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New methodology to follow the evolution of squalene by-products during model compound vulcanization studies

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Abstract

In this work, a new analytical methodology is proposed to separate squalene from its by-products after vulcanization, using reverse phase liquid chromatography. Regarding previous methods described in literature, the separation between reaction products and their detection have been clearly improved. A light scattering detector has been coupled to the HPLC equipment substituting the traditionally used UV detector. With these modifications is possible to detect a larger number of compounds along the reaction in only one-shot analysis. It is even possible to discern between cross-linked squalenes bonded with different sulfur chain lengths whose structures have been elucidated by mass spectrometry. Results obtained working with this methodology helped to gain more insight into the natural rubber accelerated vulcanization process.
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1. Introduction

As it is well known, the vulcanization process as it occurs in the production of natural rubber goods is very complex because of both the large amount of simultaneously occurring reactions and the non-soluble nature of rubber samples [1]. Although the reaction scheme proposed by Morrison and Porter [2] has been generally accepted, there is still a lot of controversy about what is exactly happening in each stage [3–7]. Vulcanized rubber samples become a complex polymer network that is very difficult to work with. Thus, the study of the reaction occurring within the network is far from becoming an easy task [8–10].

Traditionally, working with the Model Compound Vulcanization (MCV) approach [11–15] turned out to be a great step forward to overcome the mentioned difficulties about the sample manipulation. This approach consists of vulcanizing a model molecule whose chemical structure resembles that of real rubber, with the advantage being its smaller size which makes it easier to study. The assumption that these model molecules behave as the polymer does during the reaction must be made.

In literature, a wide array of model molecules can be found. Nevertheless, late studies have been using squalene as a suitable model molecule to study the vulcanization of natural rubber. This compound has been previously used in studies of adhesion [16], reversion [17,18] and vulcanization mechanism dissertation [19]. Furthermore, squalene is preferred rather than other model molecules due to its larger size.

During the 1980s, reverse phase-HPLC has become one of the most common techniques used to study and identify the compounds that play an active role during the vulcanization reaction in the MCV mixtures (i.e. accelerator, active sulfurating compounds, sulfur, etc.) [20–22]. Though, RP-HPLC analysis turned out to be troublesome concerning the study of the vulcanized model molecules, the previously mentioned squalene by-products (such as modified squalene with pendant groups). Difficulties arose from the low polarity of these types of compounds, which show excessively long retention times when analyzed by using RP columns.

Attempts to fulfill the lack of knowledge about the behavior of the model molecule during the reaction have been carried out using high performance size exclusion chromatography (HPSEC) [23–25]. However, despite the fact that some insight was gained concerning the cross-linked squalene formation, the technique did not have high

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enough resolution to sufficiently separate all the squalene by-products formed. Differences between their molecular weights were even too small to be detected with the HPSEC column that specifically covers the molecular weight range of the squalene by-products.

There is still another feature that can be improved, and that is the detection technique. Most of the rubber studies have used chromatography work with a UV detector. This type of detector has been proved to be adequate when analyzing intermediate vulcanization compounds. Nevertheless, the analysis of squalene by-products usually implied a noisy baseline overlapping important peaks and spoiling their resolution. To solve this drawback, the introduction of a light scattering detector (ELSD) is proposed because in oil, fatty acid and lipid analysis its use gave rise to neater base lines and chromatogram profiles [26–29].

Table 1
Composition of the mixture studied (phr: parts per hundred rubber)

