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LASER OPTOFLUIDICS IN FIGHTING MULTIPLE DRUG RESISTANCE

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FOREWORD

The book has an inciting title, "*Laser Optofluidics in Fighting Multiple Drug Resistance*" and is dedicated to a subject of high interest that is a challenge for the biomedical specialists as well as chemists, physicists, public health experts and even outer space applicants: fighting multiple drug resistance acquired by bacteria and tumours in normal and/or extreme conditions.

The editor and the invited authors propose two action lines, each of them implying pluridisciplinary experiments and data interpretation:

1. Exposure of selected non-antibiotic medicines at UV pulsed laser beams to modify their chemical structure and generate photoproducts with enhanced properties in fighting multiple drug resistance. At the origin, the parent compounds (mainly phenothiazines, quinazolines and hydandoin derivatives) do not have significant effects on bacteria or tumour tissues, but after being exposed to laser radiation in water solutions they generate photoproducts with individual or synergistic effects on biological targets. The book shows most recent results in the action of exposed chlorpromazine and thioridazine on Gram-positive and Gram-negative bacteria and their antibacterial and antibiofilm enhanced activity. Complementary, a report about clinically used methotrexate exposed to continuous wave (UV-Vis) optical radiation emitted by lamps and then utilised on eye pseudotumours evidenced that the mixture of photoproducts has in some cases anti-inflammatory effects higher than the parent compound. Another clinically used cytostatic, 5–Fluorouracil, exposed to UV pulsed nitrogen laser beams evidenced the same effects as methotrexate.
2. The "simple" identification of the obtained photoproducts constitutes a complex problem since the photochemistry of the processes is quite complicated; consequently, many procedures are utilised with this purpose and the obtained results are described in detail in the book. One speaks about laser spectroscopy (fluorescence), mass spectrometry, thin layer chromatography, UV–Vis and FTIR absorption spectroscopy, microfluidics (surface tension, contact angles, wetting properties) measurements and many others. This is correlated with the rigorous description of microvolumetric droplets as vectors to transport medicines to targets by applying microfluidics methods and procedures. Particular attention is devoted to description of the interaction – unresonant and resonant – between a laser beam and a single droplet which is of atmost interest in biomedical applications since it allows to fast modify the content of a microdroplet and to send parts of it on the target.

In presenting results, the editor took care that the book provides more interdisciplinary and multidisciplinary information about: the laser systems used to modify pendant droplets and bulk solutions, the properties of laser beams with emphasis on those of them which are essential in reported applications, the behaviour of droplets containing medicines exposed to laser radiation in terrestrial gravity and hypergravity conditions, the micro-spectroscopy specific methods to explore droplets' content.

The hypergravity experiments and results are groundbreaking since they show that microdroplets of chlorpromazine water solutions have better wetting properties when exposed long time to laser beams compared to water at different gravity levels. In general, unexposed/exposed medicine droplets to laser radiation have better wetting ability for cotton as well as activated charcoal target surfaces.

More, new results are shown about the way in which very small concentrations of photoproducts may be seized after exposure of compounds at laser beams, such as an antibiotic like vancomycin. The developed method is based on a microfluidic approach which allows measurement of surface tension at the interface between a gas bubble and the laser exposed solution. It may be also interpreted in terms of cleaning procedure of water from pollutants found at very low concentrations.

This E-book is very well organised in a series of condensed chapters that are illustrated with high quality figures. The text is accessible and easy to read.

The approach of the multiple drug resistance combat in the manner proposed by the editor is ground-breaking and the book opens promising perspectives in solving this threatening issue nowadays in flexible, rapid and adjustable ways.

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PREFACE

This book is proposed as a synthesis of inter- and multi-disciplinary results about new and somewhat unconventional methods and means to fight multiple drug resistance acquired by microorganisms and tumours. Essentially, two are the main directions along which the book text is elaborated:

(i) Modification of non-antibiotic medicines by exposing them at un-coherent, or laser optical radiation so that from an initial compound that is not efficient in combating bacteria or tumours one obtains photoproducts which alone or by synergetic action receive bactericide or, possibly, tumouricide properties. Examples are given regarding several classes of medicines, pointing out particular compounds amongst cytostatics, phenothiazines, quinazolines and hydantoins and showing how the generated photoproducts may be identified by methods belonging to spectroscopy, microfluidics, optofluidics, chromatography and mass spectrometry.

(ii) Developing new vectors to transport medicines to targets based on optics and micro-spectroscopy methods. These vectors are microvolumetric droplets of water solutions that contain parent medicines and have two roles. First, is to allow fast modification of their content by exposing them to pulsed laser beams so that photoproducts with bactericide properties are generated *via* resonant interaction between one beam and one single droplet. The second, is splitting the droplet by its unresonant interaction with another laser beam having suitable properties, so that nano-droplets and micro-droplets are generated which contain the photoproducts and propagate at supersonic or, respectively, subsonic speeds towards the target.

The target readers of the book are medical doctors, physicists, optofluidics and microfluidics specialists, photochemists, biologists, laser spectroscopists as well as specialists in a broad area of domains ranging from delivery methods of medicines using different sorts of fabrics, to the use of multifunctional medicines in outer space missions, after passing hypergravity conditions. A particular target group is constituted by students experimenting in laser spectroscopy, biology, biomedicine, photochemistry, biophotonics and microfluidics since the book provides new and innovative information about behavior of liquid drops, foams, emulsions and bubbles at interaction with laser radiation and the possible applications of the results in the former mentioned fields.

The book is conceived not only as a coherent synthesis of new results, but also as a source of novel ideas, yet untreated, that are proposed to the readers as working variants in future research. This approach would allow, among others, a fast and flexible reaction in the fight against naturally or accidentally occurring multiresistant microorganisms and tumours, with fast enough results to allow a rapid deal with environment unexpected changes.

A particular interest is devoted to the use of laser spectroscopy and related methods for making available multifunctional drugs that may be applied for treatment of humans or for the decontamination of modules during space flights, in the conditions in which confined small spaces are used in isolation regime for long time intervals, as happens in interplanetary missions. Another subject of interest is the micro-lasers or micro-lasing droplets that emit in free space around them and may be used in a large area of biomedical and technological applications.

The editor would like to thank:

- Dr. Tatiana Tozar for valuable assistance in placing the book text in the printing house template and for detailed checking of figures, tables, list of contributors and abbreviations.
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CHAPTER 1

Introduction

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This book is triggered by the current progress in two emerging fields: (i) fighting multiple drug resistance acquired by microorganisms (in particular bacteria) by laser/optical means and methods and (ii) developing new transport vectors to deliver medicines to targets. Each of them is related to a – respectively - larger, distinct and self-consisting domain that undergoes also a current accelerated expansion.

The use of laser radiations to produce new substances from parent compounds is part of photochemistry which deals with generation of ultrapure products by interaction of molecules with photons of laser radiation. This is a chemistry in which chemical reactions are controlled instantaneously by the interaction of parent chemicals with laser beams, to yield new (photo) products, instead of using with the same purpose thermal/pyrotechnical procedures. The interaction produces either an inner modification of molecules structure which makes them more chemically active, or a break-up of molecules in radicals which interact with surrounding environment and/or between themselves and lead to new products. In both cases resulting materials are ultrapure due to the selective interaction of laser radiation with the molecular targets. This kind of procedure becomes very useful in pharmacology because it allows to produce in not too large quantities new and ultrapure medicines starting from substances already utilised in treatments.

The vectors considered in this book for possible transportation of medicines to targets are micro- and/or nano-droplets either directly generated by a capillary system or obtained by interaction of a laser beam with one single pendant droplet when the beam is not absorbed by droplets' compounds and the light pressure on it dominates the interaction. The same kind of vectors may be droplets included in aerosols, where a particular distribution of theirs function of diameters/volumes may be produced.

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Mihail Lucian Pascu (Ed.)

The multiple drug resistance (MDR) which is also called multi-drug resistance or multiresistance of microorganisms that cause infections or even diseases may be defined as a state or property of a microorganism to resist antimicrobial drugs used either as single drugs or as "cocktails" of drugs. Depending on the kind of microorganisms, the medicines with respect to which MDR is acquired may be antibiotics, antifungal, antiviral or antiparasitic chemicals having functions and molecular structures originally conceived to efficiently eradicate the targets. Examples of most common MDR microorganisms which developed resistance are: Gram-positive (*Staphyloccocus aureus*) and Gram-negative (*Pseudomonas aeruginosa, Escherichia coli*) bacteria, viruses (*HIV-Human immunodeficiency virus*), fungi (*Candida* species, *Scedosporium prolificans, Scedosporium apiospermum*), parasites (*Plasmodium falciparum* and *Plasmodium vivax* producing malaria). Drug resistance was acquired from the very first antibiotic, penicillin (widely used since 1943, but tested before 1940) for which the resistance of *Pneumococcus* was reported in 1965, but the first resistance was reported in 1940, in the testing time interval, exhibited by *Staphylococcus*. Some antibiotics for which resistance of bacteria were shown as well as bacteria and respective years when resistance was mentioned are, as presented in SwitchYard Media [1]: tetracycline (utilised in 1950)/*Shigella* (resistance reported in 1959); erythromycin (introduced in 1953)/*Streptococcus* (1968); methicillin (introduced in 1960)/*Staphylococcus* (1962); gentamicin (introduced in 1967)/*Enterocuccus* (resistance first reported in 1979); vancomycin (first used in 1972)/*Enterocuccus* (1988) and *Staphylococcus* (2002); linezolid (introduced in 2000)/*Staphylococcus* (2001); ceftaroline (introduced in 2010)/*Staphylococcus* (resistance first reported in 2011).

A more complete description and a set of MDR related definitions (such as extensive drug resistance-XDR and pandrug-resistance-PDR) are introduced in Magiorakos *et al*. [2] as a result of a study made by the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) for creating a standardised terminology introduced to describe acquired resistance profiles in *Staphylococcus aureus*, *Enterococcus spp.*, *Enterobacteriaceae* (other than *Salmonella* and *Shigella*), *Pseudomonas aeruginosa* and *Acinetobacter spp.*. Lists of antimicrobial categories were made using the expertise of the Clinical Laboratory Standards Institute (CLSI)-US, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the United States Food and Drug Administration (FDA), as well. MDR was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, XDR as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (bacterial isolates remain susceptible to only one or two categories) and PDR was defined as non-susceptibility to all agents in all antimicrobial categories.

On the other hand, one may speak about resistance of tumours and, in particular, malignant tumours to treatment with drugs belonging to several categories such as cytostatics, antibiotics, specifically designed (quinazolines, pyridinium) compounds, phenothiazines.

The delivery of medicines to targets is made using several procedures among which the local delivery of relatively large volumes (ml) and the systemic delivery through injections (usually one or more ml) are most common. In this book the introduction of drops and droplets as vectors to transmit the medicines to target tissues is described, taking into account the small volume (μl or less) of the delivered medicine which meets the needs to treat a particular system using the smallest necessary and possible quantity of drugs to avoid toxic and related to toxicity effects (photo-toxicity included). On the other hand, a single droplet in pedant/hanging/suspended position at interaction with laser beams, emitted either in pulsed regime or in continuous wave may be used in technological applications and a more general description of the optical properties of droplets is given in the book.

In general, a drop or droplet is a small quantity of liquid which may have different shapes (cylinder, sphere, ellipsoid or combinations of them in static or dynamic evolution) bounded completely or almost completely by free surfaces defined with respect to surrounding media (gases, liquids and high viscosity media immiscible with the droplet's content and keeping it confined). The droplet may be generated in a hanging position with respect to a capillary in which case it is called pendant droplet or on a surface being called sessile droplet. If instead of generating a drop of liquid in gas environment one generates a small gas volume in a liquid, one may define a bubble as shown in de Gennes *et al*. [3]. One may also speak about a droplet in an emulsion or a bubble in a foam, or one may study droplets of emulsions or foams in pendant position in air or another media, for instance. All these physical entities are studied in microfluidics and constitute basic elements for applications in pollution control, dedicated industrial technologies and even outer space experiments.

The interaction of an optical beam with a droplet, a bubble, a liquid containing a bubble or a droplet of immiscible liquid with it, is treated by a relatively new field, the optofluidics which was coagulated mainly in the last 10-15 years. Optofluidics is a mixture of photonics and microfluidics (which deals with non-solid entities) that brings together light and non-solids to provide possible advanced technologies such as fluid waveguides, deformable lenses, microdroplet lasers, new photochemistry and low toxicity biomedicine (see [4 - 6]).

CHAPTER 2

Pendant Droplets – Microfluidic Approach

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Abstract: This chapter contains basic data about the microfluidic description of pendant droplets. Results are shown regarding the surface/interfacial tension measurements performed on water based solutions following the interaction with laser radiation. A synthesis is introduced of the main methods used to produce simple or complex droplets in different media. A method to evidence surface active products obtained after exposure of medicine solutions to laser radiation is presented. It consists in measuring in real time the dynamic interfacial tension at the interface between air and irradiated solution, when solution is in bulk form. The variation of dynamic interfacial tension is an indicator of the presence of laser produced amphiphilic molecules in solution. These results belong to series of reports dedicated to new methods used to fight multiple drug resistance developed by bacteria by decreasing the concentration of active compounds with bactericide effects. In line with microfluidic approach of droplets with µl volumes, surface tension measurements on DMSO-water mixtures containing a dye are presented.

Keywords: Aerosols, Bubbles, Capillarity, Colloidal systems, Contact angle, Dynamic surface tension, Hanging droplet, Hydrophilicity, Hydrophobicity, Immiscible fluids, Pendant droplet, Sessile droplet, Suspended droplet, Vancomycin.

INTRODUCTION AND GENERALITIES

Droplets are an important component of daily life with multiple applications in various domains. A droplet "component" that plays an essential role in understanding its behaviour, is the surface it "shows" to the environment. The shape of an individual, unperturbed droplet is mainly determined by two forces: surface tension that tends to decrease the surface to volume ratio by giving a

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(quasi) spherical shape to the droplet and gravity that tends to deform it. There is an increased interest in using droplets with milli-, micro-, and nano-meter dimensions in various domains (industry, medicine, space science, *etc*.), which created new research domains such as optofluidics in which small volumes behaviour at interaction with light beams are mainly studied.

A particular field within microfluidics and optofluidics is the development of fluid materials able to be confined in a droplet and to produce effects on targets on which these droplets are sent or deposited.

Microfluidic systems are able to provide exact liquid volumes for each droplet. This can be made in an active or a passive mode. Active control uses local forces that allow manipulation of each droplet, individually in the intended direction; it can be obtained using several methods, such as: electrowetting, electrophoresis, electrostatic manipulation, pneumatic pressure and/or thermocapilarity actuation [1 - 5]. In passive control, externally generated flows are used where each flow is modified locally through capillary geometry [6]; most of these devices are focused on droplet generation from continuous flows [7].

