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In-house development of automatic iontophoresis current source for sweat chloride test

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ABSTRACT

Background: The standard method for cystic fibrosis screening is pilocarpine iontophoresis sweat chloride test. The iontophoresis device must supply reliable and accurate electrical current within the safety range. The researchers determined that an in-house development of reliable automatic iontophoresis current source for sweat chloride test would have an impact to cost and popularity of the test in Thailand. Furthermore, it will promote the development of a technological self-reliance in the future.

Objectives: Automatic iontophoresis current source was designed and fabricated to conform to IEC 479-1 electrical safety standard and CLSI C34-A3 Sweat Chloride Testing Standard. Performance of the device was tested by performing sweat chloride test in healthy volunteers.

Materials and methods: Design and develop automatic iontophoresis current source for sweat chloride test with electrical safety standards IEC 479-1 and conform to CLSI C34-A3 standard. The developed device will be tested by performing classic sweat chloride test (CST) in healthy adult volunteers.

Results: Seventy healthy volunteers (age between 18 to 40 years old) were recruited. No history or signs of illness or being treated for diseases including the lungs, liver, pancreas, and intestines were found. 27 males and 43 females are compared between control and test with pilocarpine iontophoresis using stimulated direct current of 1.0 mA for 4 minute (0.07 mA/cm^2) and 30 minutes for sweat collection. The electrical current used was far lower than recommendation by CLSI C34-A3 standard and other known studies, but still can give out weight of sweat (more than 0.077 gram) as recommended by CLSI C34-A3 standard. Mean weight of collected sweat in male volunteers are 0.35±0.10 gram and 0.17±0.08 gram in female volunteers. And there are significant differences in chloride concentration obtained from male and female volunteers that correlated with previous studies.

Conclusion: The in-house development of automatic iontophoresis current source device for sweat chloride test was successful and successfully used in the sweat chloride test in normal healthy adult volunteers. Sweat can be stimulated effectively with low iontophoresis current in normal subject. Moreover, compare with CLSI-C34-A3 recommendation, the device can stimulate sweat production far more than minimum sample weight of 0.077 gram with lower electric current and shorter duration of stimulated time.

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Introduction

Mutation of cystic fibrosis trans-membrane conductance regulator (CFTR) gene on chromosome 7 results in autosomal recessive disease-Cystic Fibrosis (CF). Ion transport abnormalities in various tissues especially in epithelium of lungs and pancreas are the mainstay of clinical manifestations.[1, 2] Levels of chloride in sweat of CF patient was also abnormally elevated.[3] Subsequently, classic sweat chloride test (CST) that utilized pilocarpine as a cholinergic stimulant of sweat gland on the skin via iontophoresis was also published.[4] Sweat chloride concentration in normal individuals are below 30 mmol/L but are higher in CF.[5] At present, CFTR sequencing can be performed as reliable diagnostic method in CF but are expensive, so sweat chloride test is still in use and considered the gold standard in screening and diagnosis of CF.

It is long believed that CF is rare in Thai population. But in recent years, there are many reports of CF in Thais.[6-9] The actual incidence of CF in Thailand is still unknown. Sweat chloride test might be the appropriate method for screening test for CF in Thailand because of lower expense and being relatively less complicate to perform.

In Gibson and Cooke CST, pilocarpine is used for sweat stimulation from sweat glands of forearm. Pilocarpine is a parasympathomimetic alkaloid mimicking acetylcholine to cholinergic receptors that result in stimulation of muscarinic receptors of eccrine sweat gland to produce sweat. Small amount of electric currents is used to drive pilocarpine through skin to eccrine sweat gland. The process of electromotive repulsion of substances is called iontophoresis. Collected sweat is analyzed for chloride concentration. In CST, iontophoresis device consists of batteries connected in series with potentiometer to manually set electric current. Ampere meter is used to monitor the electric current. This device is simple and not expensive to duplicate but electric current is not constant. The electric current depends on multiple factors such as decay of battery, potentiometer setting, electrodes placement, electrolytes in use and skin electrical resistance. Burn and blister at the sites of electrodes placement usually occur if inappropriate overcurrent setting or prolonged stimulation time are used.[10]

The aim of this project was to design and construct an automatic constant current iontophoresis device from locally available parts that can easily be duplicated. With this device, standardized CST can be performed because iontophoresis current and stimulation time can be set and automatically regulated.

