INTERNATIONAL ATOMIC ENERGY AGENCY
UNITED NATIONS EDUCATIONAL, SCIENTIFIC AND CULTURAL ORGANIZATION
INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS
I.C.T.P., P.O. BOX 586, 34100 TRIESTE, ITALY, CABLE: CENTRATOM TRIESTE

H4.SMR/775-9

COLLEGE IN BIOPHYSICS:
EXPERIMENTAL AND THEORETICAL ASPECTS OF BIOMOLECULES

26 September - 14 October 1994

Miramare - Trieste, Italy

Bioelectrets and Water

S. Mascarenhas
University fo Sao Paulo and EMBRAPA
Brazil

6. Bioelectrets: Electrets in Biomaterials and Biopolymers

S. Mascarenhas

With 14 Figures

Charge and polarization storage via the electret state has now been found in many biomaterials. The importance of the electret effect in these materials has to do with biomedical applications as well as with its possible role in more fundamental biophysical phenomena. As biomaterials, electrets have found interesting applications as antithrombogenic surfaces. Other uses have been mentioned in the literature, such as the stimulation of tissue growth in bone and special artificial membranes. The electret effect has also been found in most biopolymers of importance such as proteins, polysaccharides, and some polynucleotides. Fundamental macromolecules of biology, such as collagen, hemoglobin, DNA, and chitin, not only exhibit the effect, but may have various sources, or, to use a more biological term, "compartments", for polarization and charge storage: dipoles and ions bound to the molecules.

One of the most important aspects of electret research in biophysics is that water bound to biopolymers in the so-called structured form (also called bound water, or biowater) may also be induced into the electret state. Electret investigation techniques were used to study this most important form of water in conjunction with the biopolymer.

The electret state has been considered in various biophysical models as a basis for the understanding of membranes, neural signals, biological memory in regeneration, electrical mediation in tissue growth, and other phenomena. One of the most interesting models claimed to depend on an induced ferroelectric metastable state similar to the electret is Fröhlich's model for coherent longitudinal polarization waves in biological systems. Fröhlich waves have been invoked to explain enzyme action, and recently the electret effect was found in various important enzymes in the solid state such as tripsin, urease, and others. For biomedical applications and in molecular biophysics, the electret concept begins to open new avenues for research, which seem to justify the usage of the term *bioelectrets*.

6.1 Introductory Remarks

It is interesting to observe that the first electret was made with a material of biological origin: carnauba wax, from the carnauba palm tree of Brazil. This was the material originally used by Eguchi [6.1] to verify experimentally the

theoretical proposal of *Heaviside* [6.2], who coined the name electret. In fact, carnauba wax proved to be, for many years, the main material for electret investigations. The samples prepared by *Eguchi* in 1922 are still electrized and subject to monitoring measurements in the laboratory of Eiichi Fukada in Japan. The other pioneer of electret investigations, *Gross*, also investigated carnauba wax electrets in many of his fundamental papers (listed in [6.3a, b]). Electret research gradually moved to simpler materials like ionic and organic crystals and polymers where fundamental solid-state properties could be correlated with the electret behavior [6.4–8]. More recently, however, the electret effect was studied in materials of biological origin like proteins, and now the picture emerges that the electret effect may in fact be a universal property of biopolymers in general such as polypeptides, polynucleotides, and polysaccharides [6.9a] (see also [6.9b], which may be the first paper on TSD from a protein–hemoglobin).

For biomedical applications, polymers with good biological compatibility (such as teflon) are also considered as biomaterials, and though, strictly, they are not biopolymers, they will be treated as biomaterials in this chapter. In this way we are led to consider the electret properties of artificial polymers such as teflon and polysulfonate films which are of importance for biological or medical applications.

The techniques used to study the electret effect in biomaterials (and biopolymers) are essentially the same as for general electret research [e.g., thermally stimulated depolarization current (TSDC)], and we shall not discuss them in detail here since they are covered in other chapters of the present volume or in the literature [6.7]. Specific changes in these techniques and important details required for electret investigation in biomaterials will be explicitly discussed, however.

Some general observations are nevertheless required in relation to experimental techniques in the special case of biomaterials.

a) Materials of biological origin in general cannot easily be put in single-crystal form. For most biopolymers, for instance, fibers or powder samples are used. Sometimes (as in the case of DNA and cellulose), a film may be obtained. In this case, the orientation of the macromolecule in relation to the film may be investigated by X-rays or optical techniques, and may be important for the interpretation of the effects observed. A typical case is the natural electret effect in keratin found in highly oriented samples of biological origin [6.10], which will be discussed in detail in another section.

b) The purity and origin of samples of a biological nature become a very important parameters electret investigations, and, in general, great attention has to be given to the preparation of samples, preferably with the assistance of biochemists and biologists. Here electret investigations really need to be interdisciplinary.

c) Many biopolymers change their properties by denaturation, hydration (or dehydration), oxidation, and even as a result of exposure to light. Careful

again such properties of the materials should be known before detailed measurements are begun.

d) Most biological molecules have been studied intensively in solution, but, in the majority of cases, little is known about the true "solid-state physics" of the material, and the investigator should be careful not to assume that properties investigated in solution apply to solid samples. For instance, the collagen molecule, a triple helix, may denature thermally partially into random coils, or completely, into isolated helices (gelatin). In solution, the denaturation temperature is around 65 °C. However, in the solid state, collagen can be heated above 150 °C with no appreciable denaturation (in vacuum) [6.11].

Biomaterials of nonbiological origin, but good compatibility in vivo, have been used as electrets in biomedical applications. Examples are teflon, dacron, and other polymers. Several biomedical applications of electrets have been proposed. We discuss here mainly electrets in antithrombogenic surfaces [6.12] and artificial membranes [6.13, 14].

Electrets have also been used in biomedicine or bioengineering in different contexts not directly linked to our previous classification. For instance, in medical dosimetry, where high sensitivity do dose is required, a new form of electret dosimeter has been developed recently [6.14] (see Sect. 4.5). The development of transducers with electret films applicable in bioengineering (for example, ultrasound probes or hearing aids) will be discussed in Chap. 7. Obviously these areas also belong to the interface of electret physics and biomedical research or applications. Exotic applications such as the possible use of electret filters for biological ions and free radicals or charged traps for bacteria have been mentioned but are also not discussed here.

Our discussion will be based on themes which we believe are varied enough to give the reader a broad view of the previous research on, and present potential of, electrets in biomaterials and in biophysics.

6.2 General Concepts in Electret Research

Before we describe general results obtained in the investigation of electrets in biomaterials and biophysics, it may be useful to summarize here some general results and concepts on electrets. The reader will find a complete and detailed treatment in the other chapters of this book. In particular, we shall not discuss the action of radiation on biomaterials – a subject of great potential interest in biophysics. For the action of radiation on electrets the reader should consult Gross's chapter.

The general method of polarizing materials may be understood from Figs. 6.1, 2 (see also Sect. 2.2.2). The material is placed in a closed vessel V, where vacuum or a controlled gaseous atmosphere can be established. Metal electrodes

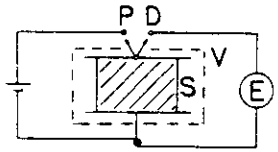

Fig. 6.1. Diagramatic representation of electret polarization and depolarization system

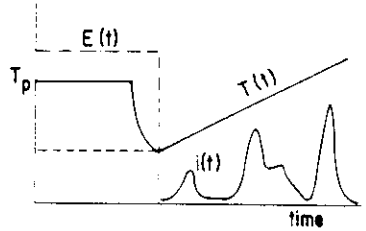

Fig. 6.2. Field (E), temperature (T), and depolarization current (i) as functions of time (t) during a typical experimental run

temperature T, the polarization temperature. The choice of t is made by a previous investigation in which the polarization stored in the material (to be defined below) is measured for several values of t. The minimum time for which the maximum stored polarization is achieved at the chosen polarization temperature T is usually used. The main concept in thermoelectrets is that the relaxation time τ for the decay (or growth) of the polarization P is a function of the temperature $\tau(T)$. $P(T)$ is then said to be thermally activated, and in general, the relaxation time is found to be a function of the form

$$\tau(T)=\tau_0 \exp(A/kT), \tag{6.1}$$

where τ_0 is a constant; A, the activation energy; k, Boltzmann's constant; and T, the absolute temperature. By varying the temperature of the sample, it is thus possible to obtain large variations in $\tau(T)$ depending on A. By lowering the temperature, decay times as long as centuries, for instance, may be obtained. An electret is defined most conveniently in terms of its relaxation time: *a substance is said to be an electret if the decay time of its stored polarization is long in relation to the characteristic time of experiments performed on the material.* If one particular experiment involves time intervals of a few seconds, then a decay time of several minutes will indicate electret behavior. On the other hand, if observations are related to time lapses of days or months, electret decay times will certainly have to be of this order or larger. In biophysics, phenomena may last from fractions of a second to years, and the characteristic electret lifetime or decay time is an important parameter to be measured. We shall see below how this can be done experimentally.

