OLD PRINCIPLE OF LIQUID ION-IMPEDANCE SPECTROMETRY (PARTICULARLY IN MILK) REALISED BY NEW COMPONENTS

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Ion impedance spectrometry enables detection of the concentrations of multiple chemical compositions in a weak solution, usually if only part of a complex signal is used and the frequency is not swept. Prior to the method described in this paper, ion impedance required rather sophisticated electronics to collect the results from an instrument, as well as the electrode polarisation minimising. We elaborated and tested circuitry that uses the newest electronics components for a chemical analysis instrument designed according to a principle patented in Latvia (No. SU-1021440A), year 1981. The newly improved ion impedance spectrometry apparatus is suited for accurate measurement of ion concentrations for commercial use by various trade professionals. The primary target was an in-flow milk tester for dairy farm milking devices (yet this is not the exclusive use).

Key words: experiment computerisation, AC-conductometry, balanced bridge circuit, electrode anti-polarisation, two-electrode-system, milk mastitometry, electronic circuitry.

HISTORY OF THE PROBLEM

Early in the 18th century, it was found that liquid chemical component concentration is linked to electrical resistance. Later it was found that, if far enough from a saturation area, concentration may be measured indirectly by the resistance between two or four submersed electrodes, and that conductance versus concentration curve was semi-linear (Lima *et al.*, 2017).

However, the electrodes become polarised rapidly (polarisation and oxidation/rusting are rather similar processes). It was discovered that, due to polarisation, four-electrode probes provide more accurate results than simpler-to-use two-electrode probes (Becchi *et al.*, 2005). Later it was found that applying AC voltage on electrodes is less aggressive against electrode polarisation than DC, and moreover, graphs of both AC active and reactive components (Fig. 1) may give a more clear view of processes occurring in tested liquids, in comparison to a simple DC circuit (Breitkopf, 2012). In 1963, Latvian inventor Helmutis Maurops started implementing a similar two electrode test system on the ba-

Fig 1. Principal response of active and reactive current components in a measured cell. The Figure does not give real data, except for the crossing point in case of milk as an electrolyte.

sis of a multivibrator circuit for a cow milk quality tester and had a rapid success. The Soviet system suspected a hidden threat in the invention and delayed granting the patent (SU-1021440A) until 1981, despite thorough testing and re-testing of milk samples from more than 1500 cows from different geographic regions. A few years later, the Rīga factory “Impulss” started to produce such rapid-analysis apparatus. Production was soon stopped due shortages of electronic components and the shift of the political/economic regime.

The patent’s concept was to use a frequency where the real impedance part has started to fall, and the imaginary part

began to rise. Concerning milk, this is a shallow quasi resonant kind of response with a 33 kHz optimum. To diminish the polarisation in a two-electrode test-cell, the inventor used a 33 Hz interval, after which the phase of current was inverted. Thus, applying an unipolar V_{cc} , there was a bipolar current between electrodes: when the selected electrode polarised in 30 milliseconds, in the next milliseconds it was polarised back. Thus, this invention was highly effective polarisation counter-measure and mastitis was detected by measuring *de facto* Na/Ca/K ion balance manifested in the AC-resistance of milk. K ions are mostly located outside somatic cells, while Na ions are located inside cells and leak out when cell membranes are broken, indicative of mastitis disease. The patented system had two multivibrator circuits with frequency ratio 1:100 and a galvanometer between the outputs. The multivibrator non-idealities, drift, non-symmetry and galvanometer insensitivities made this patented circuit rather unstable and, due to this, measure-wires hang high over the GND potential, which is hard to implement in more modern electronic systems. Therefore, one of the solutions historically was to use a four-quadrant magnetophone level 157DA1 IC detector (Anonymous, 2020b). Following the cessation of the magnetic tape-recorder era, production of this semiconductor component was stopped worldwide and similar devices could not be found. We experimented with precision full-wave rectifiers with Op-Amps, but the results were unstable.

The first Maurops' experiments with AC between two electrodes in milk (without any polarity shifting) showed visible electrolysis bubbles coming out the liquid, and it was found that the bubble-production was much suppressed specifically at 33 kHz. Later, polarity shifting was introduced, minimising bubble-creation, yet this was sufficiently suitable when the electrode materials were gold-plated (or platinum, which is too costly). His trials with sinusoidal and meandric signals showed no big difference between both, however generation and manipulation about meandric is simpler. Yet, his observations created some questions in regard to meanders seen by Fourier analysis as superposition of a basic sinus with strong weight of uneven harmonics (33% for first, 20% for second etc); anyway praxis says that a meandric signal is good enough.