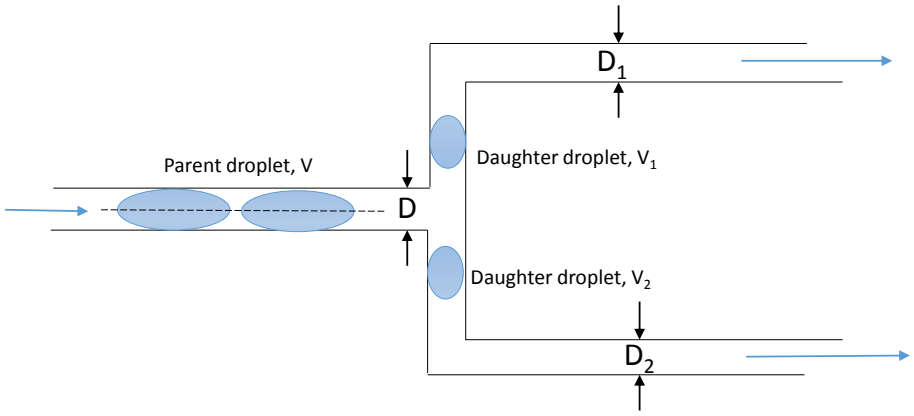

Fig. (1). Schematic of a bifurcating junction in a microfluidic device.

Passive microfluidic channels can control the droplet volume through droplet fission, fusion and sorting. The dimensions of channels are used to control the size interval between daughters and parent droplets (Fig. **1**). Microfluidic channel systems for high-throughput bio-chemical analyses, named "micro total analysis systems" (μTAS) or "lab-on-a-chip" are versatile and have multiple biomedical applications due to their capability to control droplet volume with picoliter accuracy (*e.g.* protein screening, crystal growth in mixed droplets,

electrophoresis, DNA analysis, cell growth and analysis, emulsification) [3, 5, 7 - 9].

Another method to generate droplets is represented by the use of a pumping system which sends programmed liquid samples through capillaries under computer control. The system can be used to generate droplets of a single liquid component in pendant positions (only one hanging droplet, if needed) or to mix different kinds of liquids with controlled volumes and/or concentrations of ingredients. The characteristics of the pumping system (*e.g.* syringe volume, capillary diameter) are selected depending on the volume of liquid that needs to be generated (Fig. **2a**). By adding another syringe and by changing the simple capillary with double coaxial capillary, this system can also generate layered droplets that are needed for interfacial adsorption measurements (Fig. **2b**).

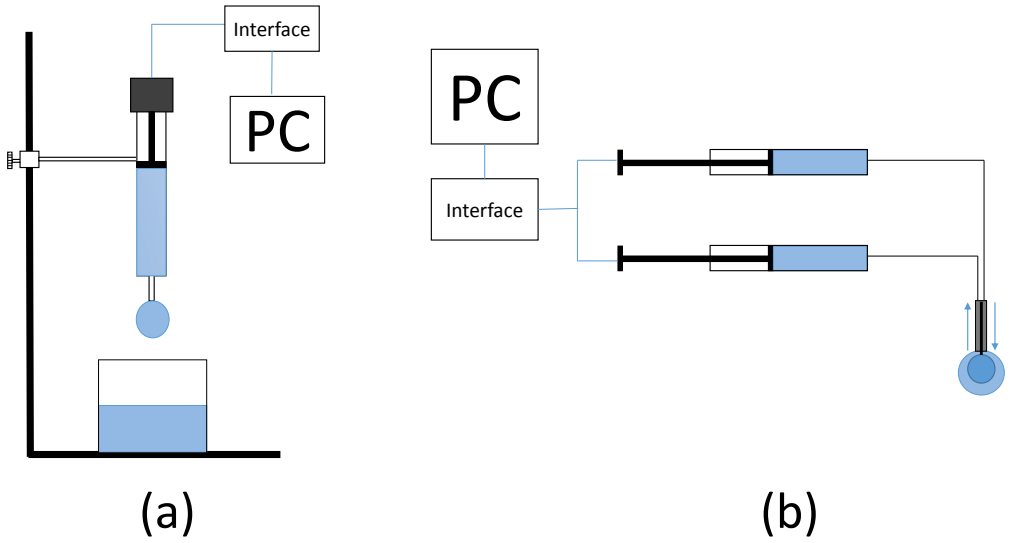

Fig. (2). Pumping system for pendant droplets generation [10]. (**a**) generation of single pendant droplets and (**b**) double syringe system for layered droplets generation.

Another way of generating complex pendant droplets is by using previously obtained emulsions. Fig. (**3**) shows a schematic presentation of a simple liquid droplet (a) and a droplet containing an oil in water emulsion (b) associated with the method to generate emulsions (c). The shape of such a droplet is (quasi)spherical and it is due to selected liquid volume (*i.e.* mass) and to liquid density, surface tension and dimensions of the capillary [11]. A more detailed presentation of this type of complex droplets and their applications is presented in Chapters 4 and 10.

CHAPTER 3

Pendant Droplets - Optofluidic Approach

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Abstract: This chapter presents a synthesis regarding the optical properties of pendant droplets in view of describing and understanding their interactions with laser and, more general, optical beams. The main methods used for pendant droplets investigation are described, from the optical point of view, at unresonant and resonant interaction with a laser beam focused or sent on it. The interaction is considered in the excitation scheme one laser pulse - one microvolumetric droplet in pendant position in air with typical volumes of the droplet in 1-15 μl range. In the unresonant interaction case the laser beam is not absorbed by droplet's material(s). Beam propagation in droplet is made according to geometrical optics rules when the separation surface between two optical media (air and water, for instance) is spherical. Total reflection of laser beam within droplet at separating surface with respect to the air can be produced and this can give a particular brightness of an illuminated droplet. At resonant interaction, the beam is absorbed by droplet's material(s) and typical phenomena may take place such as laser induced fluorescence (LIF) emission and Raman scattering. These effects are described here. LIF emitted by microdroplets of Rhodamine 6G solution in water is described and results about its amplification in the droplet considered as a spherical optical resonator are shown to the limit of obtaining lasing effects. Raman spectra of dimethyl sulfoxide and ultrapure water microvolumetric droplets are shown and a comparison between Raman beams emitted by microdroplets and bulky samples is made.

Keywords: Critical angle, Fluorescence, Hanging droplet, Immiscible liquids, Lasing, Laser induced fluorescence, Light pressure, Light scattering, Optical absorption, Optofluidics, Pendant droplet, Raman scattering, Reflection, Refraction, Refraction index, Rhodamine 6G, Total reflection.

INTRODUCTION

From the optical point of view, a droplet is an optical body and behaves like one, *i.e*. as an optical medium with a small volume, from micro-liter upward. It may be homogeneous, with isotropic properties, or may have a structured composition if immiscible materials are introduced in it, in which case the properties are not

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optically isotropic anymore.

In a liquid droplet one may introduce nanoparticles (such as TiO_2-see Chapter 17 and [1]) and then some of the optical properties may be changed with respect to the pure liquid. One may also generate droplets which contain emulsions [2] of two or more normally immiscible substances or even foams. In this case too, optical properties of droplets are those of the respective emulsions or foams. Droplets may be generated in different media and positions, but in all cases their intrinsic optical properties remain the same. Nevertheless, the use of their optical properties differs from case to case because the interaction of droplet with its surrounding media and the transmission of light from media to the droplet and back are affected by the fluidic properties of both droplet and its environment, as well as by the interface between them. In other words, the optical properties of a pendant droplet in air may be exploited in a different way with respect to the same properties of a sessile droplet. If the pendant droplet is hanging in a liquid with which it is immiscible, the interface phenomena when light is crossing it become very important. For a sessile droplet, the surface on which it is standing and the interaction with surrounding materials may regulate the input of light in the droplet as well as the output. The interaction with the surrounding media may also influence the intensity of light transmitted to and from droplets. Another factor to consider with respect to optical effects on droplets is their volume. At volumes larger than 1 μl (droplet diameter about 1.2 mm), optical properties may be treated using the known approach from standard optics and spectroscopy [3]. At microscale, *i.e*. at diameters lower than 1 mm down to nanometer scale the crossing of a droplet by light and the associated phenomena are still issues to study due to, among others, the ratio between light wavelengths and droplets dimensions, to consider only one contributing factor in a droplet's behaviour under light action.

Considering the pendant droplet as a liquid optical body with standard properties, in principle, all optical phenomena specific to an optical passive or active body may be studied on them, such as, but not exclusively: reflection, refraction, absorption, fluorescence, phosphorescence, Raman.

REFLECTION AND REFRACTION OF LASER BEAM ON A SINGLE PENDANT DROPLET

The pendant droplet may be produced in air or another environment that is immiscible with its liquid materials using a computer controlled droplet generator described in [3 - 6]. A typical example is a water droplet hanging in air on a capillary head that is hydrophobic.

The droplet is shaped under the action of its material weight and surface tension at the interface with air, but its behaviour is also influenced by the viscosity of the constituent liquid or liquid components. Normally, under the action of surface tension in air or interfacial tension in another liquid, the droplet has a spherical shape when its weight is (much) smaller than the surface tension. If the volume is increased, the droplet has an elongated, pear like shape due to the increase of its mass and it may be detached from the capillary. As mentioned above, the process is also related to droplet's material viscosity and its theoretical approach is a complex issue to deal with [7].

In Fig. (**1**) a droplet is depicted which has 1 µl volume, *i.e.* a diameter of 1.2 mm, in which an incident plane parallel laser beam is sent that covers the arcAB with the length L one eighth of the circle full length, *i.e.* 0.47 mm. At the surface of water droplet, the beam is partially reflected and most of it penetrates the droplet and is refracted according to Snell's law being bent towards the normal at the surface or propagating, for 0° incidence angle, along horizontal radius of the droplet. For a droplet with 1.2 mm diameter the time necessary for light to cross the droplet is about 5 ps (n_2 water refraction index is considered 1.334 and the speed of light in water at room temperature is taken 225×10^6 m/s). In general, function of droplet's volume and material, the lifetime of the photon in the droplet at one single pass may be different from a few picoseconds to some tens of ps. This may become important if the beam is pulsed since the time length of one laser pulse (function of the utilized laser) may vary from sub-picosecond to some nanoseconds or even longer, regardless the pulse repetition rate.

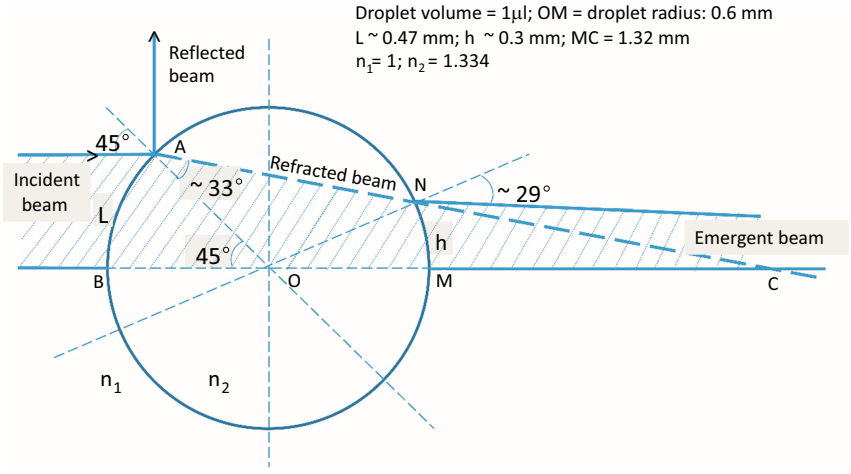

Fig. (1). Optical path of a laser ray in a transparent droplet. The input beam is plane parallel and parallel with droplet's horizontal axis; it covers 1/8 of its vertical plane cross section length, between the incidence angle 0° and 45°.

CHAPTER 4

Profile Analysis Tensiometry for Studies of Liquid Interfacial Dynamics

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Abstract: Bubble and drop profile analysis tensiometry is one of the most versatile tools for the characterisation of liquid interfaces. This technique provides the time dependence of surface and interfacial tension in a brought time range. The obtained interfacial tensions are well suited for a quantitative characterisation of adsorption layers of surfactants, proteins, polymers, and of their mixtures at liquid interfaces. Instruments equipped with an accurate dosing system allow experiments under well controlled conditions, such as constant drop/bubble volume or surface area. Also pre-programed changes are feasible, for example drop volume oscillations in a frequency range between 1 mHz up to about 0.1 Hz. This type of experiments allows determination of the dilational visco-elastic properties of liquid interfacial layers. Using a coaxial double capillary, experiments on sequential and simultaneous adsorption routes can be performed. This allows an analysis of the specific aspects of complex formation of *e.g.* proteins with other components or multilayer formation at the interface and determination of their dilational rheology. From a hydrodynamic point of view, the drop profile analysis methodology is limited to equilibrium drop/bubble profiles. Hence, when the size of a drop or bubble is changed too quickly their profiles are no longer Laplacian and the method would deliver wrong results. Therefore, the limits of PAT for a specific system need to be determined experimentally and validated by CFD simulations. Examples of CFD simulations have been presented in order to show how reliable the calculated interfacial tensions are.

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Keywords: CFD simulations, Dilational surface viscoelasticity, Drop bulk exchange, Liquid/gas interface, Liquid/liquid interface, Profile analysis tensiometry, Surfactant adsorption.

INTRODUCTION

The tension γ is one of the most easily accessible experimental parameters of liquid interfaces and it is an essential parameter that describes the work that has to be done to create a new interface of a unit area. The concept of the surface tension has been introduced by Segner as early as 1751 [1] which was later advanced by prominent scientists such as Young (1805), Laplace (1806), Poisson (1830), Worthington (1884), Bakker (1928), Brown (1947), Prandtl (1947) and Gauss (1830) [2]. The term surface tension is used for the tension of a liquid surface which is in contact with a gas phase while interfacial tension refers to the tension of the interface between two immiscible liquid phases. For simplicity any surfaces will be named interface during the entire chapter, as long as no specificities of liquid/gas surfaces are concerned.

The properties of an interface can be modified by the addition of surface active molecules which adsorb at the interface due to their amphiphilic character and decrease the interfacial tension. This adsorption effect is described by the Gibbs fundamental equation:

$$\Gamma = -\frac{1}{RT}\frac{d\gamma}{d\ln c} \qquad \textbf{(1)}$$

Here Γ is the adsorbed amount at the interface, c is the concentration of the adsorbing surface active compound, and R and T are the ideal gas constant and absolute temperature, respectively. Thus, measuring the interfacial tension as a function of surfactant bulk concentration $\gamma(c)$ the adsorbed amount Γ can be obtained from the slope of the plot $\gamma(\ln c)$. A detailed description of the history of adsorption models at liquid interface has recently been summarised [3].

Note, adsorption is a process that requires time. Therefore, measurements of the interfacial tension at a given surfactant concentration c as a function of time $\gamma(t)$ provide information about the change of the adsorption of the surfactant $\Gamma(t)$ at the given interface, assuming that the Gibbs' equation applies also under dynamic conditions. The most famous theory for describing the adsorption kinetics of surfactants at a liquid interface was derived in 1946 by Ward and Tordai [4] and is based on a diffusion controlled adsorption mechanism, *i.e*. surfactant molecules have first to be transported by diffusion close to the interface, from where they can then adsorb directly at the interface:

$$\Gamma(t) = 2\sqrt{\frac{D}{\pi}}\left[c_0\sqrt{t} - \int_0^{\sqrt{t}} c(0,t-\tau)d\sqrt{\tau} \right] \quad \textbf{(2)}$$

where D is the diffusion coefficient, t is the time, and c_0 is the surfactant bulk concentration far from the interface. This non-linear integral equation is not easy to be applied to experimental data and was therefore often the subject of theoretical work in order to allow a comparison of experimental data with this adsorption model [5].