After polarizing the material, the temperature is then lowered (see Fig. 6.2) so that the relaxation time increases and the polarization can be "frozen in" the material. When the lowest temperature has been attained (T_1), the field is switched off, and an electrometer (such as a Keithley 602 or 610 or a Cary vibrating-reed instrument) is put in series with the sample (position D of switch in Fig. 6.1). Preferably, a double-pen recorder is also used so that the current and the temperature of the sample (monitored by a suitable thermocouple) are simultaneously recorded. Alternatively, an x–y recorder may be used if the

at a constant heating rate $\beta = dT/dt$ which may vary from a few °C per minute to a few tens of °C per minute. A common rate for most biological substances is about 15 °C min^{-1}. During warm-up, the polarization stored in the material will decay when the temperature is sufficiently high. In the vicinity of this temperature, a current will be observed in the external circuit due in principle to the decay of the polarization plus other conduction components. If we disregard the conductivity for the moment, the external current will be equal to the displacement current, which for unit area is

$$i(T)=dP/dt\,.$$

This current will rise initially and then pass through a maximum at a certain temperature T_p because the polarization stored is finite. Since many subpolarizations may be present in the material, several such current peaks may appear during depolarization. The resulting current as a function of temperature is called the thermally stimulated current (TSC) or thermally stimulated depolarization current (TSDC). This function contains important information on the electret behavior and it will be one of our objects to describe such curves and the corresponding interpretations for the case of biological materials. Two fundamental questions may be asked after a TSDC is observed for a biological material:

I) *What are the physical sources of charge or polarization storage in the electret, and how can they be identified from the properties of the* TSDC?

II) *How may this storage of charge or polarization be important in biophysical phenomena or for biomedical applications?*

The first question is a question in basic physics, and a complete answer may require the applications of different techniques, such as optical measurements, dielectric constant investigations, variation of the sample parameters (e.g., doping or chemical changes), or EPR. In general, the following sources (or "compartments", to use more biological language) may be responsible for the production of charge or polarization storage:

a) dipoles of the material or related impurities or defects;
b) ionic carriers, either as impurities or intrinsic to the material;
c) electronic carriers (electrons or holes);
d) structured water bound to the material (mainly in the case of biological macromolecules).

In the case of dipoles, the relevant equations for the thermally stimulated current have been solved (assuming a nonconducting sample) by *Bucci* and *Fieschi* [6.4] and by *Gross* [6.15] in a more general form. *Bucci* and *Fieschi*, assuming a single relaxation time for the dipoles, obtain the current as

where N is the number of dipoles per unit volume, p the dipole moment, and the other symbols are as defined before. The maximum temperature of the peak T_p will be given by

$$T_p^2 = \beta A \tau(T_p)/k\,, \tag{6.3}$$

and the total polarization stored P_s as measured by the area under the peak will be

$$P_s = Np^2E/3kT_p\,. \tag{6.4}$$

For times at the very beginning of the peak, in the so-called initial rise region, the following approximation is valid:

$$i(T) = i_0 \exp(-A/kT)\,. \tag{6.5}$$

This is udeful for obtaining the activation energy for an isolated single peak. *Gross* generalized the expressions above for a distribution of relaxation times. Further theoretical developments are discussed in detail by *van Turnhout* [6.7] and in Chap. 3 above, and the reader is directed to these and various other papers [6.3b, 16] on the subject.

In the case of electronic carriers, the charge may be stored in traps which may bind these carriers. The trap distribution in energy and space is then very important. In the case of polymers, especially irradiated ones, where a large number of electrons and holes may be produced by irradiation and subsequently trapped, this will be an important case (see Chap. 4). Ionic carriers, leading to so-called space-charge storage, usually produce peaks in the TSDC at higher temperatures where ionic conductivity begins to appear (around room temperature and above for biological molecules). If the electrodes are blocking (that is, do not allow free passage of carriers between sample and the external circuit), a space charge will build up in the sample, and may be stored if the temperature is low enough so that the electrical conductivity is small. The general equations for depolarization currents arising from space charge have not been deduced in a complete form, because, in general, they lead to nonlinear differential equations in the field.

Recently *Gross* et al. [6.17] have solved some of the space-charge problems either in closed analytical form or by numerical solutions with a computer, but a complete analysis of the experimental curves is still difficult and will not be discussed here. The following observations may be made for the purpose of general interpretations of the TSDC from biological substances:

I) A dipole peak is distinguished from a space-charge peak if it shows the following characteristics:

a) Equations (6.2–4) hold, that is, polarization is a linear function of the applied field and the peak may be fitted by first-order kinetics;

c) the polarization does not depend on the thickness of the sample – essentially because for dipoles (if uniformly distributed), the following equation holds:

$$\operatorname{div} P = 0$$

provided field and polarization temperature are kept constant.

These are just general observations found to apply when simple conditions hold, such as isolated single peaks, or separable overlapping peaks (the separation technique will be discussed below).

II) A space-charge peak may:

a) show a nonlinear dependence of polarization with field (since it saturates at lower polarization levels);

b) not obey (6.2–4)

c) the peak profile and peak temperature may shift with polarization temperature [because the spatial charge distribution (through the carrier mobility among other things) will depend on polarization temperature]; in general, however, the actual shift is hardly recognizable;

d) the polarization may be thickness dependent (for the same field values).

In the case of bound water, the peaks are most easily distinguishable through their variation with degree of hydration as will be shown for many biological materials like proteins and polysaccharides.

It is very important to note that some phenomena related to peaks which are dependent on the nature of the electrodes. Charge or polarization storage in this case may be due to carrier injection from the electrodes, or from Schottky barrier formation. These will be assumed to have been previously investigated – by changing the nature of the electrodes or by interposition of a thin insulating layer (such as a mylar film) to block the junctions. Since these effects are not intrinsic to the material, they will not be considered as true sources of electret action in the sense we are discussing in this chapter, but rather as artifacts to be avoided or to be properly controlled.

Two other aspects of experimental electret work should be mentioned. The first is related to a technique of peak separation, the so-called peak-cleaning method. The second is how the various parameters like T or t may be used for further analysis and peak isolation of the TSDC.

The peak-cleaning method is used when several bands in the TSDC overlap. The overlap and the number of peaks must not be too large if the method is to be useful. Starting, for example, with two ideal overlapping peaks as in Fig. 6.3, after polarization, the sample is brought up to a temperature T smaller than T_2, but larger than T_1. This will "clean" the first peak out of the spectrum. After cooling down and warming up again, a "pure" peak at T_2 will be recorded. The activation energy and profile of peak 2 may then be properly measured. *Perlman* [6.18] discusses criteria and computer programs for analysis of TSDC with peak-

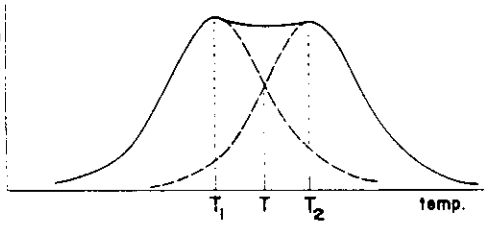

Fig. 6.3. Two overlaping peaks may be separated by warming up only to intermediate temperature T, cooling and rewarming for isolated detection of peak at T_2

knowing the particular kinetics (first, second, or higher order) of the overlapping peaks, peak cleaning may introduce changes in the cleaned peak profile. A good check will thus be to reduce the height of the cleaned peak further (by other successive warming and cooling cycles) to investigate whether peak position or profile will change as a function of the diminishing stored polarization.

Peak analysis may also be done by using a growth curve for the polarization P as a function of polarization time t. Sometimes one peak will grow faster than the other and in this way they can be separated. This is seen in the growth curves of Fig. 6.4. At time t_1, peak 1 is much larger than peak 2. The use of different T is also useful for cleaning out a higher-temperature peak without wiping off a peak occurring at lower temperatures.