Ingredients	Amount (phr)
Squalene	100
ZnO	5
Stearic acid	2
Sulfur	2
CBS	1.2

Now, the aim of the present work consists of gathering all the analytical improvements mentioned above for similar molecules to build up a new methodology for studying the squalene vulcanization reaction. The method developed is thought to include in one single analysis the study of both the vulcanization intermediate compounds and the squalene
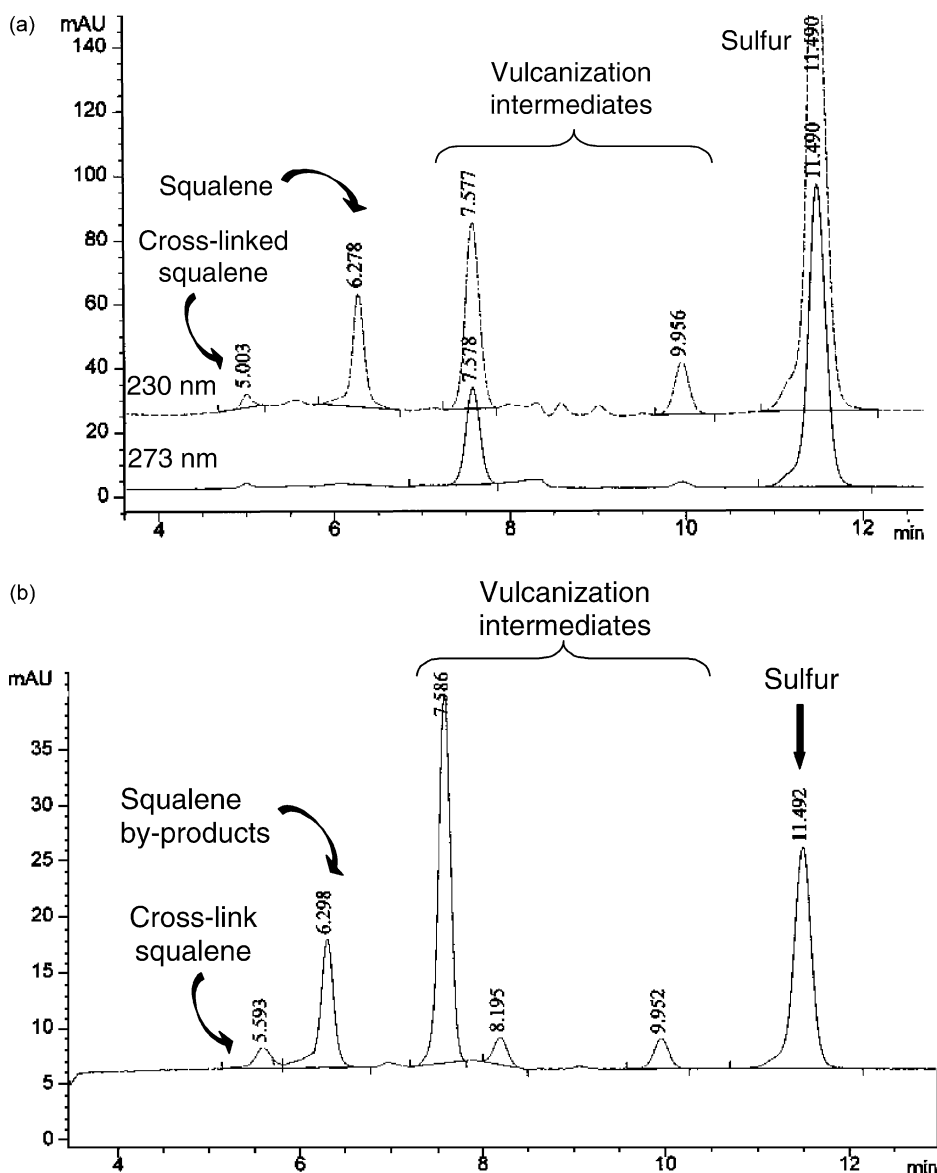

Fig. 1. (a) Detection comparison between 230 and 273 nm UV detector in HPSEC separation of the vulcanization mixture after 10 min of reaction. (b) HPSEC separation of the vulcanization mixture setting the UV detector at 273 nm after 60 min of reaction.

by-products. Coupling with a mass spectrometer was done in order to confirm the identification of all the compounds studied and make the RP-HPLC with the light scattering detector an analysis on a routine basis.

2. Experimental

2.1. Chemicals

In order to perform the study the following chemicals have been used.

The model compound chosen to simulate the behavior of NR was squalene (by Fluka). The accelerator cyclobenzothiazole sulfenamide (CBS), ZnO and sulfur (S_8) were provided by JEVSA S.A., and stearic acid by Panreac.

The mobile phase for HPSEC separation is tetrahydrofuran (THF) (Merck LiChroSorv). The solvents needed for HPLC chromatography are acetonitrile, isopropanol and hexane, all of them provided by Romil (far UV/gradient quality).

2.2. Model mixture studied

The ingredients of the model mixture studied are shown in Table 1. Only the compounds that are thought to play an active role during the vulcanization were included in the reaction mixture in order to simplify the manipulation of the samples. The accelerator used is cyclobenzothiazole sulfenamide. The vulcanization reaction was carried out in closed vials at 140 °C that contained a reference mixture.

The reaction was carried out under a nitrogen atmosphere in order to avoid oxidation of the double bonds of squalene. A continuous stirring was required to assure the homogeneity of the reaction mixture.

At previously determined times, vials were taken out from the heating bath and quickly cold quenched to stop the reaction.
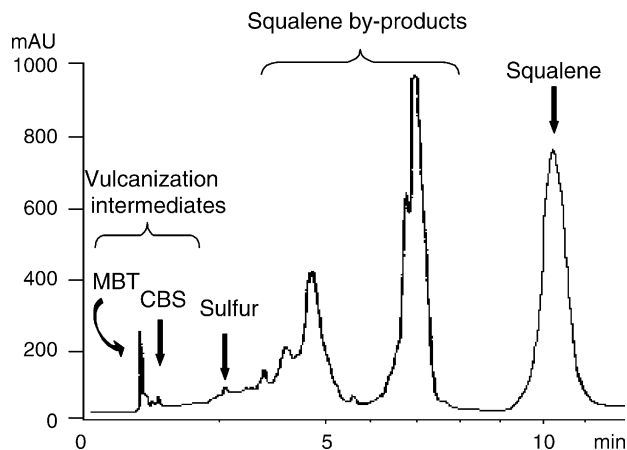
Fig. 2. HPLC separation of the vulcanization mixture (60 min reaction time sample) setting the UV detector at 230 nm.