Materials and methods

Iontophoresis voltage and current source

Versatile high precision voltage controlled programmable current source using DACs, Op Amps, and MOSFET transistors as described in Analog Devices Circuit Note CN-0151 was used as a guide in designing of constant current source (Figure 1).[11] MCP4921, 12-Bit Voltage Output Digital-to-Analog Converter[12] was used to provide controlled voltage to current source. Arduino Uno R3 microcontroller board was used to control the selected current and timing as well as all the safety monitoring. Working iontophoresis current supply circuit is shown in Figure 2. Six 1.5 volts alkaline cells were used to provide 9 volts power supply to the device. XL6009 buck and boost regulator integrate circuit[13] was used to boost 9 volts supply voltage to 16-22.5 volts which is required to generate adequate iontophoresis current. The principle circuit of XL6009 in used is shown in Figure 3. The 33uH inductor in the circuit was modified to 80uH to lower the idle current in order to preserve battery life and limit the iontophoresis current to below 10 mA. The highest skin resistance is around 6000 ohms while determined by electrodes 2 cm in diameter and placed 2 cm apart using alternating current of 1000 Hz in frequency.[14] The highest allowed skin resistance during iontophoresis with our device is set at 5000 ohms while the highest iontophoresis current is set at 4 mA. In this case, the required iontophoresis voltage would be 20 volts ($4x10^{-3}$ x 5000=20). The device will first check skin resistance with small amount of electric current, if skin resistance is more than 5000 ohms, alarm will be activated and the procedure will be aborted. If the required iontophoresis current is lower than 4 mA, then the voltage required will be lower. We plan to use low iontophoresis current of not more than 1.5 mA as in use in pilocarpine gel discs (Pilogel®).[15]

