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SKLM

Statement on the Treatment of Food using a Pulsed Electric Field

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The working group "Food technology and safety" of the DFG Senate Commission on Food Safety (SKLM) discussed the food safety aspects associated with new technologies that are being developed and used for processing food. One of these newly developed processes uses a pulsed electric field, which is currently being used only on a pilot-scale in Europe. Key objectives of this treatment include the gentle and efficient extraction of cellular constituents as well as gentle pasteurisation of fruit juices. However, the associated electrochemical processes are still insufficiently understood. The SKLM issued the following opinion on 13th March 2007, the English version was agreed on 30th March 2008..

Statement on the Treatment of Food using a Pulsed Electric Field

1 Introduction

Processing with a pulsed electric field (PEF) is a new method in food technology still being in the testing and pilot stage. It is used for inactivation of microorganisms in food or for the efficient extraction of cellular constituents.

PEF-treated food products may only be placed on the market in the European Union (EU), after having examined whether they fall within the scope of Regulation (EC) No. 258/97 [1] concerning novel foods and novel food ingredients that came into force on 15th May 1997. To be considered as novel - and thus subject to authorisation according to Article 4 of Regulation (EC) No. 258/97 - are

"Foods and food ingredients to which has been applied a production process not currently used, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism or level of undesirable substances."

Placing on the market of high pressure-treated food had already demonstrated the necessity of extensive investigations in order to decide whether the thus-treated products fall within the scope of Regulation (EC) No. 258/97 [2].

If the PEF-process leads to significant changes with effects on nutritional value, metabolism or levels of undesirable substances in the food, a safety assessment must be carried out as part of the authorisation procedure in accordance with Regulation (EC) No. 258/97 (see ANNEX 1).

No PEF-treated foods have been authorised so far in the EU. Initial trials on the pilot scale have demonstrated prospects for possible applications, e.g. gentle pasteurisation of fruit juices or increases in product yields (juices, sugar extraction, oil yield) [3-6].

2 Process engineering aspects

Pulsed electric field processing involves a sudden discharge of a capacitor to generate high-voltage pulses, which are transmitted to the food via electrodes. The configuration and geometry of the electrodes determine the course of the field lines and thus the homogeneity of the electric field. In cases of electrodes that are in direct contact with the food being treated, the selection of the electrode material and/or its coating is of considerable significance [7]. However, the specifications given on the design of different installations and the process parameters used are generally insufficient to obtain reliable information allowing the assessment of literature data [8, 9]. Such parameters include the electric field strength, the pulse shape, pulse duration, number of pulses and the repetition rate of the specific energy input as well as heating of the product and temperature distribution within the treatment cell. Additionally, in many cases it is not possible to differentiate between electrochemical effects and local thermal effects. The electrical conductivity of the product to be treated is also affected by the field distribution, which is influenced e.g. by the liberation of dissolved gases [10] or by the product's composition and consistency [11]. According to existing studies, the electric field strength as well as the specific energy input in combination with the process temperature appear to be suitable parameters in a comparative evaluation of the treatment intensity of various PEF processes. (Process engineering aspects, see ANNEX 2)

3 Microbiological aspects

The inactivation kinetics have been studied for a wide range of microorganisms in various products. Vegetative cells can be inactivated by high-voltage pulses, whereas spores remain virtually unaffected [4]. The geometry of microorganism cells influences the inactivation behaviour: smaller cell diameters exhibit a greater resistance [9; 11]. The results indicate that the inactivation mechanisms are essentially membrane-mediated.

An electromechanical model has been used to describe the processes occurring in the microorganism cells during PEF-treatment. Detailed models of electropermeabilisation also account for factors such as enlargement of already existing membrane defects and opening of protein channels by the pulsed electric field respectively. It is assumed that an initial reversible induction of pores is promoted by already existing statistically distributed membrane defects. In a second step, irreversible pores arise, which are stabilised, thus causing a permanent loss of semi-permeability of the cell membrane. Such reorganisation and permeabilisation of the membrane can take up to 20 seconds and leads to a loss of cell vitality [12-16].

