Determination of squalene by-products during model compound vulcanization studies by LC-ESI-MS using silver nitrate as a post-column reagent

B. Vega[1], N. Agulló[1], M. Ventura[2], L. Comellas[2], S. Borrós[1]*

[1]Grup d'Enginyeria de Materials (GEMAT), Institut Químic de Sarrià (IQS), Universitat Ramon Llull, Via Augusta 390, Barcelona 08017, Spain [2]Chromatography Section, Institut Químic de Sarrià (IQS), Universitat Ramon Llull, Via Augusta 390, Barcelona 08017, Spain

Determinación de los productos de reacción durante el estudio de la vulcanización utilizando escualeno como molécula modelo, mediante LC- ESI -MS y nitrato de plata como reactivo post- columna

Determinació dels productes de reacció durant l'estudi de la vulcanització fent servir esqualè com a molècula model, mitjançant LC-ESI-MS i nitrat de plata com a reactiu post-columna

Recibido: 25 de febrero de 2013; revisado: 11 de noviembre de 2013; aceptado: 28 de noviembre de 2013

En record del Sr Eugeni Gasull, al que considerem el nostre mestre i amb el que vam inciar-nos plegats en el món del Cautxú

RESUMEN

La técnica de HPLC- ESI -MS, utilizando nitrato de plata como reactivo post- columna, se ha empleado para la determinación de los productos de reacción de escualeno durante la vulcanización, (el escualeno se utiliza como molécula modelo de caucho natural). En este método, después de que todos los productos se hayan separado en la columna de fase reserva, los analitos forman complejos con el catión plata después de mezclarse, después de la columna, con una solución de nitrato de plata. Los espectros de iones positivos del escualeno, los productos de reacción del escualeno y los intermedios de reacción, mostraron la existencia de los iones $[M+Ag]^+$ y/o $[M+Ag+AgNO_3]^+$. El método descrito en este artículo supera las limitaciones inherentes a la técnica de ESI referentes a la ionización de hidrocarburos. Además los resultados obtenidos mediante esta metodología han ayudado a tener más información sobre el mecanismo de vulcanización de caucho natural con azufre. En este sentido, se han identificado los diferentes tipos de reticulación, y se ha demostrado que un incremento en el tiempo de elución cromatográfico se corresponde a un descenso en la longitud de la cadena de azufre en la reticulación. Se ha identificado también moléculas de escualeno que incorporan trozos de acelerando en su estructura.

Palabras clave: derivatización post- columna, HPLC, ionización por electrospray , escualeno, estudios de vulcanización.

SUMMARY

High Performance Liquid chromatography-electrospray ionization mass spectrometry (HPLC-ESI-MS) using silver nitrate as a post-column reagent has been used for the determination of squalene by-products during model compound vulcanization studies. In this method, after all by-products were separated by reverse-phase liquid chromatography, analytes formed complexes with silver cation by mixing with a silver nitrate solution. The positive ion ESI mass spectra of squalene, squalene by-products and intermediates of vulcanization process showed $[M+Ag]^+$ and/or $[M+Ag+AgNO_3]^+$ ions. The method described in this paper overcomes the ESI technique limitations related to the ionization of hydrocarbon. Furthermore, results obtained working with this methodology helped to gain more insight into the natural rubber accelerated vulcanization process. In this sense the identification of the different crosslink types was determined, proving that the elution time increases with the decrease of sulfur chain length in the crosslink. Squalene with pendant group was also identified.

Keywords: post-column derivatization, HPLC, electrospray ionization, squalene, vulcanization studies.

*Corresponding author: Tel: +34-932672000, Fax: +34-932056266. salvador.borros@iqs.url.edu

Resum

La tècnica de HPLC-ESI-MS, fent servir nitrat de plata com a reactiu post-columna, s'ha emprat per a la determinació dels productes de reacció d'esqualè durant la vulcanització, (l'esqualè s'utilitza com a molècula model de cautxú natural). En aquest mètode, després que tots els productes s'hagin separat en la columna de fase reserva, els analits formen complexos amb el catió plata després de mesclar-se, després de la columna, amb una solució de nitrat de plata. Els espectres de ions positius de l'esqualè, dels productes de reacció de l'esqualè i dels intermedis de reacció, van mostrar l'existència dels ions $[M+Ag]^+$ i/o $[M+Ag+AgNO_3]^+$. El mètode descrit en aquest article supera las limitacions inherents a la tècnica de ESI referents a la ionització d'hidrocarburs. A més els resultats obtinguts mitjançant aquesta metodologia han ajudat a tenir més informació sobre el mecanisme de vulcanització de cautxú natural amb sofre. En aquest sentit, s'han identificat els diferents tipus de reticulació, i s'ha demostrat que un increment en el temps d'elució cromatogràfic es correspon a un descens en la longitud de la cadena de sofre en la reticulació. S'ha identificat també molècules d'equalè que incorporen trossos d'accelerant en la seva estructura.

