Thermal Stability and Chemical Durability of PVC-Based Biomedical Devices

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ABSTRACT: Poly(vinyl chloride) (PVC)-based blood circuits for extracorporeal hemodialysis were investigated for the assessment of their thermal stability as well as their chemical durability towards ionizing radiation sterilization and environmental conditions of storage and transportation. Thermal degradation was monitored by measuring the amount of hydrochloric acid (HCl) evolved as a function of different thermal stresses. HCl was extracted from the internal lumen of the blood circuits, and then quantitatively evaluated under the corresponding form of chloride ions by chromatographic technique (HPLC-IC). Behavior of PVC heat stabilizers was evaluated as well, determining also the concentration of calcium and zinc released by the investigated materials, by flame atomic absorption spectroscopy (FAAS) technique. Electron beam irradiation revealed an impact on blood tubing higher than that of environmental storage conditions. Nevertheless, real operative cases of sterilization and storage conditions turned out to be quite safe, and all blood circuits displayed good performances in terms of thermal stability. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 102: 5378–5387, 2006

Key words: PVC; biomaterials; degradation; electron beam irradiation; HPLC

INTRODUCTION

Poly(vinyl chloride) (PVC) is nowadays the second largest polymeric material produced in volume terms, ranking second only to polyolefins. The applications of PVC range from the large and heavy sectors of building and construction to the automotive and consumer goods' fields, up to the high-technology and added-valued sectors of biomaterials. Being the world's most researched and thoroughly tested polymer, PVC still meets the favors of both scientists and manufacturers as far as medical devices exposed to either short or prolonged contact with biological fluids or their synthetic substitutes are concerned.[1–3] As a matter of fact, flexible and semirigid PVC account for a significant percentage of all plastic materials used in medical applications, e.g., catheters, intravenous fluid bags and tubing, blood and plasma storage bags, dialysis devices and equipments,[4–6] and in general, single-use presterilized components.

Despite its appeal as a material of choice as well as its ability to fulfil the demanding specifications of the biomaterial fields, PVC has always had to face a big drawback concerning its employment, i.e., its low thermal stability. PVC easily undergoes thermal degradation,[7,8] and its main reaction is known as dehydrochlorination (DHC). DHC leads to the disruption of the C—Cl bonds in the polymeric chains, resulting in the evolution of hydrochloric acid (HCl) and in the formation of long polyene sequences in the backbone of the polymer. Several events, features, and characteristics have been addressed to as hypothetical causes for PVC to degrade, and a great deal of works and publications has been issued for such topic to be developed.[9–12] Furthermore, it has been assessed that structural chain irregularities (e.g., allylic chlorine, tertiary hydrogen and chlorine atoms, end groups such as double bonds, etc.) significantly increase PVC degradation rates at the beginning of the process.[13–15] However, even when such irregularities and defect sites are not present, DHC does occur and holds on, but with a different extent.[9] In fact, thermal degradation is thought to be an intrinsic property of PVC and the removal of the above-mentioned defect sites improves the stability of the resin, though it can not completely eliminate degradation.[16]

Thermal stability of PVC-based materials becomes even a more intriguing topic as far as the applications of this polymer in the biomedical field are concerned, from the raw materials to the final products. As a matter of fact, the only heat stabilizers which are allowed to be added to the PVC-based blends by the major part of national laws and international Consensus bodies are the metallic soaps of calcium and zinc,[17] because of their low toxicity with respect to

other kinds of stabilizers. The mode of action of Ca- and Zn-based metallic soaps, as well as of other similar stabilizers, has been the subject of interest and research of many scientists, and therefore, it has been extensively reviewed and treated as well.[9,18–23] Even if both the chemistry and the mechanism of protection seem quite complex, it is generally assumed that while Zn-based carboxylates act as primary stabilizers protecting defect sites like labile chlorine bonds, on the other side, at a rough level, Ca-based carboxylates play the role of HCl scavengers, thus slowing down the DHC reaction rate.[6]

Dealing with PVC-based biomedical devices, the improvement of the knowledge on thermal stability of PVC is considered extremely meaningful. Indeed, such devices have usually to face many thermal and mechanical attacks along their processing, and thermal degradation might carry on a decrease in the products' performances and finally an alteration of the materials' biocompatibility.[24]