CIRCUIT ELABORATION

We realised the importance of extreme precision in both multivibrators by means of on/off timing equivalence, and that the output signals for both electrodes must be frequency divided by a factor of two, which is the standard method to obtain supersymmetric signals. That conceptually may be solved by means of the IC named 155TM2/561TM2 — a universal TT trigger (means as D-C as well the R-S rules inside). However, considering the need for volume production of the milk testing apparatus, little-known Soviet era components must be replaced by components available worldwide such as CD4013 (datasheet CD4013, Anonymous 2020c). For frequency division, the meander generator circuit will add the trigger. As each IC chip contains two trig-

gers, the oscillator should be made by the other half of the same trigger. TM2 has a sufficiently large input amplification factor, and thus the R-S trigger based generator may work well with two resistors of 100 kOhm and two timing capacitors and two diodes (Anonymous, 2018). But it is not the case for CD4013 (we applied firstly a DIP version), which demanded that both resistor values be slightly under 75 kOhm for higher frequency and 20 kOhm for the lower one. For the China produced SMD VSOP size CD4013, its amplification was even low, and thus the 20 kOhm and lately 10 kOhm were able to begin generation only sometimes with a random pattern. Therefore, we chose a sounder circuit topology using a phase-reverting FET transistor in output, as Johnsson proposed (Johnsson, 2010 and 2017). However, as his footnote about difficult igniting at small power was true, we chose the 2010 modified version, where a difficult start problem was solved even at V_{cc} of 3V3 voltage. We choose power so low because of the ARM processor (Atmel 328) in the system, to avoid the demand for multiple feed voltages, and the same due to other components in our system like the FSK radio data transmission line and even the display. It must be warned as it is not obvious: in a non-5V fed Atmel-328, there must be a 2X lower CLK frequency, which means using a 8 MHz quartz instead of a 16 MHz. Both generators are adjusted to twice as large frequencies as those demanded at output for the tested cell, because of the frequency dividers. However, if we would use a two voltage power for Atmel (3V3 and 5V0) and full speed consequently, our display would react much faster. Now it seems sometimes irritatingly slow to rewrite the figures.

The last but not least problem was how to measure the very low differential mode current (microampere, millivolts) via test-electrodes having many orders higher synphase (i.e. common mode rejection) of the voltage component ($2 \times 3V3$). Our experiments with a rather soundly constructed instrumental-amplifier circuit on the basis of high accuracy op-amps having a CMR factor even as large as 120 dB was a failure. Thus, after years of searching for the best available IC, we obtained a circuit capable to translate the upper shoulder current accurately into GND-bound output current in no respect the CM rippling (Fig. 2). It was a highly specific IC named INA-170 (datasheet iNA-170, Anonymous 2020d), elaborated for measuring SMPS bridge circuit upper shoulder current. We used it according to the datasheet instructions. Thus, the crucial breakthrough was gained to work on modern day element basis showing the well anti-polarisation-secured two electrode current in the test-cell. The OpAmp DC "mid- V_{cc} " ground is organised by floating reference voltage source on the basis of reciprocal Zener TLV431, which is the twin-brother of widely known TL431 but with half as low output voltage (1.25 Volts).

After iNA-170 stays the tiny-noise Op-Amp realising the integrator with a proper amplification factor, whose purpose is to convert the 33 kHz AC to a measurable relatively stable DC. After this integrator may stay the digital voltmeter panel; or other option is to acquire the reading with local

Fig 2. The proposed circuit where R7 or alternatively R9 is an electrode resistance. Depending on this choice the device will measure conductance or resistance. R7 value may be adapted for target concentration.

ARM ADC input, however, in the case of Atmel-328 the 8 bits system able to be boosted to 10 bits, the ADC resolution (alias 1000 gradations) is limit. This is sufficient for practical need.

For the case of testing milk samples, further discussion is not needed, but some other chemical mixtures can be tested with the intent to observe both real and reactive currents in tested cells. Then we can label such test impedance spectrometry, if the frequency is swept. For milk this is very useful, but this circuit permits such special reading if implement synchronous detection (aka PLL driven detection) circuit (explanation of SD, 2020) between voltage on-the-cell and current in-the-cell. Moreover, the reactive component is time-shifted (forth or back direction). Thus, as to iNA-170 decoded signal, if having stable value it means the reactance is absent, but if there is fast-changing component over each pulse plane top, it means that the difference to the base (i.e. forehead of pulse) value is reactive component impact, and base value itself is an active resistance. As we use the integrator, we see in output the absolute (resistance plus reactance) value.

