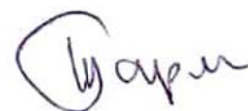


Federal Agency for Education
SAINT PETERSBURG STATE UNIVERSITY OF INFORMATION
TECHNOLOGIES, MECHANICS AND OPTIC

**Study of Effect of the AIRES Fractal
Resonator
on the State of Erythrocytes in Human
Blood**

Paper Supervisor

V.A. Tarlykov



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INTRODUCTION

The objective of this paper is to determine the nature of the AIRES fractal resonator effect on the functional state of erythrocytes in human blood.

The agent of influence was the AIRES fractal resonator, which is a fractal-matrix topological layout (designated as Sh3_16ort_clon3) made on a 7.7 x 7.7 silicon board. The main functional element of the fractal resonator is a pattern. The width of the topological pattern lines is 1 mcm.

The object of study is blood from patients diagnosed with multiple myeloma. Multiple myeloma is a widespread malignant disease of the hemic system, with its frequency rising steadily. This disease is notable for diversity of forms and variations, extremely variegated symptoms, and it is caused not only by marrow failure and bone disorder, but also by the tumor producing a specific monoclonal immunoglobulin or its enzymes.

Multiple myeloma is known as a "disease of advanced age" (patients' average age is 62 years); patients under 40 make up 2 to 3%, and 80-year-olds get sick 10 times as often as 50-year-olds. The median survival is approximately 50 months.

According to the clinic of the Russian Research Institute of Hematology and Transfusiology, life expectancy of multiple myeloma patients has steadily increased over the last 25 years and today is approximately 5 years on the average[1].

I. OSMOTIC FRAGILITY

Rheologically, blood can be treated as a liquid milieu containing particles of different shapes, sizes and properties. The bulk of blood cells are erythrocytes and therefore they play the leading part in changes in the rheological properties of blood. Parameters describing the most important properties of blood are viscosity, aggregation and erythrocytes deformability (ED).

Owing to high sensitivity to changes occurring in the organism, erythrocytes are a convenient object for evaluation of the organism's physiological status.

One of a cell's vital parameters is its reconfiguration in response to outside impact on the cell membrane caused by both external and internal environment. ED can be said to reflect, to a degree, viability of erythrocytes circulating in blood flow.

Resistance (degree of stability) to different types of influence can be used as an ED assessment tool. One of those types of influence is osmotic swelling of erythrocytes. Osmotic fragility is understood as the degree of their resistance to haemolyzing effect of hypotonic solutions.

Key parameters of the curve of hypoosmotic swelling are the coordinate of the minimum – the spherulation point; the amplitude of relative erythrocyte radius change during swelling. These two parameters describe the elastic properties of erythrocyte membrane, its ability to be deformed.

According to this research, as multiple myeloma patients undergo treatment, a positive trend emerges in changes of rheological blood parameters. The curve of osmotic swelling of erythrocytes in comparison of the patient's state before and after treatment changed both qualitatively and quantitatively [2-6].

The positive trend of the course of treatment given, according to earlier research [2-6], matches the shift of the spherulation point towards smaller hypoosmotic swelling values: increase of the relative variation value of erythrocyte radius during swelling.

1.1. Change of erythrocyte radius in hypoosmotic solutions

Let us consider the erythrocyte behavioral model in solutions of different osmolarity (i.e. with different NaCl sal content).

In blood circulation, erythrocytes take various shapes as they collide with each other and vascular walls. Without external mechanical impact in an isotonic solution (with a NaCl content of 0.85%, which is normal for the organism), the equilibrium shape turns out to be the biconcave disc meaning that erythrocytes are discocytes. As an unstressed biconcave discocyte swells into a sphere, the cell membrane is subjected to very small extension strains, but great surface curvature change. The central areas of an erythrocyte disc are deformed into the polar regions of a sphere with very little membrane expansion. Large expansion occurs mainly in the peripheral areas in the equatorial region of the biconcave.

As osmolarity of the solution changes, the erythrocyte is transformed as follows:

1. As osmolarity increases (hypertonic solution), the erythrocyte shrinks.

2. As osmolarity decreases (hypotonic solution), the erythrocyte's volume increases in two stages owing to water coming inside it (Fig. 1):

