Gas chromatography to detect bacteria-based self-healing agents in concrete

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Abstract. In the concrete industry, legal disputes might occur when a built structure performs worse than it was supposed to during the design phase. When dealing with underperformance of self-healing concrete (e.g., no regain of water tightness after cracking), these disputes might likely be related to questioning if healing agents were actually included in the material or not. In this study, a methodology based on Gas chromatography is proposed to detect and quantify poly-lactic acid based-healing agents in cementitious materials. The applicability of this technique for this purpose has been demonstrated on mortar and concrete powders with and without healing agents. The amount of material needed to conduct the experiment is less than 200 mg, making the technique suitable for on-site application to limit any destructive action as much as possible. The application of gas chromatography to detect and quantify healing agent inclusions in concrete has the potential to be extended to other additives, depending on their composition.

1 Introduction

Concrete is the most widely used construction material worldwide. Depending on the specific application, a concrete mixture can include many agents and additions able to modify and improve its characteristics, such as the inclusion of air entrainment agents, viscosity modifiers or supplementary cementitious materials. Specifically, to increase the durability of concrete structures, self-healing agents gathered high attention from both industry and the scientific community during the last decades. Due to the relatively low tensile strength of concrete, cracking is an unavoidable phenomenon affecting concrete structures during their service life. However, concrete has an intrinsic autogenous ability to heal cracks [1]. Autogenous healing occurs through continuous hydration of cement particles and through precipitation of calcium carbonate resulting from carbonation of the matrix [2]. Nevertheless, autogenous healing has a healing capacity limited to cracks 0.1–0.2 mm wide [3-5]. Self-healing capacity of concrete can be improved thanks to several methods [5]: among others, biogenic concrete has proved to be a promising solution to improve water tightness and potentially the service-life of constructions [6]. Besides recently developed technologies [7-11], poly-lactic acid based healing agents have been widely studied over the last decades and their application in practice recently took place [12-13].

The versatility of concrete makes the material demand impressive, resulting in an unquantifiable number of structures constructed with it, annually. Due to the time, social and economic interests related to realize most of the infrastructures, legal disputes are often an inevitable occurrence when making certain intervention. Among other reasons, legal disputes are often related to the time of delivery, the costs of the intervention, and the performance of the building. When a structure is underperforming compared to the expected needs and requirements, disputes related to the concrete composition might occur. In the case of self-healing concrete mixtures, underperformance of the material might raise doubts about the presence of the added-in self-healing agents, as well as their dosage in the matrix. Depending on the healing agents added in the mixture, some materials properties might be influenced (e.g., strength, air void content, transport properties) [5]. In so doing, analysing the cast concrete properties might give insight about whether the inclusion of healing agents occurred. However, these would be only indirect measurements, not evidently confirming the presence of any healing agent nor giving the possibility to quantify it. Hence, in this paper a methodology based on Gas Chromatography (GC) has been applied to detect and quantify poly-lactic based healing agents in both mortar and concrete specimens. GC was invented by Martin and James in 1952 [14] and has become one of the most important and widely applied analytical techniques in modern chemistry. Separations are achieved in GC by a series of partitions between a moving gas phase and a stationary liquid phase held in a small diameter tube (the column) after a mixture is injected as a narrow band. A detector then monitors the composition of the gas stream as it emerges from the column carrying separated components, and the resulting signals provide the input for data acquisition. A chromatograph provides a spectrum of peaks for a sample representing the analytes present in a sample eluting from

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the column at different times. The retention time for an individual species can be used to identify analytes at a specific temperature, and the area under a peak is proportional to the amount of analyte present in the chromatogram. GC can be applied to the analysis of mixtures, which contain compounds with boiling points from near zero to over 700 K, or which can be heated sufficiently without decomposition to give a vapour pressure of a few mmHg [15]. Even though GC has been applied in various research areas such as the petroleum industry [16], biochemistry [17] and food and flavour studies [18], to the authors' knowledge this is the first study in which GC has been applied for the detection of self-healing agents in construction materials. Besides describing the experimental procedure and the results obtained in this study, further potential applications of GC in the concrete industry are also discussed.

2 Materials and methodology

A total of seven pulverized samples have been analysed by a gas chromatograph (GC, model 6890N, Agilent, USA). The tested samples included different amounts of healing agent powder (HA_PLA 1.5 g/L and HA_PLA 15 g/L), plain mortar with no healing agents (CTRL_plain_mortar), mortar with added PLA healing agent particles (PLA_REF_mortar) and three concrete mixtures (TEST1-3_concrete). Mortar specimens were cast with blast furnace slag cement (BFSC, CEM III/B 42.5 N, ENCI, Rotterdam, Netherlands), a water to cement ratio (w/c) of 0,5 and fine siliceous aggregates (0.125–2 mm), with mixing proportions of 1:0.5:3, respectively. The composition of the PLA healing agents was 97.6% of lactic acid, 2% of nutrients and 0.4% of bacterial spores. In the PLA_REF_mortar, 2.6% by mass of cement of PLA healing agents were also added to the mixture during casting. BFSC concrete was also used to cast the elements from which the test powders were obtained, which also included 5-7.5 kg/m^3 of PLA healing agent particles. Further specifications related to their mix design remain confidential.

