

Study on Fluorimetric Properties of 1,2-Dihydro-2,2,4-trimethylquinoline

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The title compound, 1,2-dihydro-2,2,4-trimethylquinoline (TMQ), is a quinoline family member and is among others used to prevent the oxidative degradation of polymers. TMQ has been characterized by its fluorescence emission. In this work the effects of solvent, acidity, continuous irradiation and the influence of some interferents upon the fluorescence intensity of TMQ were investigated. Under the selected conditions (in propan-1-ol solution at $\lambda_{\text{excitation}}/\lambda_{\text{emission}} = 334 \text{ nm}/412 \text{ nm}$), the fluorescence intensity is proportional to the concentration of TMQ over the range $0.05\text{-}5 \mu\text{g ml}^{-1}$ with a correlation coefficient of 0.9963. The proposed method was applied to the determination of TMQ in synthetic mixtures of polymer additives and specific rubber samples.

Keywords: 1,2-Dihydro-2,2,4-trimethylquinoline, Antioxidant, Fluorimetry, Rubber mixture

INTRODUCTION

Numerous papers have described the estimation of various types of antioxidants used in polymer manufacturing [1-11]. The need for their analysis is very important, particularly in connection with medical plastics and food packaging where the levels of such potentially toxic substances must be accurately known.

Many sophisticated techniques have been proposed for the analysis of these antioxidants, including laser desorption/Fourier transform ion cyclotron resonance mass spectrometry [1], supercritical fluid extraction combined with GC-MS [2], pyrolysis-gas chromatography [3-4] and HPLC [5]. However, these techniques still do not satisfy all

requirements for routine analysis, mainly because of their complicated process design, time consumption or the need for expensive instruments.

While many analytical laboratories do not have such expensive instruments, almost all of them have at least a spectrometer. Thus, an alternative for the quality control of a kind of rubber mixture (having a known composition) could be represented by a spectrometric or fluorimetric method. It should be mentioned that one of the parameters verified in the quality control of rubber mixtures, with certified formula, is the content of the antioxidant (taking into account its toxicity). This is the reason for which, in the past years, we have proposed a series of spectrometric methods for the determination of some rubber additives (especially antioxidants) in specific real samples [6-9]. Because of the low

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level of the antioxidants normally used in polymeric materials, they cannot be analyzed directly by common spectrometric methods. These methods are also not specific enough [10,11].

For this reason, in our previous studies [6-9] we have proposed the derivative UV spectrometric methods for the determination of some antioxidants, in the presence of other polymer additives (accelerators, plasticizers) commonly used in polymeric mixtures. One of these antioxidants studied was 1,2-dihydro-2,2,4-trimethylquinoline (TMQ). As we have reported, it can be determined in the presence of other polymer additives by the first derivative UV spectrometry [9].

As it is known, quinoline derivatives are among the few classes of compounds in which a large proportion of the members exhibit sensible fluorescence. Apart from this specificity, fluorescence techniques are far more sensitive than absorption spectroscopic techniques and it is, therefore, surprising that more use of fluorimetric methods has not been made in the analysis of this class of antioxidants.

In the present paper, we propose a fluorimetric method for the determination of TMQ in the presence of other polymer additives such as diphenylguanidine (D) and hexamethylenetetraamine (H), as vulcanization agents, and stearic acid (SA), as plasticizer, without the separation of the additives from each other after extraction from the polymeric mixture.

EXPERIMENTAL

Reagents

All chemicals used were of analytical reagent-grade (Merck, Darmstadt, Germany). One type of elastomer was used, namely nitrilic rubber (NR). The specimen of elastomer was manufactured specifically for this study. The rubber extraction was made in propan-1-ol. Hexamethylenetetraamine (H) and diphenylguanidine (D) were selected as vulcanization accelerators. The antioxidant used was 1,2-dihydro-2,2,4-trimethylquinoline (TMQ). Stearic acid (SA) was used as plasticizer. The solvents used, chloroform, methanol, ethanol, propan-1-ol and amilic alcohol were all of spectroscopic-grade purity (Merck).

