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ORIGINAL PAPER

Survey of chemical residues and biological evaluation of photochemically pre-vulcanized surgical gloves

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Abstract Latex allergies arise from the presence of latex proteins as well as noxious rubber additives (mainly accelerators and activators used in conventional sulfur-accelerated vulcanization processes) in medical devices (e.g., medical gloves, catheters) made from natural rubber latex. As a new approach the ultraviolet (UV) light-initiated pre-vulcanization of natural rubber latex makes efficient cross-linking feasible without using any toxic, mutagenic, or irritating chemicals. The cross-linking in the latex particles is accomplished via the thiol-ene addition reaction in the presence of a polyfunctional thiol and a photoinitiator. The new process is carried out in a falling film photoreactor on a pilot scale which provides a continuous irradiation of the latex emulsion. The UV technique is suitable for an easy up-scaling and represents the entrance into large-volume industrial production. The surgical gloves are then made by a conventional coagulant dipping process comprising good physical properties and high ageing stabilities. The aim of this study was to evaluate the biological properties and skin compatibility of UV-pre-cured gloves in skin sensitization, skin irritation studies, and cytotoxic tests. In addition the biologically available chemical residues in the gloves were characterized by UV–visible spectroscopy, elementary analyses, and high-performance liquid chromatography coupled with mass spectroscopy. The results of the studies revealed that UV-cross-linked surgical gloves exhibit good skin compatibility together with low cytotoxicity and residual chemical levels in the range of 60 and 120 $\mu g/g_{glove}$.

Keywords Chemical residues · Extraction · Latex allergy · Material science · Photochemistry · Surgical gloves

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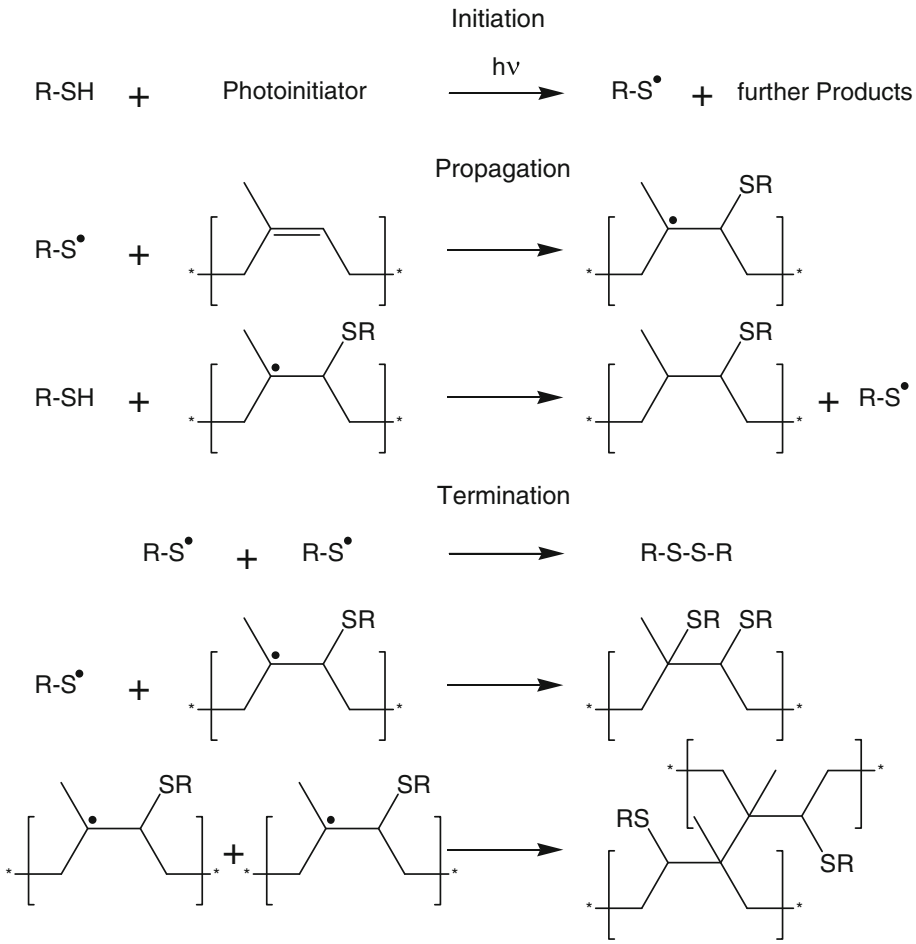
Introduction

Natural rubber (NR) latex is used in a wide variety of medical devices such as catheters, bandages, or surgical and examination gloves. However, the unique physical properties of dipped NR latex articles such as high elasticity, strength, and recovery are faced with the rising emergence of latex allergies. Latex allergy was first recognized in the late 1970s and since then has become a serious operational health problem not only among operating room personnel and health-care workers but also janitorial staff and rubber industry workers [1–4].

