Food and Chemical Toxicology 93 (2016) 51–57

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

In vitro toxicity assessment of extracts derived from sol–gel coatings on polycarbonate intended to be used in food contact applications

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ARTICLE INFO

Article history:
Received 4 March 2016
Received in revised form
28 April 2016
Accepted 29 April 2016
Available online 30 April 2016

Keywords:
Ames test
Micronucleus assay
Endocrine disruption
HepG2 cell line
Sol–gel coating
Food contact coating
Polycarbonate
Migration
Extracts

ABSTRACT

Polycarbonate is a widely used polymer in food contact applications all around the world. However, due to the potential release of Bisphenol A (BPA) during repeated washing cycles, its use becomes compromised as BPA is known for being an endocrine disruptor for rodents. In order to tackle this issue, sol–gel coatings based on organoalkoxysiloxane were developed on PC, to act as a physical barrier. To this end, two sol–gel systems based on tetraethylorthosilicate (TEOS), methyltriethoxysilane (MTES) and 3-glycidyloxypropyltriethoxysilane (GPTES), three common sol–gel precursors, were prepared. The coatings derived from the latter two systems were then studied with regards to their potential toxicity *in vitro*. Migration tests were performed in food simulants, and the maximal migration was obtained in ethanol 10% (v/v) for one system and in isooctane for the other one. *In vitro* genotoxicity was assessed with the Ames test (OECD 471) and the micronucleus assay (OECD 487), and no genotoxic effect was observed. Moreover, the estrogenic activity of the extracts was studied with a transcriptional activation assay using transient transfection in human cells; none of the extracts was found estrogenic. These negative *in vitro* results are highly promising for the future use of these new barrier coating formulations onto food contact materials.

1. Introduction

Polycarbonate (PC) is a widely used polymer in a variety of applications such as electronics, optics, automobile or even construction, which clearly shows the diversity of the use of PC (over two million tons produced each year). As a food grade material, PC is also very present in the alimentary field (bottles, containers …), the latter benefiting from its transparency, lightness and exceptional impact resistance at a reasonable cost (Wu et al., 2008). Unfortunately, PC displays poor scratch and solvent resistance along with a fairly high sensitivity to hydrolysis, which significantly limit the polymer's lifetime. In addition, the potential release of bisphenol A (BPA), a component monomer of PC, is currently of toxicological concern. It is worth pointing out that while the use of BPA in food contact materials is permitted in the European Union (EU) under Regulation (EU) No 10/2011[2], it became restricted in January 2011 when the European Commission adopted regulation (EU) No 321/2011/EU (Commission implementing regulation (EU), 2011), that prohibits to use PC in infant feeding bottles manufacturing.

To mitigate PC hydrolysis, thus BPA release, one could envision modifying PC's bulk directly, to achieve higher chemical stability. Although no such studies are reported in the literature, it is well-known that loading specific additives during PC formulation (Fabbri et al., 2008), or preparing PC copolymers (Li and Shimizu, 2011; Zhang et al., 2010) can successfully tune PC properties. However, these compositional modifications typically impact other useful properties of the material as well. Another path to achieve the same is surface modification *via* the deposition of protective

Abbreviations: BNC, binucleated cell; FBS, fetal bovine serum; FCM, food contact materials; GPTES, 3-glycidyloxypropyltriethoxysilane; IF, induction factor; MEM, minimum essential medium; MN, micronucleus; MTES, methyltriethoxysilane; PBS, phosphate buffered saline; OECD, organization for economic co-operation and development; TEOS, tetraethylorthosilicate.

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http://dx.doi.org/10.1016/j.fct.2016.04.025
0278-6915/

films: barrier coatings with improved mechanical properties (as compared to raw PC) could largely increase PC's lifetime and prevent BPA leakage, maintaining PC suitable in food contact applications.

In that regard, dense oxide-like barrier coatings are excellent candidates as high mechanical properties can be obtained using industry-compatible techniques such as Plasma-Enhanced Chemical Vapor Deposition (PECVD) (Bursikova et al., 2007), Diamond-Like Carbon (DLC) deposition (Damasceno et al., 2002), plasma-ion assisted deposition (Schulz et al., 2008), atmospheric plasma (Cui et al., 2014) or the sol–gel route (Lionti et al., 2013a, 2013b; Le Bail et al., 2015a; Le Bail et al., 2015b). Due to the rapid, low-cost and easy to implement nature of the latter technique, we focused our efforts on the preparation of hybrid silica coatings by sol–gel, on PC. ORMOSILs (organically modified silica, i.e. class II hybrid O/I silica precursors), which display a non-hydrolysable Si–C bond ensuring chemical linkage between inorganic and organic networks, were used as starting precursors (Sanchez et al., 2005). ORMOSILs particularly fit our specifications well due to the dual nature of hybrid O/I silica, the latter offering elevated hardness and resistance through the inorganic part, as well as softness/flexibility or even specific characteristics (hydrophobia, anti-microbial resistance …) through the organic one. It is worth noting that, among the commercially available ORMOSILs, only ethoxy-ending precursors (as opposed to methoxy-ending ones) were used in the sol preparation, in order to release ethanol (harmless) as a sol–gel secondary product rather than methanol (highly toxic).

