

Porous glasses as a host for solid state tunable lasers in the visible as a means for fluorescence diagnostics and photodynamic therapy

RENATA REISFELD*, ELI YARIV

Department of Inorganic Chemistry, The Hebrew University, Jerusalem 91904, Israel.

Lasers prepared by incorporation of the active dyes into porous glasses are described. Background of the use of lasers for photodynamic therapy and diagnostics is outlined. The role of hematoporphyrin and hypericin in creation of singlet oxygen, which destroys the malignant tissues, is explained. The lasers, based on sol-gel technology, incorporated by several perylimide and pyrromethane dyes emit in the suitable range in which the hematoporphyrin is excited. These compact lasers may replace, in the future, the vapour and the liquid lasers. The synthesis and the spectroscopic properties of the lasers are described here.

1. Introduction

During the last years, we have been able to design and develop solid state dye lasers that are tunable in the visible part of the spectrum. The literature search on use of lasers in medicine and especially for treatment of cancerous cells by photodynamic therapy (PDT) and fluorescence diagnostics brought us to the conclusion that lasers, when properly designed, can be utilized in these two important areas. In what follows, we shortly summarize the principles of PDT and fluorescence diagnostics, including the latest references, and then, describe the preparation and performance of the solid state lasers based on sol-gel technology.

2. Photodynamic therapy

Photodynamic therapy is a modality for the treatment of a variety of cancerous diseases. It involves the localization of photoactive chemical (photosensitizer) followed by activation with appropriate light source. The photoactivation leads to the generation of active molecular species (singlet oxygen), which are ultimately toxic to biological substrates (cells and tissues) that encounter them. Due to the high absorption of wavelengths around 570–630 nm by endogenous molecules clinically used for sensitizers, are typically required to absorb at wavelengths around 570–630 nm. The clinical application of hematoporphyrin derivatives was firstly

* Enrique Berman Professor of Solar Energy.

described by DOUGHERTY [1], [2]. Recent developments of hematoporphyrin derivatives are found in several reviews, for instance, refer to [3]. Quite recently, a new photosensitizer, hypericin, absorbing around 560 and 590 nm has been reported to be efficient in producing the singlet oxygen [4].

The new technique of PDT involves administering a light-sensitive drug and then irradiating the diseased area with suitable light to cause the drug to produce a toxic form of oxygen. This promising new use of lasers in medicine offers many potential benefits to patients [5]. In PDT technique the laser radiation is absorbed by a photosensitizer injected into the patient, which is selectively retained in the malignant tumour cell. The excited photosensitizer molecules of hematoporphyrins transfer energy to the oxygen molecules, which are promoted from their ground state $^3\Sigma_g$ to the excited singlet state $^1\Delta_g$. Singlet molecular oxygen is known to be a strong cytotoxic agent that violently oxidizes the surrounding (tumour) tissue. The common clinically used photosensitizer is hematoporphyrin derivative (HPD), known commercially as Photofrin II, with two absorption peaks at 405 nm and 630 nm. The latter peak is used for the PDT since the living tissue shows good light transmission at this wavelength. The laser sources for PDT-HPD used today are Ar-pumped liquid dye laser emitting at 630 nm (output power of milliwatts) and a gold vapour laser emitting at 628 nm (50–100 KW peak with 5–10 Hz repetition rate and about 30 ns duration). A more compact and easy to operate, smaller system would be advantageous [6].

Also lasers such as pulsed atomic neon laser, emitting at 585.3 nm, have potential application for PDT of human cancer. Its wavelength coincides with the absorption spectrum of many medical photosensitizers and has a relatively good transmittance through biomedical tissues, therefore it is possible to increase the treatment depth and to improve the effect of PDT [7].

3. Fluorescence diagnostics

The use of laser induced fluorescence (LIF) for tumour and plug diagnostics is a useful tool for noninvasive way of diagnostics. The LIF studies are performed in laser regimes where no change in the tissue is induced. The photon energy must be chosen below the limit of photodissociation for DNA in order to avoid mutagenicity [8]. The LIF can be applied by use of photosensitizer [9] or applied directly to excite the fluorescence of pathological tissues in order to detect the pathological changes [8]. The optimum laser wavelength is preferred in the green or red part of the spectrum. Other uses in medicine can be found in paper [10].