Allergies to NR latex products involve the type I latex hypersensitivity which is an immediate hypersensitivity caused by natural rubber proteins from *Hevea brasiliensis* and the type IV latex hypersensitivity, a delayed contact dermatitis to rubber additives. The contact allergy typically occurs in two phases, 24–96 h after exposure. In the first step, the induction phase, antigen-specific T cells are developed which are directed to the antigen exposure. During the second step, the elicitation phase, the allergic contact dermatitis is exhibited when reexposure to the antigen takes place [5–7].

Since the 1920s, accelerators such as dithiocarbamates and thiurams have been used in the sulfur-vulcanization

Scheme 1



processes to lower the reaction temperature and to reduce the reaction time. According to Depree et al. about 2.5% of the health-care workers worldwide are sensitized to noxious accelerators such as thiuram and dithiocarbamate derivatives or 2-mercaptobenzothiazole [8–11]. The vulcanization, i.e., the cross-linking of the free polymer chains, is important for the final properties of the rubber products. As a result of the formation of a three-dimensional polymer network, increased tensile strength, elasticity, and ageing stability can be obtained. Because of the risk of type IV allergies caused by residual accelerator levels in NR latex devices, alternative curing methods have been developed. However, cross-linking with peroxides or electron beam radiation suffers from other significant drawbacks such as poor ageing stability, high production costs, and operational safety concerns [12–16]. Recent work has further shown that a photochemical cross-linking of epoxidized NR latex in the presence of a photoinitiator and acrylates can be accomplished on a laboratory scale [17].

Taking all these factors into account, we have developed a new cross-linking process for NR latex based upon a UV-initiated thiol-ene reaction. The application of thiol-ene polymers and thiol-ene-based networks in the field of medical devices including dental and bone cements has already been reported in previous publications [18–21].

The new technology is particularly aimed at the substitution of skin sensitizing and noxious processing chemicals (e.g., acrylates, rubber accelerators) in the manufacture of surgical NR latex gloves [22, 23].

The mechanism of the thiol-ene reaction is provided in Scheme 1. In the initiation step a photoinitiator present in the latex matrix is excited by UV light which is followed by a bond cleavage to yield free radicals. In the presence of thiols, there is sufficient hydrogen transfer from the thiol to the free photoinitiator radicals resulting in the formation of thiyl radicals. Once formed the thiyl radicals are capable of reacting with the C=C double bonds in the polyisoprene generating thioethers. The termination reactions involve radical coupling leading to disulfides, thioethers, and covalent carbon–carbon bonds. By using multifunctional thiols, cross-linking is accomplished by this reaction [24].

As a result of the low transmissivity of latex the UV-initiated pre-vulcanization is carried out in a falling film photoreactor on a pilot plant scale. The concept of a falling film ensures a homogenous and continuous UV irradiation

of the latex formulation in thin films (<5 mm film thickness). The process is distinguished by its low energy consumption, making pre-vulcanization within minutes at room temperature feasible, whereas conventional sulfur pre-vulcanization techniques require several hours at 50–80 °C. UV-pre-cured surgical gloves are then produced by a conventional coagulant dipping process on a semi-industrial production line. The gloves have good physical properties and high ageing stabilities clearly exceeding the quality requirements of EN 455-2 (2000) and ASTM D 3577 [25, 26].

The present work is focused on the evaluation of the skin and biocompatibility of UV-pre-vulcanized surgical gloves by acute dermal irritation studies with rabbits, a nine-dose Buehler test, and cytotoxicity tests in compliance with ISO standards. In addition to these dermatological studies the extractable and bioavailable amount of cross-link chemical residues (photoinitiator and thiol) in UV-pre-cured latex gloves was investigated with analytical techniques such as high-performance liquid chromatography coupled with mass spectroscopy (HPLC–MS), ultraviolet–visible (UV–Vis) spectroscopy, and elementary analyses.