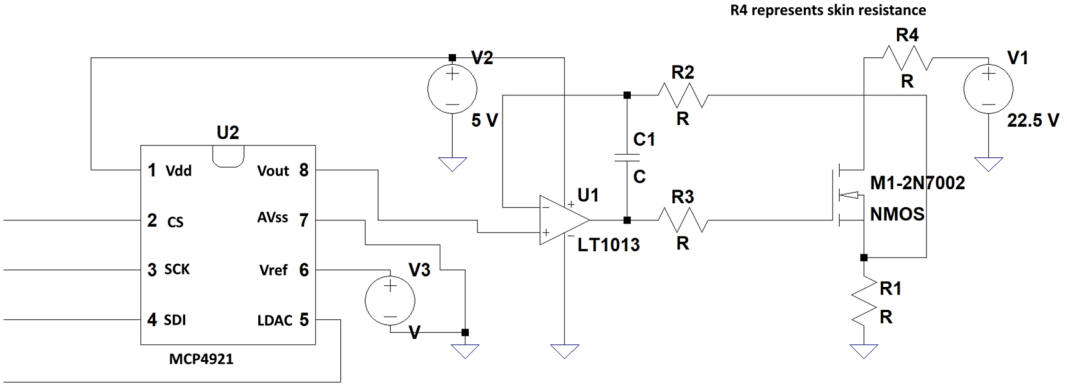

Figure 1. *Microcontroller controlled constant current source.*

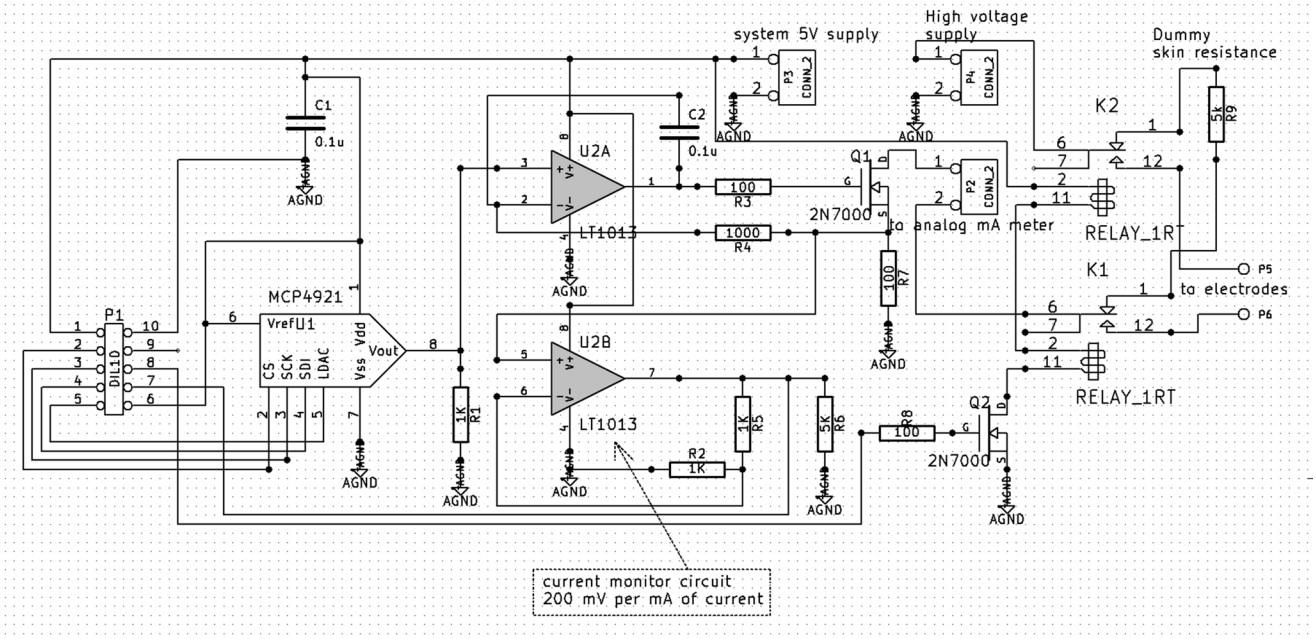

***Figure 2**. Working iontophoresis current supply circuit.*

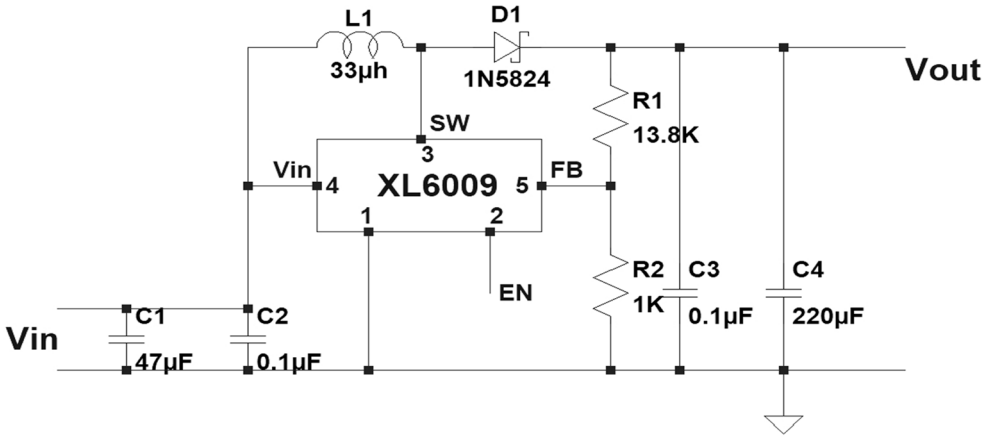

***Figure 3**. XL6009 typical application circuit (Boost converter).*

Electrodes Fabrication

Electrodes are made of 0.28 mm thick 5.1x5.1 cm square copper plate with exposed surface area of 3.75x3.75 cm as described and recommended in CLSI guideline. If iontophoresis current of 4 mA is used, current density would be 4/(3.75x3.75) = 0.28 mA/cm^2. Effective current density can be as low as 0.14-0.21 mA/cm^2. [16]

Controller Circuit

Arduino Uno R3 microcontroller board is used to control this automatic iontophoresis device. Arduino Uno board is well known in DIY hobby and very easy to program using C-style language.