4. Solid state laser based on sol-gel technology emitting in the visible part of the spectrum

Non-liquid hosts for lasers are preferable to the exciting laser for PDT and LIF due to their simplicity and lack of toxic solvents.

Among the expected advantages of solid state visible laser are medical applications, such as PDT, which is a new approach to treatment of malignant tumours, and LIF diagnostics. Both techniques need lasers emitting in the red region of the spectrum.

Until recently, liquid dye lasers were the main systems used to achieve tunability in the visible, and the only commercial choice for tunable lasers between 400 and 660 nm. However, in the last few years an intensive effort has been made to produce embedded organic dyes in various solid matrices, with the goal of achieving solid-state dye laser devices that may replace the liquid dye lasers, *e.g.*, laser dyes were incorporated into silica-gels, xerogels, alumina gels, ormosils, and composite glasses. A solid-state dye laser has advantages over a liquid dye laser by being a non-volatile solvent, inflammable, non-toxic, and by its compact size and mechanical stability. Still, for applications that require high powers, at either cw or pulsed high-repetition-rate operation, the problem of heat dissipation is a serious impediment to their utilization. In liquid dyes, on the other hand, a jet or a flowing solution are handy practical ways of solving the heat problem. In both cases, photostability is a feature of prime importance in selecting a laser dye [11]. Lasers obtained by impregnation of the perylimide dyes into sol-gel glasses, where the dyes are enclosed in the pores of the glass, seem to be so far the most photostable system.

In 1989, we succeeded for the first time in preparing a photostable tunable laser by impregnating the orange perylene derivative (perylimide) dye BASF-241 dissolved in MMA into a silica-gel. The method of Pope and Mackenzie, which allows polymerization of MMA in the pores of the glass, was applied here. The dye which is orders of magnitude more stable than the conventional laser dyes impregnated in the glass provided an efficient solid-state laser material. This laser was tunable in the range 568–583 nm.

The lasing range picking within 613 nm was extended by impregnating the red perylimide dye into composing silica gel PMMA glasses. This wavelength is important for medical PDT and diagnostics. Human blood and tissue absorption is small in the red, allowing the preferential absorption of light by a photoactive cancer therapeutic agent, such as hematoporphyrin derivative, which concentrates in tumours. The properties of red perylimide dye (RPD) in this system were described in papers [6] and [12]. The chemical formula of RPD is presented in Fig. 1 [13]–[16].

The perylimide dyes were dissolved in a methylmethacrylate (MMA) monomer to form solutions of different concentrations in the range 10^{-6} – 10^{-3} mol/litre. Highly porous silica-gel bulk glasses (density about 0.7 g/cm³) were prepared with the sol-gel method and were dried by slow heating (100 °C/day) from room temperature to 500 °C. Then, the bulks were immersed in the dye-doped solution of the MMA monomer, which was simultaneously catalyzed by the addition of 2% benzoyl peroxide. The MMA-dye solution thus diffused into the silica-gel glass pores and polymerized therein. After this process of dye impregnation, the bulks were re-immersed in an MMA-dye solution, which at this stage was catalyzed for full polymerization by 0.5% benzoyl peroxide, and kept in a sealed container at 40 °C

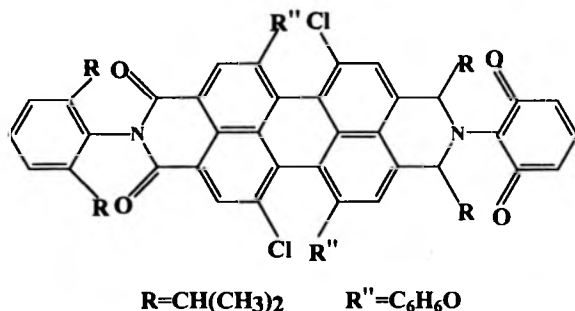


Fig. 1. BASF LFR 300 – molecular structure.

for about a week. The samples were then withdrawn, cleaned and polished to obtain parallel-piped slabs of approximate dimensions $10 \times 10 \times 3 \text{ mm}^3$, with clear smooth surfaces. For this class of glasses we give the name composite glass.

The density of the composite glass was $d = 1.447 \pm 0.005 \text{ g/cm}^3$ and the refractive index $n = 1.472 \pm 0.003$.