2.3. HPSEC analysis

0.1 g of the cold quenched sample was dissolved in 10 ml of tetrahydrofuran for 5 min in an ultrasonic bath at room temperature, and filtered with a 0.45 μm Nylon filter in order to remove insoluble particles that would damage the column. The injection volume was 20 μl.

HPSEC experiments were carried out in a Hewlett-Packard HP1090, using a Waters HR 0.5 Styragel (7.8 mm × 300 mm) column with a UV detector set at 230 and 273 nm. The mobile phase used was tetrahydrofuran at a flow of 1 ml min^{-1}.

2.4. RP-HPLC analysis

0.1 g of the cold quenched sample was dissolved in a 72:17:11 acetonitrile, isopropanol and hexane mixture for 5 min in an ultrasonic bath at room temperature, and filtered with a 0.45 μm Nylon filter in order to remove insoluble particles that would damage the column. The injection volume was 10 μl.
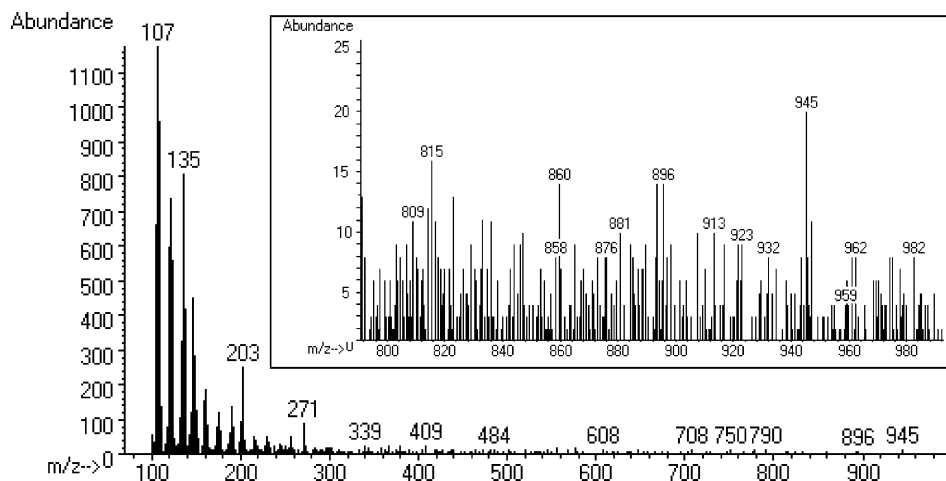
Fig. 3. The mass spectrum of the 4.2 min peak shows the molecular mass at 945 amu.

HPLC experiments were conducted with a Hewlett-Packard HP1050 chromatograph. An Ultrabase C-18 (5 μm, 150 mm × 4.6 mm) and a Waters Symmetry® C-8 (5 μm, 150 mm × 3.9 mm) column was used with an UV detector set at 230 nm, and the mobile phase flow was 1 ml min⁻¹. The experiments were carried out using the following elution mixture: acetonitrile, isopropanol and hexane (72:17:11).

