

## Estimation of Aldehyde Groups in Natural Rubber with 2,4-dinitrophenylhydrazine

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*A method is described for the estimation of the aldehyde groups in natural rubber. Rubber is reacted in solution with an excess of 2,4-dinitrophenylhydrazine; the solvent is a mixture of tetrahydrofuran-toluene. After removing the excess of reactant, the amount of hydrazone formed is estimated by measuring the absorbance of the derivative in tetrahydrofuran at 360 nm. Calibration is made with the 2,4-dinitrophenylhydrazone derivative of acetaldehyde. The 'acetaldehyde equivalent' of the aldehyde groups in clonal rubbers ranges from  $2 \times 10^{-3}$  to  $6 \times 10^{-3}$  mole per kilogram rubber.*

Natural rubber tends to harden spontaneously on storage under dry conditions<sup>1,2</sup>. This 'storage hardening' can be inhibited by adding reagents such as hydroxylamine or semicarbazide<sup>3,5</sup> to the latex before coagulation.

From the observation that the reagents that inhibited storage hardening were also compounds that react with carbonyl groups, Sekhar postulated that carbonyl groups on the rubber molecule were responsible for storage hardening<sup>6</sup>. Since 5,5-dimethyl-1,3-cyclohexanedione (dimedone), an aldehyde specific reagent, also inhibits storage hardening, he further suggested that the carbonyl groups were aldehydes.

Recently it has been suggested that the postulated aldehyde groups are in fact epoxy<sup>7</sup> or lactone-ring<sup>8</sup> residues. However the evidence that such groups are responsible for storage hardening is lacking.

Sekhar also estimated the aldehyde content of rubber by the addition of different quantities of dimedone or hydroxylamine to latex and the derivation of the critical concentrations at which hardening in the dry rubber was completely inhibited<sup>6</sup>. The method was simple and quantitatively estimated the number of aldehyde groups per rubber mole-

cule. However, the method seems to have overestimated the values since dimedone and hydroxylamine can react with other non-rubber substances in latex.

Percy *et al.*<sup>9</sup> reacted latex or a solution of rubber with [<sup>14</sup>C]-dimedone. After removing the excess dimedone, they determined the aldehyde content by measuring the radioactivity of the rubber. A summary of their unpublished work is given in the *Appendix*.

The 2,4-dinitrophenylhydrazone derivative of aldehydes and ketones show intense absorption maxima in different solvents in the region 350 – 370 nm<sup>10</sup>. This offers a sensitive method for the measurement of the aldehyde groups in rubber and was suggested by Percy *et al.*

### EXPERIMENTAL

In 5 ml toluene in a glass tube 0.2 g of rubber was dissolved. In 4 ml tetrahydrofuran (THF) 0.05 g of 2,4-dinitrophenylhydrazine (DNP) was dissolved and mixed with the rubber solution. If some of the DNP (which is only slightly soluble in toluene) precipitated, more THF was added to give a homogeneous solution. After 24 h, a tiny drop of concentrated hydrochloric acid was added and the

solution flushed with nitrogen. The sealed tube was placed in a water bath at 40°C and the temperature quickly raised to 50°C. The bath was maintained at below 60°C throughout the reaction. After 3 h the solution was cooled and the rubber precipitated by adding the solution drop by drop into 100 ml ethanol. The rubber was pressed dry between filter papers, cut into small pieces, then re-dissolved in 10 ml toluene and reprecipitated in 150 ml ethanol. This procedure was repeated two more times to remove all unreacted DNP. The rubber was finally dried in vacuum. A 0.4% to 0.6% solution of the rubber was made in THF and the optical density measured in a 1 cm cell at 360 nm using a Hilger and Watts UVISPEK Photoelectric Spectrophotometer. The blank was a solution of the same original rubber treated similarly but without the DNP.

Occasionally the final solution for optical density measurement was cloudy, probably due to the presence of gel particles. In such cases, the solution was clarified by centrifugation before measurement.