Results and discussion

The photochemical pre-vulcanization of natural rubber latex is aimed at the manufacture of medical gloves, especially surgical gloves, without using any conventional sulfur processing agents such as activators or accelerators. Therefore, allergic contact dermatitis associated with accelerators such as dithiocarbamates, thiurams, or mercaptobenzothiazole can be avoided.

When introducing a new technology and material for biomedical application, key properties such as biocompatibility, sterilizability, processibility, and costs have to be considered to ensure the safety of the users (e.g., healthcare workers) and patients. In our previous work it was shown that the physical properties of sterile UV-pre-cured gloves meet the quality requirements demanded in the production of surgical gloves [25, 26]. In addition the UV process is distinguished by its low energy consumption providing low production costs. However, probably the most important characteristic is the biocompatibility of the device. The biological testing involves skin sensitization, irritation studies, and cytotoxicity tests. Furthermore, it is important to assess the identity and levels of any processing substances that may leach out of the material during use.

According to their material safety data sheets (MSDS) the chemicals (photoinitiator and thiol cross-linker) used in the UV pre-vulcanization are not carcinogenic, mutagenic, or toxic, and do not cause skin sensitization. As a result of their high solubility in acetone the chemical levels obtained with the Soxhlet extraction procedure may be considerably higher compared to the bioavailable amount arising from skin contact. In that case the extraction procedure does not fully reflect the bioavailable levels of chemical residues but can give evidence of the free, not covalently bound cross-linking chemicals. To avoid oxidation reactions (e.g., formation of polysulfides) the extraction was carried out under inert conditions.

For the quantitative detection of the residual TriThiol levels in UV-pre-cured gloves UV–Vis spectroscopy was used. The thiol moieties were derivatized with 4,4′-dithiopyridine to give disulfides and a thione anion which can be detected at 368 nm in acetonitrile. As a result of the high absorbance coefficient of the thione anion the method has a detection limit down to 5 nmol/dm^3 in plasma [27].

Elementary analysis was further used to analyze the total sulfur content, represented by the free and oxidized thiol groups of the TriThiol, in the glove extracts. In both analytical methods, one must consider that NR latex contains proteins with thiol groups, and other sulfur moieties. In addition a reference extraction of non-cross-linked NR latex gloves containing no rubber additives was carried out. The total sulfur and TriThiol content of the reference glove extract obtained by UV–Vis spectroscopy and elementary analysis was then subtracted from the analyte concentrations of the cross-linked glove extracts. The extractable chemical levels of non-cross-linked gloves and UV-pre-vulcanized gloves are summarized in Table 1.

The spectroscopic results revealed that the TriThiol residues in UV-cross-linked surgical gloves do not exceed 60 $\mu g/g_{glove}$. However, from the total sulfur content determined by elementary analyses a residual TriThiol level of 120 $\mu g/g_{glove}$ was calculated. The difference between the TriThiol concentrations obtained from both analytical methods gives an indication that polysulfides are generated due to the UV cross-linking and dipping process. The lower amount of free thiol groups leads then to an apparently lower analyte concentration in the UV–Vis spectroscopy because the sulfide moieties cannot be detected with this method.

Table 1 Quantification of extractable rubber chemicals by UV–Vis spectroscopy, elementary analysis, and HPLC–MS

Glove type	TriThiol ($\mu g/g_{glove}$)		Lucirin TPO L($\mu g/g_{glove}$)
	UV–Vis analysis	Elementary analysis	HPLC–MS
Non-cross-linked glove	0.6	0.6	–
UV-pre-vulcanized surgical glove	60	120	60

In our study HPLC–MS was used for the quantitative determination of the photoinitiator levels in the glove extracts. It was found that 60 $\mu g/g_{glove}$ Lucirin TPO L can be extracted from UV-pre-cured NR latex gloves. Work is still ongoing to get further information on the cleavage products of the photoinitiator and will be further discussed in another paper (in preparation).

Although the extractable chemical levels are far lower compared to residual accelerator levels found in sulfur-cured commercial surgical gloves [8], a further reduction of bioavailable thiol and photoinitiator concentrations is the aim of further studies. The variation of leaching parameters such as leaching time and temperature in wet-gel leaching and post-manufacture washing processes may be one approach to efficiently reduce the chemical levels in UV-cured gloves. In addition, it has to be considered that due to the high solubility of the chemicals in acetone the bio-available amount of the residual chemical is not reflected by a 24-h Soxhlet extraction using acetone as solvent.