The light scattering detection was carried out with a PL-ELS 1000 detector. The nebulization chamber and evaporation chamber temperatures were 40° and 80 °C, respectively. A 1 ml min⁻¹ air gas flow was used as nebulizing gas.
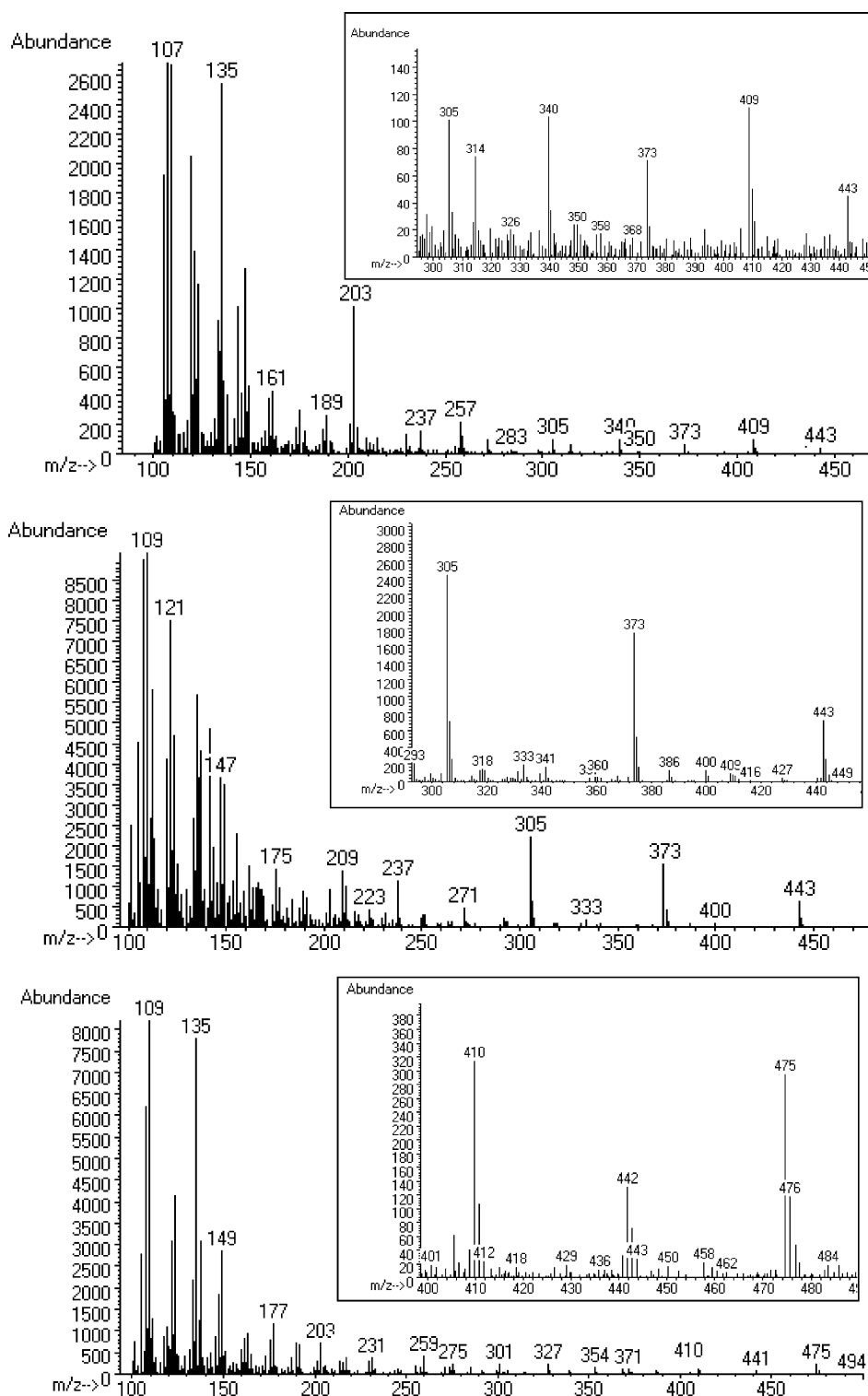

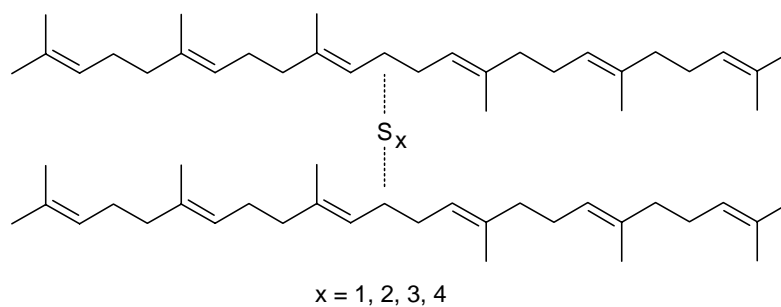Fig. 4. Mass spectra of the 5.5, 6.5 and 7 min peaks.

Fig. 5. Cross-linked squalene structures.

2.5. Mass spectrometer

The identification of the compounds was accomplished by coupling the chromatograph with a 5989A HP Mass Spectrometer (electron energy: 70 eV; MS source: 250 °C; MS quadrupole: 110 °C) through a particle beam Hewlett-Packard HP 59980B LC/MS interface (desolvation chamber temperature: 55 °C; helium inlet pressure: 40 psi).