Measurements of dynamic interfacial tensions can be performed by various experimental methods. Some of these methods are particularly dedicated to the short time adsorption process, such as the capillary pressure tensiometry. A special version of this methodology is the maximum bubble pressure tensiometry, which however is only suitable for liquid/gas interfaces. A recently published book was dedicated to various interfacial tension methods, mainly based on drop and bubble interfaces, and discusses many scientific and even technical aspects of these methods [6] so that no details will be presented on how other methods compare with the PAT that is described here extensively.

This chapter, however, is focussed only on the workhorse in modern surface science laboratories, the bubble and drop profile analysis tensiometry. It was first proposed as routine method for measuring the tension of liquid interfaces by Neumann and his group [7]. Here the history of this method is shown, as well as the state of art of its theoretical background and practical implementation, and some examples to demonstrate the potential of its application to various liquid interfaces. Note, however, although PAT is a very suitable method for studies of contact angle and wetting properties of solid surfaces, this topic is not presented here (see [8]).

DEFINITION AND HISTORY OF PROFILE ANALYSIS TENSIOMETRY

For more than a century the dimensions of large sessile drops have been used to determine the surface or interfacial tension of a liquid. Quincke in 1858 [9] practiced this method by measuring the height of a sessile drop above its equatorial diameter. It was necessary to use very large drop, which in turn was not so easy experimentally, so that some correction factors were proposed to compensate for the finite size of the used drops. Much later, in 1963, Padday [10] applied the drop-height method to determine the spreading coefficient of a liquid drop on a plane solid surface.

CHAPTER 5

Pendant Droplets: Overview of Dynamics and Applications

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Abstract: This chapter provides an overview of dynamics and applications of pendant drops. The pendant drop tensiometry is widely used in measurement of interfacial tension and thus it is described in details including the historical development and the state-of-the-art. The sensitivity of the method is discussed and the recent advancements are presented. The stability and breakup of the pendant drop are also discussed in the general context of jet instability. The multiphysics effects including the electric field, viscoelasticity and surfactants are briefly reviewed focusing on their influence on pendant drop instability.

Keywords: Drop breakup, Drop shape analysis, Electrical field, Non-Newtonian effects, Pendant droplet, Surface tension measurement, Surfactants.

INTRODUCTION

Drop formation is a ubiquitous phenomenon in daily life, science, and technology. This is critically important in various applications such as ink-jet printing, DNA micro arraying, cell and organ printing, spray combustion, spraying of agricultural chemicals, and many others. The study of drop formation started from the late seventeenth century. In early studies, Mariotte [1] and Savart [2] observed that a liquid jet becomes unstable and eventually breaks into small droplets. Later, Plateau [3, 4] and Rayleigh [5, 6] did pioneering works on liquid jet instability and breakup, and consistently explained the reason for the jet instability. Early experimental investigations were mostly motivated by engineering applications thus focused on instability of fluid jets leaving a nozzle at high speed, slow dripping under gravity, and liquid bridges. Since the early times, the pendant droplets have been widely used to measure the interfacial tension and to study the transport properties of interfaces.

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Pendant droplet is a droplet which hangs from a nozzle or some other surfaces as shown in Fig. (**1**). In an equilibrium state, the shape of a droplet is determined by the force balance of surface tension and gravity (Fig. **1d**). Surface tension tends to make drop spherical while the gravitational force tries to elongate it. In the absence of other external forces, the shape of a pendant drop depends on the Bond number (*Bo*.) and the contact angle (α.). The Bond number measures the relative importance of the gravitational force compared to the interfacial force and is defined as $Bo = \Delta\rho g R_0^2/\gamma$, where $\Delta\rho$, g, R_0, and γ are density difference across the interface, gravitational acceleration, characteristic size of the droplet (*e.g.*, drop radius at the apex), and surface tension coefficient, respectively. When *Bo* is small, *i.e.*, $Bo << 1$, the droplet takes a semi-spherical cap as shown in Fig. (**1a**). As *Bo* increases, the droplet elongates in the direction of gravitational acceleration (Fig. **1b**) and eventually breaks up when the gravitational force overcomes the surface tension (Fig. **1c**).

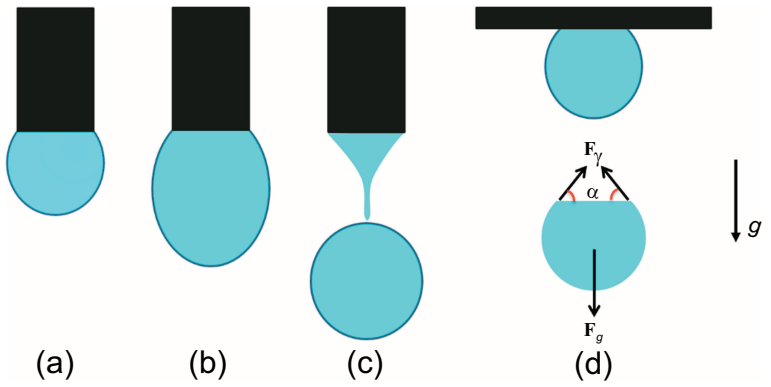

Fig. (1). Schematic illustration of a pendant droplet in various Bond number regimes. (**a**) A semi-spherical pendant droplet hanging from a nozzle at low *Bo*, *i.e.*, $Bo << 1$. (**b**) An elongated pendant droplet hanging from a nozzle at moderate Bond number. (**c**) Breakup of a pendant droplet ($Bo >> 1$). (**d**) Force balance for a pendant droplet hanging on a wall where α, F_γ, and F_g are the contact angle, the surface tension and the gravitational force, respectively.

There is a wide range of methodologies designed to measure surface and interfacial tension. Some common measurement methods are depicted in Fig. (**2**) [7 - 10]. Among these methods, the pendant drop tensiometry has been found to be the most versatile and robust method. Worthington was the first who proposed the measurement of interfacial tension using the shape of a pendant drop [11, 12]. Later Bashforth and Adams [13] produced tables for axisymmetric sessile drop profiles for different values of surface tension and radius of curvature. Then, Fordham [14] and Mills [15] developed similar tables for pendant drops. Maze and Burnet [16, 17] proposed a method using a nonlinear least-squares optimisation technique for the determination of surface tension of a sessile drop.

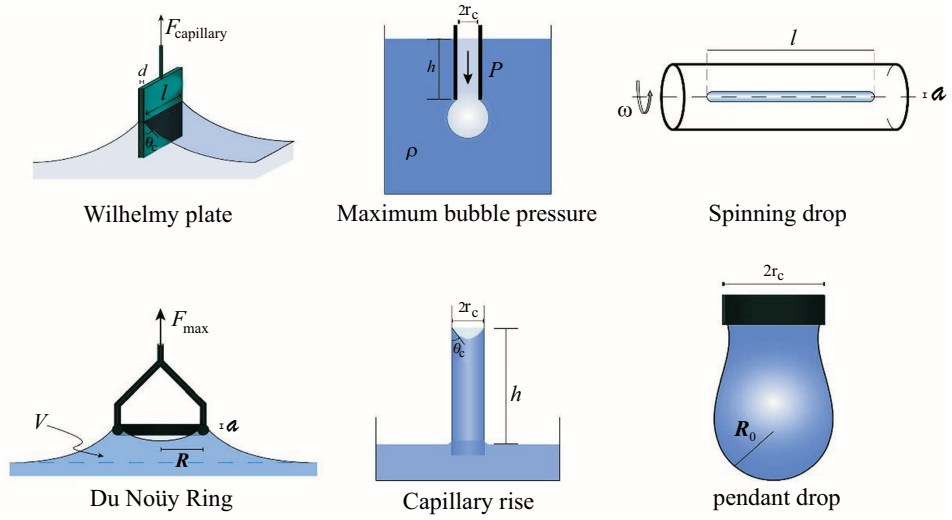

Fig. (2). Schematics of various experimental techniques used to measure interfacial tension (adapted from [10]).

Rotenberg *et al*. [18] developed a more robust technique called an axisymmetric drop shape analysis (ADSA), which fits the experimental shape with a theoretical drop profile. They used the Newton-Raphson method [19] for fitting the theoretical drop profile to the experimental data. Since then many adaptations and improvements have been carried out for the ADSA method [20]. Cheng and coworkers [21, 22] improved the ADSA method by utilizing an automatic image processing technique. del Rio and Neumann [23] found that using the Newton-Raphson method for optimisation is computationally expensive and furthermore the method may fail to converge if the initial guess is poor. Therefore, they combined the Newton-Raphson method with the Levenberg-Marquardt algorithm [19] to improve the likelihood of convergence. Hoorfar and Neumann [20] reviewed advances in computational methods of the ADSA algorithm including a close examination of potential sources of error and dynamic effects of experimental setup and fitting software.

In some cases, the edge detection of the experimental drop required by the ADSA is not possible mainly due to optical limitations. To overcome these difficulties, Cabezas *et al*. [24] proposed a new drop-shape methodology called theoretical image fitting analysis (TIFA) designed to avoid the edge detection in the ADSA. Unlike the ADSA method which involves a two-step procedure, TIFA achieves the interfacial tension measurement in one-step combining the image processing with the fitting procedure. In addition, TIFA matches the whole experimental

CHAPTER 6

Multiple Drug Resistance: An Up-Date

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Abstract: Multiple Drug Resistance (MDR) is acquired by bacteria, viruses, fungi, parasites and malignant tumours at interaction with antibiotics/medicines of old or new generation. Here is presented an up-date of MDR reported on bacteria, viruses, fungi, parasites and tumors, in this last field with emphasis on ophthalmology and neurology/neurosurgery. Multidrug resistant organisms exhibit *in vitro* resistance to one or more antimicrobial agents. One cause is the increasing use and misuse of antibiotics on humans and animals. Whereas particular bacteria are naturally resistant to some antibiotics, MDR occurs in other cases by accumulation of resistant plasmids and/or of genes, each gene determining resistance to a specific agent. The action of efflux pumps able to pump out more than one drug type is also a possible mechanism involved in MDR. In general, MDR is the most important "process" by which tumors acquire resistance to drugs during chemotherapy. Bacterial resistance to antibiotics used in ophthalmology has been reported since more than 10 years showing that several bacteria resistant to antibiotics were found in isolates from ocular infections. In neuroscience, development of new therapies to treat brain infections is more difficult. The most important cause of failure in developing new drugs for treating brain diseases is the existence and action of blood brain barrier (BBB). Brain tumors have usually poor prognosis and due to BBB, drug delivery to brain tumors is difficult. Some studies mention that BBB is involved in drug restriction to different brain neoplasias. The chapter concludes about the need to improve the arsenal conceived to overcome MDR acquired by different biological targets.

Keywords: Blood brain barrier, Cancer, Chemotherapy, Chemoresistance, DNA, Efflux pumps, Fungi, Gram-negative bacteria, Gram-positive bacteria, Hepatitis viruses, Herpes viruses, HIV, Malaria, Multiple drug resistance, Ophthalmology, Plasmodia species, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, *Staphylococcus aureus* (MRSA), Tuberculosis.

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DEFINITION

Multiple drug resistance (MDR), also known as Multiresistance or Multi-drug resistance is a condition developed by microorganisms such as viruses, bacteria, fungi or parasites, whereby they acquire resistance to distinct antimicrobials (antibiotics, antiviral, antifungal or antiparasitic drugs) [1]. There are different degrees of MDR, starting with extensively-drug resistant (XDR) and including pandrug-resistant (PDR) organisms [2]. The European Centre for Disease-Prevention and Control (ECDC) and the Center for Disease Control and Prevention (CDC) in the United States cooperated in finding a standardised international terminology for defining and describing acquired resistance profiles in microorganisms that are more likely to develop it (*Staphylococcus aureus, Enterobacteriaceae, Enterococcus spp., Pseudomonas aeruginosa* and *Acinetobacter spp.)* [2]. Epidemiologically, significant antimicrobial categories were built - up for each bacterium.

Consequently, the following definitions are used:

- MDR: acquired non-susceptibility to at least one agent in three or more antimicrobial categories.
- XDR: non-susceptibility to at least one agent in all but two or fewer anti-microbial categories (bacterial isolates remain susceptible to only one or two categories).
- PDR: non-susceptibility to all agents in all antimicrobial categories.

Besides the mentioned microorganisms, a special place is occupied by extended-spectrum β-lactamase (ESBLs) that induces multidrug resistance of Gram-negative bacteria and of tuberculosis. To ensure correct application of these definitions, is indicated that bacterial isolates are tested against all antimicrobial agents that belong to the mentioned antimicrobial categories (Fig. **1**). It is also recommended to avoid selective reporting and suppression of results [2].

MULTIPLE DRUG RESISTANCE – GENERALITIES

Multiple drug resistance has become a serious public health issue, as more and more antimicrobial drugs became inefficient in treating the infections caused by infecting agents. When discussing about bacteria, there are microorganisms from both Gram negative and Gram positive category which are incriminated. Since this problem grows with each year, so does the need for accurate and complete definitions of MDR that describe and classify resistant bacteria, so that epidemiological surveillance data can be collected and compared across all healthcare settings and countries. Multi drug resistant organisms (MDROs) are labeled as such because of their *in vitro* resistance to one or more antimicrobial

agents. Infections with MDROs can lead to inadequate treatments and can be associated with poorer results on patients. On the other hand, it is mention the restrained number of antimicrobial agents that are available or that are currently developed [2].

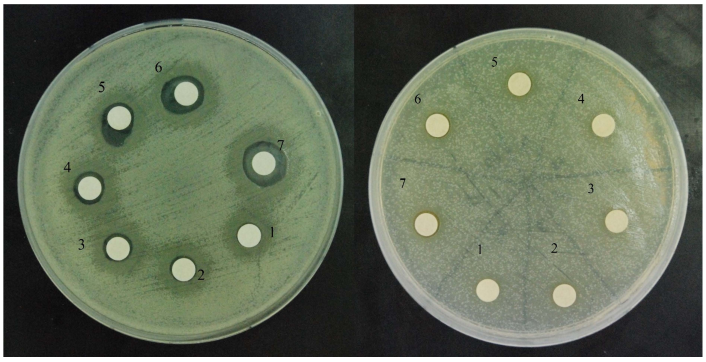

Fig. (1). Resistance test: bacteria are streaked on the culture media within Petri dishes. (Left) 30 min irradiated 20 mg/ml Chlorpromazine with 266 nm laser beam - bacteria susceptible to the antibiotic in each disc (dark, clear rings signify that bacteria have not grown); (Right) 240 min irradiated 20 mg/ml Chlorpromazine with 266 nm laser beam - bacteria are fully susceptible to solution; volume of solution applied on discs 1: 5 µl, 2: 10 µl, 3: 15 µl, 4: 20 µl, 5: 25 µl, 6: 30 µl, 7: 35 µl.

MDR Acquired by Bacteria

Drug resistance is and has been an acquired pattern whereby microorganisms adapted, evolved and survived for thousands of years. During the Twentieth century, life expectancy has dramatically increased along with the discovery of antibiotics; antimicrobial therapy acquired critical importance in fighting infectious diseases produced by bacteria. However, even from the very beginning, resistance occurred and bacteria found more modalities to resist antibiotics and other antimicrobial drugs. One cause of the development of resistance to drugs is the increasing use and misuse of already produced antibiotics in human and veterinary medicine, as well as in agriculture and related fields.

