Extraction of Cochlear Non-linearities with the Bispectral Analysis

An Application to TEOAEs in Styrene-exposed Workers

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Summary
Objectives: Transient Otoacoustic Emission (TEOAEs) are low intensity sounds generated by active mechanisms in the cochlea and elicited by broadband and short acoustic stimuli. TEOAEs are a quick, non-invasive, and very reliable measure to objectively assess the peripheral auditory system. In the current study, we present a recent technique to extract and evaluate non-linearities in TEOAEs, and apply it for the preliminary analysis of TEOAE recordings of a group of worker of a fiberglass manufacturing facility exposed to styrene solvent.

Methods: Bispectral analysis was applied to TEOAEs to extract the quadratic frequency couplings (QFCs) in TEOAEs. Amplitude of QFCs was calculated in a group of 7 styrene-exposed workers (14 ears) and compared with normative results obtained on normal hearing young adults.

Results: Difference in amplitude of QFCs were found between workers and controls. In workers, TEOAE non-linear components were found in the 1.5 – 4 kHz frequency range, whereas in control subjects they were found in a lower frequency region, ranging from 1 to 3 kHz.

Conclusion: The amplitude of QFCs highlighted differences in TEOAEs recorded among workers and controls.

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1. Introduction

In the last few years, the need of objective evaluation of hearing functionality encouraged the development of fast and reliable techniques, usually based on otoacoustic emissions (OAEs) evaluation. OAEs are low intensity sounds generated by active mechanisms in the cochlea and arise in the ear canal when the eardrum receives vibrations transmitted backwards from the cochlea through the middle ear [1]. OAEs can be measured in the external auditory canal by a probe containing a miniature microphone.

Among the different types of OAEs, Transient Evoked Otoacoustic emissions (TEOAEs) are widely used in clinical practice as they can be measured in the great majority (about 98%) of normal-hearing subjects [2] and they are quick, non-invasive, and very stable measures to objectively assess peripheral auditory system (i.e., the cochlea) functionality. TEOAEs are elicited by broadband and short acoustic transient stimuli, such as clicks. In clinical practice, short broadband stimuli are preferred to investigate cochlear functionality, because these stimuli can elicit, in a single OAE recording, responses from several sources along the cochlea, tuned to different characteristic frequencies [3]. TEOAEs are extensively used in neonatal hearing screening [4] and in adults for both clinical and research purposes [5 – 9].

For screening and basic hearing assessment, simple parameters, such as the amplitude of the emission, the signal to noise ratio, and the reproducibility in the whole signal or in specific frequency bands, are typically extracted from TEOAEs [3]. These parameters proved to be very useful in clinical practice but may not be sensitive to detect possible subtle dysfunctions or sub-clinical injuries.

In this study, we analyze TEOAE recordings with a higher order spectral analysis technique – the bispectrum. The bispectrum itself has been widely used for the analysis of several biomedical signals [10], such as EEG [11 – 12] and ECG [13]. We use bispectral analysis to extract non-linear characteristics from TEOAEs. Non-linearity is a very important and peculiar feature of TEOAEs, as the healthy cochlea is known to have highly non-linear behavior. The first sign of cochlear damage is just that the system becomes more linear because of progressive loss of compression in the cochlear amplifiers. As TEOAEs are the by-products of active and non-linear cochlear amplification mechanisms [14], they can be effectively used to estimate the degree of non-linearity of the active mechanisms in the cochlea.

The approach we propose is based on calculating the amplitude of quadratic frequency couplings (QFCs) in TEOAEs by means of the bispectral analysis. QFCs can
be viewed as direct consequences of cochlear non-linearities; they are generated when a TEOAE component at a generic frequency $f_i$ is the sum of two independent components at frequencies $f_1$ and $f_2$. In our recent works [15–16], we developed a method based on bispectral analysis to extract QFCs from TEOAEs, characterizing and validating it on synthetized and real TEOAEs. In [16] we further proceed into a deeper characterization of our proposed approach for the analysis of TEOAEs by providing normative data and assessing the test-retest repeatability of non-linear components in normal hearing young adults at different intensities of the eliciting stimulus.

Finally, in the present study, we aimed to preliminary assess the feasibility and the performance of the proposed approach in the evaluation of cochlear functionality when exposed to ototoxic agents (such as, styrene). As a consequence, it is beyond the scope of this work to perform an epidemiological investigation of the effects of styrene on non-linearities in TEOAEs.

