Physiol. Res. 42: 277–282, 1993

SHORT COMMUNICATION

A Method with Enhanced Sensitivity for Temperature Measurement in Living Tissues

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Received January 5, 1993
Accepted April 16, 1993

Summary

A system for the evaluation of temperature changes in living tissue at a dimensional level of a single cell is described. A glass micropipette the tip of which is filled with semiconducting glass (Rech *et al.* 1992), is used as a microsensor. The changes of conductivity of the sensor due to variations of temperature are evaluated by electronic circuitry based on the measurement of an AC current of sinusoidal waveform flowing through the sensor. Temperature changes in the range of 0.01 K can be detected in this way.

Key words

Microsensor – Temperature measurement – Electrical admittance measurement

Introduction

We have already described (Rech *et al.* 1992) an impulse system for temperature measurements in a microvolume of biological tissue. This system was able to discriminate temperature changes of about 0.1 K. To further improve this resolution we have developed a system which exploits the following advantages of measurement with sinusoidal waveforms:

1. The character of the measured impedance of the sensor (a bipole with non-homogeneously distributed parameters) allows only a sampling evaluation using small pulses. The bandwidth necessary for the evaluation of these small pulses is much greater than that for a sinusoidal signal of the same duration. Therefore, the signal to noise ratio (S/N), which limits the resolution of the system, is improved when using a sinusoidal signal.

2. The power dissipated in the sensor due to the measuring current flow causes selfheating of the sensor which introduces an error. Therefore, this power has to be minimized to a value acceptable with respect to the resulting S/N ratio while the peak value of the measuring current is the same in both cases. The effective value of the sinusoidal waveform is approximately equal to 0.7 power of the rectangular pulse. Moreover, by a sampling process the power is exploited only in the sampling interval, the major part of which is lost for the measurement.

3. A narrow-band frequency system suppresses the interferences (additional noise) outside its passband. This is advantageous when considering the bioelectric signals and interferences generated in the environment (power net).

In principle, the temperature is measured by evaluating the real part of the impedance of the microsensor by using a sinusoidal signal at a constant frequency. The tip of the microsensor, which is filled with the semiconductor, is immersed into a conductive tissue. It therefore represents a transmission line with short-circuited input and non-homogeneously distributed parameters (Dittert *et al.* 1988). The frequency-independent part of the impedance of such a transmission line, which is represented by an exponential function, resembles the measured parameter. In pilot measurements of the temperature in a physical environment (in gallium) (Rech *et al.* 1992) microsensors with conus-shaped tip were used. In this case of the tip geometry, the transmission formula can be solved in a closed form. Both real and imaginary parts of the impedance depend on the temperature, on the depth of the immersion of the tip in the tissue and on the frequency of the measuring signal. However, for the chosen frequency of the measuring signal, the frequency-independent part of the microsensor impedance can be replaced by its real part. The output signal of the i/u converter makes it possible to measure the admittance between the ground and one terminal of the sensor. This signal is filtered and separated into the real (U_{RE}) and imaginary (U_{IM}) parts by synchrodetectors. The real part of the signal is sampled at the instants of the maximum amplitude of the signal, i.e. at the half of the period of the measuring sinusoidal signal. The imaginary part is sampled in the same manner, integrated in time and applied to the control of the feedback capacitance C(u) of the i/u converter as to achieve the minimal amplitude of the U_{IM}. In this case, the maximal U_{RE} value represents the function of the temperature except of a known error (<1 %).

Table 1
Complex admittance Y [Ω^{-1}] of the microsensor with a conus-shaped form of the tip.

$$\mathrm{Re}(Y) = G_v(1+x') \frac{p^2+Q^2-(M^2+N^2)}{(p+Q)^2+(M+N)^2} \qquad A=(1+x')^{|\gamma|\cos\varphi}-(1+x']^{-|\gamma|\cos\varphi}$$

$$\mathrm{Im}[Y] = G_v(1+x') \frac{2(QM-PN)}{(P+Q]^2+(M+N)^2} \qquad B-(1+x')^{|\gamma|\cos\varphi}+(1+x']^{-|\gamma|\cos\varphi}$$

$P = |\gamma|(B\cos\varphi\ \cos\psi\ -\ A\sin\varphi\ \sin\psi)$
$Q = |\gamma|(A\cos\varphi\ \sin\psi\ +\ B\sin\varphi\ \cos\psi)$
$M = (1/2)A\cos\psi$
$N = (1/2)B\sin\psi$
$G_v = \sigma\pi r_0 tg\alpha$
$\sigma = \sigma_0 \exp B_k[(1/310)-(1/(273+\vartheta))]$