In 1998, in the United States, 80 million prescriptions of antibiotics for human use were filled [3]. This means 12,500 tons of antibiotics per year. Animal and agricultural use of antibiotics is added to human use. On the other hand, agriculture accounts for more than 60% of antibiotics consumption in U.S. and this adds 18,000 tons per year to the antibiotic charge sent towards the environment [3]. Data recently available show an alarming picture: nowadays, 70% of bacteria that cause infections in hospitals are resistant to at least one drug that belongs to the most commonly used antibiotics for treatment. Some microorganisms are resistant to all approved antibiotics and can only be treated with experimental drugs that may be very toxic [3]. All these facts are continually

CHAPTER 7

Laser Beam Properties

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Abstract: Since the interaction of a laser beam or otherwise called laser radiation with droplets in pendant position is a key process in the data reported in the book, the current chapter is dedicated to the description of the main properties of a laser beam of the kind used in presented experiments. Basically, laser emits electromagnetic radiation with particular properties described briefly in this chapter, such as: coherence (spatial and temporal), directivity coupled with mode structure, narrow (lower than 0.1 nm) spectral width, time structure of the emission (pulsed or continuous wave-cw), polarisation state (which is in most cases linear or closed to linear), Gaussian distribution of intensity in beam cross section, high beam energy, power and brightness.

Keywords: Astigmatic beam, Asymmetric beam, Axial mode, Beam FWHM, Beam power, Beam TFW, Beam waist, Brightness, Coherence length, Coherence time, Directivity, Fluence, Gaussian laser beam, Laser active medium, Mode structure, Monochromaticity, Optical resonator, Polarised beam, Polarisation state.

INTRODUCTION

The laser beam interaction with pendant droplets or bubbles depends on two main factors: (i) laser beam/radiation characteristics and (ii) composition and structure of the drop's/bubble's content such as: atoms, atomic and molecular ions, simple and complex molecules, molecular combinations, aggregates and supramolecular structures and in some cases, even more complex constituents.

In this chapter, the characteristics of a laser beam are described since they play a distinct role in producing the effects on pendant droplets in terms of shape and volume modifications, vibrations and material expulsion, as well as structural changes of substances/medicines contained in solutions presented in droplets form.

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The laser is an emitter of electromagnetic radiation having specific properties, among which the wavelength in optical spectral range (*i.e*. in visible and near infrared). The laser extended theoretical bases were introduced by Einstein [1] and Kastler [2] who had contributions in early research stages and then by Basov and Prokhorov [3, 4], Gordon, Zeiger and Townes [5], Bloembergen [6], Schawlow and Townes [7] and, again, Schawlow [8]. Maiman [9] reported the operation of the first laser that was a ruby laser. The distribution of spectral ranges within which laser radiation is emitted by different types of lasers is shown in reports such as [10]. Nowadays, laser emission covers an electromagnetic range larger than, strictly speaking, optical domain, starting from γ rays and going up to far infrared and even radio waves.

Beam characteristics at the output from a laser depend on emitting active medium, optical resonator (cavity) in which the active medium is embedded, some intra-cavity elements (such as pin-holes, optical filters, beam splitters, polarisers, prisms, diffraction gratings, optical Fabry-Perot etalons or interferometers, opto-mechanical systems *etc*.) and even energy delivery mode used to pump the active medium (which may work in pulsed or continuous wave-cw-regime) [10, 11]. Laser active media may be pure gases or mixtures of pure gases, liquids (containing either a single component or mixtures of solutions), solid state materials, semiconductors and plasma [12].

On the other hand, laser radiation used to interact with a target, such as a pendant droplet, has characteristics that derive from the laser beam emitted by laser itself, but they are finally controlled with optical and/or electro-optical components and systems used to process the beam and to tailor its properties to best respond experimental needs. In general, the processed beam properties at the place of interaction contain, in a significant degree, information included in it at emission from the laser. It is not possible to fully modify the properties of the beams outputting from laser and to avoid in this way some inconveniences regarding beam properties with respect to particular needs for applications and one has to always find the right compromise in terms of emitted beam characteristics *versus* beam properties required in a particular application.

PROPERTIES OF THE LASER BEAM/RADIATION

The laser beam properties considered in the following are: monochromaticity and related bandwidth, coherence state, directivity and mode structure, energy, power and brightness, polarisation state and time structure.

Monochromaticity and Related Bandwidth

The monochromaticity describes the spectral distribution of laser radiation correlated with its intensity that has a peak at a wavelength where the most part of radiation is emitted. In other words, the largest amount of laser beam energy is found at a restrained number of wavelengths around that of the maximum intensity of the beam. This intensity drops down to zero very fast near the peak's wavelength (but it does not behave like a δ-function) and this property shows the laser beam high spectral purity. As a consequence, laser beam spectral width is much narrower than in the case of incoherent optical emitters. An example of spectral distribution of laser radiation is shown in Fig. (**1**). In single axial mode amplification within the optical cavity, only the dominant line centered on λ_0 is emitted. Since stimulated emission is enhanced by successive reflections of radiation on the limiting mirrors of the optical cavity, the single longitudinal mode may coexist with other axial modes. The intensities of these modes decrease when the distance on wavelength scale increases (towards larger as well as lower values) with respect to the wavelength of the main peak of the laser line. The group of such adjacent lines generates a Lorentzian envelope which describes more correctly laser beam intensity distribution function of wavelength.

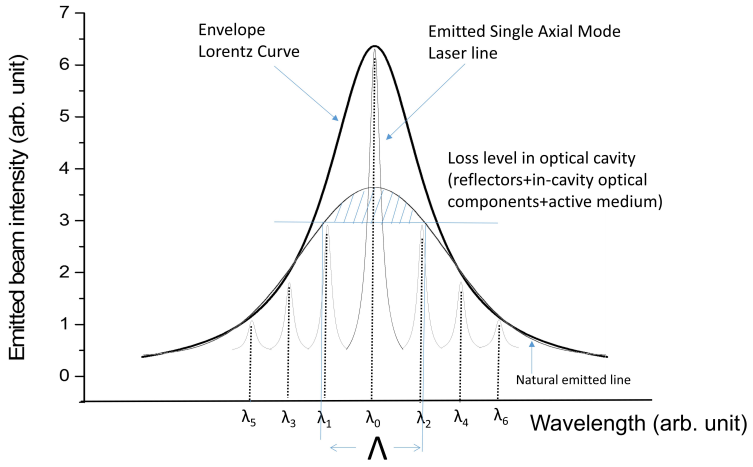

Fig. (1). Spectral distribution of the laser monochromatic beam; λ_0-laser beam wavelength; Λ-spectral bandwidth within which the gain is higher than losses.

At the same time, spectral distribution that describes the monochromaticity is determined by the shape of emitted line outputting from the active medium and by resonator axial modes. It is connected also to the relation between the gain and

CHAPTER 8

Unresonant Interaction of Laser Beams with Pendant Droplets

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Abstract: The unresonant interaction between a single droplet and a single laser beam takes place when the beam is not absorbed by droplet's material; it leads to specific effects induced in the droplet. The experiments reported here are made on droplets consisting in water solutions; 532 nm laser beam is used to produce unresonant interaction since water absorbs very little in green. The results on unresonant interaction, evidence several effects: (i) damped deformations and vibrations, (ii) expulsion of micro-jets of water that propagate with supersonic speeds and production of micro- and nano-droplets that are springs from mother droplet with different lower speeds, (iii) production of horizontal pillars of liquid and of cylindric channels within the droplet leading to appearance inside it of air bubbles. The water droplet interaction with a laser beam focused in its equatorial plane on droplet's surface, produces a velocity gradient of water inside the droplet that may be experimentally evidenced. The velocity gradient increases with increasing beam energy and does not depend on droplet volume. The surface tension and liquid viscosity characteristics influence the effects produced by the laser beam on droplet and the propagation speeds of liquid material formations emitted out of them. Studies on unresonant processes have shown that if produced on a droplet that contains a laser dye, they may be accompanied by effects induced when the interaction is resonant, *i.e.* the laser radiation is absorbed by droplet material. Such a "combination" depends on laser beam energy, solvent, viscosity and liquid surface tension.

Keywords: Laser ablation, Laser dye, Microfluidics, Microdroplets, Nano-droplets, Non-resonant interaction, Optical radiation pressure, Pendant droplet, Resonant interaction, Rhodamine 6G, Resonant interaction.

INTRODUCTION

Milli-, micro- and nano-droplets are used more and more for technological and biomedical applications and this triggers complex experiments regarding new methods to produce and more accurately characterise them. The optical and, in

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particular, laser methods utilised to study or even generate droplets constitute the bases of instruments conceived to get new data about small and very small dimension droplets. These techniques are a significant part of optofluidics that is a relatively new and dynamic field developed mainly in the last 10-15 years [1, 2]. Recent literature reports reveal new results about laser beam interaction with micro-volumetric droplets which contain single liquids or mixtures of immiscible compounds. The droplets are generated in different surrounding environments such as liquids or gaseous media, in particular in air [3 - 6].

In most studies, droplets are quasi-spherical or hemi-spherical. They contain materials in liquid phase positioned on transparent surfaces that may be chosen for hydrophobic or superhydrophobic properties, or may have repulsion properties with respect to other liquids such as oil, in which case they are defined as oleophobic or superoleophobic. When sessile, a droplet interacts with a laser beam that is transmitted through its supporting piece [7]. Sometimes, droplets are produced, fixed and/or moved at the interior of microchannels organized as structures that are built for their manipulation by light. In other cases, the droplets are introduced in single- or two-phase flows for use in optical chips [8, 9].

Other data are reported on micro-droplets that are free falling in air, levitating, hanging on a capillary tip or embedded in other liquid with respect to which the droplet material is immiscible. Most part of these results is related to fluorescence emission of droplets or even to lasing when droplets are optically pumped by laser beams [10 - 16]. The droplet's components such as dyes, or more general, fluorophores and their solvents shape the behaviour of droplets at interaction with laser beams. Other factors that may influence droplet's evolution at interaction with a laser beam are: (i) the environment in which it is embedded, and (ii) its structure (the droplet may contain one compound only, or a core and one or more covering thin layers of immiscible materials [17]). The most used solvent is ethanol, but in some cases polymeric materials are also utilised [15]. Water is a quite common solvent used in droplets since its properties (optical, physico-chemical, fluidic) are relatively well known. Though, water behavior at interaction with laser beams is not entirely known and studies of its interaction with them when irradiated in bulk [8, 18, 19], or as droplets are needed.

As mentioned above, the laser beam-droplet interaction may be resonant or unresonant [4]. Here, resonant means that the laser beam is absorbed by molecular species included in the droplet; these are excited, normally, on the first excited singlet state by absorption of one or more photons of incoming beam. If absorbent concentration is properly chosen, photons are absorbed immediately after entering in the droplet and no more photons are available to propagate within it. A result of exciting molecules on singlet states could be droplet's temperature increase

produced by (i) nonradiative transfers of molecules within the singlet states or (ii) intersystem (singlet states -triplet states) crossing with slight energy losses.

Unresonant means that the beam wavelength is such that it is not absorbed by droplet's molecular compounds or the beam-droplet interaction processes are not dominated by absorption phenomena [4]. This takes place in droplets made of a single material, such as water, the interaction producing: (i) effects originating in light pressure, (ii) application of electrostrictive forces on droplet's material as a result of laser action and (iii) thermal effects within droplet's mass [20, 21].

Hanging droplets make possible to individualise the effects of a single laser beam on a single droplet in different environments. They may present a free modification of their shapes and, at the same time and within some limits related to their material's viscosity, surface tension and weight, they may remain connected to the capillary. In this chapter, in the first place are shown results about the interaction of liquid aqueous microliter droplets with 532 nm pulsed laser beams. At this wavelength, the interaction is dominated by unresonant phenomena produced on the micro-droplet. The beam average energy is stepwise changed and the pulse full time width at half maximum (FTWHM) is 6 ns. Laser beam impact with a pendant micro-droplet produces droplet deformations as well as its mechanical vibrations. Droplets may loose some material after being collided by the laser beam if its energy is high enough and conditions in which such phenomena are produced are also described in this chapter. The effects are studied on single droplets and pulse by pulse. They are function of (i) laser beam energy, (ii) laser beam focus dimensions and position, (iii) exposure geometry, (iv) content of the droplet, (v) interaction of droplet's material with the capillary's surface on which it is pendant. The droplet shape and its changing due to collision with the laser beam are registered with a high speed recording camera used in tandem with a continuous, cold, white light source that illuminates a diffusing screen opposed to the camera. Following droplet interaction with laser beams are produced (i) nanodroplets (nl volumes) propagating at high speed, and (ii) micro-jets and micro-droplets that move at lower speeds. After interaction, the pendant droplet may have smaller volume/diameter than the parent droplet and micro-bubbles of gas (most probable air) are generated within its material/volume. Emission of nanodroplets that propagate freely around the parent is used in nanoscience, nanotechnology and nanomedicine where nanodroplets of materials are displaced within controlled gaseous media and fall on target surfaces that have characteristics which should be modified in controlled and reproducible ways.

Another kind of experiments reported here is the investigation of the behaviour of microdroplets made of solutions of laser dyes in solvents, at resonant interaction with laser pulses. The results show new ways to produce combined modifications

CHAPTER 9

Resonant Interaction of Laser Beams with Pendant Droplets

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Abstract: Resonant interaction of a laser beam with a single droplet takes place if the beam is absorbed in the droplet. Results are shown regarding the resonant interactions of pulsed laser beams with microliter droplets in pendant position which contain solutions of a laser dye-rhodamine 6G, phenothiazines (chlorpromazine, promazine), hydantoin derivative [(5Z)-5-(3-chlorobenzylidene)-2-thioxo-4-imidazolidinone)] and antibiotics (vancomycin), respectively, in water. A description of the electronic structure of rhodamine 6G and chlorpromazine using Gaussian09 and GaussView 5.0 software is made. Exposure of droplets to laser radiation leads to generation of new photoproducts that may have different properties if compared to parent compounds. Such modifications are evidenced by laser induced fluorescence and thin layer chromatography. In irradiated chlorpromazine water solution, out of hundreds of photoreaction products, 5 were identified: promazine (PZ), promazine sulfoxide (PZ-SO), 2-hydroxy promazine (PZ-OH), 2-hydroxy promazine sulfoxide (PZ-OH-SO), chlorpromazine sulfoxide (CPZ-SO). For rhodamine 6G solutions, the main results include the increasing intensity of LIF spectra in droplet with respect to bulk, even if the volume of the cuvette for bulk measurements is much higher than droplet's volume. This behaviour may be explained by the confinement of light inside droplet, where total internal reflection at surface occurs, the droplet being associated with a spherical micro-optical resonator.

Keywords: Chlorpromazine, Droplet, Fluorescence, Hydantoins, Laser dye, Lasing, Laser induced fluorescence, Promazine, Rhodamine 6G, Vancomycin, Whispering gallery modes, WGM.

INTRODUCTION

In Chapter 8, the unresonant impact of droplets generated in pendant position, with laser beams of different energies is presented. Deformations and mechanical

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vibrations are produced and losses of material under the form of nanodroplets and/or microdroplets may be produced. Nanodroplets may propagate at high (possibly hyper- or super-sonic) speeds and microdroplets propagate at lower, although high enough, speeds. Pendant droplets of smaller dimensions than the initial/parent one as well as micro/nano gas bubbles within the microdroplets volume may be also produced. These effects could constitute a basis of a new method to deliver medicines to targets.