Preliminary Evaluation of the Device

In order to test the performance of the iontophoresis device, a simple circuit of variable resistance was made from 0-5000 ohms potentiometer to simulate skin resistance and electrodes circuit. After the iontophoresis current was set, potentiometer was connected instead of electrodes circuit and the resistance was varied to simulate varying skin resistance while current was monitor. The current provided by iontophoresis device should be constant.

Sweat chloride test

After the project was approved by institutional ethic committee, the device was assembled and 70 healthy adult volunteers (age between 18 to 40 years old) were recruited. 27 males and 43 females were compared between control and test with pilocarpine iontophoresis. The mean (SD) average age of male and female were 29 (6), 31 (6) respectively (p=0.64). No history or signs of illness or being treated for diseases including the lungs, liver, pancreas, and intestines were found. CST was performed; sweat was collected and analyzed according to CLSI recommendation. The 1.0 mA iontophoresis current with duration of 4 minutes and 30 minutes for sweat collection was used. In each volunteer, skin of each forearm was prepared by cleaned the test area with 70 % Ethyl alcohol and Distilled water, then wipe dry

with a gauze. Electrodes with pilocarpine solution were attached according to CLSI standard while iontophoresis current was applied only to right arm site as test site. The left arm site was used as control site (Figure 4). For the test site, Gauze soak with 2% Pilocarpine at the Positive electrode place on lower arm area. And gauze soak with 0.05 mol/L $MgSO_4$ at the Negative electrode place on upper arm area. Then switch on the iontophoresis current source device at 1.0 mA for 4 minutes. After that, the electrodes were taken off and the areas were cleaned with distilled water. The areas were then wiped dry with dry gauze. Pre-weight 5.1 cm x 5.1 cm filter paper (Whatman® No.1, Whatman International Ltd, Maidstone England) were placed at the areas of positive electrodes in the tested site and also at the controlled site for 30 minutes (Figure 5.) After that, 4 digits weighing scale was used to determine the weight of the filter papers so that the amount of stimulated sweat were recorded for analysis.

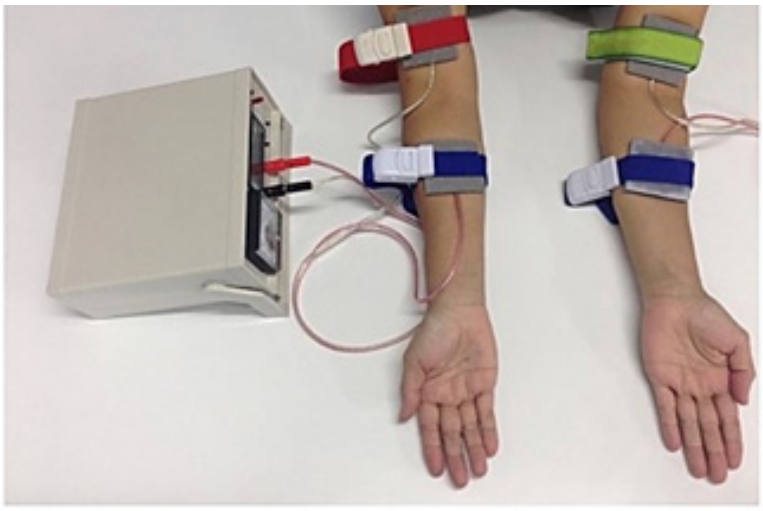

Figure 4. *Sweat stimulation with pilocarpine iontophoresis. The iontophoresis current source device is seen on the left of the picture. Positive electrodes with gauze soak with 2% pilocarpine were placed on the lower arms. Negative electrodes with gauze soak with 0.05 mol/L $MgSO_4$ were placed on the upper arms. Right arm was the tested site (near the iontophoresis device) while the control site was in the left arm.*