The calibration graph was obtained by adding known quantities of the 2,4-dinitrophenylhydrazone derivative of acetaldehyde to 0.6% rubber solutions and measuring the optical density.

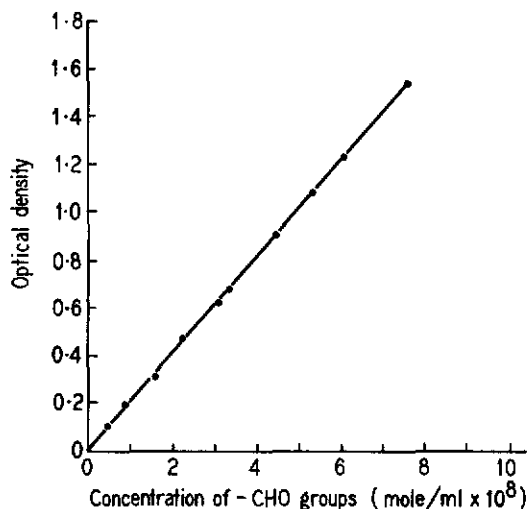
## RESULTS AND DISCUSSION

### Calibration

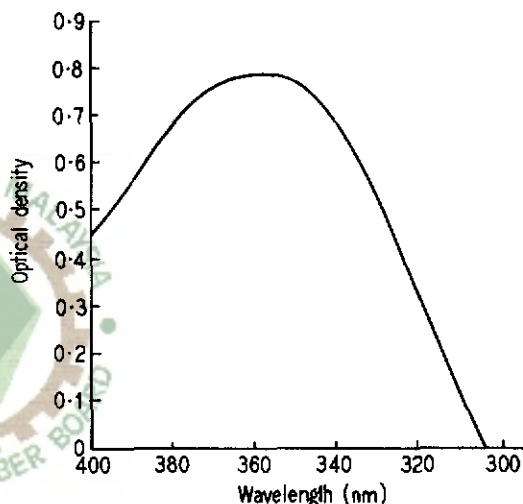
The calibration graph is shown in *Figure 1*. The graph is linear for  $-CHO$  concentrations up to  $8 \times 10^{-8}$  mole  $ml^{-1}$ .

If the concentration of the DNP derivative of rubber =  $A$  kg  $ml^{-1}$ , and the concentration of  $-CHO$  groups corresponding to the optical density of the solution at 360 nm (from the graph) =  $B$  mole  $ml^{-1}$ , concentration of  $-CHO$  groups in rubber =  $(B/A)$  mole  $kg^{-1}$ .

*Figure 2* shows a typical absorption curve for the DNP derivative of rubber. The maximum is close to the absorption maximum of the acetaldehyde derivative in THF (358 nm).



*Figure 1. Calibration graph of DNP derivative of acetaldehyde in rubber solution.*



*Figure 2. UV absorption spectrum of DNP derivative of rubber in THF.*

Strictly speaking, the actual quantity measured is the 'acetaldehyde equivalent' of the aldehyde groups in rubber. For simplicity this is referred to as aldehyde groups in the subsequent discussion.

DNP can react with ketones as well as with aldehydes and the method would estimate the total carbonyl groups in rubber. However, as shown below, when rubber was first reacted with dimedone, then with DNP, the amount of hydrazone formed was small. Thus the concentration of ketone groups (if any) in freshly prepared rubber is much less than the aldehyde groups.

*Concentration of DNP, Temperature and Reaction Time*

The effect of varying the concentration of DNP is shown in *Table 1*. The reaction is not

TABLE 1. EFFECT OF CONCENTRATION OF DNP ON THE ESTIMATED VALUE OF ALDEHYDE CONTENT  
(Reaction time = 3 h at 50°C)

Concentration of DNP (% w/w on rubber)	Aldehyde content ( $\times 10^3$ mole/kg rubber)
0.5	2.81
1.0	3.31
2.5	4.39
5	4.63
10	4.56
25	4.67
50	4.56

quantitative and a great excess (at least 5% w/w) of DNP is required for maximum reaction. In practice a concentration of 25% was used.