We have already mentioned that care must be taken with electrode effects. It is always very important in electret research to be sure that the TSDC changes polarity with a reversal of the polarization field E_p. If asymetries arise (with a nonoriented sample, such as a polycrystalline material) artifacts must be looked for, and the experimental conditions controlled until satisfactory results are obtained. When using gaseous atmospheres, it is almost certain that "reversed" peaks will appear if no guard rings are used or surface and electrode conditions interfere. These are peaks with the "wrong" sign in relation to the polarization field (homocharge effects). Finally, thermal gradients in the measuring system may induce thermoelectric effects or depolarization effects, resulting from a nonuniform temperature distribution (e.g., some effects found by Gross in certain charged insulators). For this reason, it may be useful to obtain a TSDC with no field applied before extensive measurements are done. Also, the use of a calibrating sample such as a single crystal of divalent doped alkalihalide with a known amount of dipoles is a very useful way of checking the measuring system. Some of the alkali halides have sharp, isolated peaks in their TSDC with well-known parameters like activation energy and T_p. The reader should, however, consult the review by *Fieschi* [6.18] before using these samples, because some precautions must be taken in the thermal treatment of the samples for dispersing the aggregated dipoles.

Finally, in working with biological materials, in most cases a polycrystalline pressed sample will probably be the only form available for experiments. In this situation, Maxwell–Wagner losses may be present. This is certainly one of the main criticisms of the use of polycrystalline samples. Whether peaks are due to Maxwell–Wagner losses rather than to intrinsic dipolar effects can be seen by

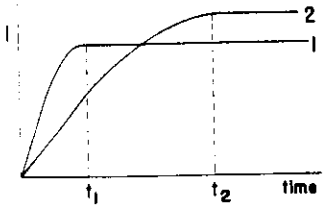

Fig. 6.4. Peaks may show a growth curve of polarization P as a function of time, and separation may be achieved by using different polarization times, such as t_1

pressure. Maxwell–Wagner peaks will shift and change profile or area under these changes. In any case, it is not always simple to separate Maxwell–Wagner losses from intrinsic bulk properties of the material under investigation, and this is a point always to be kept in mind with biological samples.

6.3 Other Dielectric Techniques Complementary to TSDC

To obtain a complete picture of the system under analysis, certain complementary techniques should be used. Of these, mention measurements of dielectric constant and absorption, usually done by an ac bridge method. These techniques are extensively discussed in the literature and will not be examined here. It has been asked why the term electret is introduced if, after all, only an extremely low-frequency dielectric relaxation measurement is performed with TSDC using a dc technique? In principle, this is a valid point. However, for extremely long relaxation times, say of the order of days, the ac bridge technique would be useless, and the electret really merits the new name because a new kind of metastable equilibrium has been attained by the material with important physical implications. Since the fundamental mathematical demonstration given by Gross that electret behavior is not due to a dielectric anomaly, but, in broad terms, can be understood by consistent use of the superposition principle under nonisothermal conditions, the study of electrets has been shown to belong to ordinary dielectric theory. It must also be said that, more recently, new ac bridges going down to extremely low frequencies (sometimes below 0.01 Hz) have been developed. These will probably prove to be very useful in electret research, especially with biological materials. A comparative study of these techniques is given by *van Turnhout* (Ref. 6.3b, p. 97, [6.19]). In the case of alkali halide electrets, a simultaneous analysis of TSDC and dielectric absorption has been done for many systems. For biological materials, a complete analysis and comparison remains to be done for most materials and especially for biopolymers.

Another technique to be used is isothermal polarization and depolarization [6.20]. If one applies a constant dc voltage to the sample, the current rises and then decays to a residual conductivity at constant temperature. By integrating the area below the $i(t)$ function and correcting for the residual conductivity the area below the $i(t)$ function and correcting for the residual conductivity current, the absorbed charge can be measured. This measurement of the stored

conditions. We have applied this technique to investigate the stored polarization in urease, an important enzyme, with interesting results (see Sect. 6.7). Charge and discharge current analysis under isothermal conditions may also be used to identify polarization kinetics by dipoles or by the filling (emptying) of traps by electronic carriers, as was done in the case of naphthalene [6.21].

Piezoelectricity is an important property of many biological molecules, as has been shown by the pioneering work of *Fukada* [6.22]. In principle, it can be shown that an electret will also show piezoelectricity, as has been demonstrated by Gubkin, and, more recently, in a very important series of studies by *Broadhurst* et al. [6.23a] (see also Chap. 5, and [6.22].) *Zimmerman* and colleagues in our laboratory also found an induced piezoelectricity in electretized materials [6.24]. Piezoelectricity has been interpreted as due to degrees of freedom in the macromolecule connected with dipolar relaxation, but a complete analysis is still lacking, and only general correlations have been found for biological materials. In this case, the comparison is made between the TSDC and the temperature-dependent curve of either the real or imaginary part of the piezoelectric constant. Since the piezoelectric constant also depends on elastic properties, the temperature dependence of these complicate the interpretation and the comparison. A definite comparison has been made in the case of the natural electret, keratin [6.24]. At the present stage, these investigations, and the correlations between electret and piezoelectric behavior, are still at an early stage, and though the subject represents a fertile line of research, it will not be further pursued here (see, however, Chap. 5); we will, however, discuss the specific case of the simultaneous use of these techniques with keratin, where some results of interest have been obtained.

6.4 Proteins

Proteins are polymers of amino acids. The 20 natural amino acids divide themselves in hydrophilic and hydrophobic classes. Thus, in general, naturally occurring proteins contain dipolar residues, and can in principle be induced into the electret state. Of course, artificial polypeptides could also be investigated, and, in this case, typically nonpolar compounds like polyglycine will not present dipolar peaks, though they might present other forms of polarization storage. Incidentally, a systematic investigation of artificial polypeptide electrets has not yet been done. The electret effect has been found in many proteins, fibrous and globular, but in the present section we discuss only the particular examples of collagen and gelatin [6.25]. Enzymes are also proteins but, due to their importance in biological phenomena, we discuss this case separately. Also the case of keratin, because it exhibits the so-called native, or natural, electret state (obtained without application of a field, as by the *Costa-Ribeiro effect* [6.26] or by biological growth [6.10, 24]), and because of the simultaneous presence of piezoelectricity, will be discussed in another section.

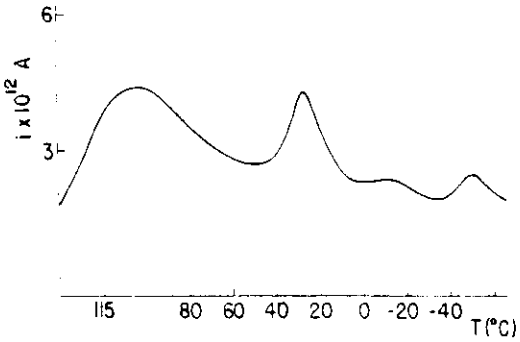

Fig. 6.5. TSDC for gelatin

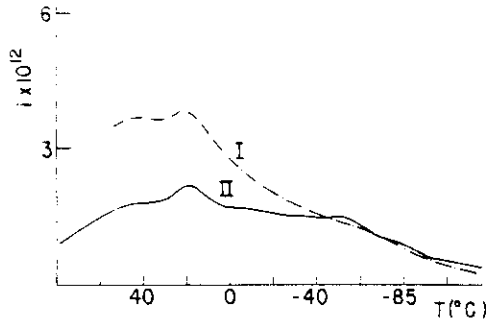

Fig. 6.6. TSDC for collagen (I), and after partial denaturation (II)

Collagen and gelatin are known to possess dipolar groups. The appearance of dipolar orientation components in the TSDC of these materials was thus expected. Such was the case shown by results (Fig. 6.5) for gelatin. Collagen also shows typical electret behavior (Fig. 6.6). Immediately one is struck by the fact that the gelatin spectrum is much richer than that of collagen at lower temperatures. However, if we thermally denature collagen in vacuum in the solid state, its electret spectrum is found to change gradually to that of gelatin (curve II of Fig. 6.6). In fact, the TSDC spectrum has proved to be a very sensitive tool for the characterization of the denaturation state of collagen through the presence of the low-temperature electret bands. The more prominent peak in Fig. 6.5 at higher temperatures was shown to be due to a space-charge effect, and the low-temperature peaks, to a molecular dipole orientation mechanism. This was done, as previously discussed, by the investigation of T_p for the peaks as a function of T_0. If the heating rate is kept constant, a dipole peak will generally not change its position with variable T_0. However, a space-charge peak mechanism for electret behavior will give peaks with T_p often dependent on T_0, as has been shown for several electrets of ionic crystals [6.18], ice [6.27], and polymers [6.15]. We could thus separate in the richer gelatin TSD spectrum the peaks due to molecular dipole orientation from the peak due to space-charge formation.