Fractions of vegetative cells resistant to PEF have not yet been observed; however, sub-lethal damages have been described in single cases. The combination of PEF with other processes/process parameters can be used to achieve synergistic effects on the inactivation of microorganisms [3, 17-20].

In contrast to the electroporation process used in molecular biology for transferring genetic material into cells, a PEF-mediated transformation of vegetative microorganisms in foods is unlikely. On the one hand, the density of competent cells is too low, and on the other, there is hardly any replicative DNA present. Furthermore, there is no selection pressure. Transformation of plant or animal cells in food is not relevant on account of their low survival rate or lack of regenerability.

4 Chemical aspects: Modifications of food constituents

To date, only few results of impact studies on high-voltage pulses on food constituents are available. PEF-processes are accompanied by electro-chemical reactions/conversions and electrolysis of water. The resulting direct/indirect changes may influence the composition of the food and thus its quality. However, the occurrence of undesirable chemical reactions cannot be excluded, particularly in cases of adverse processing conditions (see Annex 3).

Thus, as a matter of principle, the intense electric fields occurring during PEF-treatment are expected to produce reactive decomposition products of water as well as reactive oxygen species [21]. Furthermore, direct or indirect formation or release of toxic substances from electrode material is possible. Impairment of food quality, e.g. by degradation of quality determining constituents or by formation of undesirable substances cannot be excluded.

Initial investigations on the modification of quality determining constituents and of sensory properties by PEF-treatment are available for fruit and vegetable juices. PEF-treated orange juice contained higher concentrations of flavors and vitamin C compared to heat-treated juice [22]. In contrast, carotinoid and flavanone concentrations in orange juice were not affected by PEF [23]. Improved colour and sensory properties have been described for tomato juice treated with PEF compared to a heat treatment [24]. Apple juice, which had been subjected to pulsed electric fields during production, showed no differences compared with conventionally produced juices with respect to the investigated quality parameters, such as phenol concentration, total acid concentration and cloud stability [25]. Substantial equivalence, as defined in Regulation (EC) 258/97, can be assumed.

Results on the inactivation of *enzymes* by PEF are inconsistent [16, 26-33]. The causes for enzyme inactivation are not yet understood. An effect of external electric fields on the protein structure as well as electrochemical reactions are being discussed. A 62% reduction of a *Bacillus subtilis* protease activity in simulated milk ultrafiltrate by PEF was observed [34]. In contrast, the activities of lipoxygenase, polyphenol oxidase, pectin methyl esterase and peroxidase at room temperature were only slightly reduced by PEF-treatment [35]. Inactivation of these enzymes observed in liquid milk products, fruit juices and vegetable juices was attributed to thermal effects [36].

It has been shown that PEF-treatment did not significantly change the gelling properties of chicken protein. Also, PEF-treatment did not cause aggregation or unfolding of ß-lactoglobulin and ovalbumin [37].

To date, there have only been a few investigations on the shelf-life of PEF-treated foods [24, 38].

5 Allergenicity aspects

The allergenicity of food constituents can be influenced by technological processes: it is frequently lowered and rarely increased [39, 40]. Heat treatment generally leads to significantly greater modifications of food constituents than PEF-processes [41, 42]. No increase in allergenicity due to processing procedures was found in foods with a high proportion of water, e.g. fruit and vegetables. On the assumption that, compared to cooking, the PEF process leads to relatively insignificant changes in food proteins, its influence on the allergenicity can be expected to be slight, at most. Studies carried out on celery point in this direction. The allergenic potential of celery after treatment with high-voltage pulses lay between that of untreated raw samples and that of samples that had been boiled or cooked in the microwave [43].

If cells are not lethally injured by the PEF-process, a stress-induced production of secondary metabolites and pathogenesis-related proteins (PR) by the defence system of the plants cannot be excluded [44]. Since PR proteins are of high allergenic potential, the process must be designed in a way to avoid their formation as a result of PEF-treatment .

However, given the lack of scientifically substantiated studies, general statements on the modulation of the allergenic potential by PEF-treatment are not possible at present.

6 Safety aspects / evaluation criteria

At present, the knowledge of the implications of the PEF-process on different food matrices is insufficient to perform a general safety assessment of the process. Therefore, products or product groups treated by the PEF-process must be assessed on a case-by-case basis.