The effect of the reaction time and the temperature of reaction is shown in *Table 2*. Though the estimated aldehyde content is slightly higher at 80°C, there is considerable degradation of the rubber. At a 25% concentration of DNP, a reaction time of 24 h at room temperature followed by 1 h to 3 h at 50°C gives almost maximum reaction, with little degradation of rubber.

These results do not show whether the reaction is complete, that is, whether DNP reacts with all the aldehyde groups in rubber. It is not unreasonable to assume that almost complete reaction would occur since a great excess of DNP is used. The estimation of the aldehyde content with DNP in a sample of rubber by Percy *et al.*<sup>9</sup> gave a value close to that found by the dimedone method (see *Appendix*).

*Reproducibility*

*Table 3* gives the results of carefully repeating the measurements six times on the same sample of rubber. The reproducibility is not particularly good, and the results can differ by as much as 10% between measurements. This is not completely unexpected

TABLE 2. VARIATION OF REACTION TIME AND TEMPERATURE ON ALDEHYDE CONTENT AND THE INTRINSIC VISCOSITY OF RUBBER IN SOLUTION  
(at DNP concentration of 25%)

Time of reaction (h)	50°C		80°C	
	Aldehyde content ( $\times 10^3$ mole/kg rubber)	Intrinsic viscosity in toluene at 30°C (dl g <sup>-1</sup> )	Aldehyde content ( $\times 10^3$ mole/kg rubber)	Intrinsic viscosity in toluene at 30°C (dl g <sup>-1</sup> )
1	4.59	6.9	5.04	6.1
2	4.40	6.9	4.67	5.6
3	4.67	6.8	4.84	5.1
5	4.79	6.1	4.90	4.2

TABLE 3. REPRODUCIBILITY OF THE METHOD. VALUES OF SIX REPLICATE MEASUREMENTS

Estimation No.	Aldehyde content ( $\times 10^3$ mole/kg rubber)
1	4.11
2	3.90
3	4.19
4	3.75
5	3.82
6	3.98

Mean =  $3.96 \times 10^{-3}$ 

S.D. = 0.156

Coefficient of variation = 3.94%

considering the low concentration of aldehyde groups. The error probably arises mainly from the loss of a small and variable amount of low molecular weight rubber during the purification procedure.

*Pre-treatment of Latex with Dimedone and/or Hydroxylamine Hydrochloride*

When latex is treated with dimedone or hydroxylamine hydrochloride, or a combi-

nation of both, the aldehyde content of the dry rubber is almost reduced to zero (Table 4). Thus DNP appears to react with the same groups that react with dimedone or hydroxylamine. The fact that dimedone-treated rubber does not react with DNP may be surprising since dimedone has been reported to give hydrazone derivatives<sup>11</sup>. An investigation showed that there was no stable hydrazone formation under the conditions of the present method when dimedone was reacted with DNP.

*Effect of Purification of Rubber*

Synthetic poly(isoprene) shows a very low aldehyde content. In contrast, natural rubber from which the proteins and other non-rubber substances have been removed<sup>12,13</sup> shows a relatively high aldehyde content (Table 5). This shows that the aldehyde groups are part of the rubber molecule or are so strongly attached that they cannot be removed by usual methods of purification. Deproteinised natural rubber<sup>14</sup> (prepared using the enzyme 'Suprase') or DPNR, as it is popularly known, also shows a much higher aldehyde content than synthetic poly(isoprene).