Paraules clau: Derivatització post-columna, HPLC, ionització per electrospray, esqualè, estudis de vulcanització.

INTRODUCTION

Although the sulfur-accelerated vulcanization of natural rubber is a widely used process to produce some everyday life products, its mechanistic complexity has given rise to many contradictory publications. Basically there are two difficulties scientists have to deal with when studying the vulcanization mechanism: the nature of vulcanized rubber samples, which are insoluble in most common solvents and the simultaneity of reactions. In order to simplify the study, a modeling approach has been traditionally used [1-2]. This modeling approach for vulcanization is known as Model Compound Vulcanization (MCV) and consists of vulcanizing a model molecule (an olefin) whose chemical structure is similar to real rubber but with a lower molecular weight, which permits applying well established analytical techniques. Furthermore, the vulcanized model-compound mixtures usually do not contain all the ingredients present in the real rubber formulation, making it easy to focus only on the products that have an active role in the process. This method covers the whole process of vulcanization; from the formation of the active sulfurating agents, the precursor formation, until cross-link shortening and formation of the "final network" [1].

Among the large variety of model compounds described in literature [2-4], squalene has been used in vulcanization studies by our research group [5-7]. The main advantage that this model compound presents in comparison with others lies in its higher molecular weight, consisting of six units of isoprene. Moreover, samples are still soluble in most of the common solvents which enables the application of a wide range of analytical techniques allowing a complete characterization of both squalene and the curing agents during the process [8].

During the 1980s, reverse phase high performance liquid chromatography (RP-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. Late studies in our research group have developed a RP-HPLC method [9] to follow the evolution of squalene by-products as an improvement of a previously used size exclusion chromatography method (HPSEC) [10,11]. In this method electron-impact (EI) mass spectrometry was used to confirm the identification of all the compounds studied and a particle beam (PB) interface was needed to couple the RP-HPLC with the ionization source and the mass spectrometer. This interface transforms the analyte to the gas phase necessary to the electronic impact analyzer, causal of the ions generation. The results obtained were very interesting and some squalene reactive product could be identified. The main advantage of this method, in comparison with the HPSEC method previously used, was the possibility of discern between crosslinked squalenes bonded with different sulfur chain lengths. Nevertheless, electronic impact produces strong fragmentation which gives a lot of structural information but hinder the identification of the molecular ion. Although fragments give some information about the molecule structure, it would be interesting to find another MS technique with a softer ionization in order to discern between the different crosslinked squalene (with a very similar structure) and to assure the complete identification of the chromatographic peaks.

Currently, atmospheric pressure ionization (API) techniques for LC-MS are of increasing importance. These ionization techniques produce soft fragmentation and provide molecular weight information. Although at first sight atmospheric pressure chemical ionization (APCI) seems to be the most suitable technique to ionize these non polar or less polar compounds (crosslinked squalenes), spectra of hydrocarbon mixtures can be difficult to interpret due to the complexity of the competing mechanisms leading to the formation of several possible ions depending on the structures and sizes of the analyzed molecules [12]. Another API technique, electrospray ionization (ESI), is suitable for compounds that exit as ions in the LC eluent. Further, ESI is also adequate for non-ionic compounds that can produce complexes [13]. Recently, metal cations as zinc or silver, known as electron acceptors, have been used to form the cation complex with some hydrocarbons and help in this way its ionization to make it possible its analysis with ESI-MS [12-14]. Silver is known to be a suitable cationizing agent for squalene and squalene by-products in a MALDI-TOF method developed by our group [15]. In this paper a novel method based on the use of silver as a post-column reagent for this RP-HPLC-ESI-MS is proposed.

Now, the aim of the present work is to develop a new methodology to identify and characterize the squalene by-products formed in the vulcanization reaction and compare the results obtained previously with EI [9] with the results obtained with ESI.

EXPERIMENTAL

Materials

In order to perform the study the following chemicals have been used. The model compound chosen to simulate the behavior of natural rubber was squalene (Sq) (Fluka). N-cyclohexylbenzothiazol-2-sulfenamide (CBS) was used an accelerator and ZnO as an activator. These two products and sulfur (vulcanizing agent) were provided by JEVSA S.A. (Barcelona, Spain). Stearic acid (Panreac) was also used as activator. The solvents used for chromatography were acetonitrile (J.T. Baker, HPLC ultragradient analized) and 2-propanol (Panreac, HPLC-gradient-UV-IR). Silver nitrate (Sigma-Aldrich) was used as cationizing agent for the post-column derivatization.

Vulcanization of squalene

The ingredients of the mixture studied are shown in Table1. Only the accelerator system (accelerator + activators) has been taken into account, since they are the compounds that are thought to play a more active role during the vulcanization in order to simplify the manipulation of the samples. The accelerator used was N-cyclohexylbenzothiazol-2-sulfenamide. The vulcanization reaction was carried out at 140ºC, in closed vials that contained the reference mixture, in a microwave reactor CEM Focused Microwaves Synthesis System, Model Discover. More details about this method were described in a previous work published by our research group [16].