The technical parameters of the PEF-process must be described in a way that a reliable assessment of the product safety of the treated foods is possible. On the one hand, this applies to the process itself (electric field strength, specific energy input, duration, temperature, pH, design of the high-voltage cell, etc.). On the other hand, the most complete profile possible must be developed regarding PEF-induced chemical/biochemical/microbiological changes in the food to allow a scientific comparison with traditional and/or authorised food treatment processes.

In particular, studies should focus on the extent of detrimental process-related changes caused by electrolysis of water or by generation of reactive oxygen or other species.
Further issues that should be addressed include possible impacts on the allergenicity of foods and undesirable effects on proteins in terms of generation/stabilisation of proteolysis-resistant conformations.

7 Final comments

The PEF-process promotes permeabilisation of cell membranes thus allowing the efficient extraction of cellular contents and the inactivation of microorganisms in food. This opens up new possibilities with respect to utilisation of raw materials and reduction of microbial contaminants.

The number of substantiated studies is limited in all areas, and a consistent evaluation of the PEF-process is hindered due to the lack of standardisation of process parameters. Development of criteria to assess the process or PEF-treated foods requires, among other things, characterisation of suitable indicator substances and measuring parameters. According to the assessment criteria given in Chapt. 6, products treated with the PEF-process require a case-by case assessment.

8 Literature

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ANNEX 1

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Legal stipulations

Before food products that have been treated by the PEF process can be placed on the market in the European Union (EU), they must be examined as to whether they fall within the scope of Regulation (EC) No. 258/97 concerning novel foods and novel food ingredients that came into force on 15 May 1997. Foods that are to be considered as being novel - and which are thus subject to an authorisation according to Article 4 of Regulation (EC) No. 258/97 - are *"foods and food ingredients to which has been applied a production process not currently used, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism or level of undesirable substances."*

Placing on the market of food treated by high-pressure had already demonstrated that extensive investigations are necessary in order to decide whether the thus-treated products fall within the scope of Regulation (EC) No. 258/97 [2].

If the corresponding investigations prove that application of the PEF treatment causes only slight changes in the composition or structure of a food and if these changes do not have a negative impact on the nutritional value, metabolism or level of undesirable substances, then such foods will not be regarded as being novel within the purposes of Regulation (EC) No. 258/97 and thus will not be subject to authorisation or labelling requirements.

In case the PEF process leads to significant changes with effects on the nutritional value, metabolism or levels of undesirable substances in the food, the results of the investigations can be used as a basis for the authorisation procedure according to Regulation (EC) No. 258/97.

At present, the knowledge of the implications of the PEF process on different food matrices is insufficient to perform a general safety assessment of the process. This means that products or product groups treated by the PEF process must be assessed on a case-by-case basis.

Guidelines for the use of the PEF process and for the necessary safety assessments should be formulated.

Assessment criteria for applications under Regulation (EC) No. 258/97

The recommendations of the European Commission concerning the scientific aspects and presentation of information required for supporting applications under Regulation (EC) No. 258/97 can be used as an orientation guide with respect to assessment criteria. Accordingly, for the safety assessment of foods produced with a non-conventional process and which are regarded as novel under Regulation (EC) No. 258/97, information and investigations of the following aspects are considered necessary:

- *Specification of the novel food (NF)*
- *Effect of the production process applied to the NF*
- *History of the organism used as the source of the NF*
- *Anticipated intake/extent of use of the NF*
- *Information on previous human exposure to the NF or its source*
- *Nutritional information on the NF*
- *Microbiological information on the NF*
- *Toxicological information on the NF*

Literature

1 Regulation (EC) No. 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. Official Journal of the European Communities No. L 43/1, 14.2.973.

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ANNEX 2

Background paper on the process engineering aspects

Stefan Töpfl[1]/ Volker Heinz[2]

Generation of high-voltage pulses

High-voltage pulses are usually produced by a generator with capacitative storage of the necessary electrical energy. This unit essentially consists of the following components: a high-voltage charger to supply electrical energy, an energy storage capacitor and a high-voltage switch that transfers the stored energy via a protective resistor into the treatment cell and thus to the material being treated.