3. Results and discussion

When a vulcanization sample from MCV approach using squalene is analyzed by HPSEC after 10 min reaction to study the squalene by-products generated gives a chromatogram as the presented in Fig. 1a. Squalene by-products definition includes the different molecules that squalene can produce during vulcanization, from any chain modification to cross-linked squalene. In this chromatogram, a little peak
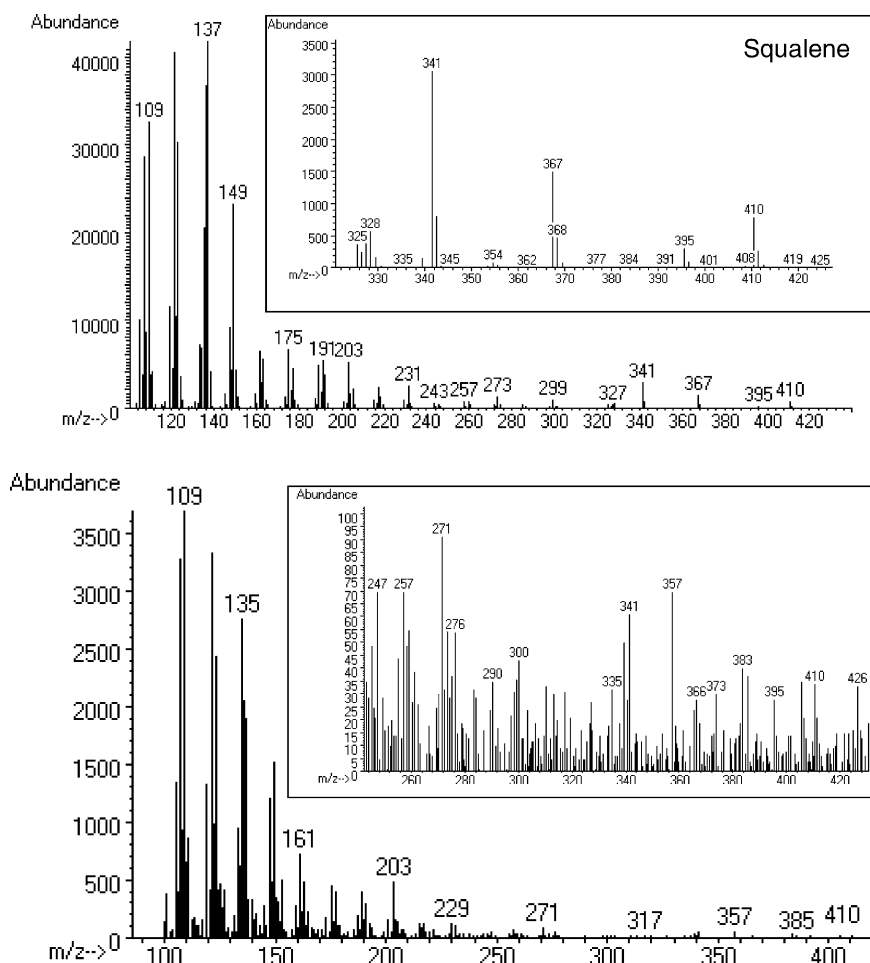

Fig. 6. Squalene and squalene by-product (eluted at 4.7 min working with the novel HPLC methodology) mass spectra.

of cross-linked squalene and a peak of squalene can be observed at 5 and 6.3 min, respectively, when UV detection is set a 230 nm. However, setting the UV lamp at 273 nm, the squalene peak is missing and only cross-linked squalene is observed.

With the evolution of the vulcanization reaction, at a higher reaction time of 60 min, other squalene by-products are generated. These squalene by-products have a molecular mass close to squalene, and despite the good column performance in this MW range, the overlapping with squalene could not be avoided (Fig. 1b). Then, that requires one to use 273 nm as a UV wavelength and hinders the utilization of any universal detector.

In a first attempt to avoid the overlapping of squalene with its by-products at 230 nm, the same mixture was analyzed by RP-HPLC using the analytical conditions described in

Section 2. Fig. 2 shows the resulting chromatogram where all the compounds of interest can be detected. It can be observed that not only squalene ($t_r \approx 10$ min) separates from its by-products (5 min $< t_r <$ 8 min), but also the vulcanizing agents and intermediate vulcanization compounds appear well defined (1 min $< t_r <$ 5 min). As it is indicated in the same figure, this latter group of compounds was confirmed by mass spectrometry identification detecting the molecular mass of 2-mercapto benzothiazole (MBT) and cyclobenzothiazole sulfenamide. MS also confirmed the presence of sulfur.