In this work the effects due to the thermal degradation of PVC-based circuits for extracorporeal hemodialysis are presented. Starting from the blending processes of the raw materials and arriving to the production of a finished item, this kind of devices has to withstand several steps of melting, and eventually a sterilization process. Nowadays, a lot of different sterilization methods and techniques are available. Among these, although the most widely exploited is probably the ethylene oxide (EtO) exposure of materials in autoclaves,[25] ionizing radiation sterilization techniques, namely γ and electron beam (or β) rays, are growing in popularity and in competitiveness. Thermal effects imparted by ionizing radiations (mainly by β-rays) are believed to depend primarily on the specific heat of the irradiated materials and on the internal energies of both preexisting and newborn chemical species.[26] Moreover, once the whole production stage is concluded, thermal stability and chemical durability of these biomedical devices have to be assessed along their entire claimed shelf life. This implies that the influence of the environmental conditions wherein the packaged devices will be stored before their employment have to be accurately evaluated. Clearly, storage conditions shall be assumed as greatly milder than those of processing and radiation sterilization. Nevertheless, the commitment both for the assessment of safety and for the evaluation of performances of biomaterials has to be prolonged along the entire shelf life of medical devices, thus including even those items and conditions which could appear milder than others. As a matter of fact, PVC is known to undergo thermal degradation even at relatively low temperatures.[27]

Any of the previously mentioned stages could *a priori* be considered a potential source of harmful heat stress. In particular, in this work, the individual influence of electron beam irradiation and storage conditions of the sterilized devices on the heat stability of the blood tubing have been focused and evaluated.

The generation and the subsequent release of HCl has been evaluated as markers of DHC through the monitoring of corresponding chloride ions (Cl^-) concentration in proper extracting solutions. In the same extracting media, calcium and zinc concentrations have been determined as well, by flame atomic absorption spectroscopy (FAAS).

EXPERIMENTAL

Materials

The experimental framework for gathering of the samples was based on a group of commercially available single-use set of blood circuitries for extracorporeal hemodialysis belonged to the same production lot. An individual set is constituted by an arterial and a venous blood line. The major part of the blood contacting materials of both blood lines of the circuitry set consists of either flexible or semirigid PVC-based materials. This means that the employed formulations usually include, among the species added to the PVC resin, a proper plasticizer (usually esters of organic acids), Ca and Zn carboxylates as thermal stabilizers and other processing aids, e.g., epoxidized oils used as lubricants.

Blood lines sampling

For the evaluation of the effects due to β-rays, all the circuits were sterilized using an accelerated electron source working at an energy of 10 MeV, specifically dedicated to these purposes. The blood circuits were processed sterilizing the products by a single scan passage running in ambient air. All the circuits which were to be sterilized received a β-rays dose of 25 kGy, accounting for the thermal effects which are likely to be expected as materials' response to a common electron beam treatment.[28–30] This complies also with several international issues regulating the analysis and the evaluation of degradation products for biomedical devices.[31]

For the real environmental storage conditions to be simulated as close as possible, a set of different thermal conditionings was performed in a thermostatic oven to generate the subsequent thermal effects. Five different thermal conditions were selected, namely temperature values of 25, 35, 45, 55, and 65°C. The first values (i.e., from 25°C to 45°C) should account for a very close simulation of real thermal conditions, as they can be usually provided by normal storage, transportation, and shipping stages. On the other side, the last values, 55°C and 65°C, were clearly selected to simulate extreme situations, pursuing the

aim of the description and the evaluation of the so-called *worst case* situation. Furthermore, to stress the effects due to the β-sterilization process, all the experiments were carried out on both not sterilized and sterilized samples even if only the latter ones are obviously employed in the real-case dialysis sessions. Finished packaged blood lines, sterilized and not sterilized, were sampled and then conditioned for 24 h using a thermostatic oven at the five above-mentioned temperatures. To verify the experiment reproducibility, three independent lines, relatively to each temperature condition and radiation dose, namely a nonsterile condition and sterilization at 25 kGy, were subjected to the simulated degradation procedure.

Experiments on arterial blood lines were performed following the same operative scheme, but considering only the highest and the lowest temperature values.

Extraction of PVC degradation products

The amount of HCl evolved from the artificially-degraded PVC-based materials was assumed as a close, direct, and straightforward marker for the extent of DHC to be evaluated, and thus, of the thermal degradation to be estimated. If thermal instability is one of the biggest problems when processing PVC and PVC-based materials, this concern grows higher when dealing with PVC-based biomaterials. Actually, leaching of degradation by-products (i.e., HCl for DHC) or of other soluble or volatile substances to biological fluids might change the original biocompatibility of biomedical devices which was initially assessed for the raw materials, the final formulations as well as for the design of the devices. Hence, an experimental procedure for the extraction and the collection of these species, evolved within the lumens of the blood lines, was developed.