To assess the biocompatibility the UV-cured surgical gloves were evaluated by irritation studies and skin sensitization tests in compliance with ISO standards. During the skin irritation study no symptoms of systemic toxicity in the animals were observed and no mortality occurred. The skin examination of the animals revealed that all areas treated with the test material and the negative control and all control areas were normal before the application and at each observation time. No erythema, eschar, or edema formation was observed. The calculated primary irritation index was 0.0, classifying the UV-cross-linked glove as non-irritant to skin. The results of the nine-induction-dose Buehler test were decisive in grading the potential skin sensitization. In this study the areas treated with test material were normal for all animals of the negative control group and the test material group. No test animal was regarded as irritated or sensitized. According to the results of the skin sensitization test, UV-pre-vulcanized surgical gloves are considered non-irritant and non-sensitizing under the test conditions of this study. Because of the results of the nine-induction-dose Buehler test, and Directive 2001/59/EC, UV-cured surgical gloves do not need to be labeled with "R43 may cause sensitization by skin contact".

The toxicity of medical gloves made from natural rubber latex has already been reported in previous studies [28, 29]. It was shown that dithiocarbamate- as well as mercaptobenzothiazole-type accelerators used in the sulfur vulcanization exhibit strong cytotoxicity depending on the residual levels in the gloves. In the field of radiation cross-linking it was further demonstrated that gamma-cross-linked NR materials exhibit a lower toxicity than conventional sulfur-vulcanized devices. It has to be further considered that not only the rubber additives but also the raw material itself, the natural rubber, harbors a cytotoxic potential. According to standardized procedure and also in the present cytotoxicity test, non-cross-linked natural rubber not containing any rubber chemicals is used as positive control. The scores of the control samples and the glove extract are provided in Table 2. Herein, the results of the positive and negative control demonstrate the sensitivity of the cells.

The full strength extracts of UV-cross-linked gloves exhibit a mild cytotoxicity, whereas the non-cross-linked positive control causes severe cytotoxic reactions. The considerable reduction of potential cytotoxicity can be explained by the cross-linking, leaching, and sterilization processes the UV-pre-cured glove was undergoing. Natural rubber latex proteins which exhibit cytotoxic reactions are removed from the gloves during the hot water leaching. In addition UV and gamma-irradiation cause a degeneration of the residual proteins leading to a further reduction of the protein levels.

With respect to the cytotoxicity of the glove extract, visual examinations of the cells under the microscope showed that they were only occasionally lysed with cell medium at a dilution of 1:2 indicating a slight cytotoxicity. In dilutions of 1:4 and above, the cells had normal morphology, were able to reattach onto the tissue culture plate, and showed the same cell growth as non-treated cells of the negative control sample.

UV-cured surgical gloves cannot be considered biocompatible based only on in vitro cells but the low potential

Table 2 Evaluation of the cytotoxicity of UV-cross-linked surgical gloves (pre-cured with 1.0 phr (parts per hundred of rubber) Lucirin TPO L and 1.0 phr TriThiol)

Sample	Dilution in complete cell medium	Score sample 1	Score sample 2	Score sample 3	Average
Negative control	Original extract	0	0	0	0
Media control	Original extract	0	0	0	0
Positive control	Original extract	4	4	4	4
UV-pre-cured surgical glove	Original extract	2	2	2	2
	1:2	1	1	0	1
	1:4	0	0	0	0
	1:8	0	0	0	0
	1:16	0	0	0	0

cytotoxicity together with the good skin compatibility and low chemical residues provides evidence of considerable advantages against conventional sulfur-cured medical devices.

Experimental

Materials and chemicals

Natural concentrated rubber latex (high ammonia, 60 wt% dry rubbers content) was purchased from a Malaysian supplier. The photoinitiator ethyl-2,4,6-trimethylbenzoyl-phenylphosphinate (Lucirin TPO L) was obtained from BASF. The cross-linking agent trimethylolpropane tris(3-mercaptopropionate) (TriThiol) was from Bruno Bock Thiochemicals. Acetone, acetonitrile, and dichloromethane were used in analytical grade and were supplied by Sigma–Aldrich. For HPLC–MS analyses water and acetonitrile of gradient grade from Acros Organics (Geel, Belgium) were used. All other reagents were from Sigma–Aldrich and were used without further purification.

