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# Experimental methods for registration of electrical activity of the heart on cell and whole organ level

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## Abstract

Modern recording systems for registration of electrical activity of the heart are based on application of voltage sensitive dye (VSD) into the examined tissue. Excited VSD's undergo changes in their electronic structure, and consequently their fluorescence spectra, in response to changes in the surrounding electric field. Fast VSDs are consistently sensitive probes and can be used in electrophysiological studies. We have proved that optical recording employing di–4–ANEPPS dye utilized in our laboratory is suitable registration method of electrical activity of isolated hearts perfused according to Langendorff. The heart is exposed to VSD diluted in Krebs–Henseleit solution to the concentration of 2μM.

**Keywords:** heart, electrical activity, electro–optical recording systems

## Introduction

Recently, electro-optical recording systems are often used to record electrical activity of isolated cardiomyocytes and the isolated heart perfused according to Langendorff (Bachtel et al. 2011). The systems are usually based on application of voltage-sensitive dyes into examined tissues. Generally, the VSD should exhibit large fluorescence and/or absorption changes that vary with changes of the membrane potential (Salama 2001). Further, its optical responses must be specifically related to the changes of membrane potential and not to ion concentration, transmembrane currents, or membrane conductance. Staining of the heart with the dye should not result in pharmacological or toxic effects to the preparation. The response time of the dye has to lay in microsecond or submicrosecond range. For reliable and repeatable recording, the dye should be optically stable in its local environment and not prone to photobleaching upon an exposure to intense light (Novakova et al. 2008). Such systems can be extended with traditional touchless electrodes to record electrograms simultaneously with optical signals as recordings of electrical activity of individual myocytes.

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## Materials and Methods

The voltage sensitive dye bound to the cell membrane responds to the membrane potential change. A dye suitable for the measurement of electrical activity in cardiac preparations must respond to the potential changes with the time constant below 1/100 s. Dyes from ANEPPS group (amino–naphthyl–ethenyl–pyridinium) are the most constantly used in cardiac preparations (Nygren et al. 2003). Styryl-derived ANEPPS fast voltage-sensitive dyes were widely used for this purpose. These electrochromic dyes utilize CT mechanism from the electron-donating N,N-dialkylamino group to the electron-deficient pyridinium core in ground and excited states. As a result, the electrochromic dye responds to the membrane potential change by a spectral shift as shown in Fig. 2 (Kolarova et al. 2010). In an ideal case, the emission spectrum shifts bathochromically and does not change its shape or amplitude. The fluorescence intensity decreases with rising membrane potential. The combination of observed bathochromic shift and decay of the fluorescence intensity upon increasing membrane potential is used in so-called fluorescent emission ratio measurements.

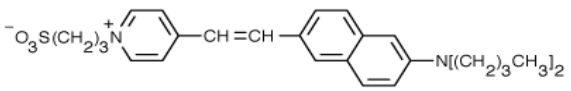


Figure 1: Aminonaphthylethenylpyridinium dye di-4-ANEPPS structure

Di–4–ANEPPS is utilized in our laboratory for recording of monophasic action potentials (MAPs) by optical method in various animal models. Most often employed experimental model is isolated heart perfused according to Langendorff. The heart is exposed to

VSD diluted in Krebs–Henseleit solution to the concentration of 2μM (Novakova et al. 2005).

The described system employs a flexible bifurcated fiber cable with illumination fibers positioned in a circle and a detection fiber positioned in the center of the cable (Provaznik et al. 2004). The optical probe is attached to the preparation to suppress motion artifacts without a need of focusing. The input end of the cable with six illumination fibers is connected to a light source. The output (detection) fiber is connected to a light detector that senses the beam of emitted light.

A halogen light source was chosen as a source of an excitation light. This cold light source with high intensity light output is designed for fiber optic applications with stable light output. The light source contains a built-in IR filter, which prevents a preparation from heating, and a band-pass filter (560 nm +/- 30 nm), which selects light at excitation maximum of the used dye.

The changes in dynamics of transmembrane potential result in amplitude modulation of the emitted light. This is detected by a photodiode detector with a high-pass (>610 nm) filter. The output signal of the photodiode detector is preamplified so that the two stage amplifier adjusts the signal to input range of data acquisition card (±1 V). The electrical circuits include also an analogue anti-aliasing filter (low-pass filter fc=2 kHz) and a high-pass filter (fc=0.05 Hz) to suppress DC offset.

Orthogonal ECG signals are recorded from six silver-silver electrodes positioned on the inner surface of the bath. All signals from the light detector and electrodes are simultaneously digitized by 12-bit AD converters at 4000 samples/sec rate. The digital signals are stored on a hard disk for further off-line processing (noise suppression, peak detection, visualization and analysis).

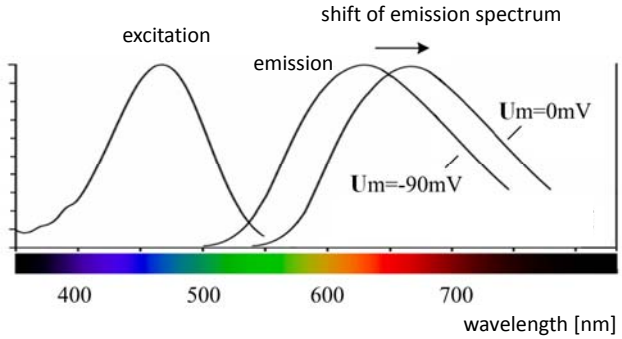


Figure 2: Optical characteristics of voltage sensitive dye di–4–ANEPPS

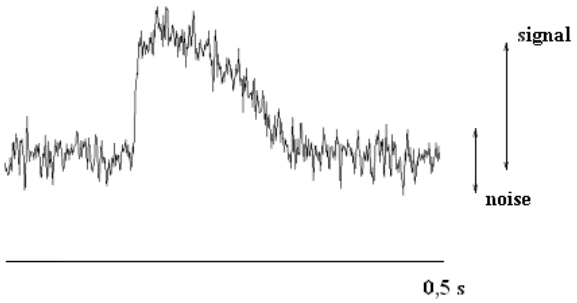


Figure 3: Raw monophasic action potential recorded by optical method from the spontaneously beating heart.

## Discussion and conclusion

The possibility of recording of the transmembrane potential of excitable cells by optical methods was first suggested in 1968. The first cardiac application was then reported in 1981. Since then the method has been improved and numerous VSDs have been tested (Dillon et al. 1998). Several problems had to be solved before these new dyes were accepted for everyday laboratory practice (Svrcek et al. 2009). One of the most important tasks was to minimize side effects of the dye on the preparation in the absence and presence of light (Salama 2001; Salma et al. 2005). Most prominent pharmacological effect of VSDs on cardiac tissue is so-called photodynamic or phototoxic damage (Zochowski et al. 2000).

We have proved that optical recording employing VSDs is suitable registration method of electrical activity of isolated cardiomyocytes and isolated hearts. Certain electrophysiological changes are present in the hearts due to presence of dyes. Most prominent pharmacological effect of VSDs on cardiac tissue is photodynamic or phototoxic damage. The exact mechanism of these effects remains unexplained. Formation of free radicals or direct interaction with the voltage–gated calcium and/or potassium channels may result in altered conductivity and the time–dependent gating. However, these changes are mostly reversible and thus myocardium can be stained with di–4–ANEPPS and be considered a reliable model for electrophysiological studies.

## Acknowledgement

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