Concerning the squalene by-products, their appearance along the reaction time follows the expected tendency, i.e. they began to be detected after 20 min of reaction when the cross-linking between squalene molecules is believed to start. All these compounds appearing within this range
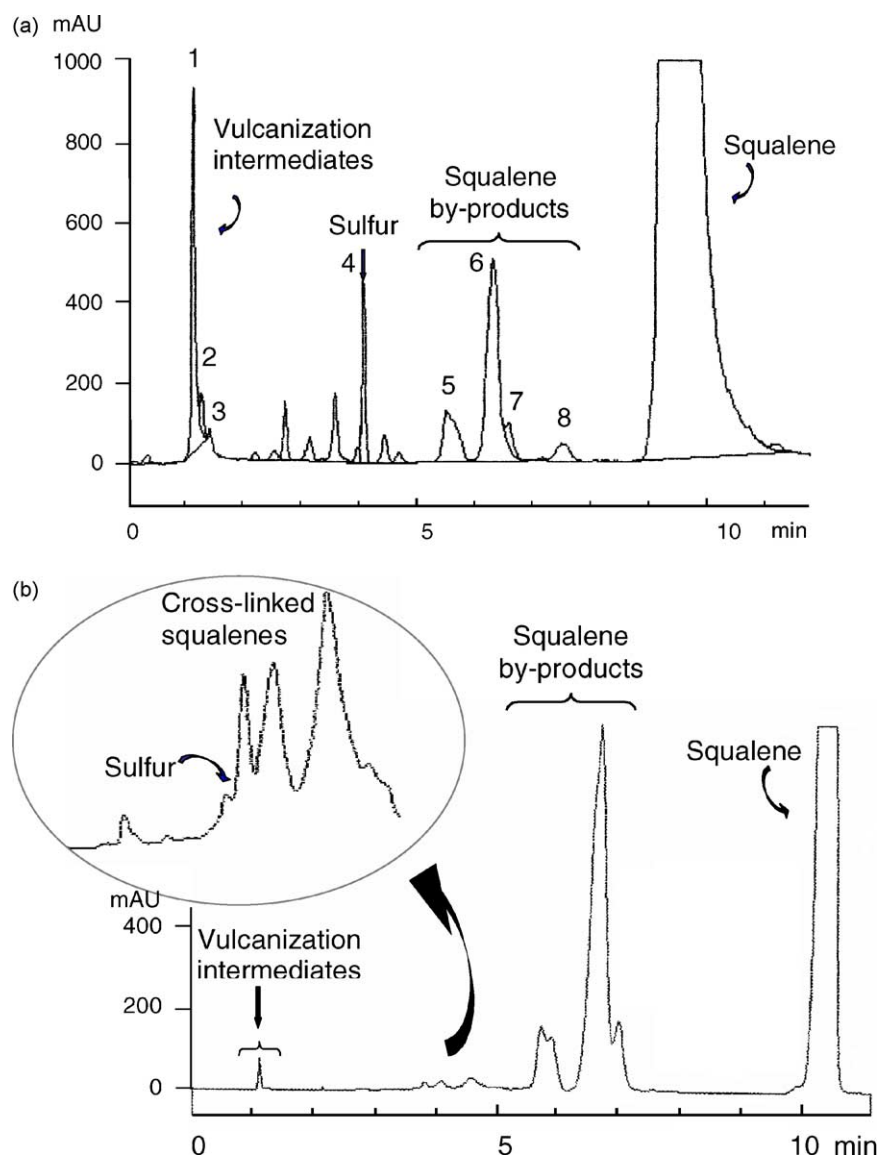

Fig. 7. (a) HPLC separation with light scattering detection (20 min reaction time sample). (b) HPLC separation with light scattering detection (60 min reaction time sample).

of retention time are confirmed to be squalene by-products since their mass spectra showed the characteristic fragmentation pattern of squalene.

Focusing on the mass spectra of the chromatographic peaks at 4.2, 5.5, 6.5 and 7 min, different fragments can be observed with higher molecular weights than squalene. In Fig. 3, the mass spectra of the 4.2 min chromatographic peak shows the molecular mass at 945 amu. This signal corresponds to two squalenes cross-linked with a four-sulfur atom chain. Regarding the other mass spectra from Fig. 4, the cross-linked squalene bonded with three, two and one sulfur atoms can be identified, respectively. For example, after 7 min of elution the mass spectrum shows the fragments 409 and 443 amu corresponding to squalene and squalene plus sulfur. Both values have their own fragmentation, which are characteristically from the squalene chain: $C_5H_9{}^+$ and $C_2H_3{}^+$. These cross-linked squalene molecules have been eluted in the RP-HPLC from the longer sulfur bond to the shorter one (see the structure in Fig. 5).

Among these cross-linked squalene peaks there is one at 4.7 min. that also corresponds to a squalene by-product. In this case, there have not been molecular weights detected higher than the molecular weight of squalene. In Fig. 6, the mass spectrum of this product is compared to the known spectrum of squalene. Signals at 109, 121 and 149 amu were observed in both spectra, however, the fragmentation of these two products exhibits some differences. As it has been described in a previous work, this is due to the transformations that can occur in the squalene chain in addition to the

cross-linking process [16]. Then, as previously mentioned, the peak eluted at 4.7 min corresponds to modified squalene and the last one, eluted at 10 min, to the original squalene chain.

As a result, it can be stated that not only squalene has been separated from their by-products, but also the different cross-linked fractions have been separated depending on the sulfur bond length.