All the sampled lines were recirculated for 4 h with a volume of 100.0 ± 0.5 mL of ultrapure deionized water produced by using a Millipore Milli Q 185 Plus equipment.[*] A constant flow rate value of 350 mL/min was always ensured during all the experiments' time length. After the 4-h recirculation, the blood circuits were emptied and the corresponding eluates collected for the subsequent analyses. Then, the HCl evolved as a consequence of the occurrence of DHC was monitored and estimated as corresponding Cl^- ions dissolved in the aqueous media. Moreover, an exhaustive description of both anionic and cationic species present in the eluates was accomplished.

Identification and quantification of anionic degradation products

Anionic products identification and quantification were performed by means of a HPLC equipped with an ionic conductivity detector (HPLC-IC). For the analysis of the anionic species, a Gynkotek P580 Bio-Analytical pump and a Rheodyne injection valve equipped with a 20-µL loop were used. Chromatographic separations were achieved on a 250×4.6 mm ID SARASEP AN1 column for anionic exchange packed with 9-µm particle size. During all the chromatographic analyses, the separation column was always thermostated at $35.0°C \pm 0.1°C$ by an Eppendorf CH-30 model column heater. The system was also equipped with a mobile phase conductivity suppressor Alltech ERIS 1000HP model Autosuppressor, set with a high capacity suppressor cells Type 312, supplied by Alltech. The final detector is a Λ1 conductivity detector Sykam S3111 model. Operative elution parameters for the chromatographic analyses are reported in Table I, while operative conditions for the suppression of the mobile phase conductivity during HPLC measurements are presented in Table II. Three repeated injections were performed for each different eluate. The HPLC-IC system and the chromatographic signals were controlled and monitored using a Chromeleon© v. 4.12 software, distributed by Softron. Identification of the anionic species was achieved by using anionic standard solutions. Quantification procedure was accomplished by preparing adequate calibration curves of proper standards diluted water. In particular, single anion PrimeAg-plus[TM] reference solutions from Romil and CertiPUR[®] anion multielement Standard II from Merck were used for the Cl^- identification and quantification.

Quantification of cationic species

The only metal ions present in the original plasticized PVC formulations should be calcium and zinc, coming from the carboxylates heat stabilizers additives system. Hence, the only metallic cations which have a chance to be detected within the eluates, bearing in mind the experimental procedure which had generated such solutions, should be Ca^{2+} and Zn^{2+} extracted and dissolved in the recirculating

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[*]From here onward, unless otherwise specified, water shall be intended as water prepared in the just-described fashion, either when used as pure solvent or when used for the preparation of aqueous solutions.

TABLE I
Operative Analytical Parameters
for HPLC-IC Measurements

Elution mode	Isocratic
Mobile phase	$NaHCO_3$
Mobile phase concentration (mM)	2.0
Flow rate (mL/min)	1.0
Run time (min)	20

TABLE II
Operative Parameters for the Eluent Conductivity Suppression Conditions during HPLC-IC Measurements

Regeneration time (min)	13
Eluent flushing time (min)	2.0
Eluent Concentration (mequiv./L)	2.0
Flow rate (mL/min)	1.0
Regeneration voltage (V)	−6.3

deionized water. Such species were then evaluated on an aliquot sampled from the blood lines eluates by means of a 3300 model Perkin-Elmer FAAS. Every analytical measurement for both cations was triplicated per each different eluate. Operative analytical parameters for the FAAS analyses are reported in Table III. Quantity of the metallic species was obtained using adequate calibration curves of proper standards diluted in water. Ca and Zn standards were Merck Titrisol® 1000 mg L^{-1} single standard solutions traceable as SRM from NIST for AAS, as $CaCl_2$ in 6.5% HCl and as $ZnCl_2$ in 0.06% HCl, respectively. For further information on the mass and charge balance within the eluates to be gathered, pH measurements were performed on the fresh solutions, i.e., as soon as they were collected from the blood circuits after recirculation, by using a model 215 Denver Instrument pH-meter, equipped with a Schott Geräte BNC glass electrode no. 42. The pH-meter was calibrated using two Merck CertiPUR certified secondary standard reference buffer solutions ($C_8H_5KO_4$ and KH_2PO_4/Na_2HPO_4) directly traceable to primary SRM from NIST/PTB at pH $(25°C) = 4.00$ and pH $(25°C) = 6.86$, respectively.

RESULTS AND DISCUSSION

General considerations

Figures 1 and 2 depict the comparison between typical chromatographic plots collected following the HPLC measurements when either β-radiation or temperature conditioning treatments had been fixed, respectively.