TriThiol working standard solutions were prepared by the appropriate dilution of a stock solution (538 μg/cm^3) containing 1,000 μg/cm^3 triethylamine in acetonitrile to yield final concentrations of 5, 12, 25, 38, and 71 μg/cm^3.

Manufacture of UV-cross-linked surgical gloves

In the first step of the pre-vulcanization process Lucirin TPO L as photoinitiator and TriThiol as cross-linking agent were emulsified in deionized water. The emulsion of the chemicals was added to 40 kg high-ammonia natural rubber latex (40 wt% dry rubber content) and stirred by means of a magnetic agitator in a storage vessel at room temperature for 2 h. In this latex formulation the concentration of Lucirin TPO L and TriThiol reached 1.0 phr (parts per hundred of rubber). The UV pre-vulcanization of the latex formulation was carried out in a tailor-made falling film reactor comprising a reactor tube with an inner diameter of 135 mm (Fig. 1). The UV lamp was a medium pressure Hg lamp (Heraeus) with an arc length of 25 cm and arranged centrically in the reactor tube. An eccentric screw pump conveyed the NR latex formulation continuously from the storage vessel to the top of the falling film reactor at a rate of 1.3 dm^3/min. A continuous falling film was obtained with a film thickness of less than 5.0 mm. The falling film was UV irradiated with a light intensity of 1.1 W/cm^2 comprising wavelengths between 240 and 460 nm. After the pre-curing in the falling film reactor 0.5 phr phenolic antioxidant was added to the pre-vulcanized NR latex.

From this latex, surgical gloves were produced on a semi-industrial scale using a tailor-made dipping machine.

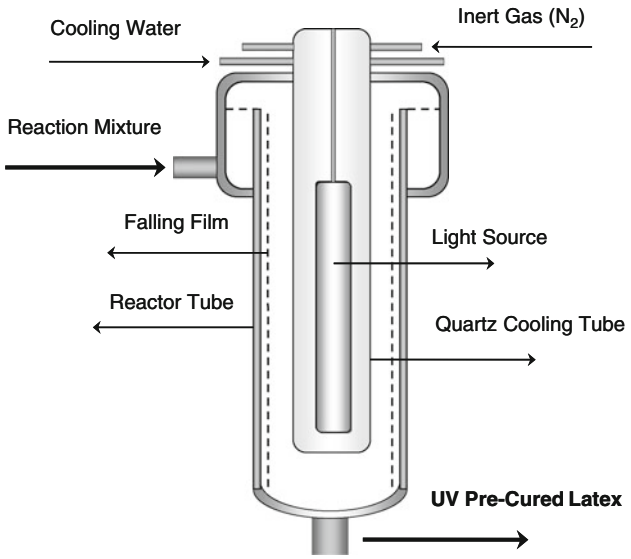


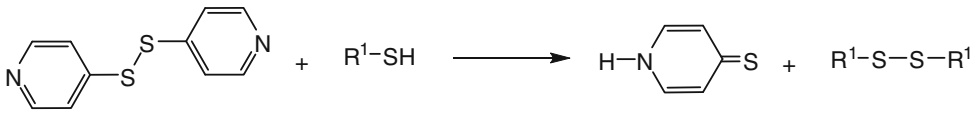
Fig. 1 Concept of a falling film photoreactor used for the photochemical pre-vulcanization of NR latex on technical scale

The porcelain hand formers, on which the latex films were shaped, were cleaned with acid and alkaline solutions. After neutralizing the formers in hot water they were dried in an oven. In the next step the formers were lowered into a coagulant solution containing calcium salts (coagulant), calcium carbonate (release agent), and surfactants. The formers were heated to dry the coagulant and were then immersed in the UV-pre-cured NR latex. The thickness of the latex gloves obtained from coagulant dipping ranged from 0.2 to 0.3 mm. After withdrawal of the formers from the pre-cured latex, the gloves were dried to ensure sufficient wet-gel strength before the leaching processes were carried out. In the next step the gloves were dipped in hot water (leaching) to remove residual processing chemicals and to lower the protein levels in the final products. After packaging the surgical gloves were sterilized by using gamma rays from a ^{60}Co source (25-kGy nominal dose).