When the switch is open, the capacitors are charged via the charging resistor R_{charge} up to voltage U_0. When the switch closes, the electrical energy Q_0 is discharged into the discharge circuit. The time profile of the pulse is primarily determined by the capacitance (C), the inductance (L), as well as the ohmic resistance of the conductors (R_{conn}), of the protective resistor (R_{prot}) and of the treatment cell (R_{TC}). Depending on the required pulse shape (exponential/rectangular), different high-voltage switches can be installed to repetitively discharge the energy storage capacitor. Possible switch designs include spark discharge or switch systems based on a tube (thyratron, ignitron etc.) as well as thyristor-based semiconductor switches. Generation of pulses with a rectangular shape requires a semiconductor switch (IGBT, closing and opening function) or a pulse-forming network whereas the other types can be used to generate pulses with an exponential decay. Furthermore, the type of selected switch dictates the maximum voltage and repetition rate. Voltages of 10 – 100 kV are required for food-processing applications. Repetition rates of up to several kHz are required, depending on the throughput rate of the product. Not only the switchable power, but also the service life and reliability of the switching device are of key importance, particularly for industrial-scale applications. At present, there are insufficient data available with respect to the robustness and potential applications of the corresponding switching devices on the industrial scale.

Process parameters

Electric field strength E. The electric field strength E is a key parameter for the use of high-voltage pulses. For plane parallel electrodes, it is calculated from the ratio between the applied voltage and the electrode gap. The formation of a pore in the cell membrane

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presupposes the induction of a transmembrane voltage of ~ 1 V, for which the externally applied field strength must exceed a critical value, E_{crit}. This lies in the range of 1 – 3 kV/cm for plant and animal cells and in the range of 10 – 20 kV/cm for microorganisms. The value of the critical field strength depends on the size of the cell, on its shape and on the orientation of the cell within the electric field as well as on the electrical properties of the membrane, of the cytoplasm and of the medium. It has been demonstrated that particularly for microorganisms with a small cell diameter, such as *Listeria innocua*, a higher electric field strength is required to cause electropermeabilisation. A further increase in the electric field strength into the range of 50 kV/cm improves the process efficiency. However, if this value is exceeded, the dielectric breakdown strength of the product must be taken into account.

Duration of treatment, specific energy input and pulse shape. The pulse duration multiplied by the number of pulses is used in numerous publications as a measure of the treatment intensity. The pulse duration for exponentially decaying pulses is the time until the voltage has decayed to 37 % of the maximum value. Increasing the number of pulses or the pulse width automatically increases the duration of treatment. In general, this has been reported to increase the efficiency of the process; however, there are also some reports of a maximum attainable inactivation in the form of delayed inactivation rates. The number of incoming pulses per volume element has only been unambiguously determined for batch processing; mean values must be used for continuous processing. Depending on the geometry of the treatment cell, the field strength may be below the critical value in certain regions of the treatment zone. This must be taken into account when considering the residence time and treatment intensity.

In addition to the duration of treatment, the specific energy input W is often used as an intensity parameter, which also allows an estimation of the expected costs, which can be calculated on the basis of the electrical energy stored in the capacitors, the pulse frequency and the mass flow rate of treated material. Together with the specific heat capacity of the product, W can also be used to estimate the expected heating due to dissipation of the electrical energy. From the process engineering point of view, the use of the required energy input to achieve the desired inactivation rate can be regarded as a suitable intensity parameter because it also allows an estimation of the costs and a comparison with other processes. However, the process cannot be completely described on the sole basis of the duration of treatment or the specific energy input because it does not include any information on the parcelling of the energy into individual pulses or the number of pulses per volume element.

The process temperature is also a crucial factor because an increase in the temperature due to the conversion of the phospholipid layer from a gelatinous state into a fluid state has a strong synergistic effect on the efficiency of the process. It has been demonstrated that a combination of this treatment with gentle heating can drastically reduce the required electrical energy.