Later solid state dye lasers were made by incorporating photostable laser dyes in organically modified silicate (ORMOSIL) and composite glass matrices. Three different types of pyromethene (PM) were used: PM 567, PM 580 and PM 597. Their formulae are presented in Fig. 2. The ORMOSIL glass samples were prepared in a one step process at room temperature with sol-gel technology, which led to the formation of hybrid organic/inorganic materials. The composite glass samples are made in a biphasic process: a) preparation of porous silica gel, b) impregnation of the dye dissolved in methyl methacrylate into the silica gel and polymerization of the MMA.

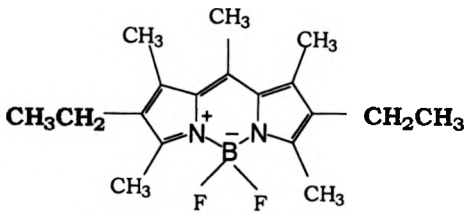
A number of various glass compositions were tested and their efficiencies and half output energy lifetimes were measured. The optimal slope efficiency for the lasers pumped transversally by frequency doubled Nd-YAG laser approaches 42% in PM 597 in composite glass [17]–[19].

Organically modified silicates usually exhibit lower porosity and enhanced mechanical properties which allow cutting, grinding and polishing prior to heat treatment. A typical ORMOSIL gel network contains a significant amount of organic functionalities, which offers great flexibility with respect to the chemical compatibility of the gels with dye to be incorporated.

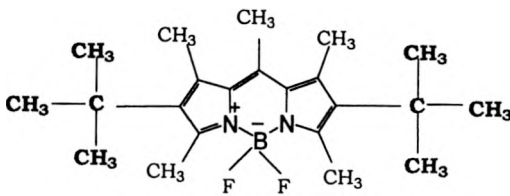
Various organic laser dyes have been incorporated into ORMOSILs derived from polydimethylsiloxane and tetraethoxysilane. These sol-gel derived PT-ORMOSILs proved to be good hosts for the laser dyes in terms of stability and optical gain [20].

The mechanical and optical properties of glasses prepared by the sol-gel are being improved constantly by modifying the sol process and using a variety of organofunctional silicane alkoxides. Modified alkoxide precursors, $\text{RSi}(\text{OEt})_3$ or $\text{RSi}(\text{OMe})_3$, where R is a non-hydrophobic group, such as methyltriethoxysilane (MTEOS), vinyltriethoxysilane (VTEOS), amyltriethoxysilane (ATEOS), 3-(trimethoxysilyl)pro-

PM 567



PM 580



PM 597

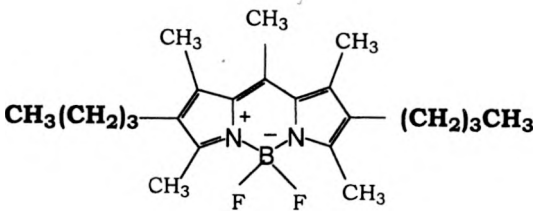


Fig. 2. Molecular structure of pyrromethene dyes.

pymethacrylate (TMSPMA), methyltrithoxysilane (MTEOS), 3-glycidoxypropyl-trimethoxysilane (GLYMO) or methyltrimethoxysilane (MTMOS) lead to organic-inorganic hybrid matrices.

The permanent organic group causes the mechanical tensions to decrease during the drying process. Functionalized alkoxides $F-R'-Si(OEt)_3$, where F is a chemical function such as an amino or isocyanate group and R' is an alkyl spacer, allow covalent graft onto the xerogel matrix to avoid phase separation and consequently to increase the concentration of the guest molecules. After drying, optically clear and dense inorganic-organic hybrid xerogels (30 mm in diameter and 15 mm thick) were obtained and described recently [21].

5. Measurements

For the laser measurements, a transversally pumped dye laser system was built. The pump ray is the second harmonic generation of Nd:YAG laser (Lumonics HY600), emitting 8 ns pulses at 532 nm. By the cylindrical lens we obtain a pump ray of

approximately 5 nm in width and 1 mm in thickness. The dye laser cavity consisted of two plane parallel mirrors 7.5 cm apart, each being a 50% broadband reflector. The energy of output and input pulses was measured by Ophir Laserstar detector. Every value was taken by averaging energy values of more than 20 pulses. The experimental setup is presented in Fig. 3.

