Investigations into the Determination of Hydroxylamine in Viscosity-stabilised Natural Rubber

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The current method for the determination of hydroxylamine in viscosity stabilised (CV) rubber relies on the use of 0.75 M sulphuric acid as the extractant. This work demonstrates that equally complete extraction can be accomplished using much milder extraction conditions. An ion chromatographic method for determining hydroxylamine has been developed and found to give results comparable to the established UV colorimetric method. The new chromatographic procedure has been used to clarify the concept of 'free hydroxylamine and to test whether the established colorimetric procedure was, indeed, determining 'free' hydroxylamine. The opportunity has also been taken to establish whether the newer grades of CV rubber now being produced have 'free' and measurable hydroxylamine levels comparable with those of the established grades. Preliminary attempts to extend the work to vulcanised compounds are also described.

The necessity for hydroxylamine testing was initially driven by the requirements of the German Bundesgesundheitsamtes (BGA) food contract regulations¹, but the test is now regularly used simply to establish whether or not samples of rubber have been viscosity stabilised. However, with increasing emphasis on 'health and safety' and 'chemical substance profiling', it is becoming essential to fully characterise raw materials, especially with regard to those potentially hazardous chemicals which have been deliberately added.

A consequence of health and safety legislation is that, as well as being concerned with providing precise and unbiased results, analysts are concerned to ensure that the procedures they adopt produce minimum amounts of waste and that whatever waste is produced is of as low a toxicity as possible.

Ever since the production of CV rubbers started there have been discussions concerning the presence, or otherwise, of 'free' hydroxylamine in the treated rubber; 'free' hydroxylamine generally being taken to mean hydroxylamine in the form of simple salts rather than as a reaction product such as an hydroxamic acid. There has been agreement, amongst workers from MRPRA², RRIM³ and elsewhere⁴, that free hydroxylamine is absent, but until recently the analytical techniques available have not been able to discriminate 'free' hydroxylamine between and 'hydroxylamine' present either in the form of labile compounds, or bound into the rubber in an acid-labile form, as both the extraction and the reaction leading to colour formation involve the use of strong acids or bases giving a potential for hydrolysis of labile compounds.

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In addition to providing a less hazardous procedure, chromatographic separation combined with acid-free methanolic extraction may be capable of determining the true free hydroxylamine content. An additional hydrolysis step, after methanolic extraction, then demonstrates whether the hydroxylamine is present as an acid labile compound, the most well-known form being that of an hydroxamic acid.

The first step in any procedure for the analysis of hydroxylamine is its extraction from the rubber. Previous extraction procedures have used either sulphuric acid^{5,6} or acetic acid¹ with both in a relatively concentrated form (0.75 M)and 0.5 M, respectively). Published work by Krajcinovic and Vranjican⁷ has demonstrated that hydroxamic acids are hydrolysed under conditions much milder than those used for hydroxylamine extraction. The actual conditions used were approximately 1 M hydrochloric acid at room temperature for 3 h. This compares with the 0.75 M sulphuric acid at 100°C for 16 h used in the standard hydroxylamine test. There must therefore be a strong probability that any hydroxamic acids initially extracted from CV rubber by either of the above acids would be hydrolysed to the corresponding hydroxylamine salt during the extraction and subsequently be determined as 'free' hydroxylamine. The exact fate of other hydroxylamine compounds would depend on their relative sensitivity to hydrolysis by acid.

Ion chromatography is a technique which is well suited to measuring the concentrations of low molecular weight cations without resorting to derivatisation. The use of this chromatographic procedure eliminates the need to acidify the solution (acidification is necessary for the first step in the reaction between hydroxylamine and 8-hydroxy quinoline, the reaction used to quantify hydroxylamine in previous studies of rubber extracts) and presents the possibility of the determination of a true 'free' hydroxylamine content.

Now that this chromatographic alternative to the colorimetric method of determining hydroxylamine is available, the opportunity has been taken to investigate extraction procedures based on less hazardous materials. Previous work⁶, to improve the robustness of the colorimetric method for determining hydroxylamine, was carried out with SMR CV and GP grades of natural rubber. For this work the additional grades 10 CV and 20 CV, now being produced commercially in Malaysia, were included. Several extractants were investigated to determine whether ion chromatography was capable of detecting and quantifying lower levels of hydroxylamine and also whether hydrochloric acid, at a 0.07 M concentration, as used in tests to demonstrate the potential for extraction of toxic materials in toys⁸, would be capable of extracting measurable amounts of hydroxylamine from raw CV rubbers.

Davey and Loadman⁶ found no significant levels of hydroxylamine in vulcanisates after curing, but did show evidence of an interference from both calcium and zinc on the colorimetric procedure. Some of this work was repeated using ion chromatography and extractants selected to maximise potential extraction of hydroxylamine from the vulcanisate.