The actual degradation experiment was specifically tailored with the aim of evaluating the HCl evolution from the PVC present in hemodialysis circuitries via the quantification of the corresponding Cl^- ions. On the other hand, it shall be highlighted that the materials under investigation are complex mixtures of several components. As a matter of fact, another chromatographic signal was always detected besides the one pertaining to the chloride ions; however it has not been matched with the proper anionic species yet. In fact, we assumed as a matching criterion for the identification of unknown species the comparison of the peak form and of the retention time between signals pertaining to analytical samples and to standard solutions. Hence, according to the operative conditions above listed, it was not possible to assess a reliable correspondence between our unknown species and the vast majority of the inorganic anions which can be separated by our chromatographic column (other halides, sulfates, nitrates, phosphates, etc.). Thus, it is very likely that such species could be an organic residue bearing one or more negative charges. A possible explanation accounting for the presence of this anionic species within the eluates might take into consideration the leaching or the extraction by lixiviation of fragments formerly belonging to the molecules constituting the original formulations. On the other side, the generation of such species as a by-product of the superimposed degradation might be invoked. If the latter hypothesis held true, then the present negative-charged species could be, in turn, generated by the direct simulated degradation occurred to the materials. As previously described, the materials with which the tubing and the other parts of the blood lines are made of, are the results of very complex formulations, involving, among other substances, plasticizers, heat stabilizers, and costabilizers. If, on one side, thermal degradation of PVC is quite a well-known phenomenon, on the other side, all the other additives could be similarly suspected to undergo a sort of degradation, if thermal injuries occurred on the blood circuits. Indeed, if the main product of the

TABLE III
Operative Analytical Parameters for FAAS Measurements

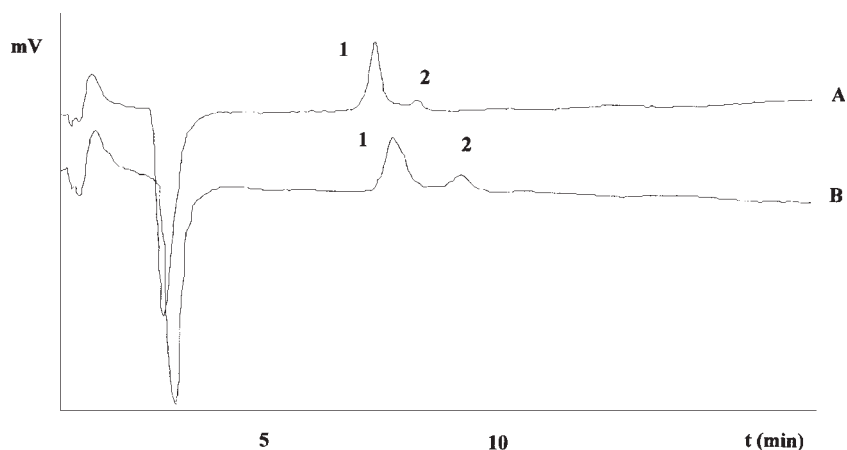| Element	Calcium	Zinc
λ (nm)	422.7	213.9
Slit (nm)	0.7	0.7
Lamp current (mA)	12	12
Burner type	5 cm Titan	10 cm stainless steel
Flame	N_2O/acetylene	Air/acetylene
Measurement mode	Working curve	Working curve
Signal mode	Background correction	Background correction
Integration time (s)	5	5
Standards concentration range (mg L^{-1})	0.1–1.0	0.1–1.0

Figure 1 HPLC-IC chromatographic plots of the eluate collected from the not sterilized venous lines. Plot A: thermal conditioning at 25°C. Plot B: thermal conditioning at 65 °C. Peak 1: unidentified ion. Peak 2: Chloride ion.

PVC degradation exerted by DHC is HCl, then it could be likely that thermal degradation of the previously mentioned additives might result in the generation of negative-charged residues such as the one which was detected in the current experiments. The second issue which can be pointed out as a possible source for the generation of the unknown species, is the environment wherein the β-radiation sterilization exerts its action, i.e., ambient air. In fact, the large amount of energy transferred during sterilization under the exposure of atmospheric oxygen, may result not only in the increase of DHC rate,[32] the so-called thermal oxidative degradation of the target materials, but also in the generation of newborn oxidized species[25] which could be subsequently extracted