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

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 microwave oven in order to follow the evolution of the vulcanization intermediates during the vulcanization process and quickly cold quenched to stop de reaction.

RP-HPLC analysis

0,1g of the cold quenched sample was dissolved in 10 mL of a 70:30 acetonitrile/2-propanol mixture for 5 minutes in an ultrasonic bath at room temperature, and filtered with a 0.45μm Nylon filter in order to remove insoluble particles that would damage the column. The injection volume was 20μL.

HPLC experiments were conducted with a Waters Separations Module 2695 chromatograph. A Teknokroma Kromasil 100 C-18 (5μm, 250 mm x 4 mm) column was used with an UV detector set at 230 nm, and the mobile phase flow was 0.8mL min^{-1}. At 230 nm the squalene molecule as well the squalene by products can be detected with enough sensibility as we have previously reported [9,16]. The experiments were carried out using the following elution mixture: acetonitrile/2-propanol (70:30). The UV detection was carried out with a Waters 996 Photodiode Array Detector Millipore detector. 2mM silver nitrate solution was added post-column at a flow rate of 0.2mL min^{-1} by using a T-connector and an isocratic pump, which results in an overall concentration of silver ions of 0.4mM. After the liquid junction, the HPLC flow rate was 1mL/min.

Mass spectrometry

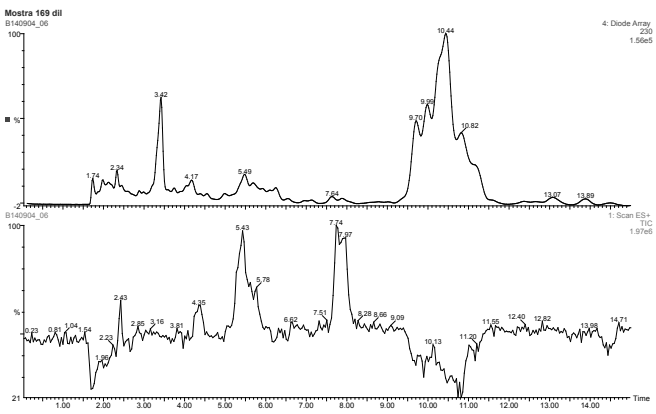
The identification of the compounds was accomplished by coupling the chromatograph with a Micromass ZMD detector single quadrupole mass spectrometer equipped with an electrospray ionization source. Nitrogen as the nebulizing gas and the drying gas was generated from compressed air. The nebulizing gas pressure was 50psi and the drying gas was held at 500 L/h. The drying gas temperature was 400ºC and the source temperature 100ºC. The ion spray needle voltage was kept at 4,5kV and the orifice voltage at 5, 20 and 40V. The range m/z 400-1200 was scanned over 2s. The experiments were carried out in the positive ion mode.

RESULTS AND DISCUSSION

First of all, the original RP-HPLC method [9] to analyze squalene by-products has been modified in order to remove n-hexane, since ESI does not allow the use of non-polar eluents. The original mobile phase, acetonitrile/2-propanol/n-hexane (72:17:11), was replaced by acetonitrile/2-propanol (70:30) obtaining a good resolution in the cross-linked squalenes peaks. However, the resolution of these peaks has been diminished in comparison with the one obtained with the original conditions, due to the polarity increase in the eluent. A drawback of the method developed, apart from the lack of resolution caused by the mobile phase polarity modification, is that the sulfur isotopic abundance (4% of the signal at M+2) is shielded by silver isotopic abundance. Therefore, in this method only the molecular weights are detected but no the isotopic abundances.

As stated before, samples do not present ionization, neither with ESI nor with APCI, in none of the work modes (positive and negative) tested. For that reason, the post-column method with silver nitrate derivatization has been developed to form silver adducts (ESI positive mode) in order to facilitate the ionization. Figure 1 displays the dramatic change in the ionization obtained with silver derivatization.

a)



b)

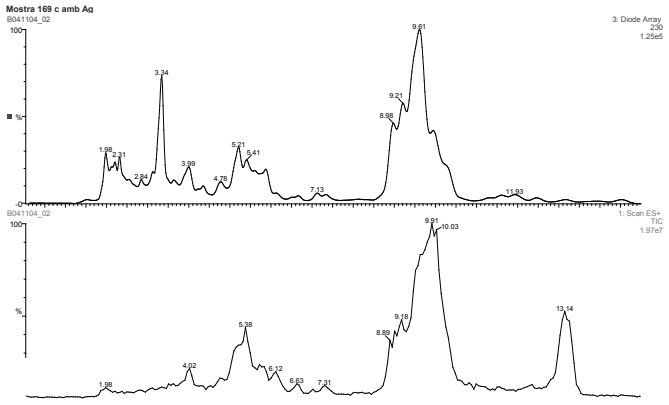


Figure 1. (a) Chromatogram at 230 nm (above) and mass spectrum in positive mode (below) without silver nitrate derivatization. (b) Chromatogram at 230 nm (above) and mass spectrum in positive mode (below) with silver nitrate derivatization. An outstanding increase of the ionization of the cross-linked squalens (peaks between peaks 9 and 11 minutes) can be observed.