Influence of the medium

The properties of the medium being treated, particularly the electrical conductivity and presence of particles with differing dielectric properties, can influence the process efficiency. Furthermore, as is the case with other methods of conservation, it is obvious that the product properties, such as pH value, water activity and the content of antimicrobial substances, also affect the inhibition of microorganisms. The (temperature-dependent) electrical conductivity of the product has a direct impact on the voltage built up in the electrodes because it, along with the geometrical arrangement of the electrodes, determines the resistance of the treatment cell. The ratio between the resistance of the treatment cell and the protective resistor integrated into the pulse generator determines the fraction of utilisable power within the treatment cell. If this resistance of the treatment cell is low, that is, for a high product conductivity, a considerable portion of the energy can decay in the protective resistor and is thus lost. The product conductivity is thus a limiting factor for the applicability of the process. Because the conductivity of the majority of products is predetermined by the composition or the recipe, this can only be adjusted by careful selection of the electrode geometry.

The presence of air bubbles or particles having different dielectric properties compared to water can interfere with the distribution of the electric field and thus has an unfavourable effect on the homogeneity of the treatment process. Particularly in the outer zones of fat droplets, a significant drop in the electric field strength can occur due to concentration of field lines inside the droplets. This must be taken into account when selecting suitable process parameters to ensure complete inactivation of all vegetative microbes and thus ensure the maximum product safety. A similar effect can also be observed in the presence of air or gas bubbles whose outer regions may have a reduced field strength so that the product may be undertreated, thus resulting in inadequate inactivation. This effect can be countered by degassing the medium prior to treatment or by treating under a counterpressure. However, electrochemical reactions that liberate gases may also take place.

Design of the treatment cell

The treatment cell, in which the product is exposed to the electric field, essentially consists of an arrangement of at least two electrodes separated by insulating material. In continuous systems, the product is pumped through this cell. Most of the systems used to date are based on three different geometric layouts – parallel plates, coaxial cylinders and co-linear cylinders. The arrangement of the electrodes determines the pattern of electric field lines and the homogeneity of the field distribution. The parallel arrangement of two plates with a large surface area can provide the best possible homogeneity; however, this layout means a low load resistance of the treatment cell, which is thus unsuitable for large-scale applications, in particular. Likewise, in spite of the homogeneous field distribution, a co-axial arrangement of two cylinders is associated with an unfavourable ratio between the electrode surface area and electrode gap and thus produces a low load resistance. From the electrical engineering point of view and in terms of the process scalability, a co-linear arrangement is usually the best solution. In this case, the medium being treated flows through cylindrical electrodes. A design with three electrodes - one central high-voltage electrode and two earthed electrodes - appears to be particularly favourable. This layout has two treatment zones, and the connections of the externally accessible electrodes are earthed and thus zero potential. Owing to its large diameter, a co-linear arrangement allows the treatment of highly viscous media that contain solids. The geometry of the electrodes and insulators must be optimised to produce a homogeneous field distribution to ensure that all volume elements are treated uniformly and to a sufficient degree. The serial arrangement of several treatment zones can be used to ensure that all volume elements are exposed to an electric field of the required intensity.

Selection of electrode materials

In addition to the basic requirement that the materials used for the electrodes and insulators are suitable for use with foods, the selection of suitable electrode materials presents a challenge. Account must be taken of the particular requirements arising from the transfer of electrons at the electrode/medium interface. Electrochemical reactions can be expected that affect both the product being treated and the electrodes. Stainless steel, which is generally suitable for use with foods, has limited suitability as an electrode material. Oxidation occurs at the anode. If stainless steel electrodes are used, this leads to formation and liberation of Fe^{2+}/Fe^{3+} and other components that can result in undesirable contamination of the food. Particularly the content of heavy metals in the electrode and their transfer into the product tends to rule out stainless steel as an electrode material. Studies on the use of graphite have shown very good results to date; however, the (mechanical) loss of material during continuous operation has not yet been assessed. At present, there is not sufficient information available on the use of alternative non-corroding materials such as glassy carbon, Ir/Ti or Pt/Ti

Annex 3

Background paper on the chemical aspects

Hans J. Schäfer[3] /Bernhard Tauscher[4]

Basic principles

Electrochemical reactions on the electrodes of an electrochemical cell take place with a pairwise charge transfer at the electrolyte/electrode phase boundary. One of the two electrodes, the anode, gains one or more electrons (oxidation) from a molecule or an ion in the solution (electrolyte). The other electrode, the cathode, loses one or more electrons (reduction) to a different molecule or ion. At low current densities between the electrodes (in the order of a few mA/cm^2), the species that is the most susceptible to reduction (oxidation) is selectively reduced (oxidised). At higher current densities (a few A/cm^2), the diffusion-controlled limiting current is reached for all electroactive species, and their reaction then becomes concentration-dependent. The latter is the case at the high current densities that occur for a short time during treatment in a pulsed electric field.

