

## INDEX

<b>1. INTRODUCTION</b>	1
1.1. LIGHT.....	1
<b>1.1.1. Definition .....</b>	1
<b>1.1.2. Historical overview .....</b>	2
1.2. LASER .....	9
<b>1.2.1. Definition and historical overview .....</b>	9
<b>1.2.2. Classification of lasers .....</b>	12
<b>1.2.3. Lasers Applications .....</b>	13
1.2.3.1. <u>Non-medical applications</u> .....	13
1.2.3.2. <u>Medical applications and mechanisms</u> .....	13
<i>1.2.3.2.1. Thermal lasers .....</i>	14
<i>1.2.3.2.2. Non-thermal lasers .....</i>	14
1.2.3.2.2.1. The photochemical effect .....	18
1.2.3.2.2.2. Karu's mechanism due to absorption in mitochondrial cytochrome chain .....	18
1.2.3.2.2.3. The photophysical effect .....	19
1.2.3.2.2.4. The bioelectric effect .....	19
1.2.3.2.2.5. The hormonal explanation .....	20
1.2.3.2.2.6. Light biomodulation and non-explained processes	20
1.3. LIGHT-MATTER INTERACTION .....	20
<b>1.3.1. Reflection .....</b>	21
<b>1.3.2. Refraction .....</b>	21

<b>1.3.3. Transmission</b> .....	22
<b>1.3.4. Scattering</b> .....	22
1.3.4.1. <u>Scattering processes due to interaction of an electromagnetic field with a chemical bond: interaction with bound electrons</u> .....	22
1.3.4.2. <u>Scattering processes due to interaction of an electromagnetic field with a chemical bond: interaction with free electrons</u> .....	22
1.3.4.3. <u>Rayleigh scattering: a quantum mechanics process to explain the interaction in transparent media</u> .....	23
1.3.4.4. <u>Mie Scattering</u> .....	24
1.3.4.5. <u>The Compton Effect</u> .....	25
1.3.4.6. <u>The Raman Effect</u> .....	25
<b>1.3.5. Absorption and interaction</b> .....	26
1.3.5.1. <u>Absorption of electromagnetic radiation: electrically conductors, opaque or magnetic media (metallic media)</u> .....	27
1.3.5.1.1. <i>Electronic excitation (atomic energy levels)</i> .....	27
1.3.5.1.2. <i>The Photoelectric effect</i> .....	30
1.3.5.1.3. <i>Absorption of light in polyenes</i> .....	31
1.3.5.2. <u>Interaction of electromagnetic irradiation with mater</u> .....	31
1.3.5.3. <u>Difference between light interaction (polarization) and light absorption due to chemical conjugated bonds</u> .....	34
<b>1.4. ATP: A KEY MOLECULE</b> .....	35
<b>1.4.1. Energy currency</b> .....	35
<b>1.4.2. ATP synthesis</b> .....	37

<b>1.4.3. ATP and electromagnetic energy .....</b>	37
<b>1.4.4. ATP as a dipole .....</b>	39
<b>2. OBJECTIVES</b>	41
<b>3. MATERIAL AND METHODS</b>	42
<b>3.1. TEMPERATURE MEASUREMENT: CONTROL .....</b>	42
<b>3.2. ATP IMPURITIES: CONTROL .....</b>	42
<b>3.3. THE LUCIFERINE-LUCIFERASE REACTION .....</b>	43
<b>3.3.1. Reagents .....</b>	43
<b>3.3.2. The reaction .....</b>	43
<b>3.3.3. Luminescence detection .....</b>	43
<b>3.3.4. Light sources .....</b>	44
<b>3.3.5. Technical procedure and experimental groups .....</b>	44
<b>3.3.6. Statistical study and data analysis .....</b>	46
<b>3.4. ABSORPTION OF ATP .....</b>	46
<b>3.5. THE HEXOKINASE REACTION EXPERIMENT .....</b>	47
<b>3.5.1. Reagents, concentrations and handling .....</b>	47
<b>3.5.2. Light sources, power, dose parameters and irradiation procedure</b>	47
<b>3.5.3. Experimental groups .....</b>	48
<b>3.5.4. The hexokinase Reaction .....</b>	49
<b>3.5.5. The indicator reaction .....</b>	50
<b>3.5.6. Experimental procedure .....</b>	52
<b>3.5.7. Spectrophotometer for kinetic measurements .....</b>	51
<b>3.5.8. Dose-response measurement .....</b>	51