In the current study, we use the amplitude of QFCs as a quantitative parameter to evaluate non-linearities in TEOAEs, and apply it to preliminary analyze possible modifications of non-linear components of TEOAEs recorded in a group of workers of a fiberglass manufacturing facility exposed to styrene solvent.

In literature, evidences that exposure to organic solvents (e.g., styrene) either alone or in combination with noise has ototoxic effects, have been largely described [7, 17–19]. In these last studies, the effect of styrene exposure was investigated with a complete set of auditory tests, including pure tone audiometry [17, 19], speech recognition tests [18], and OAE-based tests [7], to estimate the damage at the level of the cochlear outer hair cells (OHCs).

In this study, TEOAEs recorded from a group of styrene-exposed workers are analyzed in terms of amplitude of QFCs, as a preliminary evaluation of styrene-exposure effects on cochlear non-linearities. Results are compared to normative data, as described in [16].

2. Materials and Methods

A schematic flowchart of the TEOAE analysis procedures used in this study is shown in Figure 1. TEOAE recordings were analyzed with two methods: the scaled periodogram (upper branch of the flowchart) and the bispectrum (lower branch of the flowchart). From the scaled periodogram, we calculated the energy in ten 0.5-kHz-wide frequency bands from 0 to 5 kHz; from the bispectrum we calculated and integrated the amplitude of QFCs in the same frequency bands. The following Sections provide details on the tested subjects and the recording and analysis procedures implemented in our study.

2.1 Subjects

TEOAEs were recorded for both ears in a group of seven workers (33–48 years-old) employed in a fiberglass product manufacturing facility in central Italy. These data were collected in a multicenter project of the Italian Ministry of Health aimed to objectively analyze hearing functionality and hearing loss susceptibility in workers exposed to noise and/or ototoxic agents (project n. RF-2009–1470310).

The present study deals with only one of the two cohorts of workers involved in the above mentioned project, namely that one consisting of subjects exposed to styrene only. These workers have been monitored during the working shift both for noise, with personal sound level meters (Quest DLX-1, Quest Technologies, Wisconsin, USA) and for styrene exposure, with personal diffusive air samplers (Radiello, Sigma-Aldrich, Missouri, USA). All the workers considered in the current study are assigned to artifact molding task; measurements of noise exposure during the working shift revealed that they were exposed to mild level of noise, below the action level of 85 dBA set by the Occupational Safety & Health Administration (the measured noise levels were: $L_{ex,8h,mean} = 83$ dBA, $L_{ex,8h,max} = 84.2$ dBA, and $L_{ex,8h,min} = 80.7$ dBA). During working shift all workers were exposed to high levels of styrene, approximately on the order of the Threshold Limit Value (TLV) set by the
American Conference of Governmental Industrial Hygienists (ACGIH) (equal to 20 ppm). The working shift is organized on 8 h for all the subjects but one, who is a part-time worker.

Audiometric thresholds (PTA) were evaluated at 0.5, 1, 2, 3, 4, 6, and 8 kHz, starting from the level of 20 dB HL, which corresponds to the standard definition of normal hearing. At 1, 2, 3, and 8 kHz all the 14 ears showed hearing thresholds lower than 20 dB HL; at 4 and 6 kHz 9 out of 14 ears showed hearing thresholds between 20 and 25 dB HL, whereas the remaining ears had thresholds lower than 20 dB HL; at 500 Hz, 6 ears had thresholds lower than 20 dB HL, two ears had threshold between 20 and 25 dB HL, and 6 ears showed thresholds between 25 and 30 dB HL. Therefore, no audiometric thresholds exceeded 30 dB HL at any frequency and all examined ears could be classified as normal or affected by very mild hearing loss only at specific audiometric frequencies.

A population of 27 young adults (23–30 years old), which were not exposed to noise and styrene, was considered as the control group. Inclusion criteria were based on otoscopy, audiometry by air and bone conduction, tympanometry, acoustic reflex testing, and on a screening questionnaire concerning medical and otological history. Audiometric thresholds of 20 dB HL or lower at each of the tested audiometric frequencies were required. The ear which performed best in the audiometric and audiological tests listed above was selected, for each participant, and was considered for further analysis.