$|\gamma| = (1/2)[1+(4\omega_0\tau)^2]^{1/4}$
$\varphi = \mathrm{arctg}((4\omega_0\tau)$
$\psi = |\gamma|\sin\varphi.1N(1+x')$
$x' = xtg\alpha/r_o$
$\tau = (c'/(\sigma\pi tg^2\alpha))$
$c' = (2\pi\epsilon_0\epsilon_r/\ln k)$

ϑ [°C]	measured temperature
r_0 [m]	radius of the tip of the microsensor
α [grad]	angle between the axis and inner surface of the cone
σ [$\Omega^{-1}m^{-1}$]	specific eletric conductivity of the semicoductor
x [m]	depth of immersion of the microsensor
c' [F(m]	elementary transverse capacitor
G_v [Ω^{-1}]	frequency independent part of the conductivity
B_k [K]	constant (≃4270)
k [/]	ratio of external/internal radii of the tip of the micropipette

We have used the rationalized form of the overall impedance of the microsensor as the first approximation in Rech *et al.* (1992) since the estimation of the behaviour of the system during transients and their duration can be based on the contribution of the dominant pole of the impedance of the microsensor. The output signal is sampled in the time intervals which follow the transient, i.e. under the conditions of a D.C. measurement so that the influences of frequency-dependent parts of the microsensor are negligible.

Method and Results

The admittance of the microsensor determined using a sinusoidal measuring signal is represented by the following equation

$$Y = \mathrm{Re}(Y) + j\mathrm{Im}(Y),$$

where Y [Ω^{-1}] is microsensor admittance, Re(Y) [Ω^{-1}] is the real part of Y, and Im(Y) [Ω^{-1}] is the imaginary part of Y.

Assuming that the profile of the microsensor tip is linear (conical), the elements of the above equation are described in Table 1. The principal scheme of the designed synchrodetection system for measuring the temperature of the microsensor is shown in Fig. 1. The measured admittance is connected with the input of the i/u converter. The i/u converter consists of an operational amplifier A_1 with FET inputs (WSH117). The feedback of the converter is made by a parallel combination of the resistor R_f and the voltage-controlled capacitance C(u). The capacitance C(u) is the result of the serial combination of a fixed value capacitance C_f and a broadband amplifier A_2 (MBA145), the gain of which is controlled by the voltage U_1. The output voltage of the converter U_o is amplified and passes through the narrow-band second order filter BP which is tuned to the frequency of the measuring signal ω_o [s^{-1}]. The passband of the filter produced by the circuit UAF31 is approximately 60 Hz. The U_v and the $U_{v'}$, i.e. the direct and the inverted output voltages of the filter, represent the input signal of a pair of synchronous detectors PD_1 and PD_2, the output voltages of which are denoted as U_{IM} [V] and U_{RE} [V]. The former represents the imaginary part of the overall transfer function and the U_{RE} represents the real part of this transfer function. As has already been mentioned, the integrated signal U_{IM} controls the gain of the amplifier A_2 as to minimize the U_{IM} value.

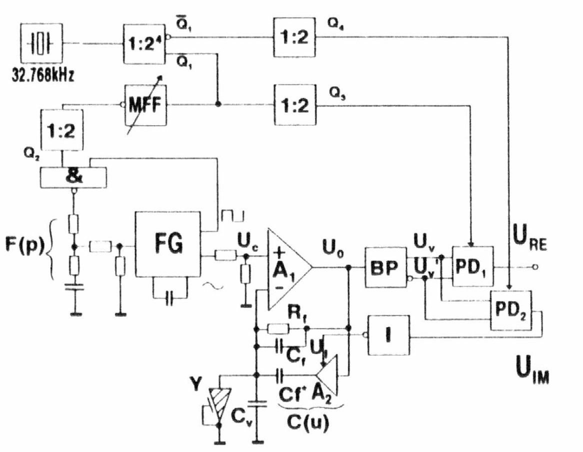

Fig. 1
Schematic diagram of the system for temperature measurement (A_1, A_2 – Amplifiers; BP – Bandpass filter; FG – Function generator; I – Integrator; MFF - Monostable flipflop; $PD_{1,2}$ – Phase-sensitive detectors; Y – Micropipette temperature sensor; & – NAND type integrated circuit; Q_1-Q_4 – Outputs of dividers).