Experimental evidence now points to the presence of the electret state in all materials containing dipoles or ions. These are not the only source for electret behavior, for if the protein or any other biopolymer is hydrated, electret behavior

6.5 Bound Water (Structured Water or Biowater)

The presence of water bound to proteins and other biopolymers is considered to be of fundamental physical and biological importance. Recently a conference on the subject was organized by the New York Academy of Sciences and the interested reader may consult the volume published [6.28] for further references. The fascinating history of the bound-water problem begins in 1938 with the basic work of *Teller* et al. [6.29]. In the following decades Bull, Pauling, Bernal, Szent-Gyorgyi, and a score of distinguished physicists, physical chemists, biochemists, biologists, and biomedical researchers became interested in the problem. A number of methods have been used to look for these properties and in particular to elucidate the structure of water bound to biopolymers: for example, NMR, infrared spectroscopy, circular dichroism, X-ray diffraction, and dielectric absorption. The particular case of water bound to collagen or gelatin has been studied intensively, and *Berendsen*, *Grigera*, and *Mascarenhas* have given reviews of the field [6.30].

Studies of water bound to collagen (or gelatin) may be important for the understanding of phenomena ranging from the structure of bound water (like the so-called question of icelike phases) to the interaction of water with the conformation of the macromolecule. Also the influence of hydration on solubility, cross-linking, and mechanorheological properties of collagen and gelatin have been studied by several authors. Recently, a study of dielectric absorption of hydrated collagen has been presented by *Tomaselli* and *Shamos* [6.31]. From results described in [6.5], the TSDC of collagen and gelatin in the dry state could be interpreted in terms of electrical energy storage in two compartments: dipoles and ionic charges. One is thus led to suspect that dipoles of water, if bound in a structured way to the macromolecule, could have energy levels for dipole rotation in the local field of the biopolymer macromolecule. Thus bound water might also exhibit the electret state. Of course we would have to limit ourselves to low hydration levels, where the resistivity of the samples would be high enough for the application of the polarization field. But these lower hydration levels are known to be related to the filling of primary hydration sites when structured binding occurs. If this is so, bound-water energy levels for dipole rotation could be studied in a most direct way. That such is indeed the case is shown by results with gelatin of Fig. 6.7. First one obtains the TSDC of the dry material. In the case of gelatin, this can be done most conveniently, because the sample can be safely warmed up to 150 °C in vacuum without any further denaturation or oxidative pyrolysis. If the sample is then left to equilibrate to a hydration degree of approximately 10%, the TSDC shows new electret bands. That these bands are due to bound water can be seen in different ways:

a) difference curves of TSDC with succeeding lower hydration, obtainable by continuous pumping of the sample, indicates a difference spectrum where the bands occur;

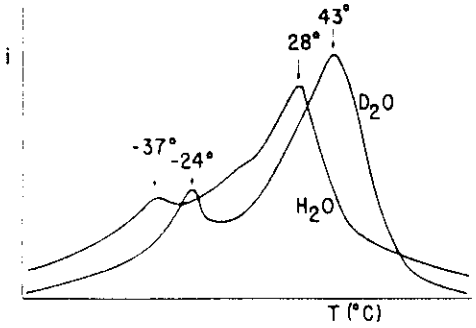

Fig. 6.7. Shift of bound-water peaks in gelatin due to heavy water

c) upon drying, the two bands disappear, and the original gelatin TSDC is obtained; this transformation can be done several times in a reversible way;

d) finally, as we discuss in more detail below, the bands can be shown to shift the maximum temperature if the hydration is done with heavy water (Fig. 6.7).

The cleaning technique to separate bound-water bands from the underlying TSDC was applied, and a quantitative analysis made of the peaks. It was found that the *Bucci–Fieschi* equations could be applied for the second peak where complete separation was possible. The activation energies of the first and second peaks were also obtained with the help of the initial rise technique as will be discussed below. The effect is present in collagen, where a similar set of two bound-water bands were found and with DNA, and many other biopolymers, where the TSDC also changed upon dehydration.

All these results on the electret state of bound water are presented in detail elsewhere [6.9, 25, 32].

We applied (6.2) (whole band analysis) to the higher-temperature bound-water peak. A good fit was found, indicating the first-order nature of the process (Fig. 6.8). For the lower band, the activation energy was determined by (6.5) (initial rise). The values of the activation energies (0.15 and 0.4 eV) suggested the following interpretation: the low-temperature peak is due to a single hydrogen-bonded water molecule, probably attached to polar residues or to the peptide bond. The higher temperature corresponds to multiple hydrogen-bonded water molecules, whose dipole can rotate only by breaking several bonds (corresponding to the 0.4 eV barrier). In view of the fact that the hydrogen bond is known to be cooperative, no suggestion is made for the exact number of linkages, beyond a minimum of the order of 3 bonds (if 0.15 eV is taken as an approximate value for the bond energy). This peak would then correspond to the "structured" bound-water or icelike phase, proposed to be a chainlike structure around the backbone of the polypeptide by several authors. The fact that the peak occurs near 40 °C indicates the presence of water as a structured phase, as required by the existence of a definite energy barrier even at this temperature. On the other hand, since (6.2) was found to be valid, another useful parameter can be obtained: the relaxation frequency ω_0, which can be interpreted in lattice-dynamic terms (as it has been in

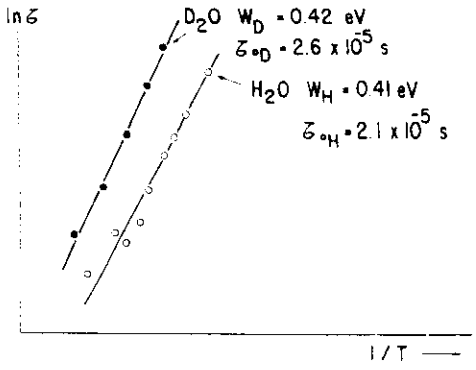

Fig. 6.8. Plot of $\ln X$ versus $1/T$ for bound-water peaks, showing validity of (6.2)

question of whether this bound water is more similar to a liquid or to a solid. The values of ω_0 (10^{-5} s) taken in conjunction with the values of the energy barrier according to the above interpretation lead us to suspect that bound water is more like a solid than a liquid (for which values of ω_0 are many orders of magnitude higher). At the same time, while the value of ω_0 is not identical to that of pure ice, it is of the same order of magnitude as the values determined by dielectric absorption in recent measurements by *G. Gross* for impure ice [6.33]. The electret state of bound water, if this interpretation is correct, may be the first direct experimental evidence for the solidlike phases of bound water structured around biopolymers. We are at present looking for the electret behavior not only of other biopolymers, but of hydrated inorganic salts and the so-called clathrate hydrates extensively investigated by *Jeffrey* [6.34a].

It should be noted that bound water may also be found in a state closely resembling a frozen gas with dipoles whose rotation is constrained. Instead of the long-range order characteristic of a solid, short-range structuring may also lead to a localized energy barrier for the rotation of dipoles. This is similar to what was found by *Onsager* et al. for a natural amorphous-ice electret [6.34b].

6.6 Polysaccharides and Polynucleotides

Besides proteins there are other classes of important biopolymers for which the electret state has been found: polysaccharides and polynucleotides. Again a systematic investigation has not been performed, and we shall take as examples the cases of chitin and cellulose (polysaccharides) and DNA (a polynucleotide).