EXPERIMENTAL

Ion chromatography was performed using a Dionex 2010i ion chromatograph in an ion interaction mode known as MPIC (Mobile Phase Ion Chromatography). MPIC uses an inert, highly cross-linked polystyrene/ divinylbenzene column packing and a mobile phase containing a relatively high molecular weight counter-ion, in this case an anion. The counter-ion adsorbs on to the column packing forming a constantly regenerated stationary phase. Separation is dependent on the relative adsorption coefficients of the counter-ion and of a complex, formed between the counter-ion and an analyte, as they compete for adsorption sites on the column.

Increasing the chain length or concentration of the counter-ion increases the retention times for an analyte, improving resolution. Octanesulphonic acid (OSA) as the counterion at a concentration of 4.5 mM gave a retention time of 8 min for hydroxylamine and provided adequate sensitivity as detailed below. Addition of a small amount of solvent to the eluent resulted in improved peak symmetry and the eluent finally selected for this work was 4.5 mM OSA/5% acetonitrile. Hydroxylamine was detected using an electrochemical detector where it was reduced on a platinum electrode at ± 1.0 volts. Under these elution conditions, the detection limit for hydroxylamine was 0.01 mg/L.

When the extracts were examined using this eluent, the hydroxylamine peak was completely separated from peaks associated with the elution of sulphuric acid and OSA, provided the concentration of sulphuric acid remained low. Problems were encountered when high concentrations of hydrogen ions, as in 0.75 M sulphuric acid extracts, were injected on to the MPIC column. Under these conditions the chromatograms showed very pronounced peaks around the void volume and a multiplet of peaks, at least three being distinguishable eluting between 4 min and 10 min, compared to the expected retention time of 8 min for

hydroxylamine. Dilution of the extract by a factor of 10 reduced the effect but did not give a return to a true baseline prior to elution of the hydroxylamine, although the actual retention time of hydroxylamine was unaffected. Further dilution of the sulphuric acid solutions was precluded by the limited sensitivity of the detector. Partial neutralisation of the excess acidity prior to injection on to the column also reduced the effect, but use of strong base could lead to localised regions of strongly alkaline solution with the potential for loss of the volatile free hydroxylamine and the use of carbonates led to a loss of hydroxylamine with the evolved carbon dioxide. Because of the potential for errors whilst making Ph adjustments, it was decided to run the 0.75 M sulphuric acid extracts after diluting 10-fold and to accept the small loss of resolution and a sloping baseline in the chromatograms.

To investigate the effect of acidity of the solution in which the hydroxylamine is dissolved on the colorimetric assay, a series of hydroxylamine solutions of varying pH's was prepared. The pH was adjusted by adding appropriate amounts of strong acid or alkali to 50 ml flasks containing a standard amount of hydroxylamine solution. The hydroxylamine content of each of these solutions was determined colorimetrically using the procedure established by Davey and Loadman⁶. The results are shown in *Table 1*. The detection limit for the colorimetric procedure was estimated at 0.02 mg/L.

To compare the efficiency of extraction of hydroxylamine from the various grades of natural rubber by different extractants, four typical extractants were chosen:

Solution number	pH of hydroxylamine	Hydroxylamine found (mg) solution	
l	0.26	0.500	
2	1.02	0.485	
3	1.99	0.475	
4	4.27	0.450	
5	6.01	0.440	
6	9.87	0.405	

TABLE 1 HYDROXYLAMINE AMOUNTS MEASURED COLORIMETRICALLY

Note: These results are derived from a calibration in which the hydroxylamine was added in 0.75 Msulphuric acid, approximately pH 0.8.

- Methanol, a common organic extractant for raw natural rubber
- 0.07 *M* hydrochloric acid, as this is used to simulate the effect of saliva in Standards⁸ concerned with the safety of toys *etc*.
- 4.5 mM OSA/5% acetonitrile, which, as the chromatographic eluent, would enable the separation step to be performed with minimal disruption to the chromatography, and
- 0.75 *M* sulphuric acid which, as the extractant used in the current method, acted as the control.

All the extractions were performed using samples prepared in exactly the same way as is used for samples in the current procedure. The thinly sheeted sample was cut into strips, placed in a round-bottomed flask with the extractant (45 ml) and then boiled for 16 h (overnight). Each of the methanol extracts was subsequently treated with hydrochloric acid (1 ml conc. HCl to 24 ml of the extract) and boiled for four hours. The extract solutions and the hydrolysed methanol solutions were analysed for hydroxylamine content by both chromatography and colorimetry. The chromatographic conditions were as detailed previously. All of the colorimetric measurements were made using the 0.75 M sulphuric acid calibration curve and the method of Davey and Loadman⁶, except that, whenever solutions extracted with solvents other than 0.75 M sulphuric acid were measured, 1 ml of 0.75 M sulphuric acid was added to the test portion prior to addition of the 8-hydroxy quinoline. An aliquot of hydroxylamine standard (0.5 mg, equivalent to 5 g of rubber containing 100 mg/kg) was subjected to the same overnight heating regime with each of the extractants, and used as a recovery check. The results of these extractions are shown in Table 2