Resonant interaction of a laser beam with a microdroplet (microliter volume droplet) in pendant position takes place when it is absorbed by droplet's components. Actually, radiation is absorbed by molecules contained in the microdroplet and this process has important consequences on their content. In order to produce resonant interaction, the laser beam must have a wavelength at which it is absorbed by one or more types of molecules contained in the droplet. To properly study the resonant interaction, solvents in droplets must not absorb the laser beam, so that only the active substance(s) molecules interact with pumping radiation. As a consequence of beam absorption, a molecule passes from fundamental singlet state on excited singlet states, either by direct absorption of one or more photons or by fast and/or slow internal rearrangements. Another consequence of absorption of laser radiation is the dissociation of absorbing molecules and the production of new substances deriving from "parent" molecules. Interaction of photoreaction products with solvent and with the same laser pumping beam may be also important. Once excited, molecules fall back on fundamental singlet state either by fluorescence emission or by nonradiative de-excitation; in this last case, the temperature of microdroplet may increase with some degrees. On the other hand, generation of photoreaction products in microdroplets following resonant interaction with laser beams is produced only as long as laser beam is on.

In this chapter, results are presented on the resonant interaction of a pulsed laser beam with liquid droplets in pendant/hanging position that contain solutions of different fluorophores (laser dye, phenothiazines, hydantoin derivatives, antibiotics) [1]. The studies are made at energy levels which do not produce at all, or produce insignificant unresonant interactions (mechanical vibrations, droplet shape deformations and liquid expulsion *etc*.) [2].

MATERIALS

Rhodamine 6G (Rh6G), [9-(2-ethoxycarbonylphenyl)-6-(ethylamino)-2,7-dimethylxanthen-3-ylidene]-ethylazanium, $C_{28}H_{31}N_2O_3^+$, is a fluorescent dye from xanthene family. The physical properties of Rh6G such as absorption, subsequent fluorescence emission and fluorescence lifetime as well as quantum yield are

reported in literature [3 - 6]. Due to its high quantum yield and high solubility in a large number of solvents (among which ethyl alcohol and water) it is used in a large area of applications and studies. Rh6G is intensely used as a laser dye (active medium in dye lasers) but it is also used as marker for a wide range of applications in biology and as photosensitiser. Its 3D molecular structure is presented in Fig. (**1**).

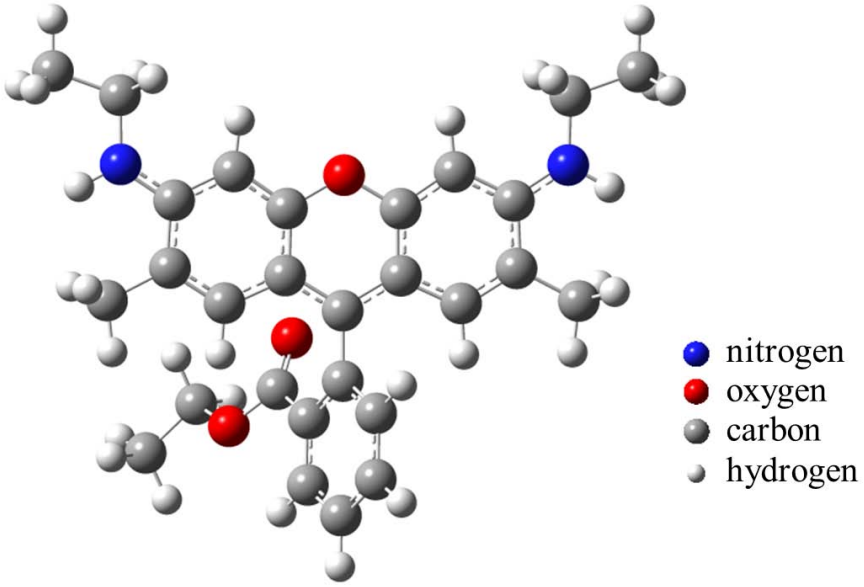

Fig. (1). 3D optimised chemical structure of Rh6G.

A phenothiazine is an organic compound having a heterocyclic system of two rings linked in the center of the ring by an -NH-group and a sulfur atom (-S-), and represents the parent molecule of many heterocyclic bioactive derivatives that have interesting biological and pharmaceutical properties. Chlorpromazine (CPZ) is a phenothiazine derivative, its primary use being in antipsychotic medication [7]. Beside this property, it was discovered that CPZ can be used with success in cancer treatment, where it induces apoptosis or inhibits proliferation in cultured cells like leukemia [8] or melanoma [9] cells. CPZ molecular structure is presented in Fig. (**2**).

Recent reports show that CPZ, dissolved in ultrapure water, exposed to ultraviolet (UV) laser beam undergoes a variety of structural modifications resulting in formation of photoproducts [10 - 12].

CHAPTER 10

Microdroplets of Laser Irradiated Drug Solutions: Surface Tension and Contact Angle

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Abstract: Precision measurements of surface tension and contact angle were performed on some medicines from the phenothiazine class (chlorpromazine, promethazine, and thioridazine) at different temperatures and concentrations in water, using the pending drop and the sessile drop methods. A specialised system DSA 100 (Krüss) was used for these measurements. Precautions were taken for an optimal acquisition of images (thermal isolation and heated windows of the chamber holding the studied droplets). Contact angles either static or dynamic were determined. Density measurements were also performed for the solutions. The same experiments were performed on these solutions after UV laser irradiation. The obtained values were discussed in comparison with the solvent behaviour and with the behaviour of other drugs (of alizarin dye class) as well. The average values of the advancing and receding angle, determined using a home developed program, are here presented for the first time.

Keywords: Chlorpromazine, CPZ, Daunorubicin, Density measurement, Doxorubicin, Drop interface, Dynamic contact angle, Laser irradiation, Nd:YAG laser, Pendent drop, Polytetrafluoroethylene, PTFE, Promethazine, PMZ, Sessile drop, Surface tension, Thioridazine, TZ, Ultrapure water, Vancomycin.

INTRODUCTION

Bubble and drop interfaces are nowadays the fundamental entities on which the operating principle of many smart products for the everyday life is based (see for example, a recent review [1]). Significant improvements of these products can be achieved by using and controlling the specific properties of interfaces between the working liquids and structures holding them. For a better understanding of the interfacial phenomena, very precise values of the surface tension are needed due to the importance of this quantity in characterising the (drug) solutions.

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Mihail Lucian Pascu (Ed.)

Several methods have been already developed to measure the surface tension of the liquids [2 - 5]. Among these, the most used are the equilibrium static methods in which still a slow flow of the liquid is able to influence the alignment of the molecules of an anisotropic fluid at the interface and consequently the measured value of the surface tension. The static methods based on the drop profile analysis present advantages as compared to the dynamic ones because they are independent of the contact angle and are based on an exact physical theory.

The pending drop method (used in this study as well) was improved in time by increasing the number of points taken into consideration for numerical computations [6 - 8] and by the technique to find the drop profile [6, 8, 9]. Actually, there are instruments to analyse the profile of a pendant drop assumed to take a shape corresponding to the equilibrium condition given by Young-Laplace equation [10]:

$$\Delta p = \sigma(\frac{1}{R_1} + \frac{1}{R_2}) \quad \textbf{(1)}$$

where σ is the interfacial tension of the two media in contact, R_1 and R_2 are the main curvature radii of the surface in a point having the coordinate z measured from an arbitrary level; $\Delta p = \Delta\rho \times g \times z$, with g representing the gravitational acceleration and $\Delta\rho = \rho_1 - \rho_2$ is the difference between the densities ρ_1 and ρ_2 of the two media. The results obtained following this procedure were comparable to those reported in the literature by using standard methods for measuring surface tension [6]. Among the advantages of the method one can mention that the calibration is simple and consists in finding the optical magnification in the captured image using metrological standards. The solid surfaces of the equipment involved in the method do not need special cleaning which is a great advantage as compared to Wilhelmy method where the used plate cleanness is crucial.

Ultraviolet (UV) light was used to control the dynamic surface tensions of mixed surfactant systems, *e.g*. containing sodium dodecylsulfate and an azobenzene derivative [11]. Light influences the dynamic surface tension of these solutions by driving the isomerization of the azobenzene moiety from cis to trans. The values of dynamic surface tension of an illuminated aqueous solution are with up to 25 mN/m lower than that of a solution not previously exposed to UV light. Furthermore, the evolution of changes induced in the molecular structure by exposure to optical radiation was studied. It was expected to induce the photo degradation (depolymerization) of large molecules and the formation of low-molecular-weight fractions [12] as observed in the case of free-falling water drops containing polysaccharides exposed to a CO_2 laser beam. The effect increases when the organic material has a significant absorbance in the range of laser lines,

because the first step is the absorption of photons by the material [13].

Some of the drugs are photosensitive compounds; when exposed to visible (white) light or to UV radiation they suffer degradation yielding new photoproducts. For example, a laser beam with high energy was applied [14] to chlorpromazine (CPZ) solutions in order to increase antibacterial activity of resulting mixture. The micro/nanodroplets containing antibiotic/antitumour medicine solutions were exposed to laser beams intending to overcome the resistance to treatments with those drugs developed in different kinds of diseases including some types of tumours. Studies on interactions of laser radiation *in vitro* with biological products indicated that the activities of enzymes, gamma globulins and blood-group substances could be altered differentially [15]. Ultraviolet laser irradiation [16] induces modifications of parental compounds [17 - 27], generating photoproducts with enhanced biological activity against bacteria and cancer cells [22, 23].

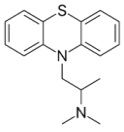
This contribution presents the results of measurements concerning the surface tension and contact angles as surface properties and the density as bulk property for drug solutions in water of some members of phenothiazine class (promethazine-PMZ, thioridazine-TZ and CPZ) in the initial form, as well as after irradiation with an UV laser beam for different time intervals. In comparison, the same characteristics were determined for drugs from other classes, much more investigated from this point of view (doxorubicin-DOX, daunorubicin-DNR and vancomycin-VCM). Drug concentrations cover the therapy interval, *e.g*. for DOX which is used in ophthalmology and cancer therapy, the concentration interval is 10^{-3}-10^{-6} M.

EXPERIMENTAL

Materials

The studied samples consisted of different aqueous solutions of some drugs from phenothiazine class and alizarin dye (Table **1**).

Table 1. Structure of the investigated drugs.

Drug/Acronym	Structure	Purchased from
Promethazine/PMZ		Sigma Aldrich (Madrid branch)

CHAPTER 11

Interaction of Laser Beams with Medicine Solutions in Bulk

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Abstract: The chapter presents a synthesis of results obtained on resonant interaction of laser radiation with medicines, in bulk, such as: cytostatics (methotrexate, 5-fluorouracil), phenothiazines (thioridazine, promethazine), quinazoline derivative (BG 1188) and hydantoin derivative (SZ-2). The effect of laser radiation on the studied samples is evaluated by analytical techniques out of which steady state absorption spectroscopy, laser induced fluorescence and FTIR spectroscopy, phosphorescence of generated photosensitised singlet oxygen and thin layer chromatography (TLC) used for qualitative photoproducts evidencing are approached. The molecular structural changes are suggested and the obtained photoproducts under laser exposure are analysed by spectroscopic means. This kind of data is related to a new method to combat multiple drug resistance acquired by microorganisms and tumours which develop new chemicals with bactericide or antitumour effects by exposing existing medicines that have low effects or are un-efficient, to laser beams.

Keywords: Absorption spectroscopy, Cytostatics, 5-fluorouracil, FTIR, Hydantoin derivatives, Laser induced fluorescence, Laser photochemistry, Methotrexate, Phenothiazine, Photosensitive medicines, Promethazine, Quinazoline derivatives, Singlet oxygen phosphorescence, Thin layer chromatography, Thioridazine.

INTRODUCTION

A problem of high importance at the moment which has to be overcome in some treatments is the acquirement of multiple drug resistance (MDR) by bacteria or, more general, microorganisms. The use of an important number of already known

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medicines became in the last decades inefficient in fighting MDR and new medicines as well as new approaches in using existing drugs to overcome MDR have to be explored. Photoactivation of some cytostatics such as 5-fluoruracil (5-FU) and methrotrexate (MTX) in order to be used as photosensitisers was pointed out in [1, 2]. Under UV laser radiation exposure, chlorpromazine (a well-known phenothiazine) exhibits molecular structure modifications in such a way that the photoproducts gain antimicrobial activity [3, 4].

Here is presented a synthesis regarding the use of laser methods to induce modifications of medicines and to obtain new photoproducts with photo-enhanced activity considering the following categories: cytostatics (MTX and 5-FU) [1, 2, 5, 6], phenothiazines (thioridazine-TZ, promethazine-PMZ) [3, 4, 7, 8], quinazoline derivative (BG 1188) [9], hydantoin derivative (SZ-2) [10, 11]. The effect of laser radiation on the studied samples is evaluated by analytical techniques such as steady state absorption and laser induced fluorescence (LIF) spectroscopy, Fourier transformed infrared (FTIR) spectroscopy, phosphorescence of generated photosensitised singlet oxygen and thin layer chromatography (TLC). These methods are used primarily for, at least, qualitative identification of photoproducts. Preclinical tests made on bacteria and tumours of these laser modified drugs are shown in Chapters 13 and 14, respectively.

MATERIALS AND METHODS

Materials

The studied compounds were selected based on several criteria: (i) the cytostatics, out of which two of the most "popular" medicines were chosen for which there are extensive applications reports; (ii) the phenothiazines were selected because this class contains photosensitive products and in some cases they have bactericide effects even if at high, toxic concentrations; (iii) the quinazoline derivative was specifically designed to fight MDR by using the mechanisms specific to the efflux pumps of bacteria; (iv) the hydantoin derivative was designed to fight MDR resistant tumours.

Methotrexate (MTX)

MTX ($C_{20}H_{22}N_8O_5$) belongs to the pterine group (folic acid, aminopterin, *etc*.) and it is an antifolate used, on one hand, in chemotherapy and on the other in treating diseases such as psoriasis and rheumatoid arthritis. MTX stops the DNA synthesis by inhibiting the action of dihydrofolate reductase (DHFR) enzyme which is responsible for the tetrahydrofolate (THF) level within the cell. THF plays an important role in purine nucleoside synthesis and in DNA synthesis.

The difference between MTX structure and that of THF consists in the presence of NH_2 radical in the pteridine ring instead of OH. These similar structures make possible the action of MTX on DNA synthesis.

Fig. (**1**) shows the 3D chemical structure of MTX optimised with the DFT method (B3LYP functional; 6-31G(d,p) basis set) using Gaussian09 [12].

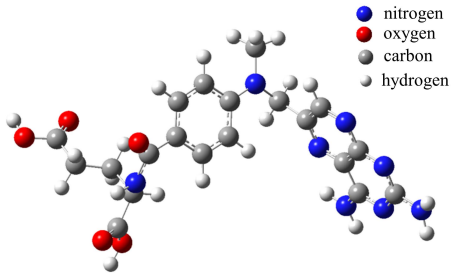

Fig. (1). The 3-D optimised structure of MTX for fundamental electronic state.

MTX used in the studied samples was produced by EBEWE Arzneimittel G.m.b.H (DE). The samples were MTX solutions prepared in natural saline at concentrations between 10^{-4} M and 10^{-5} M.

