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APPLICATION OF THE LASER INTERFEROMETRY IN STUDIES OF BIOPHYSICAL MODEL SYSTEMS

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In this paper some selected applications of laser interferometry method in studies of biophysical model systems are presented. With the aid of a laser interferometric method, specific experiments were performed, which confirm that the gravitational field significantly modifies the amount of transported substance and affects the concentration profiles as well as the time evolution of the concentration field. The laser interferometry was also used to investigation of diffusion of antibiotics (ciprofloxacin or ampicillin) into the water phase from mixtures of neutral or negatively charged liposomes, and antibiotic–liposome interactions. Differences in the diffusion kinetics of ciprofloxacin and ampicillin from liposomal solutions to the water phase were observed. Moreover, the amount of ampicillin and ciprofloxacin released from the anionic liposomal phase was higher than that from the neutral one.

INTRODUCTION

A laser interferometry is one of the methods enabling convenient and accurate examining the transport of substances. The method of laser interferometry allows a comprehensive study of diffusion (measurement of the amount and flow of the transported substance, the measurement of the diffusion coefficient of the substance) as well as visualization of the formation of the diffusion layers and the study of their evolution (Dworecki *et al*., 2005).

The method of laser interferometry was used to test various theoretical models of diffusion, studies of the substances transport through the membrane, to determine transport parameters of membranes under concentration polarization conditions (Dworecki *et al*., 2006) and hydrodynamic instability studies.

Laser interferometry is now increasingly used in biophysics, biology and medicine. Various modifications of the measurement system, the use of gel systems allow the interferometric analysis of anomalous diffusion (subdiffusion), the test release and interaction of macromolecules with biologically active substances (antibiotics, liposomes, saponins) and studies of biophysical model systems. A modification of the laser interferometric technique by immobilizing the tested molecules in agarose gel and measuring the amount of released substances allowed to determine the interactions within partially insoluble mixtures such as lipopolysaccharide (LPS)–colistin, LPS–chitosan and LPS–saponin.

LASER INTERFEROMETRY METHOD

Laser interferometry is an optical research method that uses the interference of two beams of laser light.

Main element of the measuring system which was used (Figs 1 and 2) is a Mach-Zehnder interferometer (Dworecki *et al*., 2006). The laser light after weakening by the polarizer is going through expander, which expands the beam to a diameter of a few centimeters. Such expanded beam falls on a special optical beam splitter, where it is separated into two parts. One of them is the information beam and passes through the tested system and then goes to the second beam splitter, where it meets the second beam which passes through the reference system. As a result of the interference of the two beams the appropriate pattern of interference fringes is received, which is recorded by the camera and transmitted to the image processing system. In the situation, where in the tested system the concentration is homogeneous the parallel straight interference fringes are received (Fig. 3A). In a system in which a diffusive substance transport occurs, the interference fringes are bent in areas of concentration gradients (Fig. 3B). When additionally the convective transport occurs the interference fringes are disturbed (Fig. 3C). Image processing system analyzes the run of interference fringes and determines in any point the deviation of the fringes from their straight line run $d(x,t)$ (Fig. 4). Based on the knowledge of $d(x,t)$ and the correlation between the change in the refractive index of the substance and