The timing of the logic circuitry is controlled by a crystal oscillator (32.768 kHz). The signals necessary for the control of both synchronous detectors and the sinusoidal generator are derived from adequate outputs of a multistage divider. The measuring signal U_c [V] of the i/u converter is generated by the function generator (XR 2206), the frequency and phase of which are synchronized with the signal from the crystal clock generator divided by the frequency of 1024 Hz. The synchronization is accomplished by the phase-locked loop which is realized by the filter F(p). The monostable flipflop, which is designed as MFF, sets the phase shift between the measuring signal U_c and the signal Q_3 which controls the detector PD_1 providing the signal U_{RE}. The phase shift between the control signals Q_3 and Q_4 is permanently kept equal to $\pi/2$.

The frequency of the measuring signal U_c used, makes it possible for the signal U_{RE} to represent, except for a known error, a function of the temperature of the microsensor only. If $U_{IM} = 0$, then

$$U_{RE} = U_c H \frac{1 + R_f \mathrm{Re}(Y)}{1 - (\omega_o/\omega_n)^2}$$

where H [\] depicts the gain of the passband filter BP in resonance and R_f [Ω] represents the feedback resistance of the i/u converter. The resonance frequency ω_n [s^{-1}] of the measuring system may be expressed as

$$\omega_n = \left[\frac{\omega_t}{R_f (\Sigma C + \mathrm{Im}(Y)/\omega_o)} \right]^{1/2}$$

where $\Sigma C = C_v + C_{fo} + C_f$ [F] and ω_t [s^{-1}] represents the upper frequency of the amplifier A_1.

The imaginary part of the output voltage U_{IM} equals zero if the following condition is valid

$$C(u) + C_{fo} = \frac{(\Sigma C + \mathrm{Im}(Y)/\omega_o)[1 - (\omega_o/\omega_n)^2]}{1 + R_f \mathrm{Re}(Y)} \text{ [F]},$$

where voltage-controlled capacity C(u) [F] is equal to A $(B + U_1)$, when $U_1 < 0$, $A = 5.88 \times 10^{-12}$ [F/V], and B = 3.26 [V].

The error of the measurement δ [%], which takes into the account the frequency-dependent impedance of the microsensor and the properties of the amplifier A_1 of the i/u converter (Figs 2 and 3), is expressed as follows

$$\delta = \frac{100}{1 + R_f \mathrm{Re}(Y)} \{ R_f(\mathrm{Re}Y - G) + (\omega_o/\omega_n)^2 (1 + R_f G_v) \} \text{ [\%]}$$

where G_v [Ω^{-1}] is the frequency-independent part of Y.