To assess the possibilities for extraction of hydroxylamine from vulcanisates, materials from each of the stages of progression to a vulcanisate, *i.e.* a portion of raw CV 60 rubber, a compound prepared from it (see Table 3 for formulation) and the corresponding vulcanisate, were prepared. Portions of each of these materials were extracted with four different extractants to determine which type most effectively removed any residual hydroxylamine from CV-based vulcanisates. The extractants were chosen for their expected effectiveness in removing non-rubber components from vulcanisates:

- 1,4-Dioxan as it strongly swells vulcanised rubber,
- Acidified 1,4-Dioxan to determine whether 1,4-Dioxan swollen rubber would be more effectively extracted by acid, and

Extract	ant	OSA/ MeCN	Meth (a)	ianol (b)	НСІ	H ₂ SO ₄
SMR CV 60	LC.	29	0.5	18	24	76
2	Col.	44		35	42	33
SMR CV 50	I.C.	26	0	16	21	67
	Col.	39	1	47	51	30
SMR GP	I.C.	80	26	122	103	155
	Col.	105		148	117	97
SMR 10 CV	I.C.	49	0	36	49	104
	Col.	63		94	82	70
SMR 20 CV	I.C.	51	0	57	54	96
	Col.	65		60	68	63
Hydroxylamine	I.C.	88	55	114	95	110
recovery	Col.(c)	103		121	111	81

TABLE 2 COMPARISON OF HYDROXYLAMINE LEVELS DETERMINED BY ION CHROMATOGRAPHY AND BY COLORIMETRY

The values shown are the actual concentrations of hydroxylamine in mg/kg determined for each extract solution. The results have not been corrected for the measured recoveries.

Notes:

(a) The hydroxylamine was determined prior to acid hydrolysis.

(b) Hydroxylamine determined after acid hydrolysis.

(c) The recoveries are shown as the percentage of the known addition measured by each method.

• 0.07 *M* hydrochloric and 0.75 *M* sulphuric acids for the same reasons as in the case of the raw rubbers above.

The results are shown in Table 4.

TABLE 3 FORMULATION OF RUBBER USED FOR VULCANISED RUBBER TEST

SMR CV 60	100
Zinc oxide	5
Stearic acid	2
Antioxidant 2246	2
Sulphur	2.5
CBS	0.6

The sample was pressed to a 15.2 cm \times 12.7 cm \times 1.5 mm sheet and cured 20 min at 150°C.

RESULTS AND DISCUSSION

The results in *Table 1* clearly show the changes in colour intensity, compared with the standard calibration curve, when the pH of the hydroxylamine test solution is varied. All colorimetric determinations, irrespective of extractant, were quantified by comparison with the standard calibration curve, therefore some errors due to the differences in sample pH are probable, but no attempt to estimate them was made in this study.

The results given in *Table 2* can only be considered a guide to expected levels of extractable hydroxylamine, since they refer to

Sample	Extractant	Hydroxylamine conc. in rubber by I.C.	Hydroxylamine conc. in rubber by UV
SMR CV 60	0.75 <i>M</i> H ₂ SO ₄	51.7	70.4
	0.07 <i>M</i> HCl	46.2	41.1
Compound	0.75 <i>M</i> H ₂ SO ₄	22.3	22.1
(as in <i>Table 3</i>)	0.07 <i>M</i> HCl	1.5	1.9
Vulcanisate	0.75 <i>M</i> H ₂ SO,	14.1	15.6
(as in <i>Table 3</i>)	0.07 <i>M</i> HCI	0	0

TABLE 4 HYDROXYLAMINE CONCENTRATIONS (MG/KG) FOUND IN RAW, COMPOUNDED AND VULCANISED RUBBER

Note: The extracts containing 1,4-dioxan gave variable and inconsistent results and are not included in this table (see text).

single samples from each grade. Nevertheless, they are consistent with the results of routine analyses, both in the typical values for the grades and in demonstrating that SMR GP generally has significantly higher levels of extractable hydroxylamine than any grades. The hydroxylamine values measured by I.C. from the sulphuric acid extract and shown in Table 2 are probably over-estimated. This is due to difficulties in measuring the chromatographic baseline, caused by the changes to the chromatography induced by sulphuric acid, and which have been discussed above. Although these values have been included for completeness, and to show that they still follow the same general trends as the other measurements, the absolute values have limited significance. The recovery experiments indicate the similarity between the I.C. and colorimetric determinations (102% and 104% recovery, respectively) and also the standard deviations are similar (12% and 17%, respectively). The statistics are based on single determinations of recovery using the figures in Table 2, with four values in each case, the unhydrolysed methanol results being excluded.