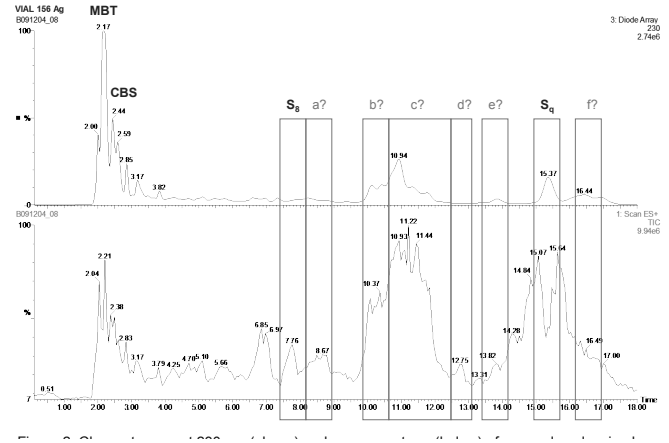


Figure 2. *Chromatogram at 230 nm (above) and mass spectrum (below) of a sample vulcanized for 60 minutes in which cross-linked and modified squalenes can be seen.*

Figure 2 shows a chromatogram at 230nm and the mass spectrum obtained with silver nitrate derivatization (m/z between 400 and 1200). This mass spectrum was obtained using a cone voltage of 5V to obtain as less fragmentation as possible. In addition figure 2 shows the chromatographic peaks that have been identified with external standards (CBS, MBT, sulfur and squalene) but also the peaks, or group of peaks, that present the same mass spectrum although their identification is not yet clear. By comparison with the results obtained in our previous paper on the identification of the squalene vulcanized intermediates,[9], 5 different fractions was assigned in the HPLC-ESI-MS chromatogram: **fraction a** was identified as cross-linked squalene with a tetrasulfídic sulfur bridge (Sq-S_4-Sq); **fraction b** was interpreted as modified squalene; **fraction c** formed by three peaks bad resolved were taken respectively as trisulfidic (Sq-S_3-Sq), disulfidic (Sq-S_2-Sq) and monosulfidic (Sq-S-Sq) cross-linked squalenes; **fraction d**, as it is seen in figure 2, is not detected at 230nm; **fraction e** was assigned as squalene with pendant group and **fraction f** was not detected either at 230nm. The development of this ESI-MS method has allowed the correct identification of the fractions, as it will be seen in the paper.

First the study of the peaks that can be identified by comparison with a standard was performed. Mass spectrum (m/z = 400-1200) of silver adduct of squalene is presented in figure 3. Squalene (Sq) has a molecular weight of 410 g/mol. Its silver adduct $[Sq+Ag]^+$ corresponds to the peak at m/z=519. Some other peaks separated by a constant distance of m/z=169 can be clearly observed. They correspond to additions of silver nitrate molecules, present in the eluent ($[Sq+Ag+AgNO_3]^+$ m/z=688, $[Sq+Ag+2AgNO_3]^+$ m/z=857 and $[Sq+Ag+3AgNO_3]^+$ m/z=1028). In literature it has been described that depending depending on the solvent and the cone voltage it is possible to find solvent adduct ions as $[M+Ag+solvent]^+$ aside from the adduct $[M+Ag]^+$ [17,18]. Comparing our results with the one described in literature [19], it can be concluded that in the samples analyzed salt adducts ions from salts present in the mobile phase are formed instead of the solvent adducts ions alone. That means that under the conditions studied, some silver nitrate adduct ions have been formed.