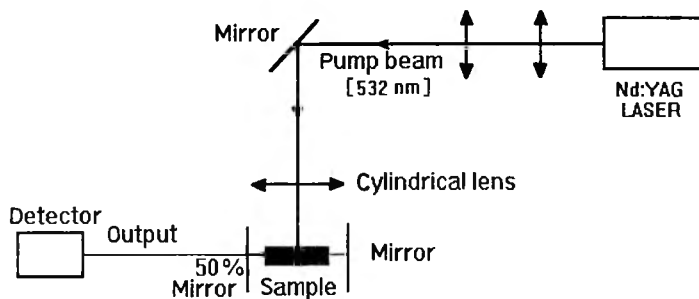


Fig. 3. Experimental setup for laser measurements.

We note here that the majority of the results published have been obtained by longitudinal pumping system and this might be essential for the results.

Excitation-emission spectra were taken by Jasco FP 750 spectrofluorimeter.

Fluorescence lifetimes of the samples was measured using Megaplus nitrogen laser (Laser Photonics) as exciting 337 nm short pulses source. The measured pulse length was 1.5 ns at repetition rate of 20 Hz. The fluorescence was detected by a 818-BB-20 photodetector (Newport) and its life curve was presented by TDS 620A scope (Tektronix). In order to obtain a "real" fluorescence curve and lifetime we used a deconvolution program which could minimize the effect of the exciting pulse length.

6. Results and discussion

6.1. Spectral properties

The excitation and emission spectra of the dyes in ORMOSIL (PM 567 and 597) and in composite glass (PM 580) are given in Fig. 4. The spectra of PM 580 in ORMOSIL and PM 597 in composite glass were also measured. There was no difference between the spectra of each dye in the two different kinds of matrices. From this figure we can easily see the similarity between the spectral properties of PM 567 and PM 580. Each dye has an excitation peak in the UV range. The main excitation peak is at 520 nm (for PM 567 and PM 580) or 527 nm (PM 597). The shoulder near the peak is attributed to dimerization and aggregation of the dye molecules [22].

6.2. Fluorescence lifetime

The results of fluorescence lifetime measurements are summarized in Table 1. We cannot find from the table any distinction between the fluorescence lifetime in

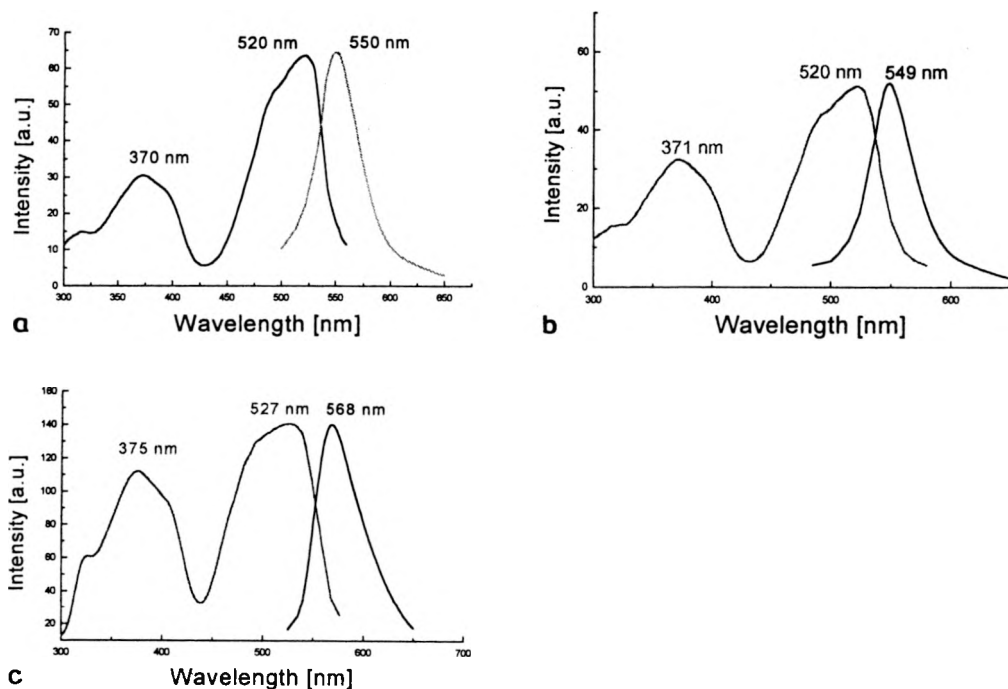


Fig. 4. Excitation and emission spectra of samples: a – PM 567/ORMOSIL, b – PM 580/composite glass, c – PM 597/ORMOSIL.