In the case of DNA, *Fukada* had already found the piezoelectric effect, indicating the existence of polarization storage sources. However, since there are many physicochemical parameters affecting the chemical and structural nature of DNA, a more systematic investigation should be undertaken. The main peak in DNA at high temperatures (50 °C) is of a space-charge nature, and thus a systematic investigation of the salt content and other physicochemical parame-

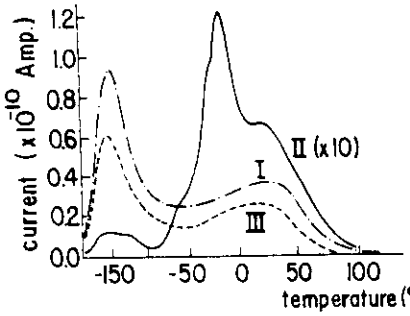

Fig. 6.9. TSDC for alpha-chitin (Dry: *I* and *III*, hum.: *II*)

For the investigation of polysaccharides, chitin will be taken as the main example. Chitin is an important structural material in biology [6.35]. Though there are several structural forms of chitin, we shall refer mainly to alpha-chitin, which can be obtained from lobsters or crab shell. Alpha-chitin forms fibers and its chemical structure is given in [6.35a]. It is seen that OH dipolar groups are present and these in principle may give rise to electret behavior. In fact, Fukada and Sasaki (personal communication) have found piezoelectricity in alpha-chitin, indicating some dipolar degrees of freedom in the material. We report here on results to be published obtained for chitin from crab shells by Fukada, Slaets, Zimmerman, and Mascarenhas to be published. Other results have also been obtained for chitin from the perisarch of tubularia by Mascarenhas, Rakesh, and Liuzzi, and from lobster tail by Mascarenhas, Rakesh, and Glimcher.

The alpha-chitin from crab shells was obtained in the form of a sheet, with the main fiber direction parallel to the sheet, as shown by X-ray diffraction experiments. The chain directions were found to be almost uniformly random within the sheet from the X-ray pattern. The electret state was found to depend also on hydration (curve II in Fig. 6.9), indicating water structuring around the fibers. After successive warm-ups in vacuum, the sample gradually dried (curves I, II), and an intrinsic spectrum characteristic of the material was obtained with two main bands in the TSDC, as shown in Fig. 6.9. The low-temperature peak was shown to be due to dipoles by investigating, as before, the maximum temperature (T_p) shift with T_0. The activation temperature for this peak was measured, and found to be very small (0.1 eV). It is proposed from an analysis of the structure of alpha-chitin (see, for instance, [6.35]) that this peak is due to orientation of the OH dipole which is internally hydrogen-bonded in the molecule. This bond is known to be weak for that particular hydroxyl group, and some of these dipoles would be free to orient under the external field. From the TSDC analysis, approximately 10^{18} dipoles per cm^3 were found to be free. This indicates that alpha-chitin is a strongly knit hydrogen biopolymer, since only a very small fraction of the dipoles are free to orient. The high-temperature peak was found to be due to space charge. As in most cases, the nature of the space charge is unknown, possible sources being protonic carriers or compensating

Following the report by *Mascarenhas* and *Malavolta* [6.35b] on the electret behaviour of cellulose, investigations have been made by on several aspects of this problem. *Talwar* and *Sharma* reported on TSD of cellulose in a study of the nature of the processes responsible for relaxation [Ref. 6.16b, p. 134]. *Pillai* and *Mollah* [Ref. 6.16b, p. 138] studied paper cellulose and the possible influence of water. *Sawatari* [Ref. 6.16b, p. 136] attributed the low-temperature broad peak appearing in cellulose at $-130\,^{\circ}C$ to molecular motion of the primary hydroxyl group of glucose in the amorphous region of cellulose.

6.7 Enzymes

Fröhlich, in a series of papers [6.36] related to the application of quantum mechanics to biology, proposed that enzyme action could be understood on the basis of its electrical polarization properties. Coherent longitudinal polarization waves coupled to the elastic field of the material constituting the enzyme would induce a metastable ferroelectric state in the system.

It has been shown [6.44] that important enzymes such as trypsin and urease exhibit the electret state, and several peaks are present in their TSDC. Each of these peaks is related to one compartment for charge and polarization storage and could in principle support *Fröhlich* waves. However, the intrinsic existence of the enzyme bioelectret is, of course, independent of the correctness of the *Fröhlich* model, being a direct experiment al fact [6.45].

The TSD for trypsin presents several peaks in the low and ambient temperature region as can be seen from Fig. 6.10. For the case of trypsin it was found by the previous methods that all the peaks below RT are of dipolar nature, while the higher temperature peak is due to space charge. An interesting effect due to bound water in trypsin was also found: hydration enhances the bioelectret behavior of the enzyme, as can be seen from Fig. 6.10 (curve I). This is probably due to the electret behavior of bound or structured water as it has been demonstrated in other biopolymers. Urease and ribonuclease were also investigated and both showed the electret state. Curves for urease and ribonuclease are shown in Figs. 6.11, 12. It is seen that the TSDC for all three enzymes are different, thereby indicating perhaps a certain structure dependence. In the case of urease, it was possible again to investigate the question of hydration and it was seen that hydration enhances the bioelectret behavior of the enzyme. For trypsin and urease, it was possible to show the reversibility of the hydration and dehydration cycle as far as the electret behavior is concerned: upon dehydration in vacuum, the TSDC changed; and upon exposure to humidity, the original TSDC was obtained. This could be done many times with trypsin with good reproducibility. This also indicates that no denaturation during warm-ups occurred for trypsin, and that the bioelectret state is a good monitor of the hydration of the enzyme.

Bioelectret behavior having been demonstrated for trypsin, urease, and ribonuclease, one should consider how it might be important for biophysical

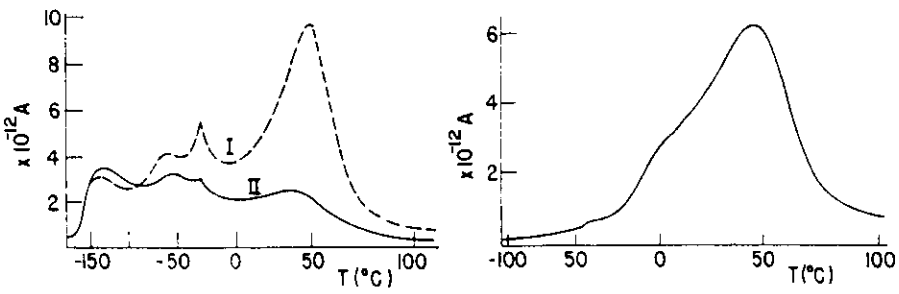

Fig. 6.10. TSDC for trypsin (*I*: hum.; *II*: partially dehydrated). Influence of hydration on TSDC of trypsin (*upper curve*: hydrated; *lower curve*: dehydrated in vacuum)

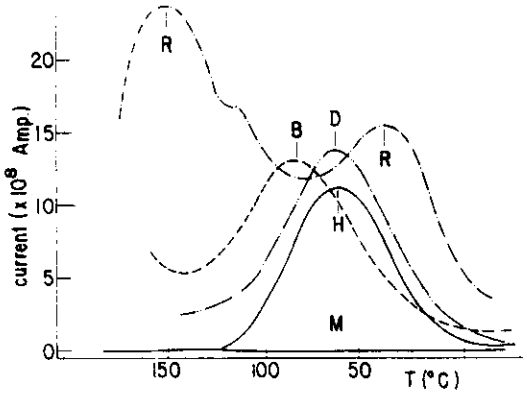

Fig. 6.11. TSDC for urease (dry)

Fig. 6.12. Electret behaviour of different femurs: *R*(rat), *B*(bovine), *D*(dog), *H*(human), *M*(mineralized)

phenomena? Though it is still too early to draw any firm indication from these results, we would like to offer some conclusions related to the solid-state physics aspects of the experiments, especially in the case of trypsin. It has been demonstrated that there are at least three sources or compartments for charge and polarization storage in trypsin: dipoles, space charge, and bound water. The dipolar part of the electret TSDC is probably due to the dipolar residues which are part of the protein constituting the enzyme. At present, however, it is impossible to make specific assignments on the basis of these results alone. The space-charge peak is probably due to ionic conduction in the molecule. Thus may be related to ions or to proton motion in the molecule. Proton semiconduction has been invoked as a possible mechanism in ice and organic materials. In the case of bound-water peaks, the fact that the peaks decrease with hydration and appear reversibly upon hydration demonstrates the importance of water for the electrical behavior of the enzyme. In the case of trypsin, a fourfold variation in the stored polarization was observed in the hydrated material.

Extrapolating from the results found with collagen and gelatin, and also hemoglobin [6.38], the increase in polarization is due to the electret behavior of hydration shells bound to the protein. In this case, water hydrates the protein in a solid icelike phase, and this phase has different energy levels depending on the orientation of the water dipole, leading to the electret behavior.