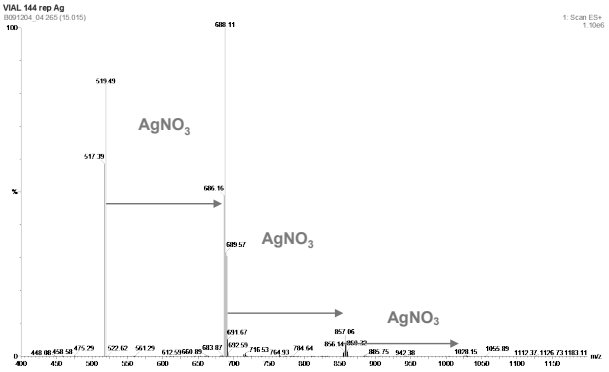


Figure 3. *Mass spectrum of the silver adduct of squalene.*

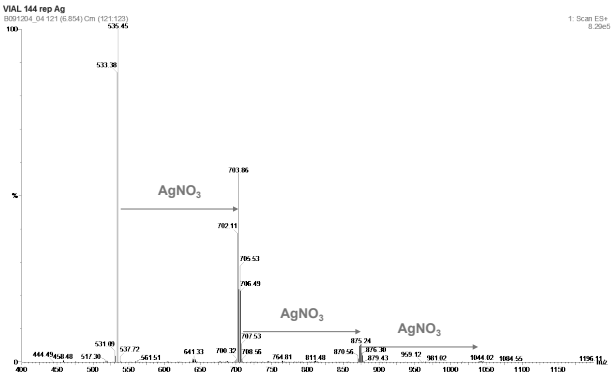


Figure 4. *Mass spectrum of the silver adduct of sulfur.*

At 6.9 minutes appears the peak of sulfur (S_8). This peak has also been identified with an external standard. Figure 4 shows the mass spectrum (m/z=400-1200) of silver adduct of sulfur. It is very similar to the one described for squalene (Figure 3). Elementary sulfur (S_8) has a molecular weight of 256 g/mol. The signal m/z=535 in the mass spectrum corresponds to the adduct of sulfur with a silver molecule

and a silver nitrate adittion $[S_8+Ag+AgNO_3]^+$. At a distance of m/z=169, and multiples some intense peaks can be also observed corresponding to multiple additions of silver nitrate molecules. This is the same behaviour described before for the squalene identification. The series detected for the sulphur molecule can be summarized as follows: ($[S_8+Ag+2AgNO_3]^+$ m/z=703, $[S_8+Ag+3AgNO_3]^+$ m/z=875 and $[S_8+Ag+4AgNO_3]^+$ m/z=1044). The silver adduct ion of sulfur $[S_8+Ag]^+$ must appear at m/z=363, outside the analyzed region.

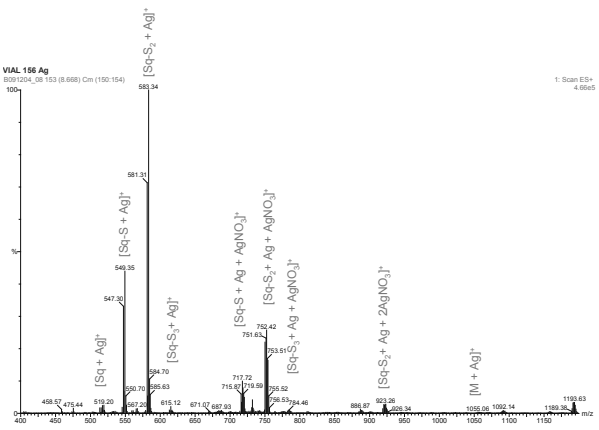


Figure 5. Mass spectrum of **fraction a** *between m/z = 400-1200. It corresponds to two squalene molecules bonded with a tetrasulfidic bond (Sq-S_4-Sq).*

Now, we will focus on the mass spectra of the fractions with the ESI-MS method.

In figure 5 the mass spectrum of **fraction a** is presented, which corresponds to the tetrasulfidic cross-linked squalene (Sq-S_4-Sq). The molecular ion corresponding to the silver adduct $[M+Ag]^+$ is at m/z=1055. Furthermore, a low signal at m/z=519 can be also seen. As mentioned before, it corresponds to the silver adduct of squalene $[Sq+Ag]^+$. Another peaks at m/z=549, 583 and 515 corresponding to $[Sq-S+Ag]^+$, $[Sq-S_2+Ag]^+$ and $[Sq-S_3+Ag]^+$ respectively can also be detected. This gradation of squalenes bonded with a different sulfur chain bridges are fragmentation products of the molecular ion and this profile is repeated in the m/z zone between 700 and 800 with the signals corresponding to the ions $[Sq-S_x+Ag+AgNO_3]^+$.

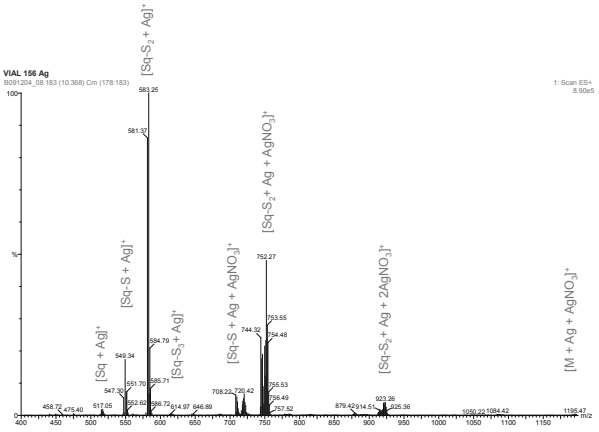


Figure 6. Mass spectrum of **fraction b** *between m/z=400-1200. It corresponds to two squalene molecules bonded with a trisulfidic bond (Sq-S_3-Sq).*