or leached during the recirculation step. Whatever the possible source of the anion could be, its aqueous dissolved form always showed quite an unstable behavior, i.e., a constant time-dependent depletion of its concentration was always observed for every analytical sample. An explanation for this experimental evidence should be probably addressed to its volatility, its reactivity, or its low solubility in the aqueous solutions. Hence, a good performance in terms of the instrumental repeatability was never achieved by the analytical procedure for the quantitative measurements of such species. Finally, it shall be pointed out that a clear as huge drawback of the incomplete qualitative identification of the unmatched anionic species is the impossibility of its
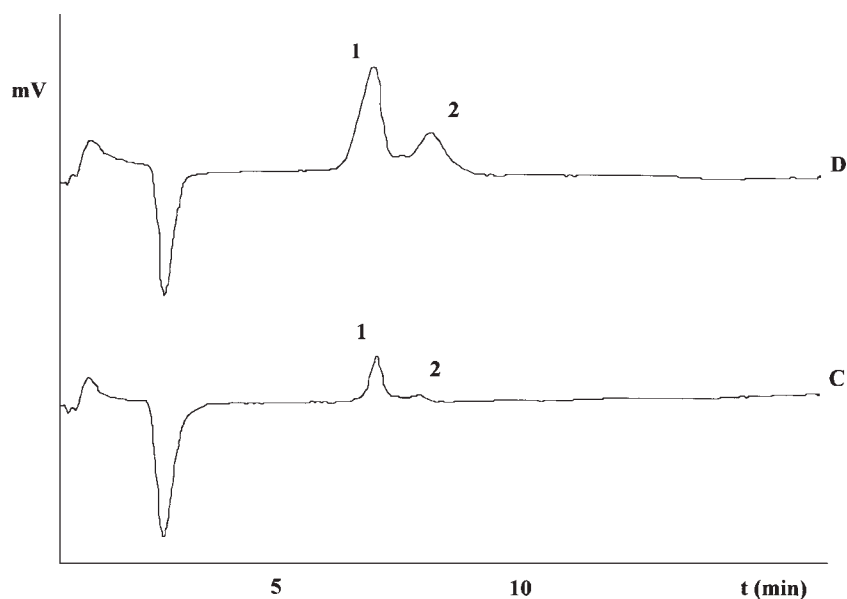

Figure 2 HPLC-IC chromatographic plots of the eluate collected from venous lines conditioned at 25°C. Plot C: not sterilized samples. Plot D: sterilized samples (Dose: 25 kGy). Peak 1: unidentified ion. Peak 2: Chloride ion.

direct quantitative evaluation through the comparison with standard solutions at known concentrations. Nevertheless, an indirect estimation of the amount of this particular species leached from the blood lines was accomplished at any rate, through some calculations on the charge and mass balance of the eluates. These results will be presented in a dedicated section later on.

Figure 3 depicts the quantitative results (values averaged over repeated injections of replicated samples) for Cl^- concentration split into the two effects imparted either by thermal conditionings or by β-treatments. Each effect will be now revised singularly, starting from the former one.

Thermal conditioning

A slight increase of the Cl^- concentration is observed as the conditioning temperature values increase. As it is evident from Figure 3, such behavior holds both for not sterilized and for sterilized blood circuits. When not sterilized and sterilized samples are considered separately, the responses towards simulated thermal influences seem to resemble quite closely each other. Thus, whether a venous line is sterilized or not, thermal conditioning effects, which were designed to simulate heat injuries coming from transportation and storage conditions of the finished devices, do not seem to deeply affect the behavior of the circuits, as far as HCl evolution is concerned. An explanation for this closeness between not sterilized and sterilized behavior could be provided, which could also account for a description of the environments where blood circuits have to be stored in before and after sterilization. Usually, prepackaged, but not sterilized, blood lines are stored within manufacturers' plants until sterilization. Obviously, the utmost care is devoted by manufacturers for the quality to be ensured till the very final step of the production (i.e., a ready-to-use blood circuit) is realized. Hence, environmental conditions at manufacturers' storage plants are considered to be safe, under control, and noneffective on the final performances of the present devices. Moreover, according to our simulation before sterilization and on the subsequent results, it can be gathered that neither transportation conditions nor storage environments at customers' warehouses and facilities are likely to generate harmful alterations on heat stability of PVC-based venous lines.