Here, we report on the toxicological testing of coatings derived from two different ORMOSILs based sols, with optimized mechanical properties and adhesion (Lionti et al., 2013a, 2013b). It is noteworthy that chemical and mechanical properties of sol–gel coatings are widely reported in the literature, but toxicology testing, which is of primary importance for food contact applications, is hardly ever carried out and reported. Since many parameters can be adjusted when making sol–gel formulations, potentially leading to coatings with different composition, structure, and properties, the compliance with the European commission regulation of each sol–gel coating derived from a specific set of sol–gel conditions, intended to be used in food contact applications, needs to be evaluated. Our first formulation is based on methyltriethoxysilane (MTES). Although numerous patents already report on the deposition of similar coatings for alimentary applications (Dubanchet et al., 2008; Jeon Bong et al., 2007; Chung Kwon et al., 2008), the nature of the substrate was different (metallic substrate) and no toxicological data were included. The second sol is based on glycidyloxypropyltriethoxysilane (GPTES) and tetraethylorthosilicate (TEOS): the possibility of using GPTES in coatings for food-contact applications was only mentioned twice before, in our previous patent (Toury et al., 2014) where no toxicology study had been intended to be conducted, and in our paper published in 2014 where we only looked at the toxicity of GPTES as a starting precursor (i.e. unreacted) (Lionti et al., 2014).

In this paper, coatings extracts derived from the two above mentioned sol–gel formulations were collected and tested, as they contain all the substances susceptible to migrate from the material to the food simulant, i.e. intentionally added substances (IAS) but also non-intentionally added substances (NIAS). Genotoxicity is the prerequisite steps required by EFSA irrespective of the migration level of the substance intended to be used in contact with food (EFSA, 2008). This paper reports the data obtained from the Ames test performed according to the OECD 471 guideline with both coatings extracts in order to detect genetic mutations in bacteria. A second genotoxicity test, the micronucleus assay, also part of the battery of tests required by EFSA, was performed according to the OECD 487 guideline on a human hepatoma cell line (HepG2 cells) in order to detect abnormalities on structure and/or in the number of chromosomes. In addition, estrogenic activity of the coatings was also tested with an *in vitro* estrogen receptor transcriptional activation (ERTA) assay which identifies chemicals that are able to activate the estrogen receptor (ERα) (i.e., ER agonists) on the human HepG2 cell line.

2. Material and methods

2.1. Chemicals and medium

Tetraethylorthosilicate (TEOS, n°CAS: 78-10-4), methyltriethoxysilane (MTES, n°CAS: 2031-67-6), 3-glycidyloxypropyltriethoxysilane (GPTES, n°CAS: 2602-34-8), Ludox AS-30 (colloidal silica, 30% (w/v) solid content, suspension in H_2O), glacial acetic acid, dimethylsulfoxyde (DMSO), cytochalasin B, all the positive controls [2-nitrofluorene (2-NF), sodium azide (SA), ICR191, 4-nitroquinoline-N-oxide (4-NQO), 2-aminoanthracene (2-AA), cyclophosphamide (CP), vinblastine sulfate (VBS)], Minimum Eagle's Medium (MEM) and 100× non-essential amino acids were purchased from Sigma–Aldrich (Saint-Quentin-Fallavier, France) and used without any further purification. L-glutamin (200 mM), heat-inactivated fetal bovine serum (FBS), phosphate-buffered saline without Ca^{2+} and Mg^{2+} (PBS) and trypsin (0.05% w/v)-EDTA (0.02% w/v) solution were obtained from Invitrogen laboratories (Cergy-Pontoise, France). Levasil 200E (colloidal silica, 20% (w/v) solid content, suspension in H_2O) was generously given by Akzo Nobel. Isopropanol was purchased from VWR. 2 mm-thick Bisphenol A-PC sheets, (molded from Mitsubishi's Lupilon S3000 UR pellets) were degreased with ethanol. A N_2/H_2-plasma treatment (atmospheric pressure, N_2/H_2 flow rate 330 mm/s), was performed on the PC substrate coated with GPTES and TEOS in order to increase the coating to substrate adhesion.

2.2. Coatings synthesis

Two different sol syntheses were done; all the steps were carried out at room temperature. MTES or (GPTES + TEOS) were mixed with acetic acid (referred to as solutions 1 and 1′, respectively). Ludox AS30 or levasil 200E and isopropanol were mixed together under stirring (solutions 2 and 2′ respectively). Solution 1 was then added to solution 2 (1 + 2 being referred to as A2 thereafter), and solution 1′ added to solution 2′ (1′+2′ being referred to as A8 thereafter), under stirring. Finally, the sols (liquid state) were left aside under stirring for 48 h to ensure full hydrolysis, and were then deposited by dip-coating (withdrawal speed of 1 mm/s) on 10 cm × 7.5 cm × 2 mm PC sheets. A2 was deposited on PC substrates degreased with ethanol; A8 was deposited on N_2/H_2 plasma treated PC substrates. Following deposition, the coated specimens were annealed at 135 °C in a ventilated oven to cure them and obtain dense hybrid silica films (solids). A final film thickness of ~4 μm was measured by profilometry for both systems.

2.3. Migration tests

The overall migration tests were carried out according to either the European standard NF EN 1186-3 for the aqueous food simulants or the European standard NF EN 1186-14 for isooctane or ethanol 95% (v/v) substitute simulants. The test conditions were selected in accordance with the European standard NF ISO 1186-1. Coated PC sheets of 0.5 dm^2 were placed in glass Petri dishes and immersed in 100 ml of the substitute food simulants under well-defined time and temperature exposure conditions: (i) 1 h at 100 °C for acetic acid 3% and ethanol 10% (v/v), (ii) 3 h at 60 °C for ethanol 95%, and (iii) 1 h at 60 °C for isooctane. To ensure a good