Before testing, mortar and concrete specimens were pulverized through a manual diamond-head driller to obtain powder with a particle size lower than 500 µm, while healing agent particles were tested as they were formulated, with a maximum particle size of around 1 mm. After formulation of the samples, each sample was mixed in a glass tube with 100 µL of liquid internal standard (IS). The internal standard consisted of a dilution of 1 g of benzoic acid diluted in 50 mL of commercial 1-propanol. After that, 1.5 mL of hydrochloric acid (HCl, concentrated commercial solution of 37%) and 1-propanol mixture (ratio 1:4) was poured into the glass tube. The combination of HCl and 1-propanol is needed to fully hydrolyse and esterify the polymer into propyl ester, the structural formula of which is reported in Figure 1.

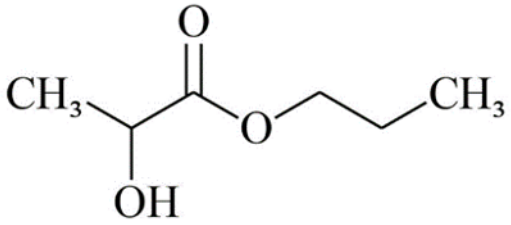

Fig. 1. Structure formula of propyl ester**.**

Then, 1.5 mL of commercial 1,2-dichloroethane (98.96 g/mol, CAS 107-06-2) was poured in the glass tube to work as solvent. The closed tubes were then heated for three hours at 100 ºC using a block heated, and vortexed every 30 minutes to ensure homogeneity of the internal content. After the three hours heating, the glass tubes were cooled down at room temperature for 30 minutes. To extract the free acids from the organic phase, 3 mL of milli-Q^{TM} water were added in each glass tube. The tubes were then centrifuged for 15 minutes at 2500 rpm to induce separation between the organic and aqueous phases. 1 mL of organic phase, at this stage confined at the bottom of each glass tube, was extracted through a pipette and then filtered through filtration drying columns. The filtration drying columns were made of pipette tips that contained a small amount of glass wool. Before the filtration, a drying agent (e.g. sodium sulphate) was added on the top of the glass wool. At the bottom of the filtration columns, the GC vials were placed to collect the filtered organic phase of each glass tube, which were tested through GC. An overview of the composition and dosages of the tested samples is reported in Table 1-2. Since the healing agents are composed by 97.6% of PLA, the PLA content values used for further analysis are those reported in Table 2.

Table 1. Overview of the sample composition analysed through GC.

Sample	Composition	Measured HA weight (mg)
HA_PLA 1.5 g/L	Healing agent	1.55
HA_PLA 15 g/L	Healing agent	14.59
CTRL_plain_mortar	Plain mortar powder	143.89
PLA_REF_mortar	SH mortar powder	142.66
TEST1_concrete	SH concrete powder	145.50
TEST2_concrete	SH concrete powder	147.89
TEST3_concrete	SH concrete powder	143.20

Table 2. Overview of the HA concentration and estimated PLA content of each sample analysed through GC (* refers to the actual content of PLA, equal to 97.6% of the HA mass).

Sample	Approximate dosage of HA	PLA content (mg)*
HA_PLA 1.5 g/L	1.5 g/L	1.51
HA_PLA 15 g/L	15 g/L	14.24
CTRL_plain_mortar	-	0.00
PLA_REF_mortar	0.575% w/w_{mortar}	0.80
TEST1_concrete	0.25% $w/w_{concrete}$	0.36
TEST2_concrete	0.25% $w/w_{concrete}$	0.36
TEST3_concrete	0.25% $w/w_{concrete}$	0.35

The former two samples (HA_PLA 1.5 g/L and HA_PLA 15 g/L) were composed of polylactic acid-based healing agent only, in order to observe where the lactic acid GC peaks occurred. No healing agent particles were added in the CTRL_plain_mortar, which was needed as a negative control reference for the tests. On the contrary, PLA healing agents were added in the specimens from which the sample PLA_REF_mortar was obtained, working then as a positive control reference. Finally, three samples (8_21-790, 12_21-790 and 14_21-790) were tested to investigate if any PLA healing agent addition was detectable in concrete powders through GC. The tests were conducted with helium as carrier gas (flow rate of 33.3 ml/minute) at a temperature of 240ºC and a HP-1 column with 100% dimethylpolysiloxane as liquid phase.