### 2.2 TEOAE Recordings

For both groups, TEOAEs were recorded by means of a standard adult probe using an Otodynamics ILO88 system. TEOAEs were elicited by click stimuli of 80 dB SPL using a 20.4 ms acquisition window. Responses were digitally sampled at \( f_s = 25 \) kHz. Tests were carried out in a silent room for styrene-exposed workers and in a sound-treated room satisfying ISO 8253-1 criteria for air conduction audiometry for controls. For the styrene-exposed workers, the TEOAEs of both ears were recorded at the end of the working shift.

#### 2.3 Scaled Periodogram

For a real, stationary, ergodic and discrete-time signal \( x(k) \), \( k = 1, 2, \ldots, N \), the power spectrum \( S(f) \) is defined as:

\[
S(f) = E[X(f)X^*(f)],
\]

where * indicates the complex conjugate, \( E[\cdot] \) denotes the expected value, and \( X(f) \) is the Fourier transform of \( x(k) \).

From a theoretical point of view, the computation of (1) involves the calculation of the expected value; therefore, multiple independent records or a data record of infinite length would be required [20]. As TEOAEs are records of finiteness, the power spectrum cannot be directly estimated using (1). Therefore, we computed the scaled periodogram, which proved to be an unbiased and consistent estimator of the power spectrum of the signal:

\[
\hat{S}_s(f) = \frac{1}{N^2} X(f)X^*(f),
\]

where \( N \) is the number of samples of \( x(k) \). \( X(f) \) is computed using a finite-length rectangular analysis window of \( N = 512 \) samples, thus obtaining a frequency resolution equal to \( f_s/N = 50 \) Hz, where \( f_s = 25 \) kHz.

For each TEOAE, the integrated value of \( \hat{S}_s(f) \) was computed in ten 0.5-kHz-wide frequency bands from 0 to 5 kHz. Finally, these integrated values were normalized on the total energy of the signal, obtaining the energy of the scaled periodogram in frequency bands \( (E_{n_1})_{f_{band}} \).

It is to note that the use of a finite length analysis window (which introduces limited frequency resolution) and of a rectangular window (which might introduce spectral leakages) should not affect to a great extent the estimation of the energy of the scaled periodogram \( (E_{n_1})_{f_{band}} \) as it was calculated by integrating the scaled periodogram in broad frequency bands of 0.5 kHz width.

#### 2.4 Bispectrum

Higher-order spectra are methods able to identify non-linear contributions in a signal [21]. If \( x(k), k = 0, 1, 2, \ldots, N \), is a real, stationary, ergodic and discrete-time signal, its \( n \)th-order polyspectrum is defined as the multidimensional Fourier Transform of the \( n \)th-order statistic. The 3rd-order polyspectrum, called bispectrum, is defined as [20]

\[
B^3(f_1,f_2) = E[X(f_1)X(f_2)X^*(f_1 + f_2)],
\]

where * indicates the complex conjugate, \( E[\cdot] \) denotes the expected value, and \( X(f) \) is the Fourier transform of \( x(k) \).

As previously pointed out for the power spectrum, the computation of the expected value in (3) requires multiple independent records or a data record of infinite length. Conversely, TEOAEs are finite data records, therefore the bispectrum cannot be directly estimated using (3). To this purpose, we used the third-order scaled periodogram \( \hat{M}_s(f_1,f_2) \) as proposed by Zhou and Giannakis [20]:

\[
\hat{M}_s(f_1,f_2) = \frac{1}{N^3} X(f_1)X(f_2)X^*(f_1 + f_2),
\]

which proved to be an unbiased and consistent estimator of (3).

The parameter \( A_{QFC}(f_3) \), defined here as the amplitude of the QFC component at a generic frequency \( f_3 \), was computed as the sum of \( \hat{M}_s(f_1,f_2) \) for all the pairs \( (f_1,f_2) \), where \( f_1 + f_2 = f_3 \). Values of \( A_{QFC}(f_3) \) were integrated in ten 0.5-kHz-wide frequency bands from 0 to 5 kHz, obtaining

\[
A_{QFC}_{f_{band}}, \quad A_{QFC}_{f_{band}} \text{ was then normalized to the total energy of } \hat{M}_s(f_1,f_2), 
\]

obtaining the amplitude of QFCs in frequency bands \( A_{QFC}_{f_{band}} \).
2.5 Statistical Analysis

The Kolmogorov-Smirnov test for normality was performed on \( E_n(f) \) and corresponding energy \( E_n(f) \) (white squares) in ten frequency bands. The magnitude of the bispectrum \( M(f_1, f_2) \) in the principal domain \([21]\) region \([f_1 \geq 0, f_1 \geq f_2, f_1 + f_2 \leq \pi]\) and normalized amplitude of QFC components \( A_{QFC}(f_1) \) integrated in ten frequency bands (i.e., within the dashed counter-diagonals).