β-Sterilization treatment

On the reverse side, a clear effect of the β-treatment was always detected on the Cl^- concentration at each experimental temperature value of conditioning. Focusing on the details of this kind of behavior, it should be highlighted that independently from the temperature value selected, Cl^- concentration for sterilized blood lines is always more than 10 times higher than the respective concentration in the nonsterilized blood lines. Evidently, PVC resin within the formulations of the investigated circuits suffers much more from the β-sterilization step than from the simulation of transportation and storage conditions, as it can also be inferred from the literature.[33] As a matter of fact, β-treatments are not only known to be the likely source of DHC,[34] but also can even result in the modification of the morphology[35] and the chemical structure[36] of the surfaces of irradiated devices, at least when sterilizing conditions become harsher. As a final consideration, quite a remarkable observation should be highlighted. The *worst case* among all the simulated treatments, i.e., a venous line sterilized and then conditioned for 24 h at 65°C, displays a chloride ions concentration slightly higher
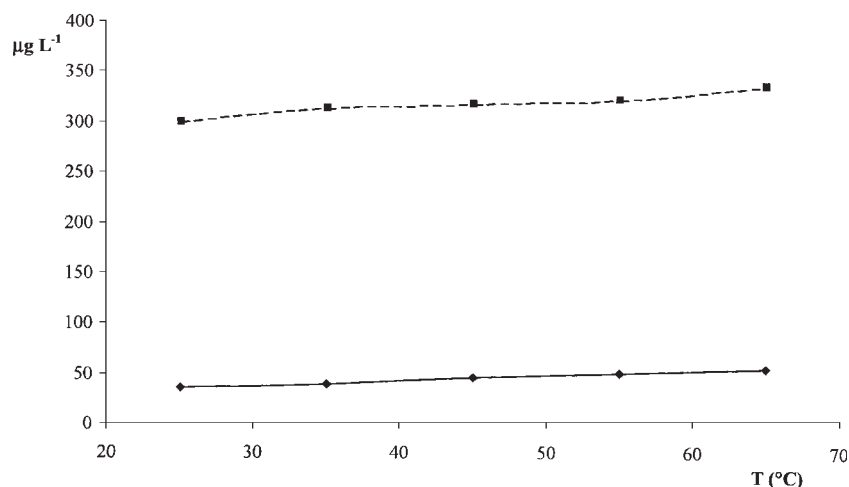
Figure 3 Chloride (Cl^-) concentration (µg/L) as a function of the increasing conditioning temperature values (venous lines). Straight line: not sterilized samples. Dashed line: sterilized samples.

than 320 µg L^{-1}, which is a fairly low absolute value.[37] Hence, even if degradation effects are absolutely non negligible, they can be safely considered mild when real-case situations are closely simulated.

Arterial lines response

Dealing with the description of the behavior of arterial lines, bearing in mind that in this case only the lower and the upper conditioning treatments temperatures were evaluated, a good agreement with respect to the venous lines was always achieved. Cl^- ions concentrations increase dependently from the temperature values, and since both venous and arterial lines were submitted to the same thermal and radiation treatments, it is likely that a close behavior could be assumed also when Cl^- concentration's trends along the entire temperature range are estimated.

Metals determination

Eluates collected from the blood lines after the degradation experiments were submitted to quantitative determination of Ca and Zn elements. Ca and Zn concentration always showed quite a good agreement when comparing arterial and venous lines responses. Hence, for the sake of simplicity, the example of Zn concentration will be discussed for the venous circuits. In fact, same results have been obtained for Ca concentration and, as already mentioned, for both arterial and venous lines. Figure 4 displays zinc concentration trends (values averaged on repeated measurements of replicated samples) as a function of conditioning temperatures both for not sterilized and for sterilized circuits. Whatever be the cation, the type of circuit, and even the condition of sterilization, a slight increase of concentration following increasing conditioning temperatures can be

detected. In particular, the dependence of metal concentration on temperature is more detectable for Ca than for Zn. In fact, such proportionality is partly hidden by a somehow low data precision, which was recorded for the present experimental measurements. Actually, an uncertainty for these numeric results was calculated, which was always higher than the one collected for the determination of anionic species.

Though, the most striking feature displayed by Figure 4 is the total absence of a detectable effect carried on the blood lines by the radiation sterilization, which in turn, was the largest effect evaluated in the previous case of anionic determinations. These large differences between anionic and cationic responses could be explained as follows. Since HCl is the main product of DHC of the PVC polymer, corresponding Cl^- ions concentration in aqueous solutions is likely to show a direct dependence on thermal treatments exerted either by simulated storage and transportation conditions or by β-irradiation. At the same time, also the other anionic species detected is likely to show a very similar behavior, being suspected to represent a product of the degradation (chemical, thermal, or mechanical) of one or more components of the PVC-based formulations. On the opposite, calcium and zinc are not really degradation products, since they do constitute the metallic soaps-based heat-stabilizing system of PVC resin. Hence, they can not be strictly considered direct markers of thermal degradation, in turn promoted either by severe environments or by ionizing radiations.