Stock solutions containing $100 \mu\text{g ml}^{-1}$ polymer additives were prepared in propan-1-ol. Working solutions were

obtained by appropriate dilutions of the stock solutions with propan-1-ol. Stock standard solutions containing $100 \mu\text{g ml}^{-1}$ TMQ dissolved in one of the mentioned solvents were freshly prepared and used to obtain secondary standard solutions. Propan-1-ol solutions of sulfuric acid having 0.01, 0.05, 0.1, and 0.5 N concentrations were prepared.

Apparatus

The fluorescence excitation and emission measurements were carried out at 25°C on a Perkin Elmer Model LS45 Luminescence spectrometer equipped with a water thermostated cell holder using a 1-cm path length quartz cuvette.

Procedures

Spectral measurements. The emission spectrum of each polymer additive was obtained by subtraction of the corresponding emission spectrum of pure solvent (background intensity).

Preparation of calibration curves. A known volume of TMQ standard solution was added to each of a series of 10 ml calibrated flasks, diluted to the mark with propan-1-ol and mixed. The fluorescence intensity of these solutions was recorded at $\lambda_{\text{ex}}/\lambda_{\text{em}} = 334/412 \text{ nm}$. Calibration graph was obtained by plotting the appropriate fluorescence value of each solution of TMQ versus its concentration.

Preparation of propan-1-ol solutions of TMQ in sulfuric acid. Five stock standard solutions of TMQ ($100 \mu\text{g ml}^{-1}$) were prepared by dissolving the same calculated amount of TMQ in propan-1-ol solutions with final sulfuric acid concentrations of 0.0, 0.01, 0.05, 0.1, and 0.5 N, respectively. Working standard solutions ($5 \mu\text{g ml}^{-1}$) were obtained by appropriate dilution of each stock standard solution with the proper propan-1-ol solution. The fluorescence intensity of the working standard solutions was measured at $\lambda_{\text{ex}}/\lambda_{\text{em}} = 334/412 \text{ nm}$.

Preparation of TMQ solutions in different solvents. Stock standard solutions of TMQ ($100 \mu\text{g ml}^{-1}$) were prepared by dissolving the same calculated amount of TMQ in one of the following solvents: methanol, ethanol, propan-1-ol, amilic alcohol and chloroform. Working standard solutions ($5 \mu\text{g ml}^{-1}$) were obtained by appropriate dilution of each stock standard solution with the proper solvent. The fluorescence

intensity of the working standard solutions was measured at $\lambda_{\text{ex}}/\lambda_{\text{em}} = 334/412$ nm.

Preparation of rubber mixtures. The rubber mixtures were manufactured with the additives in a certified formula. Thus, the ingredients were added in the following proportions: elastomer (nitrilic rubber), 100; zinc oxide, 5; silicon oxide (Zeosil 45), 40; calcium carbonate, 20; sulphur, 1.2; diphenylguanidine (D), 0.5; hexamethylenetetra-amine (H), 1; stearic acid (SA), 4; 1,2-dihydro-2,2,4 trimethyl-quinoline (TMQ), between 1 and 2. Preparation of the mixtures were done at a temperature of approximately 60 °C and the vulcanization process itself at around 160 °C, under pressure.

Extraction. Extraction was performed on weighed amounts of the rubber specimens (2.5 g, cut into small pieces and containing different amounts of TMQ, as it is indicated in the previous section), in a Soxhlet extractor for 24 h, with a defined volume of propan-1-ol, at reflux. To minimize the decomposition and loss of the antioxidant, the extraction was carried out under nitrogen. The obtained extract was quantitatively transferred with propan-1-ol in a 100-ml calibrated flask. Then, 0.5 ml of this solution was diluted to 50 ml with propan-1-ol and the concentration of TMQ was determined by the fluorimetric method.

RESULTS AND DISCUSSION

Fluorescence Spectra

In order to develop the method based on fluorescence measurements, excitation and emission spectra of TMQ in different media were recorded. Figure 1 shows the excitation and the emission spectra of TMQ in propan-1-ol solution, corrected for the blank signal. As is shown in Fig. 1, there are two excitation bands; maximum excitation was observed at 334 nm. While, there is only one emission band in the fluorescence spectrum of TMQ with a maximum located at 412 nm.