In order to improve the separation between products, a parallel work has been performed using a RP-8 instead of the RP-18 described in the experimental part. The results obtained with this reversed phase do not show remarkable differences with those described for RP-18.

As it is stated before, using HPSEC, the overlapping between squalene and some of the squalene by-products difficults the quantification when the light scattering detector is used. That is the reason why the UV detection at 275 is preferred. Nevertheless, since RP-HPLC provided a good methodology to obtain a satisfactory separation of all the products under study, the coupling with a light scattering detector was tested. The objective sought after with this change consisted of gaining sensitivity and obtaining better baselines. Moreover, the UV detection is very dependent on the squalene chain modification. The use of this detector could avoid the problem presented by the different responses presented by squalene and squalene by-products in UV detection. This feature is of major importance regarding the quantification of the products under study. Since standards of such products are not available differences in
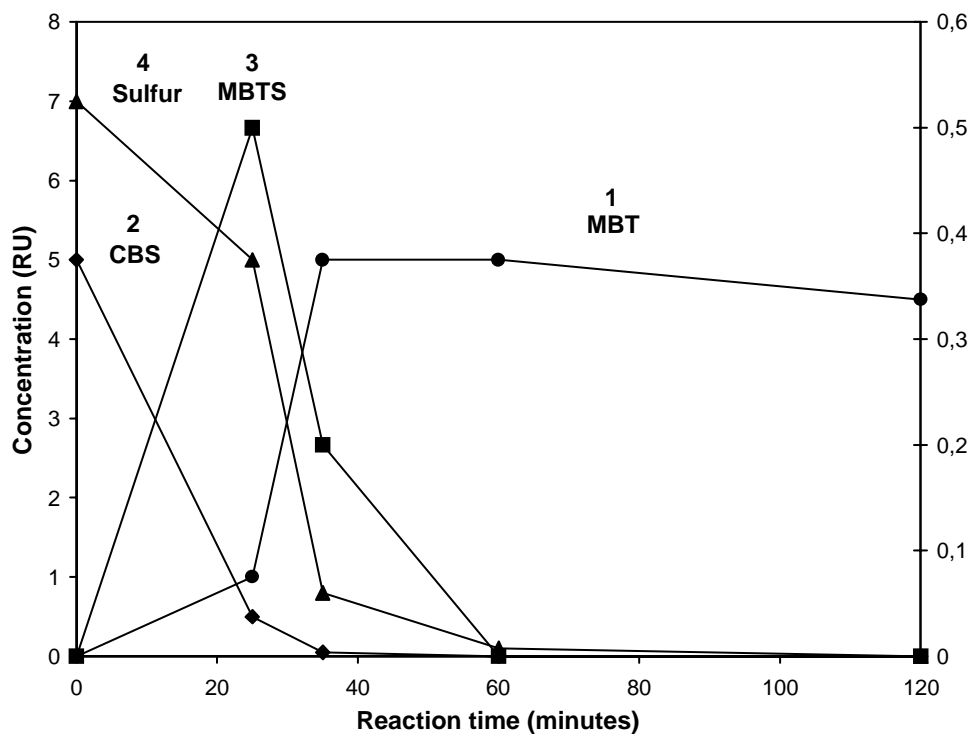

Fig. 8. Evolution of ingredients of the vulcanized mixture (CBS and sulfur) and intermediate vulcanization compounds (MBTS and MBT) in reaction time using HPLC analysis.

adsorbance could give rise to misleading results concerning quantification.

As expected, the introduction of the light scattering detector resulted in neater chromatograms that showed a notably improved baseline (Fig. 7a). The ELDS detector also shows a very good linearity in the work range of concentration (between 2 and 5 μg squalene injected), and high sensitivity.