3 Results and discussion

3.1. Identification of PLA peaks

The results of the GC test for HA_PLA 1.5 g/L and HA_PLA 15 g/L are reported in Figure 2 and Table 3. According to Table 2, the only peak that was proportional to the amount of healing agent added in the glass tube was peak 6, occurring at 10.51 minutes. As visible, the peak of HA_PLA 1,5 g/L was around 10 times smaller than that of HA_PLA 15 g/L, in proportion to the healing agent concentration of each sample. The presence of the standard, measured in each sample at around 14.60 minutes with similar peak intensity, further confirms the reliability of the comparison between the two samples. Hence, the occurrence of GC peaks at around 10.51 minutes demonstrates the presence of lactic acid healing agents in the tested solutions.

Table 3. Overview of the main peaks found through GC for healing agent only samples (in green, peaks related to the presence of lactic acid - LA; in yellow, peaks related to the presence of the internal standard - IS).

	HA_PLA 1.5 g/L		HA_PLA 15 g/L	
Peak	Time (min)	Height (counts)	Time (min)	Height (counts)
1	7.48	1278	7.48	1236
2	7.91	11358	7.91	11416
3	8.10	15988	8.10	15628
4	8.39	5.48	8.39	5.35
5	9.70	4.29	9.69	5.31
6 - LA	10.52	41.88	10.52	415.34
7 - IS	14.59	105.45	14.59	103.30

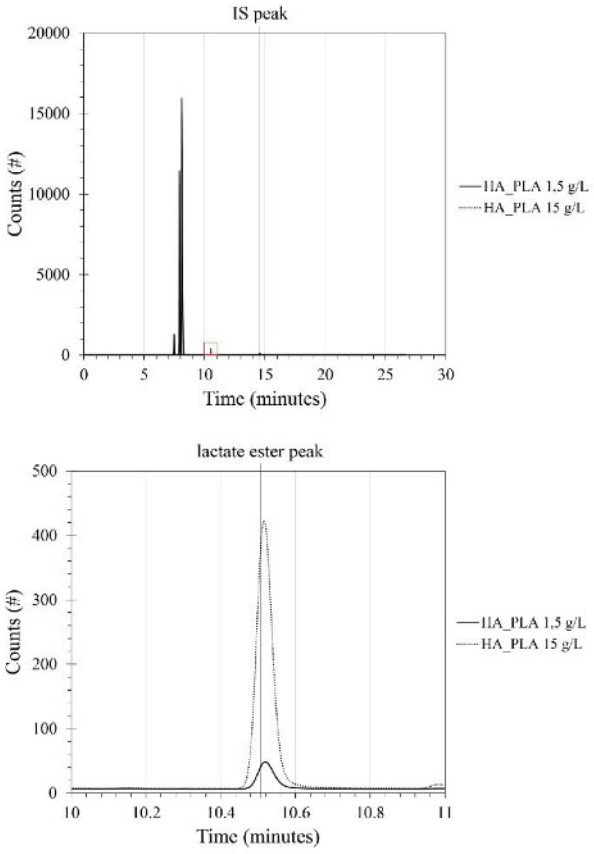

Fig. 2. (top) GC curve over the whole test duration for HA_PLA 1.5 g/L and HA_PLA 15 g/L (the red square indicates the zoomed-in area). (bottom) zoomed-in view of GC test interval where lactic acid peaks occur.

3.2 Detection of PLA in mortar and concrete samples

In Figure 3 and Table 4, the GC results of the tested cementitious powders are reported.