Figure 6 shows the mass spectra of **fraction b** which has been identified as trisulfidic cross-linked squalene (Sq-S_3-Sq). In the EI method [9] this chromatographic peak had been interpreted as modified squalene, but this method of RP-HPLC-ESI-MS with silver nitrate post-column derivatization has allowed its correct identification. This fraction is formed by two chromatographic peaks, but both have the same mass spectrum. This is due to the formation of different isomers according to the position of the sulfur bridge that bind the two squalene chains. The molecular ion corresponding to the silver adduct $[M+Ag]^+$ is at m/z=1023. This signal does not appear in the mass spectrum, but at m/z=1195 the ion $[M+Ag+AgNO_3]^+$ is clearly detected. Besides, there are peaks at m/z=517 corresponding to the silver adduct of squalene $[Sq+Ag]^+$ and at m/z=549, 583 and 515 corresponding to $[Sq-S+Ag]^+$, $[Sq-S_2+Ag]^+$ and $[Sq-S_3+Ag]^+$ respectively. These signals have also appeared in the tetrasulfidic cross-linked squalene (Figure 5), but the relation between their intensities is different. This gradation of squalenes bonded with a different sulfur chain bridges are fragmentation products of the molecular ion and this profile is repeated in the m/z zone between 700 and 800 with the signals corresponding to the ions $[Sq-S_x+Ag+AgNO_3]^+$.

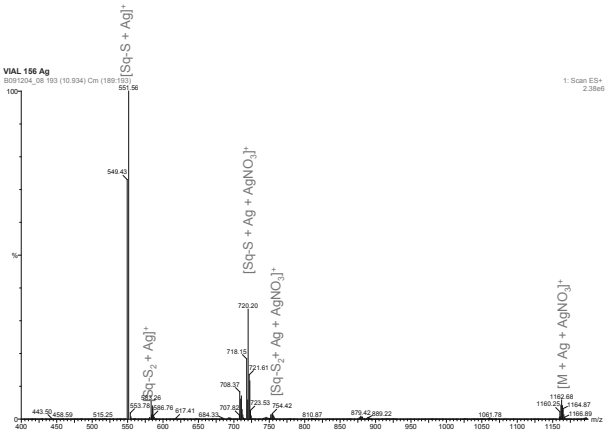


Figure 7. Mass spectrum of **fraction c** *between m/z=400-1200. It corresponds to two squalene molecules bonded with a disulfidic bond (Sq-S_2-Sq).*

In figure 7 the mass spectra of the **fraction c** is seen and it has been identified as disulfidic cross-linked squalene (Sq-S_2-Sq). As it can be stated before in our previous work [9] this three chromatographic peaks had been interpreted as trisulfidic, disulfidic and monosulfidic cross-linked squalenes respectively, but this RP-HPLC-ESI-MS method with silver nitrate post-column derivatization has allowed its correct identification. These three peaks have the same mass spectrum, since they are different isomers according to the position of the sulfur bridge that bind the two squalene chains. The molecular ion corresponding to the silver adduct $[M+Ag]^+$ is at m/z=991. This signal is not in the mass spectra, but we find a peak at m/z=1162, which is the ion $[M+Ag+AgNO_3]^+$. In this case signal m/z=517 corresponding to the silver adduct of squalene is not observed $[Sq+Ag]^+$, but a very intense signal is seen at m/z=551, which corresponds to $[Sq-S+Ag]^+$. There is also a small signal at m/z=583 corresponding to the ion

[Sq-S_2+Ag]$^+$. This can be explained due to the homolytic break of the disulfidic bridges (Sq-S_2-Sq), giving rise to a molecule of squalene connected to a sulfur (Sq-S), whose adduct with silver is m/z=551. At m/z=720 the peak of [Sq-S+Ag+AgNO^3]$^+$ can be seen.

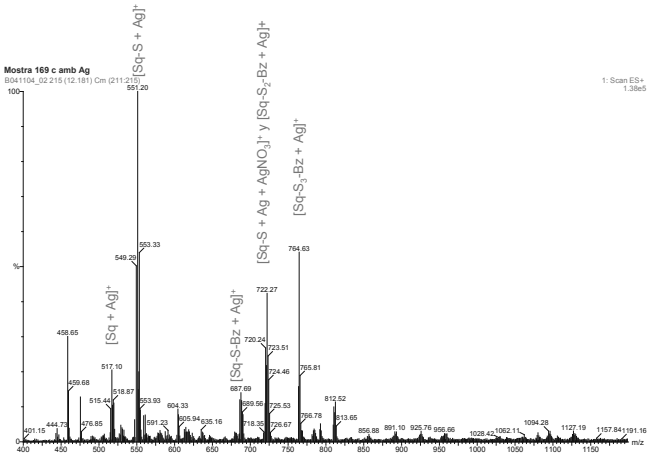


Figure 8. Mass spectrum of **fraction d** *between m/z=400-1200. It corresponds to a squalene molecule bonded with a pendant group.*

In figure 8 the mass spectrum of **fraction d** is presented and it is very similar to the disulfidic cross-linked squalene (Sq-S_2-Sq) already discussed (figure 7). This peak is detected by ionization in the mass spectrometer, but does not absorb at 230 nm, since it does not appear in the DAD signal. The signals are the same than in the Sq-S_2-Sq (figure 7), but the peaks of m/z=688, 720 and 750 appear with more intensity. This can be explained because they could correspond to the squalenes with pendant group: [Sq-S-Bz+Ag]$^+$, [Sq-S_2-Bz+Ag]$^+$ and [Sq-S_3-Bz+Ag]$^+$ respectively.