The Effect of Chemical Variables

The effect of chemical variables on the fluorescence signal of TMQ was examined at a concentration of 5 $\mu\text{g ml}^{-1}$. The influence of the nature of solvent was investigated and it was found that the fluorescence intensity is maximum when the

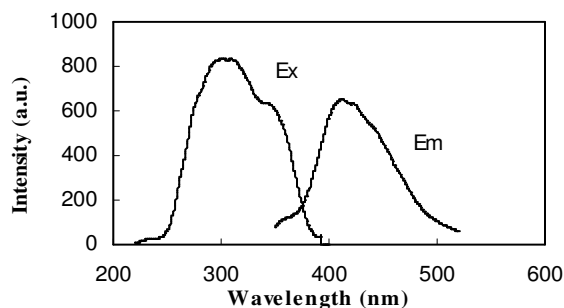


Fig. 1. Molecular spectra of 5 $\mu\text{g ml}^{-1}$ of TMQ in propan-1-ol solution. (Ex) Excitation spectrum ($\lambda_{\text{ex}} = 334$ nm), (Em) Emission spectrum ($\lambda_{\text{em}} = 412$ nm).

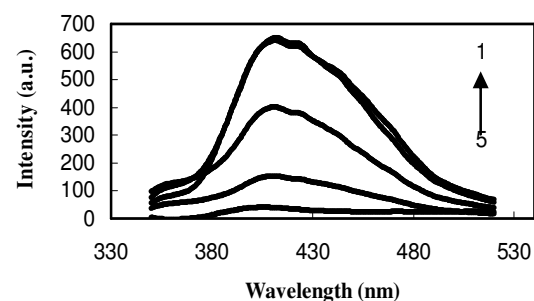


Fig. 2. Influence of the nature of solvent on the fluorescence intensity of 5 $\mu\text{g ml}^{-1}$ solution of TMQ in different solvents: (1) chloroform, (2) methanol, (3) ethanol, (4) propan-1-ol, (5) amilic alcohol.

TMQ solution was prepared in amilic alcohol. As is shown in Fig. 2, the fluorescence of TMQ in propan-1-ol is slightly lower than that in amilic alcohol. However, further experiments were made using propan-1-ol as solvent, owing to its lower toxicity compared with that of amilic alcohol.

The influence of the acidity was also tested. It was observed that the optimum value of the fluorescence intensity was reached for the TMQ sample prepared in propan-1-ol solution, without adding H_2SO_4 . The increase of the acidity leads to a meaningful decrease of the fluorescence intensity (see Fig. 3).

This behavior was predictable and should be explained as follows. Quinolines are weak bases, so that the lone pair of their nitrogen is no longer fully available to combine with protons. The lone pair on the nitrogen interacts with the

delocalized ring electrons of aromatic radicals. As it is known, delocalization contributes to fluorimetric properties of aromatic molecules. Thus, without adding H_2SO_4 , TMQ has the greatest fluorescence signal. The acidity leads to a decrease of fluorescence. This is more evident with the increase of the acidity. The reason for this behavior is the fact that, even though TMQ is only a very weak base, it reacts with a strong acid like H_2SO_4 . In these conditions, the lone pair of nitrogen is used to bind to a hydrogen ion supplied by sulfuric acid and it is no longer available to contribute to the delocalization. Therefore, the disrupting the delocalization with increasing solution acidity will decrease the fluorescence of TMQ.

Stability of the Fluorescence with Time

The influence of continuous irradiation (for 1 h) of TMQ solution on the fluorescence intensity was also investigated. The fluorescence intensity was found to be stable for 40 min, then a slight decrease was observed ($\sim 0.25\%$).

Calibration

Under the selected conditions described above, a linear relation was obtained between fluorescence intensity and concentration of TMQ in the range $0.05\text{--}5\ \mu\text{g ml}^{-1}$ (Fig. 4). The limit of quantification (LOQ, signal to noise ratio = 10:1) has been found to be $0.05\ \mu\text{g ml}^{-1}$ TMQ.

Precision

The precision of the determination was calculated by ten replicate measurements of the same sample. The coefficient of variation of these measurements was 1% for $5\ \mu\text{g ml}^{-1}$ TMQ of propan-1-ol solution.