5-Fluorouracil (5-FU)

5-FU (5-fluoro-2,4(1*H*,3*H*)-pyrimidinedione; $C_4H_3FN_2O_2$) is an antimetabolite used in the chemotherapy of a variety of solid cancer tumours. It is a chemically synthesised fluorinated pyrimidine, administered as a sodium salt; 5-FU (Sindan, ROU) solutions in natural saline (0.9% NaCl) at 10^{-4} M concentration M and adjusted to pH = 8.4 by the addition of NaOH were studied.

5-FU presents 2 tautomeric forms: the diketo (lactam) form, which is a major component, more stable and more weakly fluorescent tautomer, and the enol-keto (lactim) form (Fig. **2**), as a minor component but fluorescent tautomer.

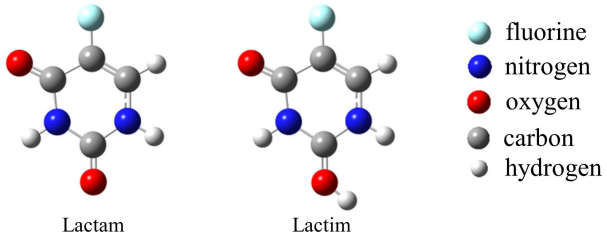

Fig. (2). The 5-fluorouracil (5-FU) tautomers structure.

CHAPTER 12

Lasers in Foams and Emulsions Studies

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Abstract: This chapter presents results regarding production of emulsions and foams and their interaction with laser beams. Foams and emulsions are considered mainly collection of bubbles, or drops of different kind with particular properties, respectively. Here, emulsions of oily vitamin A and water in which various surfactants were introduced are described outlining the conditions in which they have a longer time stability. Laser induced fluorescence emitted by microdroplets that contain emulsions of rhodamine 6G in water and oily vitamin A is shown and the spectral distribution of the fluorescence radiation is described evidencing the enhancement of fluorescence emitted by droplets with respect to bulk. Lasing conditions in droplets are discussed and the role of reflections on foam drop inner structural components is outlined. Foams based on water solutions of vancomycin, produced by droplets interaction with laser beams are described in correlation with biomedical applications. Foams of polidocanol in water produced by Tessari method are described and their use in varicose vein treatments is introduced outlining the role of their exposure in tissues to infrared Nd:YAG laser beams in connection with the more rapid positive treatment effects. Polidocanol foam stability function of several surfactants such as tween 80, glycerin and xanthan gum is presented and discussed.

Keywords: Aetoxisclerol, Colloids, Emulsion, Foam, Hanging droplet, Laser, Nanoparticle, Pendant droplet, Polidocanol, Rhodamine 6G, Sclerosing foam, Sclerotherapy, Sessile droplet, Suspended droplet, Tessari technique, Vancomycin, Varicose veins, Vitamin A.

INTRODUCTION

Droplets with submicron dimensions are of high interest for applications in various domains (pharmaceutical, cosmetic, food industry, *etc.*). The multiple applications of nano-/micro-droplets in emulsions and the need of smaller droplets

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dimensions pushed the efforts to develop better emulsification technologies. Droplets with dimensions smaller than 1 μm are suitable for biomedical applications as transport vectors for medicines to specific targets. The systems using droplets allow substances poorly soluble in water, but oil-soluble, such as vitamins or drugs to be incorporated in a lipophilic phase so that the increase of their local bioavailability takes place. This also stabilises components sensitive to enzymatic degradation, allows a slower, controlled release of components to targets over a prolonged period of time and reduces the side effects of drugs [1]. Based on small particles use in systemic treatments, one predicts that micro-/nano-emulsions uptake improves the efficiency of lipophilic substances The dispersed phase is present in the form of droplets existing in continuous phase [1].

Emulsions

Emulsions are defined as heterogeneous systems composed of an immiscible or partially miscible liquid denominated as dispersed phase, in another liquid (continuous phase) as droplets with diameters ranging from nanometres to 100 μm. A particular case is represented by oil and water mixtures, the continuous and dispersed phases being determined by several factors such as the percentage of each liquid, the type of surfactant used, *etc.* Multiple emulsions are also possible (*e.g.* O/W/O, W/O/W - where O means oil and W means water) to produce. In order to stabilise the emulsion (avoiding droplet breakage and coalescence) surfactants are used.

The two liquid phases are selected to be fully immiscible and chemically non-reactive which makes their mixture thermodynamically unstable [2]. In the case of a mixture of two immiscible liquids, a molecular exchange between dispersed phase and continuous phase is not possible, and therefore the Ostwald ripening effects are avoided.

One of the characteristics of an emulsion is the mean size and the size distribution of droplets. By selecting the proper emulsification method and/or device(s) these characteristics can be controlled [3].

Emulsions are widespread and have applications in many domains with an impact on daily life, such as: food, pharmaceutical and cosmetic industry. Applications of compound droplets are reported in the literature and are widely used in various domains. One example is the use of droplets (diameter~2.7 mm) that contain emulsions of oil in water to cool a moving steel strip and at the same time lubricate the sprayed areas [4]. By using coaxial electrified jets, stratified capsules with micro- and sub-micrometre dimensions and emulsions composed of immiscible liquids were produced (0.15 μm capsules composed of a water core of 0.1 μm and a surrounding layer of olive oil of 0.025 μm diameter) [5].

The emulsification process implies the use of energy to mix the immiscible liquids. This can be made by mechanical or pseudo-mechanical means, such as rotor-stator systems (high speed homogenisers), high pressure homogenisers, ultrasonic probes, micropore size membranes, microfluidisers, phase inversion systems, *etc.* The emulsification is based on two opposite processes: the drop breakage that produces smaller droplets out of a parent drop, and droplet-droplet coalescence [6]. The evolution of drop-size distribution during emulsification is determined by the competition between these two processes [1].

The stability of an emulsion depends on various factors such as physical properties of interfacial film, rheological properties of continuous phase, composition of the mixture (surface active substances addition). The type of added surfactants/nanoparticles, the presence of electrostatic or steric barriers on droplets, the droplet size distribution, the dispersed/continuous phase ratio, and the temperature are also factors to consider [7 - 8]. By adding surfactants to one or both phases, droplet size as well as droplet-droplet interactions are influenced. Addition of surface active substances decreases the surface tension due to surfactant molecules that migrate to interface, which fact influences droplets sizes and modifies their visco-elastic properties; therefore, this delays droplets coalescence, which leads to an increased emulsion stability [9]. In the case of mechanical methods used to mix the liquids, droplets dimensions are determined by the energy used to apply the procedure (rotating speed, pressure difference *etc.*). Under these conditions the evolution of drop-size distribution in an emulsion is mainly determined by drop breakup process. Function of surfactant concentration in bulk, the newly formed interfaces may be fully or partially covered with surfactants. Due to the function of surfactant concentration in bulk or type of surface active substance one can control drop coalescence effects, drop breakage due to the employed emulsification method and therefore the stability of the emulsion after finishing the emulsification process.

A widely accepted technique for emulsification consists in using a rotor-stator system that can mix substances in controlled conditions (rotating speed, type of rotor, *etc.*). Another emulsification method may use high pressure homogenisers that can mix the immiscible components under high pressure differences. Fluids are pushed at high speed through a small orifice and droplet breakage is produced. Each emulsification method requires energy consumption by the system. The energy may be provided in various ways: trituration, homogenisation, heat, agitation, ultrasound *etc.* There are also emulsification methods that do not require mechanical energy to be dissipated; these emulsions are generated by condensation techniques, are mainly determined by thermodynamic principles and are usually performed in the presence of emulsifying or swelling adjuvants.

CHAPTER 13

Application of Laser Modified Medicines in Fighting Multiple Drug Resistance Acquired by Microorganisms

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Abstract: The development of new medicines and alternatives to existing antimicrobial agents represents a high priority in treating infections caused by multi-drug resistant bacteria. This is reported mainly, as multiple resistance acquired by bacteria at treatment with antibiotics. The use of laser radiation to photo-generate new compounds from known medicines can be an effective method to obtain a better antimicrobial activity against infections with bacteria and fungi. The irradiation with a laser beam emitted at 266 nm of 2 mg/ml aqueous chlorpromazine and thioridazine solutions for periods of time varying from 1 to 240 min leads to photochemical changes in the molecular structure of the parental compounds and to generation of new photoproducts with enhanced antimicrobial, antifungal and antibiofilm activity. The susceptibility of broad panels of Gram-negative and Gram-positive bacteria and fungi in planktonic and biofilm state, to the unirradiated and irradiated CPZ and TZ were performed in order to highlight the possible use of these substances for the development of novel antimicrobial agents. The antimicrobial activity was evaluated by quantitative methods, *i.e*. minimum inhibitory concentration and minimum biofilm eradication concentration assays. Both CPZ and TZ irradiated solutions presented, as cocktails of medicines obtained after laser irradiation, enhanced antimicrobial, antifungal and antibiofilm activity when compared to the unirradiated samples.

Keywords: *ABC 230*, Antimicrobial activity, Bacteria, Biofilm, *Candida albicans*, Chlorpromazine, *Cryptococcus neoformans*, Fungi, *Klebsiella pneumoniae*, Laser, MBEC, MDR, MIC, Multiple drug resistance,

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Pseudomonas aeruginosa, Susceptibility, Thioridazine, *Staphylococcus aureus*, UV laser radiation.

INTRODUCTION

In 1883, Bernthsen published the first paper describing the synthesis of phenothiazine molecules [1]. Between 1883 and 1950, intense research was dedicated to synthesising biologically and medicinally active phenothiazine derivatives, which resulted in the discovery of the 10-dialkyl derivatives prepared by Charpentier [2]. These derivatives came into wide clinical use due to their multiple therapeutic properties. In 1988 it was reported that over four thousand phenothiazine derivatives were synthesised and investigated for a wide range of biological and pharmacological activities. Phenothiazine derivatives were found to have the following clinical effects: antihistaminic, sedative, antiemetic, tranquilising, antipruritic, antipsychotic, anthelmintic, anticholinergic, analgesic, antitussive, anti-inflammatory, antimutagenic, *etc*. [3].

One of the most important and widely used phenothiazine derivatives is chlorpromazine (CPZ), a drug mostly prescribed for its antipsychotic effects. The description and CPZ molecular geometry is shown in Chapter 9. Another important phenothiazine derivative is thioridazine (TZ), a piperidine phenothiazine derivative prescribed for the treatment of schizophrenia and psychosis. The description and molecular geometry of thioridazine can be found in Chapter 11.

Within the wide range of biological and medical effects, phenothiazine derivatives have been proven to possess cancer chemo-preventive effects, such as inhibition of the P-gp transport function, inhibition of PKC activity, decrease of cell proliferation, and anti-CaM activity [4 - 6]. Literature data support the idea that (i) substituents at C-2 position of the tricyclic core of phenothiazine and (ii) length of alkyl side chain connected to nitrogen at position 10 (N-10) of the thiazine ring, are influencing compounds activity against cancer cells. The type of substituents present in phenothiazine core structure plays a larger role than the length and nature of side chain [7].

Also, phenothiazine compounds are considered to increase cellular sensitivity to cytostatic drugs by reversing the multi-drug resistance (MDR) of neoplastic cells by a strong inhibiting effect on P-gp - dependent mechanism of MDR [4]. Phenothiazine C-2 position substituents that have been proven to reverse MDR include the following functional groups: $-COCH_3$, $-COCH_2CH_3$, $-COCH_2CH_2CH_3$, $-SOCH_3$, $-SO_2CH_3$, $-SO_2N(CH_3)_2$ [8].

The nitrogen atom at position 10 exerts a pyramidalisation effect on the structure of phenothiazine compounds. A high pyramidal character has been linked to increased biological activity, especially the antitumour effects on AIDS-related leukaemia and lymphomas, since the molecule becomes more reactive with the increase of these characteristics [9].

A relatively new approach in the fight against MDR is the study of laser modified phenothiazine derivatives. Most compounds that have a phenothiazine core structure are highly sensitive to light in general, showing visible changes such as colour modification of drug powder or solution when exposed to light. The colours can vary from light green, blue or yellow to dark brown. The change in colour depends mostly on the nature of the substituents at C-2 and N-10 position. By exposing these drugs to certain laser beams having particular wavelengths, it has been proven that the process of photodegradation can be directed so that the mixture that results from this exposure has antibacterial properties [10 - 13]. As for cytotoxic effects of irradiated solutions, a lower toxicity is observed compared with the unirradiated sample when tested against Murine Swiss albino fibroblast, 3T3 and human epithelial carcinoma HeLa cell lines [14].

In this chapter, photochemical modifications produced by the interaction of laser radiation at 266 nm with CPZ and TZ aqueous solutions are shown. Promising results are obtained regarding the antimicrobial, antifungal and antibiofilm activity of irradiated CPZ and TZ samples against a panel of pathogens.

MATERIALS AND METHODS

The studied compounds are CPZ and TZ (98.9%, Sigma Aldrich, DE). Solutions of CPZ and TZ having a concentration of 2 mg/ml were irradiated with a 266 nm pulsed laser beam (fourth harmonic of the Nd:YAG laser, Excel Technology, Surelite II model) having an average pulse energy of 6.5 mJ. The characteristics of Nd:YAG laser are the following: 6 ns full time width at half maximum (FTWHM), 10 Hz pulse repetition rate. The optical path length was 1 cm, the beam cross section at interface of the spectrophotometric cell with air 0.38 cm^2 and the fluence of the laser beam 17.1 J/cm^2.

The irradiation was carried out for different exposure time intervals of 1, 5, 15, 30, 120, and 240 min for CPZ and 1, 15, 60, 120 and 240 min for TZ. For these studies, 2 ml of aqueous solutions of CPZ and TZ were used, which were continuously mixed/agitated at 700 rpm with a magnetic stir bar to homogenise the samples and to prevent formation of precipitates.

The susceptibility of microorganisms to unirradiated and irradiated samples was tested *in vitro*, using as methods the *minimum inhibitory concentration* (MIC) and

CHAPTER 14

Application of Optically Modified Medicines in Fighting Pseudotumours

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Abstract: Results are shown about the applications of medicines modified by exposure to optical radiation on pseudotumours indusced in rabbit eyes. These consist, first, in characterising modifications produced on medicines by exposing them to optical/laser radiation. Selected medicines are (i) cytostatics: methotrexate, 5-fluorouracil, (ii) benzopyridine derivatives: BG 204, BG 1120, (iii) phenothiazines: chlopromazine. They were used as solutions either in water or in natural saline. The anti-tumour properties of resulted solutions are measured after applying them on pseudotumours induced in rabbit eyes by Schmidt-Erfurth method. The use of methotrexate on pseudotumours has shown that eye conjunctive and neovascularisation disappear after 1-2 treatments which consists in exposure of eye injected with unirradiated methotrexate solution to cw Hg lamp radiation. Even if the use of methotrexate fastens the recovery, one should avoid direct exposure of eye to UV-Vis radiation. 5-fluorouracil was exposed to nitrogen pulsed laser beam which was sent to the eye already impregnated with cytostatics. Eye conjunctive and neovascularisation disappeared after 1-2 treatments, recommending 5-fluorouracil to cure such pseudotumours by combining its action with that of 337.1 nm laser. BG 1120 was used only exposed to cw Xe lamp incoherent radiation, without irradiating the eyes impregnated with solutions of it. BG 1120 modified by exposure to optical radiation leads to faster decrease of inflammations associated with pseudotumours and of neovascularisation of conjunctive tissue. For chlorpromazine, the most efficient in the recovery of a pseudotumour tissue is the sample irradiated 20 min, at 10 mg/ml concentration in water.