Furthermore, as it has been explained before, results obtained along the reaction time (Fig. 7a and b) showed the behavior of the ingredients of the vulcanized mixture, their degradation to form intermediate compounds and the resulting cross-linked squalene together with several reaction by-products. These HPLC data are summarized in Figs. 8 and 9. Fig. 8 shows the behavior of both the ingredients of the vulcanized mixture (CBS and
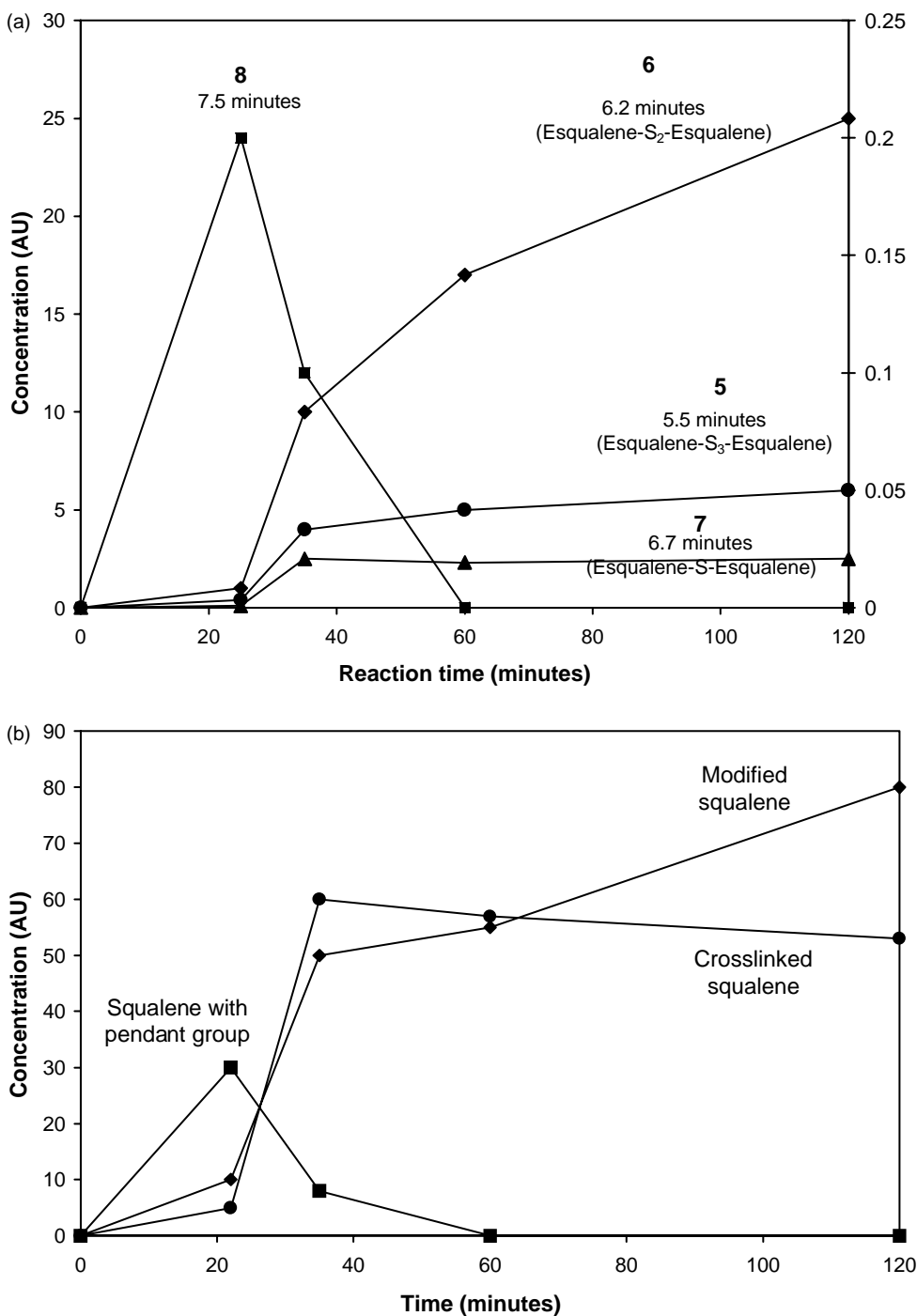

Fig. 9. (a) Evolution of squalene by-products in reaction time using HPLC analysis. (b) Evolution of squalene by-products in reaction time using HPSEC analysis.

sulfur) and intermediate vulcanization compounds such as bis-(2-benzothiazolyl)-disulfid (MBTS) and 2-mercapto benzothiazole and Fig. 9a the evolution of some squalene by-products generated along the reaction mixture. The trend of the major compounds in the mixture during vulcanization agreed with the general assumed reaction scheme for natural rubber sulfur vulcanization using sulfenamide accelerator-type, and with the results obtained from the common GPC analysis (Fig. 9b). However, a large number of squalene by-products could be detected. This capability to clearly detect several new squalene by-products is believed to give valuable hints into clarifying the exact reaction mechanism that occurs within the polymer network during the cross-link formation.

4. Conclusions

The new RP-HPLC methodology developed allows both the separation of squalene from its by-products and the detection of a larger number of squalene by-products along the vulcanization reaction. It is even possible to discern between cross-linked squalenes bonded with different sulfur chain lengths. Simultaneously, the accelerator, the vulcanization intermediate compounds and sulfur can also be detected.

The coupling of a light scattering detector gave rise to neater chromatograms, where sensitivity and resolution were improved. The use of this detector made it possible, as well, to neglect the absorbance differences between the compounds analyzed. This is of great importance since standards of such compounds are not available; therefore such differences could give misleading results when quantification is performed.

In closing, use of the Model Compound Vulcanization approach and further analysis with this methodology allows the detection of a new group of compounds, and offers the possibility of a major comprehension of the natural rubber vulcanization process.

Acknowledgements

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