange: Chem. & Ind., 13 June, 800, 1970.
5. Hagopian, M.: Environ. Sci. Technol. 3 (1969) 567.
6. Stedman, R. L., and R. L. Miller: Chem. & Ind., 15 April, 618, 1967.
7. Sato, T., T. Suzuki and T. Fukuyama: Brit. J. Cancer 16 (1) (1962) 7.
8. Michael, L. W., E. E. Menden and H. G. Petering: Environ. Sci. Technol. 5 (3) (1971) 249.
9. Nisbet, M. A., J. Johnson and S. Schmeller: Tobacco Sci. XVI (1972) 13.
10. Bilimoria, M. H., J. Johnson, M. A. Nisbet, S. Schmeller and K. K. Georgieff: Beitr. Tabakforsch. 7 (1973) 158.
11. Swain, A. P., J. E. Cooper and R. L. Stedman: Cancer Res. 29 (1969) 579.
12. Elmenhorst, H., and G. Grimmer: Z. Krebsforsch. 71 (1968) 66.
13. Georgieff, K. K., K. G. Blaikie and R. C. White: J. App. Polymer Sci. 8 (1964) 889.
14. Shine, H. J., J. A. Waters and D. M. Hoffman: J. Am. Chem. Soc. 85 (22) (1963) 3613.
15. Takeshita, T., and H. Ohe: Nippon Senbai Kosha Chuo Kenkyusho Kenkyo Hokoku, No. 106, 163, 1964.

The authors' address:

Imperial Tobacco Products Ltd.,
Research and Development,
3810 St. Antoine Street, Montreal 207,
Quebec, Canada.