ORMOSIL samples compared with composite glass samples, but we can conclude that fluorescence lifetime of PM 567 is slightly larger than the fluorescence lifetime of PM 580 and PM 597. We can also note that the fluorescence lifetime of PM 567 in ORMOSIL (8.1 ns) is longer than its fluorescence lifetime in liquid solutions, which is between 6 and 7 ns [19].

Table 1. Fluorescence lifetime of samples.

Dye	Matrix	Dye concentration*	Fluorescence lifetime
PM 567	ORMOSIL	7×10^{-4} M	8.1 ns
PM 580	ORMOSIL	5×10^{-4} M	7.2 ns
PM 580	Composite	5×10^{-4} M	7.1 ns
PM 597	ORMOSIL	5×10^{-4} M	7.0 ns
PM 597	Composite	5×10^{-4} M	7.2 ns

* Dye concentration in the initial solution.

6.3. Slope efficiency

The efficiencies obtained for all the samples under investigation were quite high, since the samples were doped with dye at concentrations which were chosen to be close to the optimal one. However, the most impressive result was obtained for

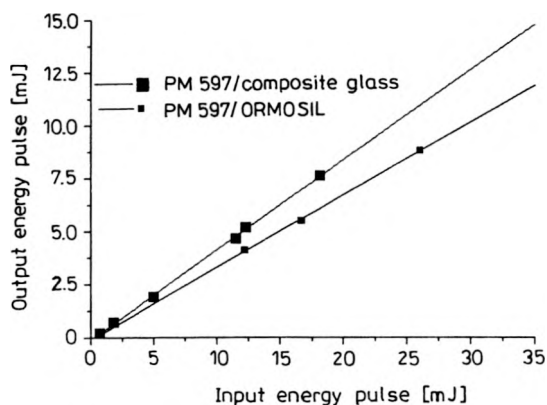


Fig. 5. Laser efficiencies of PM 597 samples.

PM 597/composite glass with 42% slope efficiency. In the second place was PM 597/ORMOSIL with 35% slope efficiency (Fig. 5), [19]. There was no significant difference between the efficiencies of PM 567 and PM 580. A possible explanation for these results is that PM 597 has its excitation peak at 527 nm which is closer to the pump wavelength (532 nm) than the excitation peaks of the other dyes (520 nm). The results are summarized in Tab. 2. A method for optimization of the dye concentration might lead to improved efficiencies for such samples [19].

Table 2. Slope efficiencies of laser samples.

Dye	Matrix	Dye concentration *	Slope efficiency
PM 567	ORMOSIL	7×10^{-4} M	32%
PM 580	ORMOSIL	5×10^{-4} M	28%
PM 580	Composite	5×10^{-4} M	23%
PM 597	ORMOSIL	5×10^{-4} M	35%
PM 597	Composite	5×10^{-4} M	42%

*Dye concentration in the initial solution.

In order to compare our solid samples with liquid pyromethene lasers, slope efficiencies of pyromethene dye/ethanol solutions of similar optical density were measured. We obtained slope efficiencies around 50% for all three dyes [19]. The results show that the efficiency in solid matrix is not much lower than in ethanol, mainly for PM 597.

6.4. Photostability

In order to investigate the photostability of the laser sample, we measured the output energy pulse of the sample after the sample had been pumped by numerous pulses of constant energy, emitted by Nd:YAG laser (the second harmonic generation). We could observe the efficiency to decay exponentially with the increasing number of input pulses as a result of photodegradation of dye molecules caused by the

pump pulses. In this measurement the samples were pumped by 3.4 mJ (68 mJ/cm²) pulses. The efficiency of PM 597/composite glass sample decreased to 50% from its initial value (half life longevity) after 23,000 pump pulses. The half life longevity for PM 597/ORMOSIL was 14,000. So, in this case, the photostability of the composite glass sample is higher than that of the ORMOSIL sample. Half life longevity for PM 580 in ORMOSIL and composite glass in the same kind of measurement was generally between 11,000 and 13,000 pulses, and for PM 567/ORMOSIL 10,000 pulses.

7. Conclusions

Porous glasses have been shown to be excellent hosts for tunable solid state lasers. The incorporation of laser dyes is governed by the porosity of the glass.