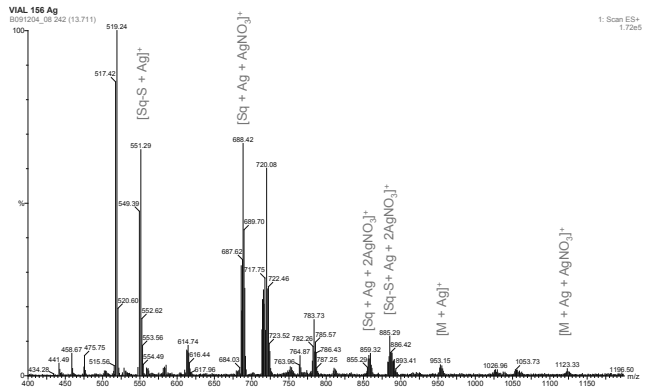


Figure 9. Mass spectrum of **fraction e** *between m/z=400-1200. It corresponds to two squalene molecules bonded with a monosulfidic bond (Sq-S-Sq).*

Figure 9 shows the mass spectrum of **fraction e** and that corresponds to the monosulfidic cross-linked squalene (Sq-S-Sq). In the electronic impact method [9] this chromatographic peak had been interpreted as squalene with pendant group, but this method of HPLC-ESI-MS with silver nitrate post-column derivatization has allowed a new identification. The molecular ion corresponding to the silver adduct [M+Ag]$^+$ is at m/z=953 and the salt adduct of the molecular ion [M+Ag+AgNO$_3$]$^+$ is at m/z=1123. In this case the signal m/z=517 corresponding to the silver adduct of squalene [Sq+Ag]$^+$ and the signal at m/z=551 corresponding to [Sq-S+Ag]$^+$ are observed. Both ions are fragmentation products of the breakage of the monosulfidic cross-linked squalene. At m/z=688 and 720 we find the adduct ions [Sq+Ag+AgNO$_3$]$^+$ and [Sq-S+Ag+AgNO$_3$]$^+$ respectively; and at m/z=859 and 885 the adduct ions [Sq+Ag+2AgNO$_3$]$^+$ and [Sq-S+Ag+2AgNO$_3$]$^+$ respectively.

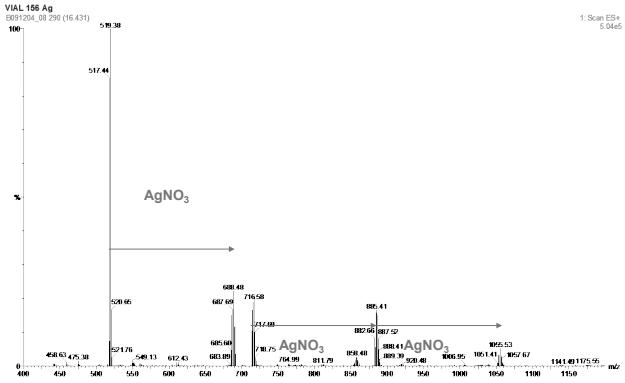


Figure 10. Mass spectrum of **fraction f** *between m/z=400-1200. It corresponds to a modified squalene.*

Finally **fraction f** (Figure 10) displays a mass spectrum very similar to the squalene mass spectrum (figure 3), but some intensities vary significantly, which could mean that this peak belongs to the modified squalene, with a similar structure to squalene.

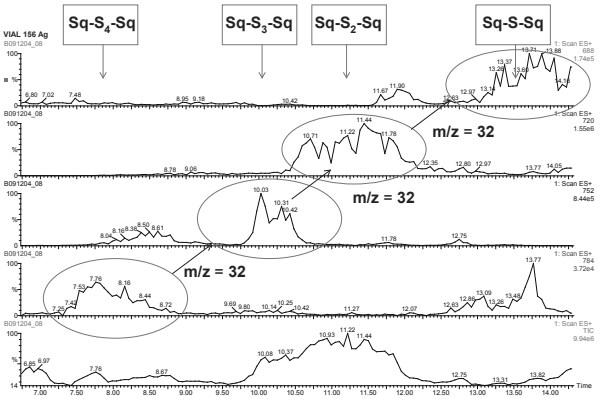


Figure 11. Mass spectrum between m/z=400-1200 of the chromatogram zone where the cross-linked squalenes are (7-14 minutes) (below). Extracts of m/z 688, 720, 752 y 784 (from above to below) where the zones of the chromatogram rich in these ions, and the gaps of m/z 32 corresponding to the sulfur lost, can be seen.