Finally, possible relations of these results with Fröhlich's model for the electrical behavior of enzymes should be mentioned. *Fröhlich* proposes that

enzymes are capable of presenting longitudinal polarization waves. The fact that several relaxation polarizations, were found for the enzymes investigated suggests that some of these relaxations may sustain Fröhlich waves. Whether the bioelectret behavior of enzymes favors the model first introduced by *Fröhlich* remains to be seen; it is, however, important to observe that enzymes are indeed able to store large amounts of electrical polarization in their structure (amounting sometimes to 10^{-7} C cm^{-2}). Also, theoretical calculations by Luiz Nunes de Oliveira (unpublished) on the field dependence of the Fröhlich model indicates a threshold value for the metastable ferroelectric transition. A detailed experimental analysis for all the individual polarizations in the TSDC of trypsin, for instance, as a function of the field should be done to see whether a threshold field, as predicted by the Fröhlich model, can be observed.

The question may also be asked: the electret state was induced in vitro in the enzyme by external applied fields: how can this happen in vivo? As discussed by *Fröhlich*, the fields provided by ion absorption in the macromolecule may be of the order of 10^4 V cm^{-1}. This is easily sufficient to induce the bioelectret state in the enzyme. The fact that the electret peak exists near and above room temperature and relevant ranges such as 30–40 °C also demonstrates that the enzyme would remain an electret at usual in vivo ranges. In fact, the question may be raised about "the electrical denaturation" of enzymes when used above the temperatures of the relevant electret peaks. At Sao Carlos we are presently extending our observations to other enzymes in an exploratory way, to gain familiarity with the general properties of the electret behavior of these important biological substances.

6.8 Thermally Stimulated Pressure and Bound Water

As has been discussed in the previous sections, the bioelectret state depends drastically on the amount of hydration. Bound water with long relaxation times for dielectric relaxation is responsible for the polarization storage in bioelectrets, and the corresponding peaks in the TSD spectrum. Another technique that can be used to investigate hydration effects is Thermally Stimulated Pressure (TSP) [6.37]. Since during the TSD measurements, the sample is continuously heated, the changing temperature also changes the degree of hydrogen. Thus there is one basic inherent difficulty with TSD measurements in hydrated bioelectrets: the hydration may be changing during the experiment. Changes in hydration may also correspond, and they usually do, to changes in conformation of the biological molecule under investigation. The same objection is valid for all hydrated electret materials, biological or not. In order to investigate this problem, we have developed a very simple technique. The partial pressure of the water vapor desorbed from the sample is measured continuously as the sample is warmed. The corresponding pressure P as a function of temperature T can be

thermocouple must be properly calibrated against absolute values of the pressure.

The water desorption can be monitored with this technique, and if the temperature derivative of the pressure $P(T)$ (i.e., dP/dT) is plotted as a function of T, peaks may be found. This type of spectrum, similar to TSD, is what we call the thermally stimulated pressure (TSP) spectrum. *Celaschi* and *Mascarenhas* have investigated lysozyme using TSD, TSP, as well as thermogravimetric analysis (to measure the change in mass during water desorption). TSP turns out to be more sensitive than thermogravimetric techniques in measuring the kinetics of the dehydration process. It is also much simpler and more convenient. In the case of lysozyme, the very interesting effect was found that bound-water dipoles, previously oriented with an external field, show electric current peaks during desorption. This electrical effect associated with desorption of water dipoles is similar but basically different from the electric effect found by *Onsager* et al. [6.38] in amorphous ice, in which electric potentials and currents were found during the growth and subsequent heating of the samples.

Assuming that TSP spectra could be analyzed in the same way as TSD and that superposition of several peaks could occur, activation energies, the number of water molecules, and other corresponding parameters could be calculated. Details can be found in [6.37].

The other cases in which TSP was used are DNA and RNA bioelectrets. As in the case of lysozyme, the isothermal polarization decay (IPD) is a very convenient means of observing the electret behavior. The reason for this is that, with IPD, the temperature being constant, there is no change in hydration. Making measurements at different temperatures, the behavior of the sample as a function of hydration can be properly investigated. The DNA and RNA cases are very interesting not only because of the importance of these macromolecules in biophysics, but because they were claimed to be ferroelectrics in the literature [6.39]. On the basis of this supposed ferroelectricity, various speculations were made as to the role of electric polarization in memory storage mechanisms [6.40]. It was later shown [6.41] that, due to nonlinear transport mechanisms, DNA could display hysteresis loops when the Sawyer-Tower technique was used to detect ferroelectricity. In the case of RNA, ferroelectricity was similarly reported [6.42], and, as in the case of DNA, it was shown by *Mascarenhas* et al. [6.43] that nonlinearity was also operative. Both DNA and RNA were found to be strong bioelectrets. TSP studies were also made, and the bioelectret state in both DNA and RNA was found to depend strongly on hydration. Since hydration has been found to be fundamental for polarization storage in bioelectrets, the use of IPD together with TSP is essential for the proper study of these systems.

The other very interesting case is that of cellulose and chitin. TSP studies indicate broad compound peaks of water desorption in these materials. The study of cellulose now seems to have attracted a great deal of attention. In investigating its bioelectret behavior, TSP should be applied simultaneously

6.9 Bone, Artificial Biomaterials, and Biomedical Applications

We shall mention here three selected topics, which will serve to demonstrate the potential of electret applications in medicine: antithrombus-formation surfaces, artificial membranes, and the electret state in bones.

Blood-Compatible Electrets

The main problem to be solved in obtaining blood-compatible biomaterials (for use as, for example, cannula, heart valves, and even entire artificial-heart systems) is that of avoiding thrombus formation (blood coagulation). Since the fundamental discovery by Sawyer, and results of Sawyer and Brattain that blood platelets are electrically charged, the idea was pursued that a negatively biased surface would inhibit coagulation.

By using metals, such as magnesium (and *not* electropositive noble metals) which are electronegative, in cannular implants in dogs, it was indeed shown that the concept would work. Unfortunately Mg was also poisonous, and could not be used for a permanent implant. Combining the well-known biological compatibility of teflon with its good electret behavior, the problem was investigated again by *Murphy* and *Marchant* [6.46] and hopeful results were reported. *Mascarenhas* et al. [6.47] following these investigations, also studied electretized cannular implants. We refer the reader to the detailed reports on the subject [6.48].

Membranes

The possible use of electret membranes has been proposed and discussed by several authors both from an experimental and theoretical viewpoint. The main idea here is that electret membranes might show different transport properties as well as different surface properties. With this motivation, *Linder* and *Miller* [6.13] investigated artificial polyelectrolyte membrane electrets. Measurements of polarization as a function of composition temperature, applied field, molecular weight of the polarizing component, and time were made on a series of membranes containing sodium polystyrenesulfonate in matrices of polyvinyl alcohol, polyacrilamide, and polyvinylpyrrolidone. The results indicate that the process depends mainly on interactions between components and not on the intrinsic nature of the components themselves. For detailed results we refer the reader to [6.13] and to other papers on membrane electrets.

Bone

TSD for several types of bone samples are shown in Fig. 6.12. It is seen that a main broad electret band appears in the range 30–100 °C. The effect seems to be a general property of all bone types investigated: bovine, canine, rat, and human

been investigated for bones as a function of several parameters like E_p, T, and nature of sample. A saturation polarization was found with fields of the order of 1 kV mm^{-1} for polarizations near 40 °C. In terms of this saturation polarization expressed in coulombs per cm^2, a practical parameter can be obtained as a measure of the capacity for storing polarization. Typical results for bone are of the order of 10^{-8} C cm^{-2}, a value comparable to the polarization storage obtainable with good electret materials. Since the results on bone have been published elsewhere (Ref. 6.16a, p. 650; [6.25, 49]), we report here only the more fundamental results. From the curves of Fig. 6.12, it can be seen that the effect is present in samples which have been demineralized but is not present in mineralized samples from which all the protein has been extracted.

The electret state is thus related to the electret behavior of collagen, which we have discussed above. It is interesting to observe that since the denaturation of collagen can be detected by the electret technique, it was possible to show that collagen in the mineralized tissue did not denature even after strong heating or mild chemical treatment. This particular behavior of collagen in bone had been previously demonstrated by *Glimcher* and *Katz* [6.50] using biochemical techniques. Here we find a useful and simple application of the electret investigations. More recently Slaets (personal communication) has found in our laboratory that the saturation behavior of bone samples may depend on hydration.