Possible changes to food constituents

Electrical currents flow during PEF treatment. Depending on the amount of current and the current density, this may cause undesirable changes in the food constituents:

Decomposition of water: Since water is always available for electrode reactions, its decomposition products from oxidation or reduction can always be expected. At the anode, these are OH radicals, H_2O_2 (a strong oxidising agent) as well as O_2. Furthermore, the generation of protons lowers the pH value in the vicinity of the anode. At the cathode, H_2 is formed and the pH value increases. Particularly highly reactive OH radicals as well as H_2O_2 can react further with other constituents of the food being treated.

Oxidation or reduction of water can produce a pH gradient in the vicinity of the electrode, which thus promotes acid/base-catalysed side-reactions.

The sensitivity of a substance to a redox reaction is indicated by its oxidation and reduction potentials. Substances are oxidised more easily than water if they contain conjugated double bonds, e.g. carotinoids, enols such as vitamin C, nitrogen and sulphur atoms with a low oxidation number. The reduction of the constituents is generally more difficult than water.

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Proteins: In addition to H^+- or OH^--induced hydrolysis or racemisation reactions, groups that are particularly sensitive to oxidation or reduction are thiol and disulphide (e.g. in cystine, cysteine). Furthermore, there may be e.g. the hydroxyl radical-induced dimerization of radicals, fragmentation or secondary oxidation reactions.

Inorganic constituents: Cations and anions can be converted to higher or lower oxidation states

Carbohydrates: The hemiacetal group is susceptible to oxidation. Reactions with hydroxyl radicals or hydrogen peroxide generated by the oxidation of water can cause modifications. Proton catalysis on the anode may lead to changes of sugar acetals (hydrolysis, transacetalisation and epimerization).

The available data on low molecular-weight compounds are limited so that no comprehensive statements can be made regarding possible PEF-induced reactions in foods.

Analytical studies must focus on whether and in what way the process affects redox-sensitive substances in foods. For example, it is conceivable that unfavourable processing conditions could lead to the degradation of vitamin C and lycopene.

If this process is to be applied to a particular food, an individual assessment should be made of whether

1. the amount of nutritional constituents is reduced,
2. toxic compounds are formed or introduced,
3. the microbiological safety is guaranteed
4. statutory limiting values are observed.

According to previous deliberations, the electrochemical components of the process can make a significant contribution to the deterioration of constituents and to the formation of potentially toxicologically harmful substances. Therefore, a number of representative foods that reflect the range of physical conditions as well as the chemical and enzymatic constituents that may occur should be assessed with respect to points 1 to 4.

Since the electrochemical intensity depends on the current density and pulse duration, investigations should be carried out on how far they can be reduced by lowering the voltage and the pulse duration but without reducing the efficiency in destroying microorganisms. In addition, the voltage at the end of the pulse should not be discharged via the cell, but through a metallic conductor located between the electrodes. To completely stop the flow of current through the cell, it is urgent to carry out trials in which both electrodes are coated with the thinnest possible (a few molecules thick) breakdown-resistant film of a) high and b) low dielectric materials. The voltage should also be pulsed to ensure a rapid (low?) mass transfer in the electrical bilayer. In this way, the electrical potential field could be adjusted without a flow of current, if necessary. Germ inhibition under these conditions should be tested.

Electrode materials

Alternative materials to metal electrodes for the PEF process are Ir/Ti (metals), glassy carbon, graphite or Pt/Ti (metals). Although these materials are ultimately oxidisable, they are more difficult to oxidise than water, form harmless side-products or their oxidised products are not soluble. It is possible that chemical reactions can be completely avoided by the use of suitable, non-conducting electrode coatings because this would furnish a purely capacitative cell without any electrode reactions.