In order to finish determining the chromatographic peaks allocation, the m/z extraction of the most characteristic peaks of cross-linked squalenes have been performed. For that reason, in figure 11 the result of this peak extraction is shown. The figure shows the extraction of m/z=688, 720, 752 and 784 (from top to bottom) where the rich zones of the chromatogram in these ions can be observed. Furthermore, some constant gaps between them can be observed due to the sulfur lost (m/z 32). In the graphic it is possible to observe that the elution order is Sq-S_4-Sq, Sq-

S_3-Sq, Sq-S_2-Sq and Sq-S-Sq, from more polar to less polar, as it was said in literature [9]. In addition, it is observed that the peak extractions results in a wide zone, that does not correspond only with the chromatographic peak. This is due to the formation of diverse isomers of the different types of cross-linked squalenes and also a consequence of the resolution decrease caused by the eluent change. Furthermore, it can be observed that some of these isomers do not absorb at 230nm, which shows that it is not possible to quantify completely the degree and nature of the crosslink process with HPLC-UV.

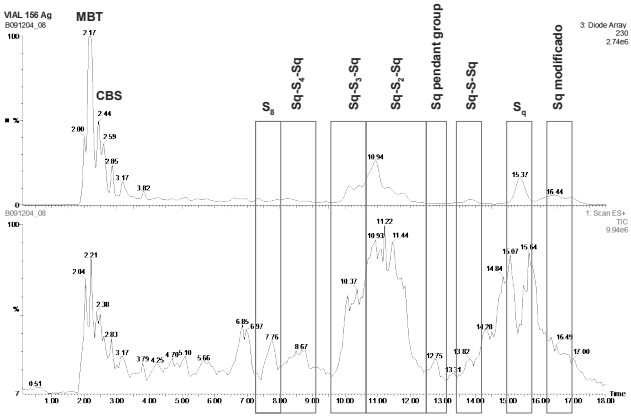


Figure 12. Peaks identification of a chromatogram at 230 nm (above) and mass spectrum (below) of a simple vulcanized for 60 minutes, where cross-linked and modified squalenes can be separated.

Finally, figure 12 summarizes the new identification obtained from the method developed in this paper, with all the vulcanization intermediates detected identified.

CONCLUSIONS

Suitable conditions have been found for the characterization of squalene and squalene by-products. For the first time, squalene, cross-linked squalenes and intermediates compounds in the vulcanization process of squalene have been identified by RP-HPLC-ESI-MS using silver nitrate as post-column derivatization.

ACKNOWLEDGEMENTS

The authors (Grup d'Enginyeria de Materials (GEMAT)) would like to thank the Generalitat de Catalunya (Research Consolidated Group grant: SGR-2009) for providing funds to support this research. Berta Vega would like to thank IQS for its economical support under a PhD fellowship.

REFERENCES

1. Agulló N, Borrós S (2000) Kautsch. Gummi Kunstst. 53(3): 131-136
2. Lautenschlaeger FK, Edwards K (1980) Rubber Chem. Technol. 53(1): 27-47
3. Van den Berg JHM, Beulen JW, Hacking JMH, Duynstee EFJ (1984) Rubber Chem. Technol. 57(4): 725-734
4. Versloot P, Haasnoot JG, Nieuwenhuizen PJ, Reedijk J, van Duin M, Put J (1997) Rubber Chem. Technol. 70(1): 106-119
5. Rodríguez S, Masalles C, Agullo N, Borros S, Comellas L, Broto F (1999) Kautsch. Gummi Kunstst. 52(6): 438-445
6. Vidal E, Borros S, Agullo N, Van Ooij WJ (2001) Gummi, Fasern, Kunstst. 54(5): 330-333
7. Borros S, Vidal E, Agullo N, Van Ooij WJ (2000) Kautsch. Gummi Kunstst. 53(12): 711-715
8. Agulló N, Study of sulfenamide and sulfenamide-thiuram accelerating systems for natural rubber vulcanization using the modeling approach, PhD (1999)
9. Vidal-Escales E, Borros S (2004) Talanta 62(3): 539-547
10. Gregg EC, Lattimer RP (1984) Rubber Chem. Technol. 57(5): 1056-1097
11. Pokorny S, Janca J, Mrkvickova L, Tureckova O, Trekoval J (1981) J. Liq. Chrom. 4(1): 1-12
12. Roussis SG, Proulx R (2002) Anal. Chem. 74(6): 1408-1414
13. Takino M, Daishima S, Yamaguchi K, Nakahara T (2001) J. Chrom. A 928(1): 53-61
14. Rentel C, Strohschein S, Albert K, Bayer E (1998) Anal. Chem. 70(20): 4394-4400
15. Cunneen JI, Russell RM (1969) J. Rubber Res. Inst. Malaya 22(3): 300-30
16. Vega B, Agulló N, Borrós S (2007) Rubber Chem. Technol. 80(5): 739-750
17. Bhalla R, Dyson PJ, Lauterwasser F, McIndoe JS (2000) Gold Bulletin (London) 33(2): 56-59
18. Guan F, Uboh C, Soma L, Hess A, Luo Y, Tsang DS (2003) J. Mass Spec. 38(8): 823-838
19. Ells B, Barnett DA, Froese K, Purves RW, Hrudey S, Guevremont R (1999) Anal.Chem. 71(20): 4747-4752