The methanol extracts from the various raw rubbers were analysed both before and after treatment with $1 \dot{M}$ hydrochloric acid and the results are shown in Table 2. The level of hydroxylamine found in the untreated methanol extract should be an indication of the 'free' hydroxylamine, whereas the hydroxylamine level after hydrolysis gives the total extractable hydroxylamine. The recovery experiment on hydroxylamine in 'neutral' methanol gives a low recovery (55%) but the 'lost' hydroxylamine is regenerated when the solution is treated with hydrochloric acid. The reason for this inability to measure hydroxylamine after overnight reflux was not investigated further. The results show that, other than the GP grade, the raw rubbers contained no 'free' hydroxylamine and that methanol extracts a similar quantity of 'hydroxylamine' to the other extractants.

With the exception of the sulphuric acid extracts, it will be noted that the colorimetric method gives consistently higher results than the chromatographic procedure. This is probably due to the colorimetric hydroxylamine test being non-specific for hydroxylamine. The basis of the colorimetric test is reaction with 8hydroxy quinoline, and many metallic cations are capable of reacting with 8-hydroxy quinoline to give coloured products under the conditions of the test^{6,9}. Many of these cations would be expected to be extracted by the acidic extractants, however, the extent of extraction is dependent on the chelating strength and the concentration of the extractant. The variation of concentration of these species with grades of SMR has been addressed previously by one of the authors¹⁰, but only with regard to the total metal content, not the extractable levels. The interferences due to calcium and zinc were specifically addressed by Davey and Loadman, with regard to vulcanised rubber⁶.

Excluding the sulphuric acid results, the results are in good agreement, given the relatively high standard deviations of the results. The presence of similar levels of hydroxylamine in the I.C. eluent extracts (4.5 mM OSA/5% MeCN) and in the hydrolysed methanol and hydrochloric acid extracts demonstrates that all three of these, relatively mild, extractants are equally capable of being used as replacements for sulphuric acid.

As stated above, the detection limits for the colorimetric and chromatographic procedures are similar at an instrumental level, being 0.02 mg/L for colorimetry and 0.01 mg/L for chromatography. However, when the detection limits in the rubber are considered, due to the extra ten-fold dilution step involved in the colorimetric procedure, the detection limits become 2 mg/kg for colorimetry and 0.1 mg/kg for chromatography.

1,4-dioxan (*Table 4*) is not a satisfactory extractant for vulcanised rubber, which could

be due to the presence of low levels of peroxides in the 1,4-dioxan reacting with the hydroxylamine. Although it would be possible to purify the 1,4-dioxan to remove these peroxides, this would introduce an additional step into routine analysis and, if a sample showed no hydroxylamine after extraction with the purified 1,4-dioxan, there would still be some doubt as to whether the absence was due to a genuine absence of hydroxylamine or whether a low level of peroxide had remained and reacted with extracted hydroxylamine.

The sulphuric acid extraction results in compounded and vulcanised rubber (Table 4) are almost identical to those reported by Davey and Loadman⁶. This present study also found some 20 mg/kg of hydroxylamine in extracts of compounded rubber. However, in this case we have not checked against compounds formulated with non-CV rubber to verify that the hydroxylamine measured colorimetrically is due to an interference. It was considered that obtaining estimates of the hydroxylamine content by two completely different methods, chromatography and colorimetry, should have given that estimate much greater reliability. However, the closeness of the agreement between the results of Davey and Loadman⁶ and those reported here do suggest that some caution is required in interpreting these results.

The 0.07 *M* hydrochloric acid, whilst being broadly in line with the sulphuric acid when extracting raw polymers, does much less well when confronted by the compounded and vulcanised materials. There could be many different reasons for the lower hydroxylamine levels measured in hydrochloric acid extracts, but no efforts to isolate them were made in this study.

CONCLUSION

The ability of 4.5 mM OSA/5% MeCN, acidified methanol and 0.07 M hydrochloric acid to effectively extract equivalent amounts of hydroxylamine, indicates that any of these extractants would be worth considering as a replacement for 0.75 M sulphuric acid for routine hydroxylamine determinations in raw rubber.

The ion chromatographic procedure combined with either of the weakly acidic extractants, or with acidified methanol extraction, is capable of determining hydroxylamine with at least one order of magnitude greater sensitivity than the present colorimetric method. This would enable the determination to be carried out with a significant reduction in the handling of hazardous materials.

The limited results obtained in this investigation for vulcanised rubber would support the conclusions of Davey and Loadman⁶ that vulcanised CV rubbers do not contain hydroxylamine which can be extracted by extractants less aggressive than 0.75 M sulphuric acid.

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