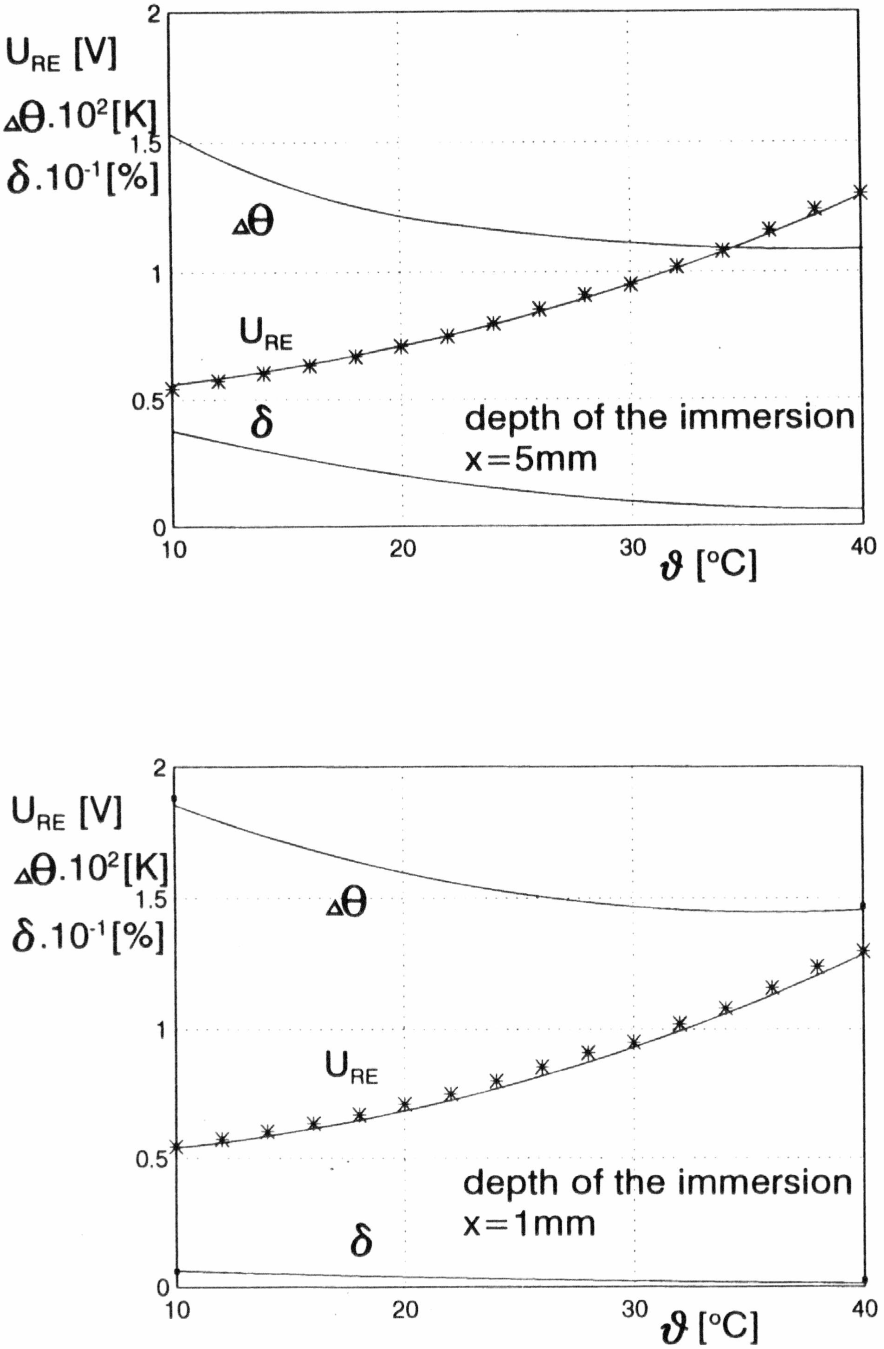

Fig. 2
Graph of computer evaluated resolution $\Delta\Theta$ [K] and systematic error ∂ [%] and experimentally determined output voltage U_{RE} [V] all as a function of temperature ϑ [°C] of the sensor immersed into a tissue (x=1 mm depth – upper panel; x=5 mm depth – lower panel).

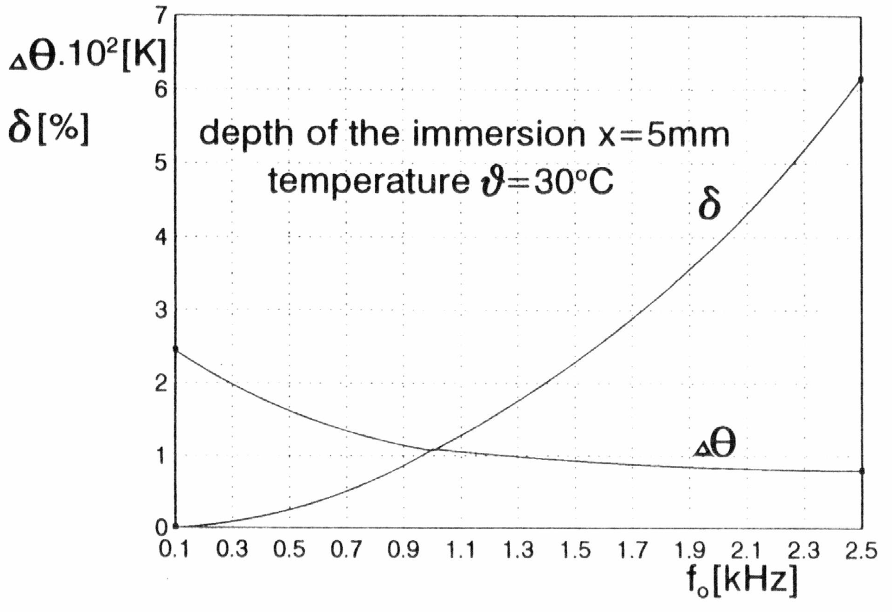

Fig. 3
Computed dependency between resolution $\Delta\Theta$ [K] and systematic error δ [%] related to the frequency of the measuring signal f_O [kHz].