Keywords: Absorption spectroscopy, Benzopyridine derivatives, Chlorpromazine, Cytostatics, 5-fluorouracil, FTIR, Hg-lamp, Laser inducedfluorescence, Laser spectroscopy , Malignant tumours, Methotrexate,

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Multiple drug resistance, Nitrogen pulsed laser, Ophthalmology, Phenothiazine derivatives, Photochemistry, Pseudotumours, Solid state laser, Xenon lamp.

INTRODUCTION

Multiple drug resistance or multi-drug resistance (MDR) is acquired by bacteria, in a broader sense by microorganisms, but it is also signalled in the malignant tumours treatment where the cancer tissues may not be controlled after a particular time moment of their development in human body, if a given stage in cancer development was reached. The tumours are resistant to treatment, in particular to chemotherapy, and new methods to treat them and other drugs are requested to overpass or even cure this illness. In this chapter, results about the use of some drugs are reported which although, normally, do not have anti-tumour effects may generate through exposure to optical radiation photoproducts that by individual or combined action may prove to be efficient at least in the treatment of pseudotumours tissues produced on rabbit eye conjunctive.

The reported results are organised in two parts: the first, contains the description of drugs exposed to optical incoherent radiation emitted by continuous mode (cw) high pressure gas lamps (Hg and Xe) or by pulsed UV lasers. The second shows the effects that are exhibited by some exposed medicines on pseudotumour tissues either when drugs are injected in the eye conjunctive and exposed to optical UV and visible radiation *in situ*, or when drugs are exposed first to optical radiation, modified by it and then injected subconjunctivally in rabbit eyes. Of course, drugs in the two categories are different. Out of the utilised drugs, cytostatics played a core place, since they are already widely used in the treatment of proliferative processes. Optical beams were used to produce molecular structural changes of such drugs and to improve their effectiveness in tumour treatments.

Other classes of medicines were benzopyridine and phenothiazine derivatives that do not have necessarily anticancer effects, but may generate photoproducts during exposure to incoherent or coherent optical radiation which may be active compounds against tumours. Results in this respect obtained on pseudotumours produced by Schmidt-Erfurth method are shown in the chapter.

MEDICINES

Methotrexate (MTX)

MTX has the chemical formula $C_{20}H_{22}N_8O_5$ and molecular weight 454.44 g/mol; it is a yellow to orange-brown odourless crystalline powder that interferes with DNA and RNA synthesis [1, 2]. The MTX is sensitive to light, oxidation and hydrolysis [1, 2]. It is soluble in diluted solution of alkali hydroxides and

carbonates, insoluble in chloroform, alcohol, ether and water and slightly soluble in diluted hydrochloric acid [3]. In therapeutic use, MTX acts as abortifacient [4], antirheumatic [5], nonsteroidal [6], antineoplastic [7], antimetabolites [8], dermatologic [9], immunosuppressive agents [10], and enzyme inhibitor [11].

MTX stops the DNA synthesis by inhibiting the action of dihydrofolate reductase (DHFR), thus reducing the tetrahydrofolate (THF) in the cell. THF has a major role in DNA synthesis [1, 12]. The difference between MTX and THF is the substitution with an amino group ($-NH_2$) of the oxygen in the pteridine ring. Photochemical studies on folic acid have shown the photo-dissociation effects of light on MTX [13] by measuring consecutive changes in absorption and fluorescence spectra with increasing exposure time to radiation; 6-formilpteridinic acid and p-aminobenzilglutamic derivatives are formed [1, 14]. Relatively recent studies and reports have proposed the combination MTX-radiation as a possible cancer treatment procedure [1, 14]. A detailed description and the molecular geometry of MTX can be found in Chapter 11.

5-fluorouracil (5-FU)

5-FU having chemical formula $C_4H_3FN_2O_2$ and molecular weight 130.077 g/mol, is an odourless, crystalline powder used for its antineoplastic activity [15]. It is soluble in water and methanol [16] and stable after exposure to environmental air [17]. 5-FU is a pyrimidine derivative belonging to the cytostatic group that includes the so called antimetabolites. It is primarily used to treat colon, breast and pancreas cancer and, not least, ocular tumours. Antimetabolites generally alter DNA or RNA synthesis by interfering with the synthesis of nucleic acids [18].

The utility of 5-FU is limited by the fact that tumour tissue acquires resistance to it relatively quick [18, 19]. Therefore, it became necessary to discover new techniques potentiating 5-FU action, the exploitation of its photo-physical properties being a recommended approach.

At the same time, the combination between cytostatics and laser therapy as an alternative to cancer treatment was tested [12, 14, 17, 18, 20, 21]. The administrated cytostatic is selectively accumulated in tumours and the tissue is then irradiated with an optical beam emitted at properly chosen wavelength [13]. The tumour is destroyed by the chemotherapy effect of cytostatics and by the resulting compounds produced *via* irradiation [22]. This therapy could also induce an immune response against the treated tumour. In addition, the reaction could even lead to disappearance of metastatic tissue. A detailed description and the molecular geometry of 5-FU can be found in Chapter 11.

CHAPTER 15

Interaction of Medicines Exposed to Laser Beams with Fabrics of Interest for Biomedical Applications

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Abstract: Photosensitive phenothiazine drugs subjected to UV laser radiation exhibit alteration of the initial parental compounds and provide newly generated photoproducts with possible bacteriostatic, bactericidal and antitumor effects. These new compounds with enhanced properties may also have outstanding wetting abilities when applied on fabrics of interest for applications, in order to develop new tools for drug delivery to patients, biological targets, or even surfaces of manmade equipment, constructions, or space ships. However, prior to biomedical applications, surface properties including contact angle measurements, require more thorough studies since such properties may control the delivery process of medicines to target tissues. This chapter shows the results obtained on the wettability of cotton, polyester and Parafilm M surfaces by laser irradiated phenothiazine aqueous solutions. In the present study, pendant chlorpromazine, promazine and promethazine droplets have been generated, then by simply bringing them into contact with the respective surfaces, detachment from a vertical capillary took place thus achieving the formation of sessile droplets. Results have evidenced the fact that some of the implemented drugs, containing the photoproducts obtained by prolonged exposure to laser radiation of the parent compounds, indicated better wetting abilities compared to their unexposed control, hence providing new promising perspectives.

Keywords: Absorption, Adsorption, Advancing contact angle, Chlorpromazine, Contact angle hysteresis, Cotton, Non-antibiotics, Parafilm M, PES, Polyester, Promazine, Promethazine, Receding contact angle, Sessile droplet, Surface tension, Surface wettability, UV laser, Wetting properties.

INTRODUCTION

Wetting processes may be regarded as a key player in many applications, such as biology [1, 2], medicine [3, 4], cosmetics [5], industry [6, 7] as well as space

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science [8 - 11]. Concerning biomedical applications, studies of biological fluids in interaction with biological/alien surfaces have a significant role in numerous treatments [12]. Bearing this in mind, wetting processes have been under investigations in (i) dentistry (*e.g.* gingival surface wettability in contact with intra-oral water [13], wetting behaviour of dental implants [14], denture base materials wettability with natural and artificial saliva [15]), (ii) ophthalmology (*e.g.* tear film wettability with or without contact lens [16], tear film breakup depending on corneal surface wettability [17]) and (iii) vascular biology (*e.g.* inhibiting the adhesion of blood components by increasing wettability of implanted materials [18]). Another interest resides in studying the interaction of medicine solutions with different surfaces implemented in medical treatments (*e.g.* medicinal bandages, transdermal patches), as well as with human skin.

One possible approach to determine the degree of wetting consists in contact angle (CA) measurements.

As shown in Chapters 2 and 10, the CA formed by a liquid drop on an ideal solid surface, *i.e.* flat, rigid, smooth, homogeneous and with no contact angle hysteresis (CAH), is governed by the thermodynamic equilibrium of the drop under the action of three interfacial tensions, described by Young's equation:

$$\gamma_{LV} \cos\theta = \gamma_{SV} - \gamma_{SL} \quad \textbf{(1)}$$

where γ_{LV}, γ_{SV} and γ_{SL} represent the liquid-vapor, solid-vapor and solid-liquid interfacial tensions, respectively, and θ is the CA arisen from the balance of the intermolecular interactions between solid-liquid [19], illustrated in Fig. (**1**).

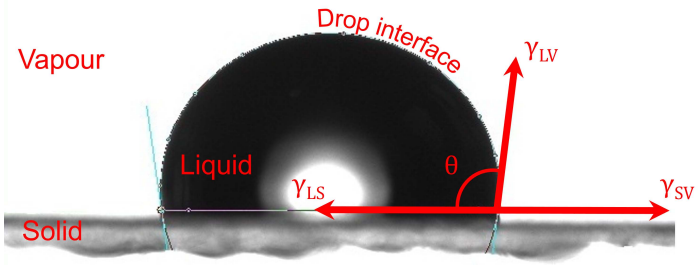

Fig. (1). Schematic representation of a liquid drop and CA formed by the intersection of the liquid-solid and the liquid-vapor interface.

A liquid drop tends to avoid contact with surface when cohesive forces between liquid's molecules are stronger than adhesive forces between solid'-liquid's molecules. Otherwise, the drop will spread onto surface [19], as depicted in Fig. (**2**).

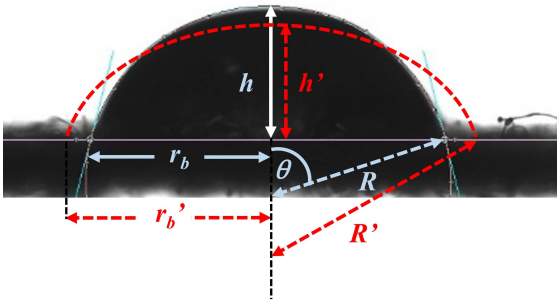

Fig. (2). Evolution of a sessile drop dimensions when spreading of a liquid drop onto a solid surface occurs.

The majority of surface and colloid scientists regard Thomas Young as the father of CA and wetting fundamentals, although most probably the earliest direct recognition, concerning wetting phenomena may be attributed to Galileo Galilei, who might be perceived as the grandfather of the field [20 - 22]. His observations of a dense, flat and thin solid floating on water allowed him to advance the idea of spreading and wetting in 1612 [19, 21]. The respective early findings were followed by the introduction of CA equation on ideal solid surfaces in 1805 by Young [23], while in 1869 Dupré correlated the Young equation with the work of adhesion [24]. The studies which followed included investigations on the shape of pendant drops, carried out by Worthington in 1881, whereas in order to analyse drop profiles he implemented the Laplace curvature theory instead of measuring the formed tangents of drop profiles [25]. However, the first experimentally measured CA results, in the time interval 1877-1898, can be associated with the work of Quincke [19]. Further research regarding CA in terms of surface forces and hysteresis can be assigned to Lord Rayleigh, who examined in 1890 the advancing and receding of a liquid drop onto a solid surface [19, 26]. Later on, Bartell, Hatch and Mack [27, 28] have carried out trigonometric calculations by considering a spherical geometry of droplets, obtaining CA from the height and diameter of small drops utilising the following equation:

$$tan\frac{\theta}{2} = \frac{h}{r_b} \qquad \textbf{(2)}$$

where h represents the height and r_b is the base radius of the drop. The first correlation between the CA and the roughness of a specific surface, by taking into consideration the area of liquid/solid interface, was defined by Wenzel in 1936 [29]. His investigations were further extended by Cassie and Baxter, who performed studies on porous surfaces by including the interfacial area fraction approach, in 1944 [30]. A review on CA history is detailed in [19].

CHAPTER 16

Microvolumetric Droplets in Air in Hypergravity Conditions

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Abstract: The interaction of laser modified medicine solutions with hydrophilic and hydrophobic target surfaces has been investigated under the effect of simulated hypergravity conditions, employing the Large Diameter Centrifuge (LDC) facility, developed by the European Space Agency (ESA). Experiments have been performed within the HyperMed project under the aegis of the ESA "Spin Your Thesis!" 2015 programme. During centrifugation, real-time video files have been recorded regarding generation of ultrapure water, unexposed and laser exposed chlorpromazine aqueous pendant droplets, followed by their detachment due to the exerted high gravitational accelerations and finally by the formation of sessile droplets on target surfaces. In this way, information about the volume of the generated droplet, the degree of wetting and its time evolution at different hypergravity levels has been obtained. Phenothiazine solutions irradiated with UV laser radiation indicate reduced surface tension, thus presenting better wetting properties. Target surfaces impregnated with medicine solutions may constitute an unconventional tool and even vector in developing new drug delivery systems. Such a wetting process under high g-level conditions may be useful in space medicine applications. Microorganisms can survive, grow and even proliferate under the effect of increased gravity. Therefore, upon launching of a spacecraft, during a long-term mission in microgravity conditions, astronauts and spacecraft surfaces may require treatment and decontamination, respectively, against onboard infectious microbes. Since non-terrestrial gravity may alter drug properties, medicine droplets behaviour in interaction with target surfaces under hypergravity conditions is the aim of the present study.

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Keywords: Activated charcoal, Aluminium, Contact angle, Cotton, Hydrophilic surface, Hydrophobic surface, Hypergravity conditions, HyperMed project, Large Diameter Centrifuge, Long-Term space mission, Microbial infections, Multiple drug resistance, Nd:YAG laser, Parafilm M, Phenothiazines, Space medicine, "Spin Your Thesis!".

INTRODUCTION

Since the access to spaceflight research is limited, different ground-based facilities are employed in order to induce outer space conditions. Gravity influences at a large extent countless processes, thus emphasising the impact of weight on life and physical systems. Ground-based simulators of both, microgravity and hypergravity, allow scientists to perform preliminary studies and test hardware as well as to determine experiments' parameters and protocols. Such an approach provides cost-effective platforms that can be implemented in gravitational research related to space missions [1, 2].

The term microgravity refers to a micro-g environment, which is also referred to as weightlessness or zero-g. However, such denomination should be avoided since neither space vehicles nor ground-based simulators can achieve and sustain real zero gravity. In microgravity conditions the net sum of all forces is not actually zero, but just very small compared to terrestrial conditions [3]. For instance, onboard space platforms like the International Space Station (ISS) or Shuttles the g-level may range between $\approx 10^{-3}$ and 10^{-6} g, depending on the location within the spacecraft and the frequency of vibration induced by aboard machinery, thruster operation for positional orientation or reboost, docking/berthing, astronauts' activity and micrometeorite impacts [1, 4 - 9]. On the other hand, the hypergravity terminology defines the condition wherein gravity surpasses that on the surface of Earth, *i.e.* exceeds 1 g. By taking into consideration that all organisms on Earth have evolved under 1 g condition, their exposure to high g-levels might lead to significant alterations of their structures and/or behavior [2].