Soxhlet extraction procedures

The UV-cured surgical gloves were extracted to determine the residual TriThiol and photoinitiator levels and the total sulfur content, respectively. In these studies surgical gloves pre-vulcanized with 1.0 phr Lucirin TPO L and 1.0 phr TriThiol were characterized. In addition a reference Soxhlet extraction was carried out to estimate the total sulfur content and the thiol residues in non-cross-linked latex gloves not containing any cross-linking agents or other rubber additives. Each sample was weighed before being cut into approximately 1 × 1-cm pieces with a pair of scissors. Sample portions of about 15 g were placed in

Scheme 2



Soxhlet cellulose cartridges and the extraction was carried out by using 200 cm^3 acetone (HPLC grade). The solvent was refluxed for 24 h under inert conditions. The solution was filtered through a microfilter with PTFE membrane (0.45-μm pore size) and the extracts were concentrated on a rotary evaporator (40 °C, 450 mbar). The extract was vacuum dried at 40 °C to constant mass, weighed, and stored at 8 °C for eventual analysis.

HPLC–MS determination of Lucirin TPO L levels

Chromatography was carried out on the Thermo Electron LCQ Advantage MAX system equipped with vacuum degasser, quaternary pump, autosampler (20-mm^3 sample loop), and UV–Vis diode array detector (all from Thermo Electron Corporation, Waltham, MA, USA). MS detection was performed on the Thermo Electron LCQ Advantage MAX LC–MS/MS Ion Trap mass spectrometer equipped with an ESI (electrospray ionization) source. The separation column was a Hypersil Gold C18 (150 × 2.1-mm ID, 3-μm particle size) obtained from Thermo Electron.

A 10-mm^3 aliquot of each sample was injected into the HPLC–MS system. The mobile phase consisted of a gradient of water and acetonitrile. After 11 min of isocratic elution at 50% acetonitrile (elution of Lucirin TPO L), the gradient was run to 100% acetonitrile within 1 min and kept at this level for 13 min to ensure complete elution of residues of the Soxhlet extract. The column was maintained at 30 °C and the flow rate was set to 200 mm^3/min.

ESI detection was carried out in positive mode. No split was applied and the total flow of 200 mm^3/min was introduced into the MS interface. Nitrogen was used as nebulizer gas and as drying gas. The spray voltage was set to 6,500 V, the capillary voltage to 5 V, and the capillary temperature to 200 °C. The tube lens offset was set to 40 V.

All analytes were dissolved in acetonitrile (gradient grade) giving 10 mg/100 cm^3 stock solutions, kept in the dark at room temperature, and filtered through a 0.2-μm PTFE filter prior to injection.

Calibration of the UV–Vis detector was performed with varying injected masses of Lucirin TPO L in the range of 0.1 and 1.0 μg. A strictly linear relationship was obtained between injected mass and the corresponding peak area with a regression coefficient of $r^2 = 0.99975$. Identification of Lucirin TPO L in the Soxhlet extract could be ensured by the aid of its MS signal of $m/z = 317$.

UV–Vis spectroscopic determination of TriThiol levels

UV–Vis spectroscopy was carried out on a Biorad Jasco V-530 spectrophotometer with a wavelength range between 190 and 1,100 nm. The dried extract together with triethylamine (1,000 μg/cm^3) was diluted in 200 cm^3 acetonitrile. A 1-cm^3 aliquot of this solution was taken and further diluted with an acetonitrile solution containing 1,000 μg/cm^3 triethylamine in a ratio of 1:100 prior to the derivatization. Aliquots (2 cm^3) of this solution were treated with 100 mm^3 of a 4,4′-dithiopyridine solution (5,000 μg/cm^3 in acetonitrile). The derivatization of the thiol groups with 4,4′-dithiopyridine yields a thione anion, a chromophore which shows a strong absorbance at 368 nm in acetonitrile. The reaction mechanism is displayed in Scheme 2.

After 15 min at room temperature 100 mm^3 of concentrated acetic acid was added to stop the reaction and the absorbance of the derivatized solutions were then measured. Aliquots (2 cm^3) of the standard solutions were treated and measured in an analogous way. A reference cell was loaded with acetonitrile to eliminate any signals from the solvent itself. Furthermore the absorbance of a solution containing the reactants (4,4′-dithiopyridine, triethylamine, and acetic acid) but not the analyte (TriThiol) was determined and subtracted from the absorbance of the derivatized standard and extract solutions.