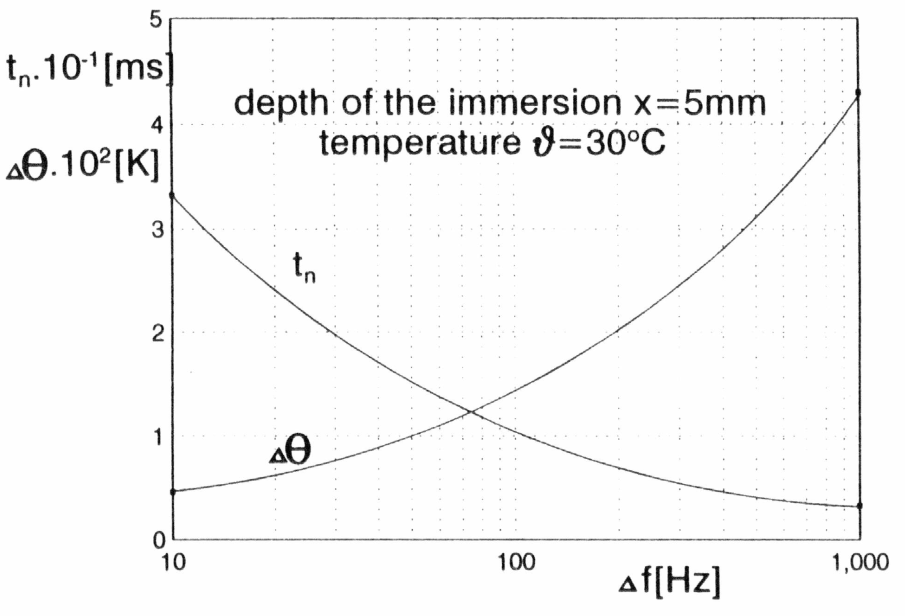

Fig. 4
Computed relation between resolution $\Delta\Theta$ [K] and transient time t_n [ms] (related to changes of micropipette immersion depth) with respect to the bandwidth Δ_f [Hz] of the bandpass filter of the system.

The real part of the electrical current i_v [A] passing through the microsensor heats it up. This results in the error of temperature measurement which is expressed as

$$\mathrm{Re}\, i_v \simeq U_c G v$$

The temperature resolution defined by equation

$$\Delta\theta = E_{no} / (\partial U_{RE} / \partial\theta) \ [K]$$

where $\partial U_{RE}/\partial H$ [V/K] is the sensitivity, E_{no} [V] is the output noise voltage.

This temperature resolution is better compared with the STEP method (Rech *et al.* 1992) in the ratio p[\], according to the following equation

$$p = e_n \left[\frac{2\pi \Delta f \omega_t R_f \Sigma C(C(u) + C_{fo})}{4k\theta(1 + R_f G_v) + i_n^2 R_f} \right]^{1/2}, \ (p \geq 10)$$

where Δ_f [Hz] is the bandwith of the bandpass filter BP, e_n [V/$\sqrt{}$Hz and i_n [A/$\sqrt{}$Hz] represent the spectral densities of the noise voltage and current at the input of the amplifier A_1. Both the current and voltage can be considered as constant in the narrow frequency band centred around ω_o.

The maximal detectable frequency of the temperature changes in a steady sensor set-up is given by the doubled frequency of the measuring signal (sampling of the local maximum of the measuring signal). In case of changes of the physical setting (e.g. change of the immersion depth) the transient time, i.e. the time interval necessary for the system to achieve the steady state (U_{IM} = 0), is determined by dynamics of the circuit which controls the gain of the A_2 amplifier. The control of the parameter U_{IM} = 0 is represented by a nonlinear differential equation of the second order. Considering that in practice the stepwise changes of the temperature will be in the range of units of K, we are allowed to neglect the nonlinearities and to determine the transient time t_n [s] of the control circuit (Fig. 4) in the following way

$$t_n = 2\sqrt{2} / \Omega_n, \ (t_n = 5 + 20 \text{ ms})$$

$$\Omega_n = \omega_o \left[\frac{\omega_o U_c A H R_f (1 + R_f \mathrm{Re}(Y))}{2\pi \Delta f T [1 + \omega_o^2 R_f^2 (C_f + C(u)))^2]} \right]^{1/2}$$

where Ω_n [s^{-1}] is the resonance frequency of the control circuit without damping, T [s^{-1}] is the time constant of the integration.

Discussion

The narrow-band frequency transfer function of the designed measuring system makes it possible to improve the signal/noise ratio (S/N) at least 10 times. Thus the temperature discrimination capabilities of the discussed system are at least 10 times higher when compared with that already published impulse measuring system (Rech *et al.* 1992). The above mentioned transfer function which is centered at 1024 Hz represents, in practice, a stop for bioelectric signals and other external sources of the noise. In case of a low amplitude measuring signal U (10 mV), the real part of the electrical current passing the microsensor is less than 1 nA which causes an error well below 10^{-3} K. Since the sensitivity in the discussed system increases with the temperature and noise increases in the same manners, the discrimination capability remains constant in the whole range of investigated temperatures.

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