TABLE 4. EFFECT OF TREATMENT OF LATEX WITH DIMEDONE OR HYDROXYLAMINE HYDROCHLORIDE ON ALDEHYDE CONTENT

Concentration of dimedone (% w/w on rubber <sup>a</sup> )	Concentration of hydroxylamine hydrochloride (% w/w on rubber <sup>a</sup> )	Aldehyde content ( $\times 10^3$ mole/kg rubber)
0	0	5.43
0.5	0	2.21
1	0	1.04
5	0	0.28
0	0.05	2.96
0	0.1	0.65
0	0.2	0
2	0.15	0

<sup>a</sup> Total solids film dried at 40°C for one week

TABLE 5. EFFECT OF PURIFICATION OF RUBBER ON ALDEHYDE CONTENT

Sample	Aldehyde content ( $\times 10^3$ mole/kg rubber)
Total solids film (unpurified)	6.4
Purified rubber	5.3
Commercial sample DPNR	4.9
Synthetic poly(isoprene)	0.3

### Aldehyde Content of Different Grades of Rubber and Clonal Rubbers

The aldehyde content of different grades of natural rubber from bulked (mixed clonal) latex is given in Table 6. SMR 20, SMR 50 and Blanket Grade Crepe were commercial samples. The measurements on the first three samples in Table 6 were made a few weeks after their preparation, and on the last three samples, an unspecified time after their preparation. The high value for Blanket Grade Crepe is probably due to oxidation and represents the total carbonyl content rather than the aldehyde content alone. SMR 50, a low grade rubber, also probably shows some degree of oxidation judging from the value in Table 6.

TABLE 6. ALDEHYDE CONTENT OF DIFFERENT GRADES OF RUBBER

Sample	Aldehyde content ( $\times 10^3$ mole/kg rubber)
Total solids film	6.9
Air dried sheet	5.4
SMR 5 (Comminuted)	7.0
SMR 20 (Cuplump) <sup>a</sup>	7.6
SMR 50 <sup>a,b</sup>	10.3
Blanket grade crepe <sup>a,b</sup>	18.5

<sup>a</sup> Commercial sample<sup>b</sup> Probably oxidised

Table 7 gives the aldehyde content of some clonal rubbers. The measurements were made soon after tapping<sup>15</sup>. The average values are all below  $6 \times 10^{-3}$  mole —CHO per kilogram rubber and are much lower than values previously reported<sup>6</sup>. As mentioned earlier, the previous method considered the amount of reactant added to latex rather than the amount reacting with the rubber alone and is likely to give high values. The results reported in this paper are of the same order of magnitude as estimated by Percy *et al.*<sup>9</sup> by their Method 2. In fact the present method is similar to the latter method in two respects:

- The reaction is carried out in solution.
- Excess reactant is removed by a solution-precipitation technique.

The ratios of the Mooney viscosities of clonal rubbers after and before accelerated storage hardening range from 1.1 to 2.2<sup>15</sup>. Such an increase is consistent with a small

TABLE 7. ALDEHYDE CONTENT OF SOME CLONAL RUBBERS FROM RRIM EXPERIMENT STATION, SUNGEI BULOH

Clone	Field	Aldehyde content ( $\times 10^3$ mole/kg rubber)
RRIM 501	48A	6.0 <sup>a</sup>
RRIM 501	60D	5.4 <sup>b</sup>
RRIM 605	48A	5.0 <sup>a</sup>
RRIM 605	64A	4.6 <sup>b</sup>
RRIM 600	48A	4.5 <sup>a</sup>
RRIM 600	60D	3.7 <sup>b</sup>
RRIM 701	64A	3.7 <sup>b</sup>
PR 251	60D	3.3 <sup>b</sup>
GT 1	60D	2.9 <sup>b</sup>
PB 86	64A	2.9 <sup>b</sup>
Tjir 1 (1971)	48A	3.0 <sup>a</sup>
Tjir 1 (1972)	48A	2.3 <sup>b</sup>
RRIC 36	60D	2.0 <sup>b</sup>

<sup>a</sup> Mean of twelve samples<sup>b</sup> Mean of eight samples

number of aldehyde groups as reported here. A high aldehyde content, such as twenty groups per molecule, would be expected to give on storage-hardening, a more highly cross-linked rubber, the bulk viscosity of which would be too high for measurement in the Mooney instrument.

# CONCLUSION

The reaction of DNP with rubber offers a sensitive method for the quantitative estimation of the 'acetaldehyde equivalent' of the aldehyde groups in rubber. Though the error is fairly large, the method is satisfactory for the determination of the relative aldehyde content of rubbers. The aldehyde content of clonal rubbers ranges from  $2 \times 10^{-3}$  to  $6 \times 10^{-3}$  mole per kilogram rubber. The low values are consistent with the increase in Mooney viscosity on storage.