Ca and Zn soaps are believed to pursue their heat protection task shifting from the initial form of carboxylates to the final one of chlorides. Focusing then on a fine scale level, Zn carboxylates shall be considered as primary heat stabilizers, while Ca carboxylates play the role of HCl scavengers.[9] The differen-
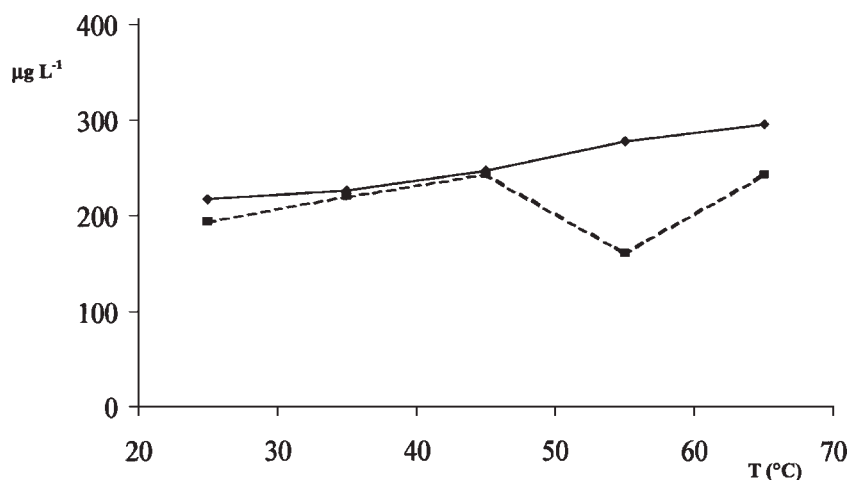

Figure 4 Zinc concentration (µg/L) as a function of the increasing conditioning temperature values (venous lines). Straight line: not sterilized samples. Dashed line: sterilized samples.

ces between the intrinsic behavior of Ca and Zn carboxylates could also account for a rationale of the more straight dependence on temperature of the concentration of the former than of the latter. As soon as Zn shifts from the form of carboxylate to that of chloride, it is restored by Ca carboxylate, which in turn changes into chloride.[38] Thus, it could be gathered that the likelihood of finding a metal ion under the form of hydrophilic chloride rather than of hydrophobic carboxylate, is higher for Ca than for Zn. Hence, such item could finally explain the concentration dependence on the temperature of the two metals, which is more straightforward for Ca than for Zn.

However, a thermal modification, rather than strictly a thermal degradation, could be described even following the evaluation of these metallic species. Ca and Zn carboxylates are not characterized by a fixed and frozen position within the structure of thermoplastic moieties in the solid state; rather, an equilibrium could be invoked, which involves the distribution of these species between the bulk and the surface of the materials with which the investigated devices are made of. Furthermore, several clues can be found in the literature, which claim that such distribution equilibrium could be strongly affected by a temperature gradient,[39,40] resulting in the migration from or towards the surfaces.

Hence, not only the complex foundings pertaining to Ca and Zn evaluation should take into account the chemistry of the carboxylates intended as single molecules, but also the just mentioned distribution equilibrium. This in turn is inclined to be displaced by the heat treatments (either thermal or radiative) imparted to the blood lines. Successively, the interpretation of the results should consider the chemical durability of the inner surfaces of the blood circuits as well. Actually, an equilibrium between a solid phase (inner walls of the blood lines) and a liquid one (recirculating solutions) is likely to be established during the 4-h-long recirculation experiment. In other words, more than, or besides, direct products of thermal degradation, presence of Ca and Zn species within the eluates could be regarded as the final synergistic effect of the instauration of a first equilibrium of distribution of the heat stabilizers within the solid matrix between its bulk and its surface, and a second one between the leaching of these species from the surfaces and their extraction carried on by the solutions. This sort of equilibrium in turn could even be displaced towards a lower chemical durability of the inner surfaces induced by thermal modifications occurred to the materials under investigation.

Charge and mass balance

pH measurements were always performed on the fresh eluates immediately after their collection from the blood lines. pH measurements showed quite a good repeatability as well as a good reproducibility, at least on the fresh eluates. A thermal degradation-dependent pH profile was displayed by all the eluates, being thus consistent with the quantitative evaluation of the anions which was described above. pH values always showed a constant decrease following the increasing embitterment of the simulated thermal degradations, both for venous and arterial lines. As a matter of fact, pH measurements ranged from values of 4.9 to $\sim$ 5.5 (averaged values), the former ones pertaining to the more severe degradation conditions, i.e., the combination of β-treatments and of the highest temperatures, and the latter describing the milder ones. It shall be pointed out here that initial values of pH, i.e., the values collected on water volumes just before their infusion into the blood lines prior to recirculations, lay around 6.2, because of the dissolution of atmospheric CO_2 into the fresh deionized water.