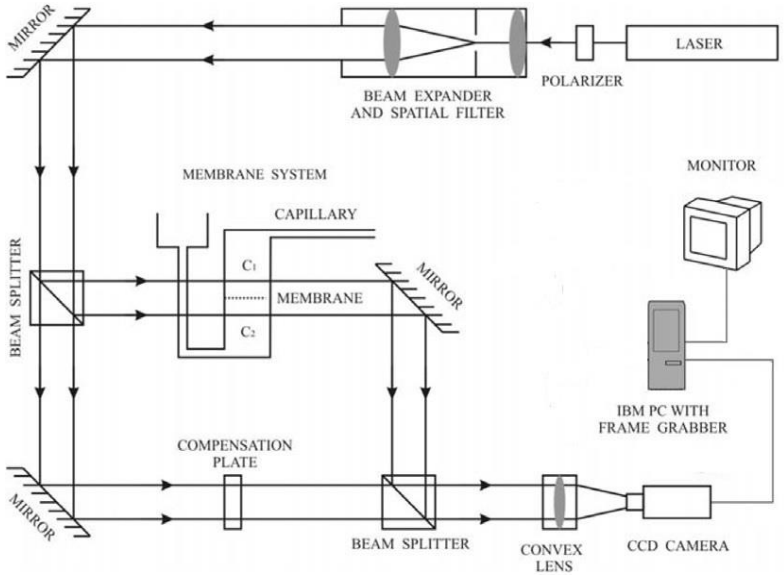

Fig. 1. Diagram of measuring system for interferometric investigations.

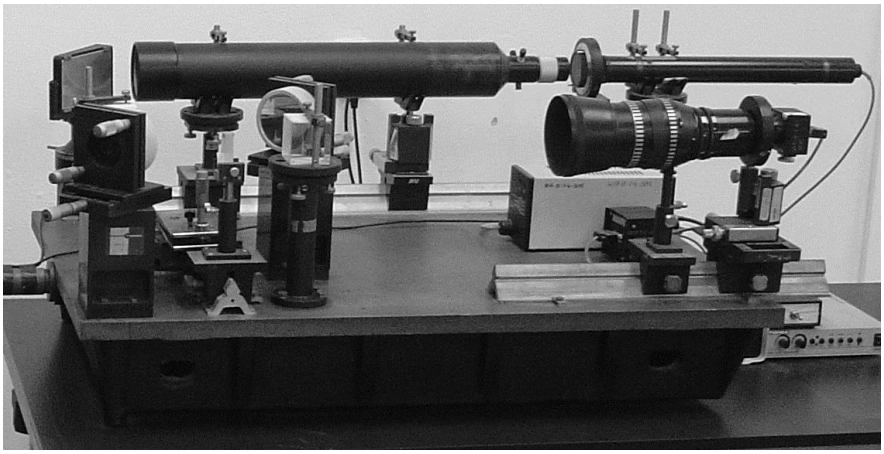

Fig. 2. Experimental set-up with Mach-Zehnder interferometer.

its concentration, the space-time distribution of concentration (i.e. concentration profile $C(x,t)$) of the tested substance is determined, according to the expression:

$$C(x,t) = C_0 + a\frac{\lambda\, d(t,x)}{hf}, \qquad (1)$$

where C_0 is the initial substance concentration, a is the proportionality coefficient between the concentration and the refractive index of the tested substance determined in a separate experiment using the interferometric refractometer, λ is the wavelength of laser light (632.8 nm), h denotes the distance between the fringes in the field where they are straight lines, f is the thickness of the solution layer in the measurement cuvette along the beam run. By recording interferograms at a given time interval, one can reconstruct the concentration profiles at different times. Thus obtained the concentration profiles are the basis for further quantitative analysis.

The amount of substance $N(t)$ which diffuses after time t through the interface (membrane) of area S from one compartment of the system to the other is calculated by integrating the concentration profile according to:

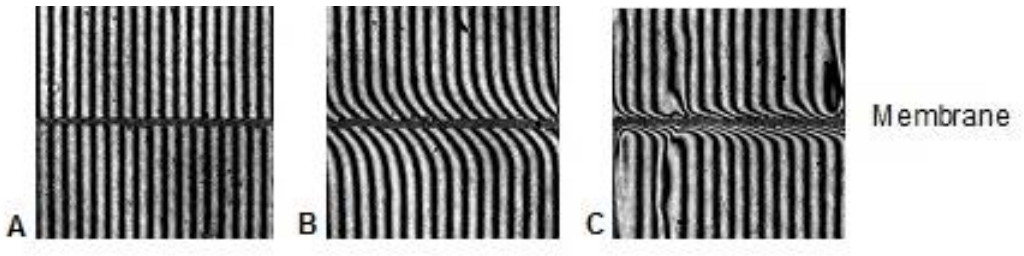

Fig. 3. The interferograms obtained for the system without (A) and with (B) concentration gradients, (C) – interferogram obtained for the system in which additionally the convective transport occurs.