6.10 Natural Electrets

Menefee [6.10] made the important discovery that the native keratin from porcupine quills presented strong thermocurrents when heated to temperatures at which melting of the alpha helix occurs. At these temperatures, dipole disordering takes place simultaneously, and it is this configurational change that induces the observed currents. Thus the existence of natural electrets has been demonstrated for a material in native conditions. *Menefee* also was able to show that the effect showed the expected symmetry, that is, reversing the orientation of the sample (in relation to its in vivo geometry) reversed the current observed during heating. Also, mounting the sample in such a way that the quill fiber axis was in the plane of the measuring electrodes, the released current was negligible, indicating a strong anisotropy. This was expected, since the dipoles groups are known to lie along the helix axis. For samples with nonoriented dipoles (that is, sample with no true permanent dipole moment, such as nylon), no effect was observed. *Menefee*'s results are shown in Fig. 6.13.

Fukada et al. [6.24] have also confirmed *Menefee*'s results and extended the observations with simultaneous piezoelectric measurements for the case of horn keratin. *Menefee* has also proposed a simple model for charge separation by helix disordering. The model presupposes an initial polarization P_0 decaying as a

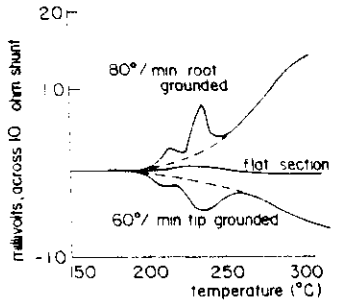

Fig. 6.13. Depolarization of native keratin [6.10]

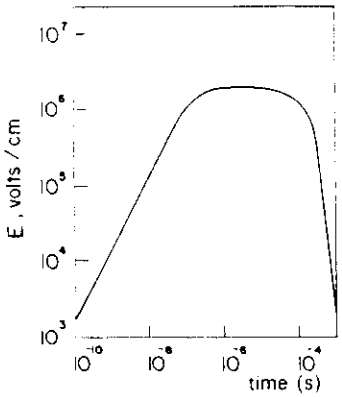

Fig. 6.14. Field from melting α-helix dipoles [6.10]

capacitance of the system C, and the sample resistance R, the following equation is obtained:

$$(2C - C_0)dV/dt + V/R = (P_0/\tau_1)\exp(-t/\tau_1), \tag{6.6}$$

where C_0 is the vacuum capacitance of the system. With boundary condition $V=0$ at $t=0$ and $t=\infty$, one can solve (6.6) for the electric field E:

$$E = [\varrho P_0/(\tau_1 - \tau_2)][\exp(-t/\tau_1) - \exp(-t/\tau_2)], \tag{6.7}$$

where ϱ is the specific resistivity, and τ_2 is given by

$$\tau_2 = \varrho(2\varepsilon - 1)\varepsilon_0 .$$

Assuming $P_0 = 3 \times 10^{-6}$ C cm^{-2}, $\tau_2 = 1.7 \times 10^{-4}$ s, and $\tau_1 = 10^{-7}$ s and using (6.7) he obtained the results of Fig. 6.14.

The assumptions above were made for a hypothetical gellike membrane consisting of alpha-helix material undergoing a transition with the relaxation time which has been assumed to apply to such polypeptides.

It is seen that fields as high as 10^6 V cm^{-1} can be calculated from the model.

calculations corresponds or not to experimental conditions. Nevertheless, the model is very appealing from the point of view of possible biophysical applications, such as the study of phenomena that may occur in membranes or in the generation of signals from sensory receptors, as in the olfactory system.

The possible relation between these natural electrets and their production in vivo invites much speculation as to the role of the electret state in biological processes at the cellular and membrane level.

6.11 Conclusions

We have not tried to cover the entire literature concerning applications of electrets in biology. The tutorial nature of this monograph required a more compact presentation which we hope will have introduced the reader to certain specific but more or less typical problems of the field. The main conclusion is certainly that the field is still in its beginnings and that much valuable fundamental work remains to be done. Another conclusion is that the interdisciplinary nature of the research on these problems imposes limitations on the organization of the investigations. For instance, in bioengineering applications of electrets as biomaterials, the cooperation of biologists or medical specialists is certainly required. Even in the case of more basic investigations, such as the electret properties of biopolymers, the cooperation of biochemists in sample preparation and adequate control of physicochemical conditions is also necessary.

Last, but not least, the use of accessory techniques such as piezoelectricity, X-ray diffraction, and optical measurements as in other areas of solid-state physics will be very important for corroborating and reinforcing possible models and interpretations.

The fact that the electret state has been found for practically all important classes of biopolymer (polypeptides, polysaccharides, polynucleotides) opens wide areas for systematic investigations. The extremely important field of structured-water electrets will certainly attract the attention and interest of many, and we think that it may prove to be one of the most fertile fields for applications in biology. It is also per se a unique method for investigating water structuring around macromolecules. Membrane electrets constitute another field of great potential. In this respect, theoretical semiquantitative calculations such as those given by *Menefee* for signal propagation in a gellike helical polypeptide membrane may be a source of further ideas and investigations.

Natural electrets, as found in keratin, may also be present in vivo in a variety of other biological materials, including bone, tendon, and other tissues. They may also be a general feature of certain biopolymers capable of presenting dipole ordering in their structure. Such may be the case for chitin. In fact, just to mention one example in this field, the possibility exists that the perisarc of tubularia (a hydra), composed of chitin, is such a natural electret and may be

involved in important biophysical effects (*Mascarenhas, Shuhan, Liuggi:* unpublished results). Theoretical work is also needed not only to predict possible electret effects in biology but also for a better understanding of basic electret behavior in biological materials, such as the structured-water problem. Also there is the practically untouched field of the action of radiation on the electret behavior of biological molecules, and photoelectret properties, which may be an interesting side field in the new and large area of photobiology.

One of the most important aspects of electret work in general is related to the nonlinear electret. This is particularly important in the case of bioelectrets. With the examples of DNA and RNA, recently investigated by *Mascarenhas* et al. [6.43], and of collagen, by *Povoa* and *Zimmerman* [6.51], it is clear that ionic transport is an essential aspect of charge and polarization storage, inducing highly nonlinear effects. These are further coupled to hydration effects, as discussed previously. *Fröhlich* has called attention to these aspects [6.52]. An important picture that emerges from the bioelectret work is that there are perhaps strong connections between conformation of the biopolymers, compensating charges, hydration, and dipolar orientation. Changing the polarization storage induces changes in conformation that are of paramount importance for the biological action of the molecule. It is in this respect that the nonlinear bioelectret is a very rich concept. Throughout this chapter, we have considered the electret as a microscopic, molecular concept, in which sufficiently polarization or charge is stored locally with a relaxation time long in relation to the phenomena under observation. This concept is important in all our considerations of bioelectrets.

The work described in this chapter leads us to the conclusion that the study of electrets in biomaterials and biophysics is a new and fascinating field of basic and applied research.

Acknowledgements. We would like to thank the Foundation for Research of the State of Sao Paulo, the National Research Council of Brazil, and the NSF for financial assistance. The cooperation of colleagues at the Institute of Physics and Chemistry, Sao Carlos, and stimulating discussions with the late Professor L. Onsager, and with H. Fröhlich, E. Fukada, and M. Kasha are gratefully acknowledged.