Values of pH were then employed for the calculation of the ionic charge and mass balance for the eluates. First of all, the evaluation of the charge and mass balance of the ions dissolved within the eluates offered a crosslinked support for a self-consistency of the various determinations of the present work. Moreover, it turned out to play a fundamental role for the quantitative determination of the anionic species which has not been identified yet. Regarding numerical values of the concentrations of Cl^-, Zn, and Ca, along with the pH values, on the basis of the mass and charge balance law, all the eluates always showed a displacement of the equilibrium towards the cationic species. The difference between cations and anions concentrations (in terms of micro-Normality, μN), could reasonably be ascribed to the presence of the unknown anionic species. Hence, the mass and charge balance within the eluates represented a valid tool for the indirect quantitative evaluation of this species. Once more being consistent with all the other determinations, the gap between cation and anion equivalents increases as simulated thermal degradation becomes more severe, both for venous and for arterial lines. Such experimental evidence implies that the release of the unidentified anionic species from the PVC-based materials grows higher as the blood lines are more stressed. As a matter of fact, the lowest concentration values of the presently unknown anion were recorded for the eluates corresponding to not sterilized blood lines treated at the lowest temperatures, and they lay at about 11 μN. On the opposite side, the highest concentration were observed for the eluates coming from sterilized blood lines treated at the highest temperatures, reaching values up to nearly three times higher, i.e., $\sim$ 32 μN.

Finally, it should be noticed that, in the same scale, the chloride ion displayed concentration val-

ues of around 1 μN and 10 μN for the milder and the more severe degradation conditions, respectively. Hence, if the unidentified anionic species bore a single negative charge or it had a molecular mass similar to the one of the chloride ion, then its real concentration in terms of $\mu g \ L^{-1}$ would result in some cases even 10 times higher for the unknown anion than for the chlorides. Though, a similar conclusion cannot be drawn, since the qualitative identification of the actual unknown species has not been accomplished yet. On the other side, it is considered more likely that the present anionic species could be an organic residue, with a molecular mass larger than the one of the chloride ion, even bearing more than a single negative charge, e.g., a conjugated base of a dicarboxylic acid.

CONCLUSIONS

High performances of PVC-based blood circuits for extracorporeal hemodialysis must be obviously ensured and guaranteed when they are performing their specific function, i.e., during dialysis treatments. Hence, tests and assays for the assessment of the quality of materials and products must be applied all over the ''life'' of a blood circuit, from the choice of the raw materials to the finished products. In particular, in this work the influences of electron beam radiation sterilization and of environmental storage conditions on the blood tubing were extensively examined.

An analytical method for the estimation of the thermal degradation effects carried on by the latter items, i.e., β-sterilization step and transportation and storage conditions, was performed.

Simulated conditions of storage and transportation resulted in very mild effects on PVC-based blood circuitries, even when *worst case* experimental conditions were applied. On the other hand, β-treatment effects are not negligible. However, an interesting detail is thought to be highlighted at this point, when bearing in mind the low absolute values detected for the Cl^- ions released by the materials and dissolved in aqueous solutions. Since an extreme situation was studied, real-case common treatments are supposed to exert much milder effects. Hence, operative conditions of β-radiation sterilization are regarded to be a safe, noninjurious, and reliable treatment with respect to the target blood circuits irradiated.

Dealing with heat stabilizers, the experimental extraction solutions were submitted to a further analysis, which consisted in the quantitative determination of Ca^{2+} and Zn^{2+} dissolved ions. From the perspective of Ca and Zn concentration measurements, a good response of blood circuits inner surfaces can be inferred, with respect both to β-treatments and *a fortiori* to transportation and storage simulated conditions.

Actually, experimental evidences claim that Cl^- ions are not the only anionic species which can be either removed or leached by an aqueous solution flowing within the lumens of the blood lines during a dialysis simulation. Such behavior would suggest that besides PVC, other chemicals involved in the materials' formulations could be inclined to thermal and chemical attacks, resulting in patterns similar to those of the resin, i.e., the release of small amounts of short residues. On the other hand, a full knowledge of the possible thermal alteration events and chemicals involved in these processes could finally result in a great improvement of the performances and of the biocompatibility of the present blood lines as well as of biomedical devices in general.

All these results, considering the materials formulations chosen in this work, seem to be perfectly suited to withstand the chemical and thermal simulated environments injuries which have been selected.

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