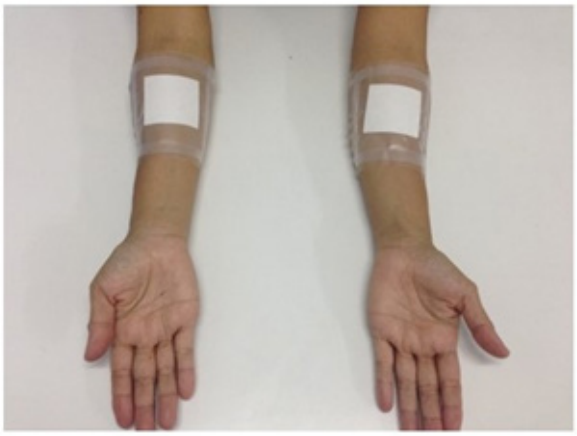

Figure 5. *Sweat collection. Filter paper was placed at the same area of positive electrode for 30 minutes.*

Sweat chloride test evaluation

After collected and weighted the filter papers from tested and controlled sites, 8 ml of distilled water were added and leave at room temperature for 40 minutes. Standard, control and test samples were prepared as described in table1. Then 0.002N Mercuric Nitrate was used in titration process as described in CLSI-C34-A3 guideline. The chloride level reference range for cystic fibrosis diagnostic were shown in table2.

Table 1 Preparation of Standard chloride concentration tube, Control tube and Test tube for CLSI- C34-A3 chloride determination using titration method.

	Standard Tube	Control tube	Test tube
Standard saline 10 mmol/L	0.5 mL	-	-
sample	-	2 mL	2 mL
3% Nitric acid	1 drop	1 drop	1 drop
Diphenyl carbazone	2 drops	2 drops	2 drops

Table 2 Chloride level reference range for cystic fibrosis diagnosis. (CLSI C34-A3 standard-sweat testing: sample collection and quantitative chloride analysis).

	Infant – 6 months, Cl- level	>6 months, Cl^- level
Non- cystic fibrosis	≤29 mmol/L	≤39 mmol/L
Intermediate*	30-59 mmol/L	40 – 59 mmol/L
cystic fibrosis	≥60 mmol/L	≥60 mmol/L

**Intermediate mean that Cystic fibrosis is possible. Should be repeated and confirmed with other method.*

Results

The iontophoresis device was constructed and can operate within the required specification as shown in table 3. To test the stability of the iontophoresis current, a 0-5000 ohms potentiometer was used to simulate electrodes circuit and skin resistance. After the iontophoresis current was set, potentiometer was varied to simulate varying skin resistance while current was monitored. Resistor of 200 ohms was used as serial current sensor. If current was set to 1 mA, 200 ohms resistor would provide 200 mV across its terminals. The potentiometer would provide 0 to 5 volts to represent its resistance. Despite the intense variation in simulated skin resistance, the current provided by iontophoresis device appeared to be highly constant as shown in Figure 6. Then iontophoresis device was used to perform CST in the volunteers. The test site significantly produced more sweat than control site. The sweat obtained from the test site are all farer than minimum sample weight of 0.077 gram recommended by CLSI-C34-A3 standard in every volunteer subjects. But there are significant differences in sweat weight and chloride concentration obtained from male and female volunteers (Table 4). The results were conformed to previous investigations in the literatures that male can produced more amount of sweat and chloride concentration than female subjects.[17-20] There was no blister and burn at the electrode sites in all subjects.