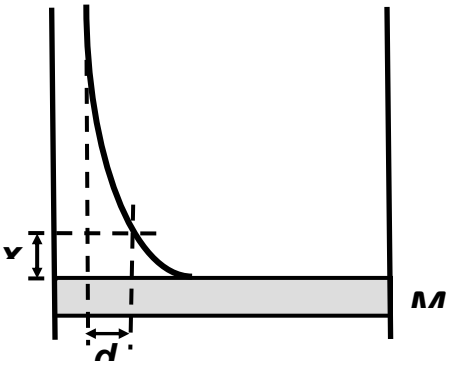

Fig. 4. Analysis of the interferometric fringe run.

$$N(t) = S\int_0^{\delta} C(x,t)\,dx, \qquad (2)$$

where δ is the concentration boundary layer (CBL) thickness.

The CBL thickness δ is determined by using the Nernst layer criterion (3). According to this criterion the layer thickness δ is defined as the distance from the interface of phases (or the membrane) to the point at which the concentration decreases k times, i.e.

$$C(x=0, t) = k\,C(x=\delta, t=0), \qquad (3)$$

with $x = 0$ being the liposomal–water interface position, k is an arbitrary parameter. In the literature data state different values of this parameter. In our previous works we used the values of k: 8.33, 12.5 and 33.3.

APPLICATION OF LASER INTERFEROMETRY IN STUDIES OF SUBSTANCE TRANSPORT

Influence of gravity on substance transport

The use of gel systems provides new research possibilities in artificial as well as biological systems. Gels and other polymeric structures due to their ability to damping of convection instabilities allow among others to study of the influence of gravitational field on the substance transport. The influence of gravity on the diffusion of substances can manifest in two ways: the gravitational field impacts immediately on the motion of the particles (migration) and buoyant forces appear which cause the onset of convection if the gravitational acceleration is not perpendicular to the density gradient. The influence of gravity on the substance transport can explain the mechanism of many effects, which are observed in biological systems under microgravity conditions (Schatz *et al*., 1992). Lack of convection in microgravity favors the formation of diffusion layers around the cells. These layers act as an additional resistance to permeation in series with the membrane resistance. This additional resistance reduces solute and water fluxes across cell membranes (Tosteson, 1978). In these cells, the uptake rate of oxygen and nutrient is changed and therefore the cell metabolism is markedly affected. The effects at the cellular level cause the changes in the molecular organization, genetics, growth, cell division and differentiation, and morphological characteristics of single cells and the whole organism. Anticonvection gel properties allow to study the transport of substances in two gravitational configurations of the measuring system (Wąsik *et al*., 2010). When the upper compartment is filled with the substance solution and the lower contains the gel (Fig. 5), the dissolved substance diffuses to the gel according to the gravitational force (configuration A). In such a situation, convection does not occur because the hydrodynamic instabilities are damped by the gel (for water, such a configuration would be gravitationally unstable because the layers of higher density are above the layers with lower density). In the reverse configuration (e.g. when the upper compartment contains the gel and the lower is filled with the aqueous solution), the substance diffuses against the gravitational force (configuration B). Here, only diffusive transport occurs and this configuration is also gravitationally stable.

The interferometric images obtained for agarose and aqueous solution of glucose in configurations A and B of the measuring system show that the run of the fringes

is not disturbed outside the layers. This indicates a lack of convection in the system.