References

6.1 M. Eguchi: Philos. Mag. **49**, 178 (1925)
6.2 O. Heaviside: *Electrical Papers*, Vol. 1 (Macmillan, London 1892) p. 488
6.3a B. Gross: *Charge Storage in Solid Dielectrics* (Elsevier, Amsterdam 1964)
b M. S. Campos (ed.): *Rep. Int. Symp. Electrets and Dielectrics* (Brazilian Academy of Sciences, Rio de Janeiro 1977)
6.4 C. Bucci, R. Fieschi: Phys. Rev. Lett. **12**, 16 (1964)
6.5 M. Campos, S. Mascarenhas, G. F. Leal Ferreira: Phys. Rev. Lett. **27**, 1432 (1971)
6.6 F. S. Sinencio, S. Mascarenhas, B. Royce: Phys. Lett. **26** A, 70 (1967)
6.7 J. van Turnhout: *Thermally Stimulated Discharge of Polymer Electrets* (Elsevier, Amsterdam

6.8 M. Perlman, C. Reedyck: J. Elec. Soc. **115**, 45 (1968)
6.9a S. Mascarenhas: J. Electrostatics **1**, 141 (1975); Ann. NY Acad. Sci. **238**, 36 (1974)
b T. Reichle, M. Nedeska, A. Mayer: J. Phys. Chem. **74**, 2659 (1970)
6.10 E. Menefee: In *Electrets*, ed. by M. Perlman (Electrochemical Society, New York 1973) p. 661
6.11 I. Yannas: In *Biomedical Physics and Biomaterials Science*, ed. by E. Stanley (MIT Press, Cambridge, Mass. 1973) Chap. 3
6.12 E. Murphy, S. Merchant: In *Electrets*, ed. by M. Perlman (Electrochemical Society, New York 1973) p. 627
6.13 C. Linder, I. Miller: J. Phys. Chem. **76**, 3434 (1972)
6.14 M. Goel, S. Meera, P. Pellai: In *Abstracts Int. Workshop Electrical Charges in Dielectrics*, Kyoto, Japan, ed. by E. Fukada (1978) p. 64
6.15 B. Gross: In *Electrets and Charge Storage Phenomena*, ed. by M. Perlman, L. Baxter (Electrochemical Society, New York 1968); Appl. Opt., Supp. 3: "Electrophotography", 176 (1969)
6.16a M. Perlman, L. Baxter(eds.): *Report Int. Electret Symposium* (Electrochemical Society, New York 1968)
b E. Fukada(ed.): *Abstracts Int. Workshop Electrical Charges in Dielectrics*, Kyoto, Japan (1978)
6.17 G. F. Leal Ferreira, L. N. Oliveira: Phys. Rev. **11**, 2311 (1975);
G. F. Leal Ferreira, B. Gross: Rev. Bras. Fisica, **2**, 205 (1972);
B. Gross et al., Phys. Rev. **B9**, 5318 (1974)
6.18 R. Creswell, M. Perlman: J. Appl. Phys. **41**, 2365 (1970);
R. Capelletti, R. Fieschi: In *Electrets: Charge Storage and Transport in Dielectrics*, ed. by M. Perlman (Electrochemical Society, Princeton, N. J. 1973) p. 1
6.19 J. Slaets: "Ultralow frequency bridge", M. Sc. Thesis (University of Sao Paulo, Sao Carlos 1976)
6.20 B. Gross: L. F. De Novd: Phys. Rev. **67**, 253 (1945);
B. Gross: J. Chem. Phys. **17**, 866 (1949)
6.21 M. Campos, S. Mascarenhas, G. F. Leal Ferreira: Phys. Rev. Lett. **27**, 1432 (1968)
6.22 E. Fukada: Prog. Polym. Sci. Jpn. Kodan-Sha **2**, 329 (1971); in *Electrets, Charge Storage, and Transport in Dielectrics*, ed. by M. Perlman (Electrochemical Society, Princeton, N. J. 1973) p. 486; Ann. N. Y. Acad. Sci. **238**, 7 (1974)
6.23a M. G. Broadhurst et al.: In *Electrets*, ed. by M. Perlman (Electrochemical Society, Princeton, N. J. 1973) p. 492
b R. L. Zimmerman et al.: J. Appl. Polym. Sci. **19**, 1373 (1975)
6.24 E. Fukada, R. Zimmerman, S. Mascarenhas: Biochem. Biophys. Res. Commun. **62**, 415 (1975)
6.25 S. Mascarenhas: Ann. N. Y. Acad. Sci. **238**, 36 (1974)
6.26 J. Costa Ribeiro: An. Acad. Bras. Cienc. **22**, 325 (1950)
P. Eyerer: Adv. Colloid Interface Sci. **3**, 223 (1972)
6.27 S. Mascarenhas: In *Physics of Ice*, ed. by N. Riehl, R. Bullemer, H. Engelhardt (Plenum, New York 1969) p. 483
6.28 C. Hazlewood(ed.): Ann. NY Acad. Sci. **204**, (1973)
6.29 S. Brunauer, P. Emmett, E. Teller: J. Am. Chem. Soc. **60**, 309 (1938)
6.30 H. Berendsen: In *Biology of the Mouth*, (American Association for the Advancement of Science, Washington, D. C. 1968) p. 145
R. Grigera: *Introduction to the Biophysics of Water* (Universitaria, Buenos Aires 1976)
R. Grigera, S. Mascarenhas: Stud. Biophys. (to be published)
6.31 V. Tomaselli, M. Shamos: Biopolymers **12**, 353 (1973)
T. Ghilardi Neto, R. L. Zimmerman: Biophys. J. **15**, 573 (1975)
6.32 S. Mascarenhas: In [6.16b]
6.33 G. Gross: In *Electrets, Charge Storage and Transport in Dielectrics*, ed. by M. Perlman (Electrochemical Society, Princeton N. J., 1973) p. 560
6.34a G. A. Jeffrey, R. McMullan: Inorg. Chem. **8**, 43 (1967)
b L. Onsager, D. Staebler, S. Mascarenhas: J. Chem. Phys. **68**, 3823 (1978)
6.35 N. Dweltz: Biochim Biophys. Acta **44**, 416 (1960)
6.36 H. Fröhlich: Int. J. Quantum Chem. **2**, 641 (1968); Phys. Lett. **44A**, 385 (1973); Proc. Nat. Acad.

6.37 S.Celaschi, S.Mascarenhas: Biophys. J. **20**, 273 (1977)
6.38 L.Onsager, D.Staebler, S.Mascarenhas: J. Chem. Phys. **68**, 3823 (1978)
6.39 J.Polonsky, Douzou, C.Sadron: C. R. Acad. Sci. **251**, 976 (1961)
6.40 P.Fong: Bull. G. Acad. Sci. **30**, 13 (1972);
B.T.Mathias: In *Proc. 3rd Conf. from Theoretical Physics to Biology*, Inst. de La Vie, ed. by M.Marois (Karger, Basel 1973)
6.41 C.Brot et al.: J. Chem. Phys. **43**, B603 (1965)
6.42 A.Stanford, A.Lorey: Nature (London) **219**, 1250 (1968)
6.43 S.Quezado: "DNA: Bioelectret, Ferroelectricity, and Non-linearity", M. Sc. Thesis, University of Sao Paulo, Sao Carlos (1978)
S.Mascarenhas et al.: "On the the problem of ferroelectricity and non-linearity of DNA and RNA", in *Riken Symposium on Electrical Properties of Polymers*, Saitama, Japan, ed. by E.Fukada (1978)
6.44 S.Mascarenhas et al.: An. Acad. Bras. Cienc. (to be published)
6.45 S.Mascarenhas: "Bioelectrical Properties in connection with Fröhlich's Theory", in *Conf. Int. Physique Théorique à la Biologie*, ed. by M.Marois (Institut de La Vie, Versailles 1975)
6.46 P.V.Murphy, S.Marchant: In *Electrets, Charge Storage and Transport in Dielectrics*, ed. by M.Perlman (Electrochemical Society, Princeton, N. J. 1973) p. 627
6.47 I.M.Spinelli, S.Mascarenhas, A.Sader: In "Rep. st Latin-Am. Cong. Med. Phys., ed. by S.Watanabe (University of Sao Paulo, 1972) p. 14
6.48 E.Fukada, T.Takamatsu, I.Yasuda: Jpn. J. Appl. Phys. **14**, 2079 (1975)
J.J.Konikoff, J.West: In *Rep. Conf. Electrical Insulation and Dielectric Phenomena* (National Academy of Sciences, Washington, D. C. 1978) p. 304
6.49 S.Haridoss, R.Bullard: Proc. Indian Acad. Sci. **24**, 34 (1978)
6.50 M.Glimcher, E.J.Katz: Ultrastruc. Res. **12**, 705 (1965)
6.51 J. Povoa, R.L.Zimmerman: To be published
J. Povoa: "Non-linear transport in collagen", M. Sc. Thesis (University of Sao Paulo, Sao Carlos 1978)
6.52 H.Fröhlich: "Non-linear Dielectric Properties of Biological Materials", in *Rept. Conf. Electrical Insulators and Dielectric Phenomena* (National Academy of Sciences, Washington D. C. 1978) p. 259