### Results

By way of example, Figure 2 shows the analysis of a TEOAE recording (Figure 2) and Figure 3 shows the analysis of a representative exposed worker (Figure 3).
2a) for a representative subject of the control group. Figure 2b shows the scaled periodogram $\tilde{M}_2^\nu(f)$ (thin bars). The main components of the signal were observed in three frequency regions, i.e., around 0.5 kHz, 0.8 kHz, and 1.2–1.3 kHz. Dividing the frequency axis into 0.5 kHz frequency bands and integrating $\tilde{M}_2^\nu(f)$ on each band, we obtained values of $E_{n_1} \big| _{f\text{-band}}$, as shown in Figure 2b with squared markers. The highest values of $E_{n_1} \big| _{f\text{-band}}$ were in the bands 0.5–1.5 kHz.

The lower part of Figure 2c shows the contour plot of $\tilde{M}_2^\nu(f_1, f_2)$ in the $(f_1, f_2)$ plane. The 3rd-order scaled polyperiodogram showed components distributed in the region $[f_1 \leq 2.5, f_2 \leq 1.5]$ kHz, with two peaks at (0.6, 0.5) kHz and (1.1, 1.1) kHz.

By integrating $\tilde{M}_2^\nu(f_1, f_2)$ on the 0.5 kHz frequency bands of $f_3$, represented in Figure 2c as dashed counter-diagonals, we obtained values of $\tilde{A}_{\text{eqc}} \big| _{f\text{-band}}$, represented in Figure 2c with circle markers. The highest values of $\tilde{A}_{\text{eqc}} \big| _{f\text{-band}}$ were in the 1–2.5 kHz range.

As another example, Figure 3 shows the analysis of a TEOAE recording (Figure 3a) of a styrene-exposed worker. Figure 3b shows the scaled periodogram $\tilde{M}_2^\nu(f_1, f_2)$ (thin bars). The main components of the signal were observed in three frequency regions, i.e., around 1.2–1.3 kHz, 1.6–1.8 kHz, and 3.2–3.3 kHz. Analogously to Figure 2, values of $E_{n_1} \big| _{f\text{-band}}$ are represented in Figure 3b with squared markers. The highest value of $E_{n_1} \big| _{f\text{-band}}$ was in the band 1.0–1.5 kHz.

Figure 4 shows mean values and standard errors of the energy of the scaled periodogram (upper panel) and of the amplitude of QFCs $\tilde{A}_{\text{eqc}} \big| _{f\text{-band}}$ (lower panel) in the ten frequency bands for styrene-exposed workers and the control group. $E_{n_1} \big| _{f\text{-band}}$ had a similar shape for both workers and controls, with main components in the 0.5–2 kHz range and a maximum in the 1–1.5 kHz band. A 2 (groups) $\times$ 10 (frequency bands) ANOVA revealed that there were significant differences across frequency bands ($F_{9,409} = 115.2, p < 0.001$) and between groups ($F_{1,409} = 4.6, p = 0.032$), and a significant interaction between groups and frequency bands ($F_{9,409} = 5.4, p < 0.001$). Statistical analysis confirmed that main components for both groups are in 0.5–2 kHz range. Post-hoc Holm-Sidak’s test highlighted statistically significant differences between groups in the 0–1 kHz range, with higher values for controls, and in the 2–2.5 kHz and 3–4 kHz ranges, with higher values for exposed-workers. $\tilde{A}_{\text{eqc}} \big| _{f\text{-band}}$ had a bell-shape for both groups. The main non-linear components were found at lower frequencies for the control group, with a peak in the 1.5–2 kHz range. Conversely, for the worker group, the main non-linear components appeared shifted toward higher frequencies, with a peak in the 2–2.5 kHz range. A 2 (groups) $\times$ 10 (frequency bands) ANOVA revealed that there were significant differences in $\tilde{A}_{\text{eqc}} \big| _{f\text{-band}}$ across frequency bands ($F_{9,288} = 115.2, p < 0.001$) and between groups ($F_{1,288} = 4.6, p = 0.032$), and a significant interaction between groups and frequency bands ($F_{9,288} = 5.4, p < 0.001$). Statistical analysis confirmed that main components for both groups are in 0.5–2 kHz range. Post-hoc Holm-Sidak’s test highlighted statistically significant differences between groups in the 0–1 kHz range, with higher values for controls, and in the 2–2.5 kHz and 3–4 kHz ranges, with higher values for exposed-workers.
The differences in $An_{QFC}|_{f_{\text{band}}}$ between groups were not significant ($F_{1.288} = 0.01, p = 0.908$), but interaction between frequency and groups was significant ($F_{2.288} = 9.5, p < 0.001$). Statistical analysis confirmed that the main non-linear components for the exposed-workers are in the 1.5–4 kHz range, whereas for controls they are in the 1–3 kHz range. Post-hoc Holm-Sidak’s test highlighted statistically significant differences between groups in the 0.5–2 kHz range, with higher values for controls, and in the 3–4 kHz range, with higher values for exposed-workers. As a final remark, it was observed that the distribution of both the energy of the scaled periodogram $E_{ns}|_{f_{\text{band}}}$ and the amplitude of QFCs $An_{QFC}|_{f_{\text{band}}}$ across frequencies of the part-time worker were fully comparable to those observed in the full-time workers.