The paper presents recent results on the efficiency and photostability measurements for such glasses. The experiments being in progress shown that yet better efficiencies and photostabilities can be obtained by improving the composition of the matrices and the experimental setup [19], [22]–[30] and diagnostics: gas and mercury adsorption and dielectric measurements.

The sol-gel technology has proven itself as a powerful method for designing new luminescent materials. Their potential is now explored in a large number of research institutions as well as by many industries. The number of the papers on the subject in the last years is overwhelming. Papers [25]–[30] are some examples of the recently published findings on sol-gel technology.

References

- [1] DOUGHERTY T.J., Annual Reports to the U.S. Food and Drug Administration, IND 12,678, 1980–1987.
- [2] DOUGHERTY T.J., Photochem. Photobiol. **38** (1983), 377.
- [3] DOUGHERTY T.J., Photochem. Photobiol. **45** (1987), 879.
- [4] EHRENBERG B., ANDERSON J.L., FOOTE C.S., Photochem. Photobiol. **68** (1998), 135.
- [5] STRINGER M.R., Physics Education **31** (1996), 90.
- [6] GVISHI R., REISFELD R., J. Phys. (Paris), Colloq. C7, **1** (1991), 199.
- [7] YANG C.H., ITOH K., TOMITA H., OBARA M.J., Appl. Phys. **78** (1995), 130.
- [8] ANDERSON-ENGELS S., JOHANSSON J., SVANBERG S., SVANBERG K., Analytical Chem. **62** (1990), 19A.
- [9] ALFANO R.R., TATA D.B., CORDERO J., *et al.*, IEEE J. Quantum Electron. **20** (1984), 1507.
- [10] DUARTE F.J., HILLMAN L.W., *Dye Laser Principles*, Academic Press, Inc., San Diego 1990.
- [11] REISFELD R., *Laser based on sol-gel technology*, [In] *Optical and Electronic Phenomena in Sol-Gel Glasses and Modern Applications*, [Eds.] R. Reisfeld, C. K. Jørgenson, *Structure and Bonding*, Vol. 85, Springer-Verlag, 1996, 215.
- [12] REISFELD R., GVISHI R., BURSHEIN Z., J. Sol-Gel Sci. Technol. **4** (1995), 49.
- [13] REISFELD R., SEYBOLD G., J. Luminesc. **48**, **49** (1991), 898.
- [14] REISFELD R., SEYBOLD G., Chimia **44** (1990), 295.
- [15] GVISHI R., REISFELD R., BURSHEIN Z., MIRON E., Proc. SPIE **14–16** (1992), 390.
- [16] GVISHI R., REISFELD R., BURSHEIN Z., Chem. Phys. Lett. **213** (1993), 338.
- [17] REISFELD R., Opt. Mater. **4** (1994), 1.
- [18] REISFELD R., Proc. SPIE **2288**, (1994), 563.
- [19] YARIV E., REISFELD R., Opt. Mater. **13** (1999), 49.

- [20] HSIN-TAH LIN, BESCHER E., MACKENZIE J.D., *et al.*, *J. Mater. Sci.* **27** (1992), 5523.
- [21] BOILOT J.-P., BITEAU J., CHAPUT F., *et al.*, *Pure Appl. Opt.* **7** (1998), 169.
- [22] REISFELD R., YARIV E., MINTI H., *Opt. Mater.* **8** (1997), 31.
- [23] RAN M.D., KING T.A., GORMAN A.A., HAMBLETT L., *Appl. Opt.* **36** (1997), 5862.
- [24] FALOSS M., CANAVA M., GEORGES P., *et al.*, *Appl. Opt.* **36** (1997), 6760.
- [25] DUNN B., NISHIDA F., TODA R., *et al.*, *Mat. Res. Soc. Symp. Proc.*, **329** (1994), 267.
- [26] ALMEIDA A.M., *J. Non-Cryst. Solids* **259** (1996), 176.
- [27] RAZAFIMAHATRATA A.D., BENATSSOU M., BOUZAOU M., *et al.*, *Opt. Mater.* **13** (2000), 439.
- [28] BAHAT A., BOUZAOU M., BAHAT M., *et al.*, *J. Non-Cryst. Solids* **202** (1996), 176.
- [29] TERKI F., PILLIEZ J.N., WOIGNIER T., *et al.*, *Philos. Mag.*, **B 79** (1999), 22081.
- [30] LAM K.S., LO D., *Appl. Phys. B* **66** (1998), 427.

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