Interferences

Although literature data indicate that only the quinoline derivatives show significant fluorescence, the possible interferences from the other polymer additives were investigated. Thus, propan-1-ol solutions of potentially interfering additives were tested first. As is shown in Fig. 5, at a level of $5\ \mu\text{g ml}^{-1}$, only TMQ is fluorescent while hexamethylenetetraamine (H), diphenylguanidine (D) and stearic acid (SA) give insignificant signals.

It is known that the determination of the antioxidant is

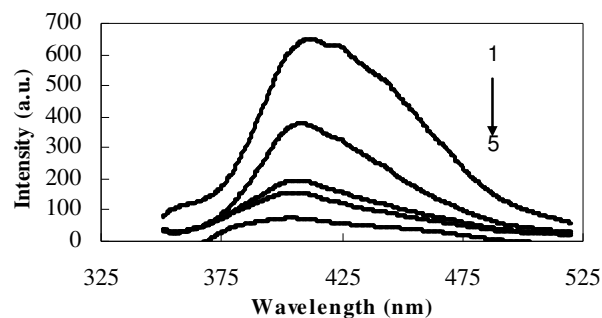


Fig. 3. Variation of the fluorescence intensity with sulfuric acid concentration of a $5\ \mu\text{g ml}^{-1}$ propan-1-ol solution of TMQ. Concentration of H_2SO_4 : (1) 0.0 N, (2) 0.01 N, (3) 0.05 N, (4) 0.1 N (5) 0.5 N.

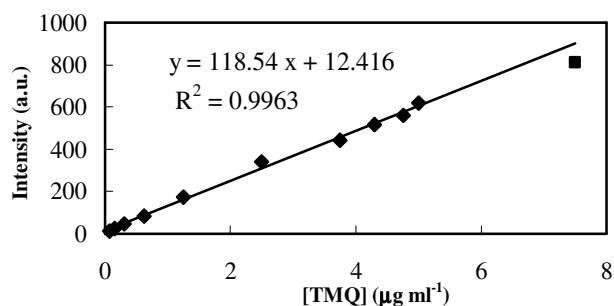


Fig. 4. Fluorescence intensity as a function of the concentration of TMQ.

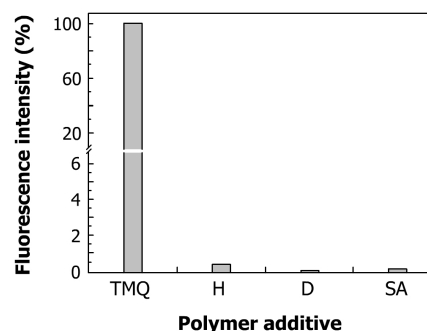


Fig. 5. Fluorescence intensity of some polymer additives; $c_{\text{additive}} = 5\ \mu\text{g ml}^{-1}$.

possible after its extraction from the rubber mixture, as described in the general procedure. Besides the antioxidant, the extract also contains other polymer additives. For this

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Table 1. Results of TMQ Determination in Binary Mixtures by the Fluorimetric Method ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 334 \text{ nm}/412 \text{ nm}$)^a

TMQ ($\mu\text{g ml}^{-1}$)		Relative error		
In sample	Found	(%)	Interferent	($\mu\text{g ml}^{-1}$)
5.0	5.02	+0.4	H	5
5.0	5.05	+1.0		10
5.0	5.07	+1.4		15
5.0	5.00	-	D	5
5.0	5.00	-		10
5.0	4.98	-0.4		15
5.0	5.00	-	SA	5
5.0	4.99	-0.2		10
5.0	4.98	-0.4		15

^aMean values for three independent measurements.

Table 2. Results of TMQ Determination in Multicomponent Mixtures by the Proposed Fluorimetric Method ($\lambda_{\text{ex}}/\lambda_{\text{em}} = 334 \text{ nm}/412 \text{ nm}$)^a

Sample No.	TMQ ($\mu\text{g ml}^{-1}$)		Relative error (%)	Interferents ($\mu\text{g ml}^{-1}$)		
	In sample	Found		H	D	SA
1	0.05	0.048	-4.1	0.10	0.10	0.15
2	0.10	0.10	-	0.50	0.50	0.75
3	0.50	0.50	-	1.00	1.00	1.50
4	1.00	1.00	-	2.00	2.00	3.00
5	2.50	2.53	+1.2	5.00	5.00	7.50
6	5.00	5.04	+1.6	10.00	10.00	15.00
7	7.00	6.12	-12.5	14.00	14.00	21.00

^aThe values are mean of three independent measurements.

reason, binary and ternary mixtures containing the antioxidant and the other polymer additives were prepared and the proposed fluorimetric method applied to the determination of TMQ. In the case of binary mixtures, the results obtained for three different interferent:TMQ ratios (from 1:1 to 3:1) are shown in Table 1. As it was expected, the vulcanization agents and the plasticizer have only a slight effect on the determination of TMQ even up to a 3 fold excess with respect to TMQ.