<b>3.5.9. Statistical analysis .....</b>	<b>52</b>
<b>3.5.10. Graphic representation and non-linear regression analysis for kinetic measurements (<math>k_m</math> and <math>v_{max}</math>) and fluorescent measurements</b>	<b>52</b>
<b>3.6. FLUORESCENCE OF ADENOSINE N-PHOSPHATE MOLECULES.....</b>	<b>53</b>
<b>    3.6.1. Reagents, concentrations and handling .....</b>	<b>53</b>
<b>    3.6.2. Spectrofluorometer .....</b>	<b>53</b>
<b>    3.6.3. Light sources and irradiation procedure .....</b>	<b>53</b>
<b>    3.6.4. Experimental groups .....</b>	<b>54</b>
<b>    3.6.5. Statistical analysis .....</b>	<b>54</b>
<b>    3.6.6. Graphic representation and non-linear regression analysis for kinetic measurements (<math>k_m</math> and <math>v_{max}</math>) and fluorescent measurements</b>	<b>55</b>
<b>3.7. THE MICHELSON INTERFEROMETER EXPERIMENT .....</b>	<b>55</b>
<b>    3.7.1. Solutions .....</b>	<b>55</b>
<b>    3.7.2. Light source .....</b>	<b>55</b>
<b>    3.7.3. Experimental setup .....</b>	<b>55</b>
<b>    3.7.4. Image analysis .....</b>	<b>57</b>
<b>3.8. THE MEASURE OF THE REFRACTIVE INDEX (n) .....</b>	<b>57</b>
<b>    3.8.1. Solutions .....</b>	<b>57</b>
<b>    3.8.2. Light sources, electrical field and irradiation procedure .....</b>	<b>57</b>
<b>    3.8.3. Refractometer .....</b>	<b>58</b>
<b>    3.8.4. Experimental groups .....</b>	<b>58</b>
<b>4. RESULTS</b>	<b>59</b>
<b>4.1. TEMPERATURE MEASUREMENT IN THE SOLUTION .....</b>	<b>59</b>

4.2. ATP IMPURITIES .....	59
4.3. LUCIFERINE-LUCIFERASE REACTION .....	59
<b>4.3.1. Exponential behaviour of V(t) .....</b>	<b>59</b>
<b>4.3.2. Luminescence measurements .....</b>	<b>60</b>
<b>4.3.3. Luminescence peak (V<sub>0</sub>) .....</b>	<b>61</b>
<b>4.3.4. Reaction rate (k) .....</b>	<b>64</b>
<b>4.3.5. Area under the luminescence curve .....</b>	<b>65</b>
4.4. ABSORPTION OF ATP .....	68
4.5. THE HEXOKINASE REACTION .....	69
<b>4.5.1. Kinetics measurements .....</b>	<b>69</b>
<b>4.5.2. Dose-response measurements .....</b>	<b>73</b>
4.6. FLUORESCENCE OF ADENOSINE N-PHOSPHATE MOLECULES ....	74
4.7. THE MICHELSON INTERFEROMETER EXPERIMENT .....	79
4.8. THE MEASURE OF THE REFRACTIVE INDEX (n) .....	80
<b>5. DISCUSSION</b>	<b>82</b>
5.1. LIGHT INTERACTION WITH ATP .....	82
5.2. ABSORPTION OF ATP .....	82
5.3. THERMAL EFFECT IN ATP DUE TO IRRADIATION .....	83
5.4. IMPURITIES IN ATP COMPOUND .....	83
5.5. KINETIC MEASUREMENTS FOR THE TWO REACTIONS .....	84
5.6. THE LUCIFERINE-LUCIFERASE REACTION .....	84
5.7 THE HEXOKINASE REACTION .....	86
<b>5.7.1. Introduction .....</b>	<b>86</b>

<b>5.7.2. Light threshold .....</b>	87
<b>5.7.3. Kinetic measurements .....</b>	88
<b>5.8. FLUORESCENCE OF ADENOSINE N-PHOSPHATE MOLECULES ....</b>	97
<b>5.9. INSTABILITY OF ATP'S TERMINAL BOND .....</b>	100
<b>5.10. OPTICAL PROPERTIES OF AN ATP SOLUTION .....</b>	102
<b>5.11. LIGHT CAN CONTRIBUTE TO THE INSTABILITY OF THE BOND</b>	104
<b>5.12. EFFECT OF LIGHT IN THE TWO PHOSPHOR-OXYGEN BONDS OF ATP.....</b>	105
<b>5.13. CELLULAR INDUCED EFFECTS BY ELECTRICAL CURRENTS AND A COMPARISON WITH LIGHT EFFECTS .....</b>	107
<b>5.14. MITOCHONDRIAL ACTIVITY IS INFLUENCED BY ATP HYDROLYSIS .....</b>	109
<b>6. CONCLUSIONS</b>	111
<b>7. REFERENCES</b>	112