RESULTS: MEASURE-SYSTEM EFFICIENCY

This is not a new analytical method. For example, “Afimilk” company explains the methods chrestomatic basics in its website (Anonymous, 2010), and numerous scientific publications explain it in even more detail, with practical aspects (Anonymous, 2020a) or in depth chemical basics (Romero *et al.*, 2016; Muchetti *et al.*, 1993). The benefits of regular conductometric tests in veterinary aspects are discussed in (Lusis *et al.*, 2017).

New at our job was the hardware realisation method by means of iNA-170 giving more simple, accurate and effective implementation of measurement physically-chemical principle in comparison with other concurrent electronics, for example, instrumental amplifier in quadrature envelope detector. Surplus, the implementation of both supersymmetrical carrier signal generators by means of frequency division to factor of 2, thus effectively diminishing carrier signal inaccuracy caused electrode polarisation as well as did the original Maup's Patent.

For small farm obstacles classical mastitometric analyses customarily are made by taking out the milk samples, thus uninterrupted sampling in the milk live flow is a rather rare thing, however, providing good knowledge of how conductance (or other parameters in measure) is/are changing during the time of milking. Thus, our proposed method decline the test-caused time loss to absolute zero (except to read the figures) and qualified laborant workforce need here is zero as well. The self-cost of complete measurement services offered by our unit may be evaluated by assumptions that the market price is 100 Eur and the expected work life is over 20 years, thus performing milking three times per day we obtain a cost of about 0.4 cents per analysis or proportionally less for many cows.

We evaluated the impact of flow speed to the conductometric results when electrodes were parallel to the flow, perpendicular to the flow, and with no flow. There was no notable difference. We evaluated the best option for measuring cell resistance obtained by estimating expected liquid-under-test resistance. For the case of milk standard substitute CircoTop-MBX (GEA Farm Technologies, Metatron MB, Austria) in a subscribed dilution of 7 ml/l and using 12 mm long rectangular gold-plated electrodes with thickness of 0.6 mm and distance of 5 mm (extracted from pcb connectors), the resistance was 1500 Ohms, contradicting the authors of many articles stating that normal milk conductance is 4.0–5.5 mS/cm (or 4–4.9 mS/cm for healthy milk and 5.0–7.5 mS/cm for unhealthy); thus we expect 75–100 Ohms at our 5 mm cell for natural pure cow milk (Fernando, 1982; Nielen, 1992). Other sample that we used was “Alma” (Tartu) pasteurised packaged milk (2.5% fat) resulting in 7000 Ohms to 5 mm. It is important to know the right figure to adapt the maximum sensitivity that is gained if $R_7 = R_9$ (where 80 kOhm is a fictious figure in the diagram). However, the dispute about the best R_7 value is off-topic, since we discuss circuitry not milk.

Method’s repeatability is determined by electrode polarisation. We applied electrodes into a flask with a 1:1 NaCl+KCl solution of less than 1 g/l until the resistance of 0.8 kOhm/cm (corresponding to 1.5 mS/cm), and continued

monitoring for a one-week-long uninterrupted trial. The initial result did not shift by more than 0.1%, which was the accuracy of the ADC reading unit of ARM (at stabilised temperature). Thus, electrode polarisation is negligibly small. Normally, under one year exploitation for one cow, the summed time of use of the electrodes may be under four “uninterrupted days”, and thus difference in measured values would be due to sample (milk) chemistry and not of the electronics. Therefore, we can trust the patent author Maurops’ extensive comparisons in the 1960s conducted on 1500 cows (~10 000 measurements) between his mastitometer and other available methods in many agricultural regions of USSR. The elaborated circuit effectiveness using real-life environment measurements was made several years earlier on the basis of a less elegant circuit solution. That includes correlation comparison with citric acid samples obtained by titration (Maurops *et al.*, 2018). The change of electronics components did not alter the electro-physics of the tested cell.

Every new electronics part or even one nominal of say resistor may change the graduation graph telling where is the invisible line between slightly ill, hardly ill and completely healthy cow. But the apparatus is not intended to say bold diagnosis, it is intended instead to tell the figure for the operator himself (with the help of his veterinary doctor) to elaborate the level — to act mild, to act hard, or not to act at all, the principle that is bolded at (Anonymous, 2020b). Thus, the precise figure of limit elaboration was not the aim of this project (however, graduation is highly possible), but elaboration of a cheap, accurate and flexible electronics circuitry in SMD PCB including ARM computer and most up-to-date electronics component technologies for use as rough indicator. Thus, the project goal was fulfilled.