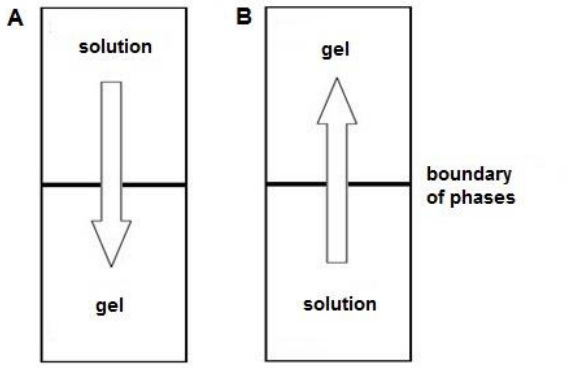

Fig. 5. Gravitational configurations of the measuring gel system.

Analysis of the time dependencies of the glucose flow $N(t)$ shows that the amount of glucose transported from the aqueous to the gel solution increases nonlinearly with time. For a given concentration of gel solution, the glucose flows differ for the two gravitational configurations of the system. The glucose flow is larger when the substance diffuses according to the gravitational field. The difference in the amount of glucose transported to the gel in both configurations increases with time. One can also note that the glucose flow is larger for the gel of lower concentration.

The interferometric investigation showed that the gravitational field plays a crucial role in substance transport and it modifies transport parameters in the system. The substance flow is evidently higher when the substance diffuses according to the gravitational field. The difference between the substance flows for the two gravitational configurations is dependent on the gel concentration.

Analysis of antibiotics diffusion from liposomal solutions

An optimized laser interferometry system without an artificial boundary of phases was used for quantitative analysis of antibiotic (ciprofloxacin and ampicillin) diffusion to the water phase from mixtures of neutral or negatively charged liposomes. The system contains a horizontally located nucleopore membrane with large pore diameter to avoid the hydrodynamic disturbances during the experimental model preparation only. This system with hydrophilic/hydrophobic solutions and large-pore membrane allows one to obtain a free interface (Fig. 6) and makes it possible to perform direct investigations of substance transport (Wąsik *et al.*, 2013).

A comparison of interferograms obtained for control liposomal solutions (without the antibiotic) and liposome–antibiotic solutions suggest a decrease of liposomal solution hydrophobicity in the presence of antibiotics.

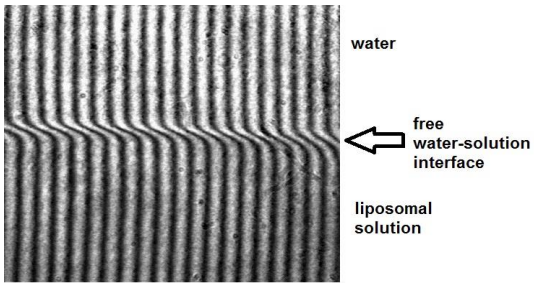

Fig. 6. Interferometric image obtained after time 120 s in the system with free water-neutral liposomal solution interface (without the membrane).

Analysis of interferograms obtained for control solution showed also that the release from control liposomal solutions was very small in comparison with the liposomal solutions with antibiotic. The amount of lipid released from control solution reached about 2 % of the total amount of substance released from solution with antibiotic. Anionic liposomal formulation was less hydrophobic than neutral ones, because the amount of liposomes released from anionic control solution was about two times higher. The diffusion of anionic and neutral liposomes into the water was negligible, and during the experiment practically only the diffusion of pure antibiotic was measured.

Time dependencies $N(t)$ of the amount of antibiotic transported from the liposomal solution to the water show differences in the diffusion kinetics of ciprofloxacin and ampicillin from liposomal mixtures to the water phase.

Ampicillin diffused more efficiently than ciprofloxacin regardless of the liposomal solution type. The amount of ampicillin and ciprofloxacin released from the anionic liposomal phase was higher than that from the neutral one. The results confirm that ciprofloxacin at neutral *pH* shows little tendency to bind neutral liposomes. It was also observed that ciprofloxacin disrupts negatively charged liposomes as a final effect of antibiotic–lipid interactions.

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