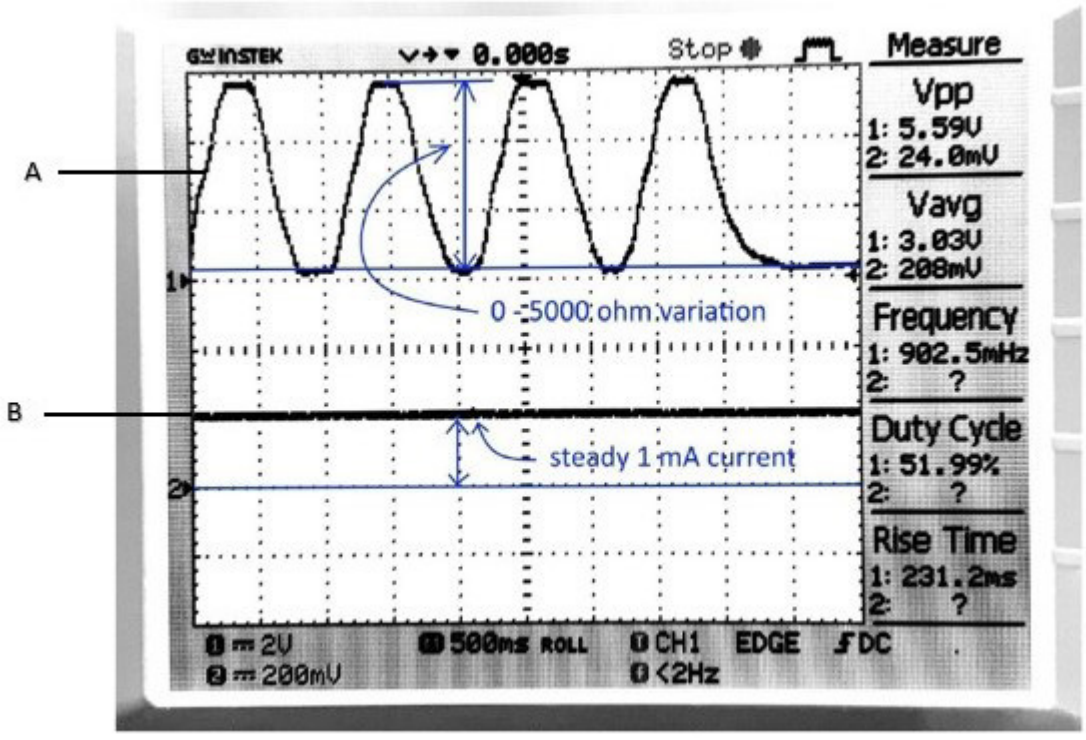

***Figure 6**. Constant iontophoresis current versus varied simulated skin resistance recorded with GW Instek GDS-1102A digital storage oscilloscope Good Will Instrument CO., LTD. Taipei, Taiwan. (A) which is seen very constant despite extensive varying of skin resistance from 0 to 5000 ohms. (B) represent set current (1 mA).*

Table 3 Specification of iontophoresis Device.

Power supply:	9 volts DC form 6 AA Alkaline cells
Electrode surface area:	3.75 cm x 3.75 cm.
Iontophoresis current:	0.5-3.0 mA in step of 0.5 mA
Iontophoresis current characteristic:	Continuous direct current
Timer:	0.5-5 minutes in step of 0.5 minutes
Skin-electrodes direct current resistance:	400-5000 ohms
Error detection:	Battery voltage below 7.8 volts: "LOW Batt Pls Replace Bat" Battery voltage below 7 volts or higher than 9.5 volts: "Error" Working voltage: below 4.7 volts or higher than 5.6 volts: "Error" Iontophoresis voltage below 12.0 volts or more than 16.5 volts: "Error" If any "Error" occurs, system will shut down.

Table 4 Age, Sweat weight and chloride concentration obtained from male and female volunteers.

	GENDER	N	MEAN±SD	*p* value
AGE (year)	Male	27	29±6	0.64
	Female	43	31±6	
SWEAT WEIGHT (gm)	Male	27	0.35±0.10	0.01*
	Female	43	0.17±0.08	
CHLORIDE (mmol/L)	Male	27	23.42±9.22	0.01*
	Female	43	14.90±6.51	

Discussion

The automatic iontophoresis device can be built using locally available parts to perform within required specification. While 0.28mA/cm^2 current density with 3.75 cm x3.75 cm exposed surface area is recommended by CLSI guideline, the developed iontophoresis device can induce sweat with only 1.0 mA current using the same stimulating 4 minutes time and 30 minutes for sweat collection. The current density is only 0.07mA/cm^2, which is far below the CLSI guideline recommendation. No blister or burn at electrode sites were found.

Conclusion

The automatic iontophoresis device can be built to perform within required specifications. While current can be set to 1 mA or higher with this device, sweat can be stimulated effectively with low iontophoresis current in normal subject. But the performance of the device in CF patient or in pediatric age group is yet to be determined.

Acknowledgements

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