4. Discussion and Conclusion

In this work, we analyzed both linear and non-linear components of TEOAEs recorded in styrene-exposed workers, and compared it to normative data described in [16]. By means of traditional spectral analysis, we obtained the scaled periodogram and defined the parameter $E_{ns}|_{f_{\text{band}}}$ to investigate the spectral content of the signal in narrow bands, while using bispectral analysis we computed the amplitude of QFCs and defined the parameter $An_{QFC}|_{f_{\text{band}}}$ to evaluate contributions of non-linear components.

We observed that the mean values of $E_{ns}|_{f_{\text{band}}}$ calculated on the scaled periodogram which contains both linear and non-linear components, were quite similar for workers and controls, with main components in the same 0.5–2 kHz range. Therefore, the distribution of the spectral components in the frequency bands was similar for workers and controls, and it was not possible to discriminate between the two groups using only this parameter.

Interestingly, the present preliminary analysis showed that QFCs amplitude, which is a measure of cochlear non-linear interactions, is quite different in exposed workers and controls. The parameter $An_{QFC}|_{f_{\text{band}}}$ revealed that the main non-linear components in workers, identified by higher amplitude of QFCs, were shifted toward higher frequencies than in the control group. These differences can be attributed to the effects of the styrene exposure only, as possible confounding effects of noise exposure can be excluded because workers were exposed to noise levels well below the action level of 85 dBA set by the Occupational Safety & Health Administration.

Many studies in literature investigated effects of organic solvents (such as styrene) on hearing functionality, but only in few cases these effects have been evaluated using OAEs [7, 22, 23]. It is not possible to make a direct comparison of our results with those obtained in these last studies because of differences in mixture of organic solvents to which workers are exposed, levels of exposure, and confound effects due to combined noise exposure.

For example, in [22] a reduction in OAE amplitude, both in TEOAEs and Distortion Product Otoacoustic Emissions (DPOE)s was observed in subjects exposed to a mixture of organic solvents (including styrene) with a rate of combined total exposure ranging (calculated considering the sum of quotients of compounds concentrations by respective exposure limit values) from 0.94 and 3.73 mg/m$^3$. In [23], authors found that at average inhaled styrene levels of about 30–50 ppm per working day over a period of about 15 years with previous exposure to higher levels above 50 ppm increased the risk for hearing impairment, but no relationship was found between parameters extracted from TEOAEs and exposure.

Results obtained so far are encouraging, but of course, due to the small size of the analyzed sample, they need to be confirmed in further analyses on a sample of larger size. Also, since non-linearities in normal-hearing subjects depend on the intensity of the eliciting stimulus [16], it may be interesting in the future to investigate whether and how non-linear characteristics of TEOAEs elicited in styrene-exposed workers change with stimulus intensity. Finally, a longitudinal study could also be interesting to assess if, and to what extent, cochlear non-linear mechanisms change during the day, in particular before and after the working shift.

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