One cannot rule out the realistic possibility of transporting living microorganisms through space. The first mention of the respective hypothesis was made as early as the 5th century BC by Anaxagoras [10], which later gained a more scientific approach through prominent scientists such as Berzelius (1834), Richter (1865), Kelvin (1871) and Helmholtz (1879) [11 - 14]. A more comprehensive study of the hypothesis was proposed by Arrhenius (1903) [15], and was discussed by Hoyle and Wickramasinghe (1974) [16, 17]. According to Arrhenius' suggestion, the transport of living bacteria from one solar system to another may be possible by the radiation pressure of its home star [15]. Although this kind of transfer might be difficult, the transfer within our solar system should not be neglected

[18]. Since in extraterrestrial environment microbes may better survive shielded, given the strong UV space radiation [19], they might be able to be transported inside meteorites, for example [20, 21]. The hypothesized process of microorganisms journey from one planet to another, consists in the ejection phase of bacteria-bearing rocks from the planet of origin as the result of an impact, where organisms would be, besides other factors, subjected to extreme accelerations. The following phase corresponds to the space travel alongside the associated environmental hazards such as vacuum, radiation and desiccation. Finally, the last phase involves the atmospheric entry, where the microbes would be exposed to heating and deceleration upon landing on Earth, for instance [20]. According to predictions made by Mastrapa and colleagues [20], a 3×10^5 g maximum acceleration would be possible due to an impact ejection from Mars. In order to fight microorganisms that were submitted to conditions during space fligths, one should have available medicines and transport vectors that contain them which have been submitted to the same conditions.

Another importance of hypergravity conditions arises during launches and reentries, the exposure being up to 3.2 g and around 1.4 g (as reported by NASA Ames Research Center), respectively (this could be much more in Soyuz systems where up to 20-40 g for a very short time can be produced). The fastest man-made object re-entry on record with successful landing was constituted by the Stardust Sample Return Capsule in 2006, with a hypervelocity of ≈ 46000 km/h. A drastic profile characterised the capsule reentry, ranging from Mach 36 to subsonic speed within 110 s, the peak deceleration lasting 40 s at 34 g at an altitude of 55 km [22].

Thus, investigations performed at high g conditions become worthwhile when one has to identify micro-organism survival and growth rate in outer space rocks, *e.g*. meteorites, asteroids, comets and planetoids, and onboard spacecrafts as well.

In order to tackle the issue of microbes endurance at high acceleration and jerk, ballistic experiments have been made by Benardini *et al*. [23], resulting in ≈75-100% survival of spores of *Bacillus* species basalt isolates after their exposure to 1.5×10^6 m/s^2 and 1.5×10^{10} m/s^3. Survival of *B. subtilis* spores and *Deinococcus radiodurans* cells during a hypothetical ejection from Mars have been determined and reported in [20]. *B. subtilis* spores subjected to extended periods of time of ultracentrifugation at 4.36×10^5 g peak acceleration, presented endurance at the respective order of magnitude of acceleration [20]. According to [24], it was observed that studied Gram-positive and Gram-negative bacteria not only survived, but also experienced robust growth at hyperaccelerations up to≈2×10^4 g, *Escherichia coli* and *Paracoccus denitrificans* proliferating even at 403,627 g.

CHAPTER 17

Lasing by Optically Pumped Pendant Droplets

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Abstract: Pendant droplets have optical properties that reproduce the characteristics of corresponding bulk materials. A particular case is the droplet hanging in air, made of a laser dye solution and optically side-pumped. Here are shown results about laser induced fluorescence (LIF) emitted by a single pendant droplet excited with 532 nm pumping pulsed laser beam when its content is solution of rhodamine 6G in water kept as such, or doped with TiO_2. By changing the geometry of fluorescent medium from bulk to pendant droplet and its volume, significant LIF changes occur. The main difference is amplification of emitted fluorescence in pendant droplets, explained by the confinement of light *via* total internal reflection in droplet. A new technique is shown, developed to distinguish temporal changes of a droplet emission in air. It was observed that the presence in the droplet of rhodamine 6G solution in ultrapure water of TiO_2 nanoparticles induces LIF spectra modifications depending on nanoparticles number density and laser beam pumping energy. A concentration of 10^{11} part/cm^3 nanoparticles favours formation of two new emission bands shifted towards blue with respect to main band. The increase with one order of magnitude of TiO_2 number density, produces the disappearance of these bands regardless the pumping energy. Data suggest that addition of TiO_2 nanoparticles to rhodamine droplet solutions influences emission spectra which can be modulated by varying nanoparticles concentration and pumping beam energy.

Keywords: Fluorescence, Laser active medium, Laser dye, Laser induced fluorescence, LIF, Lasing, Microdroplet, Nd:YAG laser, Spherical cavity, Rhodamine 6G, Rh6G, TiO_2 nanoparticles.

INTRODUCTION

As it was already presented in previous chapters particularly in Chapter 9, the resonant interaction(s) of laser beam(s) with pendant droplet(s) triggers an increased interest for further possible applications. This is correlated with the fact that pendant droplets are suitable for different applications such as lasing [1 - 4],

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sensing, molecular modifications by exposure to laser beams and transport of substances/medicines to targets [5 - 7].

A pendant droplet that contains a laser dye, may work as a particular type of dye laser, when optically pumped, if it has suitable dimensions (liquid volume and resulting diameter) and a chosen content (laser dye, solvent, concentration in the solute). Functioning of a microvolumetric droplet as a dye laser is conditioned by its proper placing with respect to the pumping laser beam in a given pumping geometry and by the pumping laser beam energy correlated with beam waist and focus dimensions and position. Given the characteristics of the pendant droplet, this kind of laser may be considered as a droplet-, or an optofluidic- dye laser. In this case, the liquid droplet represents laser active medium and absorbs the pumping beam energy emitting induced fluorescence which is further amplified. The shape of the droplet correlated with the shape of the laser pumping beam may produce multiple total internal reflections leading to a confinement of pumping and fluorescence light similar to that produced in a closed quasi-spherical optical resonator. The liquid-air surface of the pendant droplet provides the optical feedback at selected wavelengths corresponding to the morphology-dependent resonances of a spherical droplet [1] or whispering gallery modes functioning [8, 9]. The properties of the laser light such as wavelength, spatial mode structure, emission direction(s) and tunability depend on the properties of the liquid medium and on pumping conditions.

At the same time, several reports on the effect of nanoparticles on dye laser emission were made. Droplets doped with nanoparticles contain scattering formations constituted by these nanoparticles. When losses in the droplet are lower than gain due to longer optical path obtained by light scattering produced by nanoparticles conglomerates, one may obtain a random droplet laser [10]. After the first report about random laser [10], studies on the effect of different nano-scatterers present in the dye laser medium such as TiO_2 [11 - 13] and ZnO [12] nanoparticles, polymers [14], intralipids [2], emulsions [4], and CdSe nanostructures [15] were reported.

In the following some results about the studies on laser induced fluorescence (LIF) are shown and its amplification by laser pumped pendant droplets that contain rhodamine 6G (Rh6G) solutions in water and are hanging in air. A comparison is made between bulk solutions and pendant droplets of the same materials as bulk, in terms of pumping geometries of active media underlining specific phenomena obtained in droplet. In the case of pendant droplet, the emission signal is studied function of different experimental parameters such as: droplet's content, laser dye concentration, pumping geometry and fluorescence collection geometry, emitted light collection time [16].

Measurements of temporal structure/changes of droplet emission are presented, conducted by varying the fluorescence signal acquisition gate width and gate delay of the analysing spectrograph in correlation to laser pulse - droplet interaction time. By using this sampling technique it was obtained the temporal evolution of emission spectra during the interaction of the laser pumping pulse with a droplet.

Also, studies on the emission spectra of microdroplets containing solutions in water doped with TiO_2 nanoparticles are presented. The excitation is made by the second harmonic of a pulsed Nd:YAG laser in green at 532 nm, pulse duration at half maximum 6 ns, pulse repetition rate 10 pulses per second, energy varied between 6 and 10 mJ. LIF emitted spectra are analysed function of TiO_2 concentration and laser pumping energy. A comparison between emission spectra for pendant droplets generated in air and containing TiO_2 nanoparticles, and water solution droplets pumped in the same conditions is made [17]. A combined action of droplet spherical cavity/resonator that produces morphology dependent resonances (or WGM) and of TiO_2 nano-scatterers on the emission of the dye takes place, and its effect is evaluated function of droplet size, nanoparticles density and pumping energy.

Emission Spectra Function of Geometry of Fluorescent Medium and/or Geometry of Collection System

The experimental set-up presented in Chapter 9 is used for droplet's irradiation. Laser beam passes through a lens with focal length of 150 mm (Fig. **1a**). Keeping constant the droplet's position with respect to the collecting optical fibre, the emission signal is measured for different positions of L_1 with respect to the droplet.

LIF intensity depends on the distance lens-droplet (Fig. **1b**). One may observe that collected fluorescence decreases close to the focal point. In this region, the power density is increased and is localised on a small volume along the droplet diameter (on laser beam axis) and as a consequence the number of excited dye molecules is reduced. Therefore, around the focus point, the unresonant effects [18] are dominant and the droplet is destroyed. Going beyond the focal point, the intensity of droplet emission starts to increase, but with smaller rates compared to that of droplet placed diametrically opposed with respect to the focal point position.

Based on this result in the experiments performed further the distance between the lens L_1 and the droplet was set at 100 mm. In this case the beam waist is 2.5 mm and almost the entire droplet with diameter 2.67 mm (volume 10 µl) is covered. The area of the irradiated surface on droplet is a spherical cap of 0.3 cm^2.

CHAPTER 18

Spectroscopy of Microdroplets: An Alternative to the Spectroscopy of Bulky Materials

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Abstract: In this chapter data about the laser induced fluorescence and Raman spectra obtained from microvolumetric (some microliters) droplets are introduced when they are exposed to laser radiation of suitable characteristics in terms of wavelength, power, and focusing conditions. A comparison is made with the same spectra obtained for the same solutions and using the same laser beams, except focus characteristics, from bulky volumes (some milliliters) showing that, basically, the information obtained from a single droplet after interaction with a single laser beam pulse is the same with that obtained from the bulky sample. In some cases, function of optimisation degree of the excitation geometry, the accuracy of spectral data collected from a microdroplet is higher than for bulk. The equivalence of the spectral data obtained from droplets and bulk is due to the fact that the interaction takes place in small volumes of the samples in both cases. In droplet, the remaining solution components that have not interacted with the pumping laser beam are very few, whereas in bulk their numbers are higher and so, the radiation emitted at the interaction volume is perturbed by them. This experimental evidence leads to the recommendation to use in many cases interaction of laser beams with a single droplet to obtain reliable spectral data, rather than bulky samples.

Keywords: Chlorpromazine, Dimethyl sulfoxide, Droplet, Hydantoin derivatives, Laser beam, Laser induced fluorescence, Laser induced phosphorescence, Light scattering, Luminescence, Microdroplet, Microspectroscopy, Raman, Resonant interaction, Rhodamine 6G, Spectroscopy, Unresonant interaction.

GENERAL CONSIDERATIONS

Studies about interaction of laser beams with microvolumetric droplets (further called microdroplets), require understanding of the behavior of samples having

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very small volumes, of some microliters and even smaller towards nanoscale domain, at interaction with laser radiation field. In doing this, it is necessary to control, quite precisely, the mode structure of the laser beam, its waist, energy, time duration and if focused, the shape, dimensions and intensity distribution in the focus. A more accurate description of these properties is made in Chapter 7. The shape of the laser beam at input on a droplet is, in any case, critical.

Micro-spectroscopy as a term refers to the spectroscopy of samples with volumes smaller than 1 mm^3 (1 μl). The samples may be gaseous as is the case of optogalvanic spectroscopy in which atomic and/or molecular phenomena of relatively high temperature metal vapors are, typically, studied. More general, cold plasma devices use or may use very small volumes of plasma samples which are constituted by neutral atoms or molecules and of charged particles as well (electrons, positive and/or negative ions, possibly clusters, *etc*.). But very small liquid droplets may be also target samples in micro-spectroscopy, having volumes lower than 1 μl of liquid. The smallest sample volume which can be utilized is determined by experimenter's ability to generate and keep constant an as tiny as possible liquid volume, down to nanometric scale which is normally considered below 0.1 μl.

In micro-spectroscopy, samples may be groups of droplets in stationary position and jets or aerosols, but of particular interest are single droplets that interact with optical beams. In this case, as shown in this book, a droplet of microliter volume may hang on a capillary in air and the interaction may be produced with single laser pulses, repetitive pulses of laser radiation or even one or more cw beams. The target droplet may also be kept in levitated position using ultrasound sources or may be fixed in a certain position in a liquid with which its material is immiscible [1]. The use of sessile droplets for spectroscopic studies are used as an alternative [2 - 4]. The samples may be also microspheres of amorphous or crystalline materials that may provide spectral data about their components at interaction with optical beams [5, 6].

From spectroscopic point of view, droplets are optical "components" with the same properties as bulky objects made of the same material. Their small dimensions comparable with laser beam waist or focus dimensions are in most cases an advantage for performing accurate spectroscopic measurements. Up-to-date, the spectroscopic data that may be collected from a microdroplet are, mainly, related to laser induced luminescence (fluorescence or phosphorescence) and Raman scattering which may be obtained regardless of the sample volume from the origin point where the interaction of laser beam with the sample takes place. So, if a beam is focused, with focus diameter of some micrometers, the information about the sample is obtained from the volume described by focus,

independently of how large the total volume of the sample actually is. In droplets case, the linear dimensions of laser beam are very close to the dimensions of microdroplets, so that the interaction beam-droplet implies practically all droplet's volume. A negligible number of molecules in the liquid droplet remains "untouched" by laser beam, so that the radiation that is coming out from droplet contains information only from the molecules that interacted with the beam. In other words, the excess molecules in the sample may not influence the quality of recorded signal if the interaction takes place in a droplet, comparatively with a bulky sample where signal may be jammed by the molecules that surround the interaction place.

In utilising microdroplets for spectroscopic purposes, an important requirement is to conserve their volume and shape and to keep fixed their positions during measurements. As shown in Chapter 8, spectroscopic measurements based on absorption of pumping laser radiation or on its scattering by droplet's material are accompanied by mechanical vibrations of droplets as entities, their deformations or even destruction, so that it is necessary to identify and chose the optimal laser beam parameters for such applications. Given the fact that the light forces at the utilised beam energies are quite close to the weight of the droplet, unresonant and resonant effects may interfere and this process must be minimized in order to obtain reproducible spectral data from a microdroplet. Another experimental parameter is the droplet temperature during measurements on the interaction with a pulsed laser beam. This should be kept constant so that the microdroplet does not have inner modifications due to temperature gradients and is not subject of other temperature related phenomena.

EXPERIMENTAL DATA

Fluorescence Spectra

In the following, experimental data about laser induced fluorescence (LIF) emitted by droplets in pendant position in air are first shown. Several variants of experimental set-ups used to perform this kind of measurements on microdroplets are introduced in Chapters 8, 9, 12 and 17. In Chapter 8, detailed comments are made about the ratio between the unresonant and resonant interaction with laser beams; then, Chapter 9 is dedicated exclusively to resonant interaction outlining the laser induced fluorescence effects obtained in microdroplets that contain different fluorophores. An example about laser induced fluorescence emitted by chlorpromazine (CPZ) in ultrapure water solution at 10 mg/ml concentration measured in bulk and droplet is shown in Fig. (**1**). In droplet experiments, the irradiation energy was 3 mJ in order to fast accumulate resonant effects and at the same time to avoid mechanical effects specific to unresonant interaction. The

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