Behavior similar to that previously described is also observed in the case of ternary mixtures (see Table 2). In order

to test the proposed method for TMQ determination in the presence of large amounts of the other polymer additives, a ratio of TMQ-vulcanization accelerators (H+D) = 1:4 and a ratio of TMQ-plasticizer = 1:3 were chosen. As it can be seen, the proposed fluorimetric method, applied to complex mixtures of rubber additives, allows the determination of TMQ in presence of three other constituents, in the range 0.05-5 $\mu\text{g ml}^{-1}$. Moreover, the fluorimetric method has the advantage of a better limit of quantification (LOQ) of 0.05 $\mu\text{g ml}^{-1}$ in comparison with that obtained by the derivative spectrometric methods (0.25 $\mu\text{g ml}^{-1}$), as we have reported in a

Table 3. Results of TMQ Determination in real Samples^a

Sample	TMQ content (g/100 g)		
	In sample	Fluorimetry ($\lambda_{ex}/\lambda_{em} = 334 \text{ nm}/412 \text{ nm}$) ^b	First derivative spectrometry (254 nm) ^b
Vulcanized rubber	1.15	0.96 ± 0.02	0.94 ± 0.01
	0.87	0.73 ± 0.03	0.71 ± 0.02
	0.58	0.49 ± 0.03	0.50 ± 0.02

^aThe samples were manufactured according to the general procedure. ^bThe values are mean of three independent measurements.

recent paper [9].

Real samples

Once developed and in order to verify its precision, a new method must be compared with another one. We have chosen to compare the proposed fluorimetric method with the UV derivative molecular absorption spectrometric method, previously developed by us [9]. The analytical potential of the proposed method was tested by applying it to the determination of TMQ in rubber samples, having different contents of TMQ and manufactured as reported in the general procedure. The results are summarized in Table 3.

As can be deduced from Table 3, there is a good agreement between the results obtained by both methods. The expected difference between the amount of TMQ before and after extraction could be explained by possible partial decomposition and loss of sample during the extraction. This is because the antioxidants have high reactivity and low stability, as reported in a systematic study on this kind of compounds introduced in polymeric materials [11]. Taking into account the complex matrix of rubber mixtures and the low stability of the antioxidants, a recovery of about 84-85% of TMQ (by applying the proposed method) is acceptable.

Thus, for quantitative estimation of TMQ in unknown samples (containing the mentioned polymer additives), a correction factor can be applied on the results obtained by the proposed fluorimetric method. On the basis of the results listed in Table 3, this correction factor was found to be 1.20.

CONCLUSIONS

The present study was focused on the development of a

specific method for determination of the antioxidant in rubber mixtures, having known formula. The rubber antioxidant 1,2-dihydro-2,2,4-trimethylquinoline (TMQ) was found to have native fluorescence. The proposed fluorimetric method permits the estimation of the antioxidant TMQ in ternary mixtures without separation from the other polymer additives. Also, TMQ could be determined in real rubber samples after its extraction, at reflux, in propan-1-ol solution. Compared with the derivative UV spectrometric method, the proposed technique has the advantages of good selectivity and LOQ.

The procedure is simple and the results of TMQ determination are reliable, with good precision (~ 1%) and LOQ (0.05 $\mu\text{g ml}^{-1}$). On the basis of the obtained results, we can also conclude that the rubber mixtures entail careful selection of the analytical method for determination of the antioxidant in presence of other specific polymer additives. The efficiency of the fluorimetric method reveals its potential application to the determination of TMQ in complex mixtures of known polymer additives. This assumption is based on the specificity of fluorimetric methods in comparison with the absorption molecular spectrometric methods. In this respect, the approach explored in our paper may be useful for further applications.

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