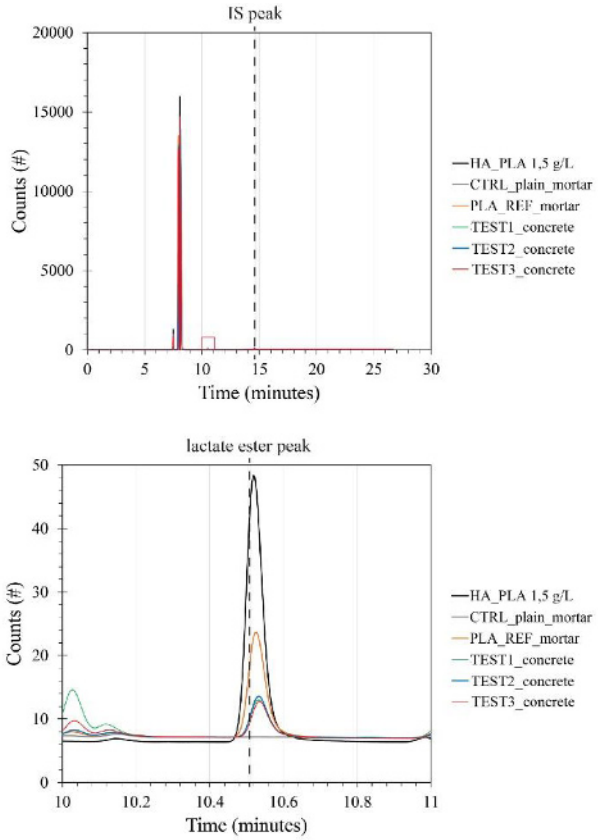

Fig. 3. (top) GC curve over the whole test duration of mortar and concrete powders (the red square indicates the zoomed-in area). (bottom) zoomed-in view of GC test interval where lactic acid peaks occur.

Table 4. Overview of the main peaks found through GC for mortar and concrete powder samples (1-LA: peak related to the presence of lactic acid; 2-IS: peak related to the presence of the internal standard).

	HA_PLA 1.5 g/L		**CTRL_plain_mortar**	
Peak	**Time (min)**	**Height (counts)**	**Time (min)**	**Height (counts)**
1 - LA	10.52	41.8	-	-
2 - IS	14.59	105.5	14.59	97.5
	PLA_REF_mortar		**TEST1_concrete**	
Peak	**Time (min)**	**Height (counts)**	**Time (min)**	**Height (counts)**
1 - LA	10.53	16.6	10.53	5.9
2 - IS	14.59	91.2	14.60	90.5
	TEST2_concrete		**TEST3_concrete**	
Peak	**Time (min)**	**Height (counts)**	**Time (min)**	**Height (counts)**
1 - LA	10.53	6.5	10.53	5.7
2 - IS	14.60	97.9	14.60	94.2

The presence of the lactic acid healing agent in the mixture can be demonstrated by the presence of the lactic acid representative peak (e.g. lactate ester), as reported in Figure 2 and Table 3. According to Figure 3 and Table 4, only the CTRL_plain_mortar sample did not show any peak related to lactic acid healing agent, while all the others had that representative peak at around 10.53 minutes, demonstrating the applicability of GC to detect lactate ester and, hence, to confirm the presence of lactic acid based healing agent in PLA_REF_mortar, 8_21-790, 12_21-790 and 14_21-790. For quantification purposes, the relation between the PLA estimated mass in each sample (previously reported in Table 2) and the lactate ester GC peak intensity is also reported in Figure 4.

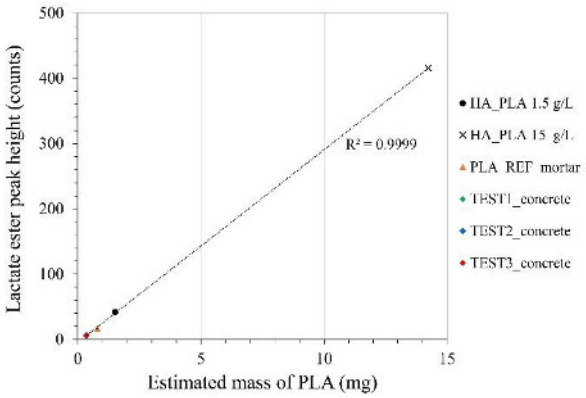

Fig. 4. Relation between lactate ester peak intensity (counts) and estimated mass of poly-lactic acid (mg).

According to Figure 4, the relation between the lactate ester peak intensity and the concentration of poly-lactic acid in each sample is evident. Despite the relatively low amount of PLA present in the concrete powder samples (e.g., less than 0.4 mg), the lactate ester peaks are not only detectable, but also representative of the actual dosage of the healing agent added in the mixture. Given the results reported in this study, GC can be an effective technique to detect and quantify the presence of, at least, poly-lactic acid based healing agents in concrete. Besides that, the applicability of GC is further increased by the potential quantification of healing agents dosage in the mixture through minimal intervention on the built structure. Also, GC could be applied to detect other added-in compounds, such as polyhydroxyalkanoate, which has been recently applied as bacteria-based self-healing agent substrate [10-11].

4 Conclusions

In this report, a methodology based on Gas Chromatography (GC) was applied to detect the presence of lactic acid healing agents in different mortar and concrete specimens. The representative GC peaks of lactic acid were firstly identified through testing a solution including the healing agent alone. After that, mortar and concrete specimens were pulverized and processed through the same methodology to which the healing agents were previously treated. The presence of lactate ester peaks was observed for all the mortar and concrete specimens with added-in poly-lactic based healing agent, but not for the CTRL_plain_mortar, which had no healing agent inclusions. Also, the intensity of the lactate ester peaks was linearly proportional to the healing agent concentration, hence potentially allowing not only the detection of any poly-lactic agents, but also the quantification of their dosage in concrete.

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