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## APPENDIX

### *THE WORK OF PERCY, E.J., BARNARD, D. AND SEKHAR, B.C.*

More than twelve years ago, Percy and collaborators carried out a thorough quantitative investigation of the aldehyde groups in natural rubber by reacting rubber with [ $^{14}\text{C}$ ]—dimedone and measuring the amount of dimedone incorporated. The following is a brief summary of their unpublished work.

Dimedone was reacted with rubber by two methods.

#### *Method 1*

[ $^{14}\text{C}$ ]—dimedone (0.1 g, *ca*  $10^5\text{d/s/g}$ ) dissolved in 0.5*N* ammonium hydroxide (5 ml) was added to latex (5 ml) which was then filmed and dried at 25°C–30°C for two days. The dry film was extracted under nitrogen with acetone or a mixture of acetone and methanol. The radioactivity of the extracted rubber was then assayed.

#### *Method 2*

Latex (1.5 ml) was dissolved in chloroform (30 ml) and shaken with water (30 ml) for 1 hour. The resulting emulsion was separated by centrifugation and the chloroform layer

refluxed for 2 h with [ $^{14}\text{C}$ ]—dimedone (0.075) and piperidine (one drop). The rubber was then precipitated (three times) from chloroform solution (30 ml) by the slow addition of methanol (1.5 litres) and the radioactivity then assayed.

The aldehyde content in rubber as determined by *Method 1* was independent of the temperature of drying and the drying medium (air, nitrogen or vacuum). However, an excess of dimedone (about 1% to 2% by weight on rubber) was necessary for maximum reaction.

In *Method 2* the results depended on the concentration of dimedone used as well as on the time for which the solution was refluxed. For maximum reaction, a 2-h reflux period was sufficient if the concentration of dimedone was at least 7% w/w on rubber.

The results obtained are summarised in *Table 8*.

*Method 2* gave consistently lower values than *Method 1*. Percy *et al.* suggested two possible reasons for this discrepancy. During the repeated solution-precipitation procedure in *Method 2*, there was a loss of lower molecular weight rubber. Thus if this lower molecular weight rubber had a high count rate, then *Method 2* should give a lower value for the aldehyde content. The second suggestion was that there is present in NR some low

TABLE 8. CONCENTRATION OF ALDEHYDE GROUPS IN RUBBER ESTIMATED WITH [ $^{14}\text{C}$ ]—DIMEDONE

Sample	Method	Concentration of aldehyde groups (with excess dimedone) ( $\times 10^3$ mole/kg rubber)
Several samples of latex including clonal latices	1	7.7 – 12.0
	2	4.2 – 5.4
Latex before purification	2	5.3
Latex purified by creaming with soap (about 25% of rubber lost on purification)	2	3.6
Commercial sample of latex containing ammonia and ammonium pentachlorophenate	1	2.8
	2	1.9

molecular weight material which can react with dimedone to give a small amount of a highly radioactive product. This is not removed from the rubber by the extraction procedure used in *Method 1* but is removed by the more powerful precipitation technique of *Method 2*. Fractionation of rubber followed by the radioactive assay of the different fractions suggested that the second explanation was the true one. This was confirmed by the application of the extraction procedure to rubber obtained by the evaporation of the solvent in *Method 2* and purification by precipitation

of the sol rubber content of the dried latex film from *Method 1* when the count rates were reversed. A highly active product of dimedone and some unidentified component of NR latex was isolated from the methanol precipitant as a white solid, insoluble in most common solvents.

For one sample of rubber, Percy *et al.* also estimated the concentration of aldehyde groups using 2,4-dinitrophenylhydrazine. A value of  $5.4 \times 10^{-3}$  mole per kilogram rubber was obtained compared to  $5.3 \times 10^{-3}$  by the dimedone method.