We compared the results obtained from a mastitometer MI-1 produced in 1999 with those made with the new apparatus. We used one fresh milk sample and one with added 0.05 gram table salt, at volume about 300 ml. We first measured the fresh milk, then the one with salt added, and then with more salt. The graduation graph is shown in Figure 3.

Fig 3. Comparison of two mastitometers, old version with 157DA1 and new version with iNA-170. Upper line – old measurer showing three possible “answers”: good 1, slightly suspicious = 2, alarm = 3. Lower line – new measurer, value in Volts. At absceyss – additive count of dirty milk droplets.

DISCUSSION

The circuit diagram obtained is highly stable and may be replicated by any average-qualified electronics engineer. The impedance tester was implemented as a part of an innovative Small Animal Farm Milking Apparatus, which is a classical vacuum-driven milking machine supplemented with:

- 1) impedance (AC semi-resonant conductometry) analyser of milk with live flow mastitis early warning with simple switching between conductometry mode or ohmmeter mode on demand;
- 2) precision digital thermometry IC that measures milk (means bovine basal) temperature for identification of any unspecified illness as an early warning indicator and even as an ovulation cycle indicator in the hands of a well-trained user;
- 3) simple flow-meter of rotating squirrel-cage giving the “machine-overmilking” criterion warning sound saying the milking must be stopped immediately, thus negating the accepted wisdom that no apparatus can be as good as milking by hand.

If any ARM controller is used in the circuit, why not to save 10 cents for trigger IC and ARM CLK-bound possibilities to generate both meander signals instead. Firstly, the reason was the heritage of previous versions of apparatus; secondly, the apparatus may be used with or without the ARM sub-versions; thirdly, the Atmel-328 in circuit has a rather serious load servicing 6 ADC channels, bidirectional radiochannel data packet workflow, making a set of calculations and decision-making tasks, so we were afraid it might overflow and this monotone signal generation job may turn out be a last droplet crushing all good.

The principles of the described circuitry may be applied to multiple tasks apart from testing of milk, such as analyses of urine samples as well as blood samples for health. Among many other potential applications is the testing of titrated citric acid solutions associated with dental surgery. After another research for an optimum frequency, there is a possibility that the circuit may solve even the much needed problem in veterinary medicine, which is the Ca ions selective concentration analysis in a simple and inexpensive manner.

During the research for this project, the idea emerged that the physical effect behind the circuit is measuring ion mobility. Simplifying for a better picture, the ions are moving to and fro in between electrodes space created by E-field of AC, yet with displacement which is hardly less than distance between electrodes. Since the viscous liquid means far harder mechanical resistance force for larger ion radius than for small ions, therefore the concentration being for all three ions in milk (NaCl, KCl, CaCl etc salts) not so much different, produces a rather high shift on AC conductance readings, when the dominant ion is substituted by non-dominant both ions from intra-cellular volume. And the resonant char-

acter of this movement explains a semi-resonant character of conductance readings by frequency in use, thus, the optimal frequency choice. It should be noted that the polarisation process may be visually observed if the chosen frequency wrong, like small bubbles stacking on the surface of electrodes, or absence when it is minimized.

Data availability statement: Surplus information on the design may be obtained by correspondence, see author mail address.

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SENAIS JONU IMPEDANCES SPEKTROMETRIJAS PRINCIPS (TAI SKAITĀ PIENAM), IEDZĪVINĀTS JAUNĀ MODERNAS ELEKTRONIKAS REALIZĀCIJĀ

Jonu impedances spektrometrija spēj mērīt dažādu ķīmisko elementu koncentrāciju mērāmajā nelielas koncentrācijas šķīdumā. Iepriekš šī metode prasīja samērā sarežģītu elektroniku, lai realizētu mēršūnas datu savākšanu un apstrādi, tai skaitā novērstu elektrodu polarizāciju. Mums izdevās izstrādāt un pārbaudīt ķīmisko analīžu instrumenta izstrādei vienkārši realizējamu shemotehniku, kas darbojas uz jaunāko elektronikas detaļu bāzes, kas atbilst Latvijas Patenta (Nr. SU-1021440A) principiem (1981). Tādējādi uzlabotais jonu impedances mēraparāts spēj precīzi mērīt jonu koncentrācijas dažādos komercpielietojumos, kaut arī mūsu primārais mērķis bija piena kvalitātes testēšana tekošā strūkļā slaukšanas iekārtā. Tomēr pielietojumi iespējami ļoti dažādiem analizējamiem šķīdumiem/vielām.