Elementary analysis determination of TriThiol levels

The total sulfur content of the glove extracts was determined by elementary analysis (C, H, N, S) using an EA 1108 CHNS-O elemental analyzer by Carlo Erba Instruments. The determination limit for sulfur with sample amounts of 2–3 mg was about 0.05 wt% with an uncertainty below 0.02 wt%. The measurements were carried out at the Microanalytical Laboratory, University of Vienna.

Acute dermal irritation studies with rabbits

The aim of this study was to examine the possible irritation and corrosion by the UV-pre-cured glove following a single application to the intact skin of rabbits. The study was conducted in conformance with ISO 10993-10 (Biological evaluation of medical devices-Part 10: Tests for irritation and delayed-type hypersensitivity, 2002) at the Austrian Institute of Technologies (AIT Seibersdorf).

In brief, the hair of three female rabbits (New Zealand White from Charles River Deutschland GmbH, D-97633 Sulzfeld) with body weights between 2.1 and 2.3 kg were clipped on the dorsal areas of the trunk 1 day before the application of the test material. Two plates of the UV-cured glove and two plates of the negative control (cellulose patches obtained from Fa. Hartmann, A-2355 Wiener Neudorf) with a size of 2.5 × 2.5 cm each and soaked with 1 cm^3 deionized water were placed on cellulose patches and were applied to two test sites each. After an exposure of 4 h the samples were removed. The skin of the animals was examined for local alterations 1 day before the administration of the test article and immediately before the administration using a cold light source KL 1500 electronic. The treated areas of the animals were examined 1, 24, 48, and 78 h after patch removal. Dermal alterations were scored and recorded by using the primary irritation scores.

Skin sensitization studies: nine-induction-dose Buehler test

The skin sensitization test according to E. V. Buehler was performed in conformance with ISO 10993-10 (Biological evaluation of medical devices-Part 10: Tests for irritation and delayed-type hypersensitivity, 2002).

In brief, 10 test guinea pigs (Dunkin Hartley from Harlan Winkelmann GmbH D-33176 Borchen), the test material group, were patched with the test material (2 × 2 cm^2) on the left flank and five test animals were patched with the negative control blank (lint patches obtained from Fa. Hartmann, A-2355 Wiener Neudorf) on the left flank. The patches were removed after 6 h of exposure and the skin of the animals was examined after a rest period of 24 h. This procedure was repeated three times per week for 3 weeks for a total of nine epicutaneous exposures. The challenge exposure was carried out after a rest period of 2 weeks. Therefore, the right flanks of the test material group and the control group were patched with the test material which was removed after 6 h of exposure. The skin of the test animals was observed 24 and 48 h after patch removal. Each animal was assessed for a sensitization response and the skin reactions were compared between the test material group and the negative control group.

Cytotoxicological studies

The in vitro biocompatibility of UV-pre-vulcanized gloves was elevated on the basis of cell cytotoxicity. The cytotoxic tests were preformed in accordance with ISO 10993-5 (Biological evaluation of medical devices-Part 5: Test for in vitro cytotoxicity, 1999) and were carried out at Nelson Laboratories (Salt Lake City, USA).

Table 3 Scoring scheme for cytotoxic reactions

Score	Reactivity	Description of reactivity zone
0	None	Discrete intracytoplasmic granules, no cell lysis
1	Slight	Not more than 20% of the cells are rounded, loosely attached, and without intracytoplasmic granules; occasional lysed cells are present
2	Mild	Not more than 50% of the cells are rounded and devoid of intracytoplasmic granules; no extensive cell lysis and empty areas between cells
3	Moderate	Not more than 70% of the cells are rounded and/or lysed
4	Severe	Nearly complete destruction of the cells

In brief, the sterile UV-cured glove was incubated with serum containing tissue culture for 24 h at 37 °C. The ratio of test article to solvent was 60 cm^2/20 cm^3. These extracts were tested undiluted and in dilutions with tissue culture medium. The extracts and dilutions of them were incubated for 24 h with freshly trypsinised mouse heteroploid connective tissue cells (L-929). The cytotoxic action was determined by visual examination of the cells, by trypsination of the cells, and testing their ability to reattach and to grow. The morphology of the cells was examined under a microscope using cytochemical stains if needed, scored on a relative scale of 0–4 (see Table 3), and the average out of three wells was calculated.

Samples with complete cell medium served as media control. The positive control was 4 g of natural rubber latex tubing per 20 cm^3 extraction medium and the negative control 4 g of polypropylene pellets per 20 cm^3 extraction medium.

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