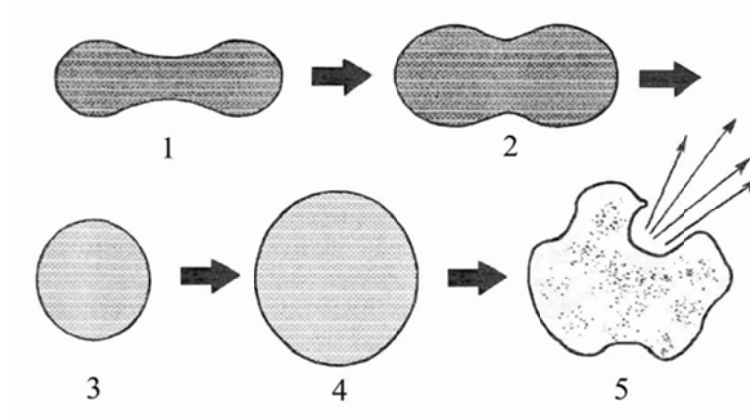


Fig. 1. Erythrocyte transformation in hypoosmotic swelling:

1 – discocyte; 2 – discocyte swelling; 3 – transformation of erythrocyte into spherocyte; 4 – spherocyte swelling; 5 – hemolysis

- a) erythrocyte transforms into a sphere with its membrane surface area unchanged;
- b) the area of erythrocyte spherical surface increases due to swelling of the cell up to the point of hemolysis (tearing of the erythrocyte membrane).

Let us consider erythrocyte behavior in hypoosmotic swelling [7]. In the first phase, internal pressure in the cell is low, and one can say with little error that osmolarity inside and outside the erythrocyte is the same. In view of the above, when creating hypoosmotic conditions by introducing distilled water in the isotonic environment with a volume of ΔV_0 the following equation should be true:

$$\frac{\Delta V_e}{V_e} = \frac{\Delta V_0}{V_0},$$

where V_e is the erythrocyte volume in isotonic environments, V_0 is the original volume of the isotonic environment, ΔV_e , ΔV_0 are volumetric gains of the erythrocyte itself and the external environment, correspondingly. Thus it turns out that relative change in erythrocyte volume precisely matches total relative change in suspension volume when distilled water is introduced. Without much error, the same can apply when suspension contains many erythrocytes.

As the distilled water volume introduced in the suspension reaches a certain value, the erythrocyte shape becomes spherical. To build a theoretical behavioral model of the erythrocyte in hypoosmotic solution, the average erythrocyte diameter is taken as 7.7 μm .

To determine the erythrocyte spherulation point on the theoretical

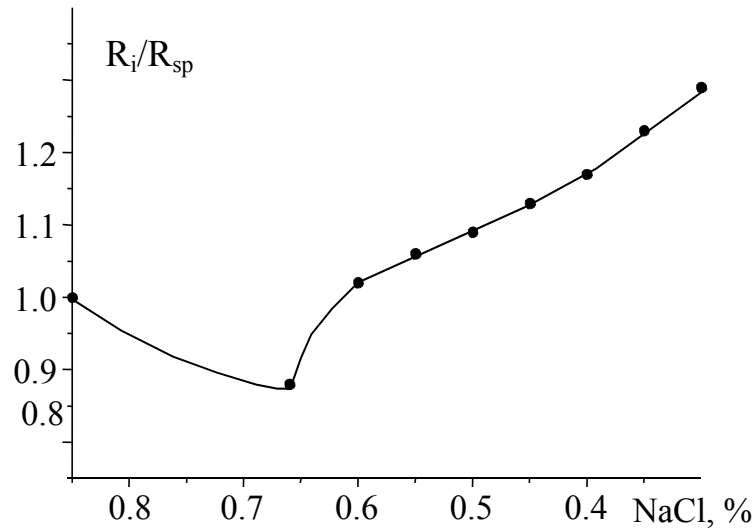


Fig. 2. *Hypoosmotic curve of erythrocyte swelling: R_i - current erythrocyte radius, R_{sp} – erythrocyte radius corresponding to the spherulation point.*

curve describing erythrocyte behavior in hypoosmotic solution, we shall equate relative change in erythrocyte volume with relative change in suspension volume and thus find the spherulation point.

Dependence of erythrocyte radius on NaCl content has a minimum that matches the spherulation point (Fig. 2).

1.2. Blood sample preparation technique

To perform blood tests, the blood has to be stabilized, i.e. blood coagulation that normally occurs after 3 to 6 minutes has to be prevented. Venous blood is stabilized by 3.8% solution of basic sodium citrate, 9:1. To prevent interference of plasma in RBC tests, the red cells should be separated from plasma and buffy coat layer by centrifugation.

Blood was drawn from the human median cubital vein with an injection needle to a dry centrifuge tube, with first drops left on the tampon the moment they are drawn to prevent tissue factor that escapes in the moment of puncture from getting in the tube. 0.2 ml of essential sodium citrate is added to the tube to avoid coagulation.

To preclude exposure of erythrocytes to substances in plasma, the blood was centrifuged.

The resulting residue was placed in normal saline with approximately 0.85% NaCl content and centrifuged in the same conditions to better wash the cells of blood plasma.

Usually normal saline with approximately 0.85% NaCl content is used to work with washed erythrocytes, it creates osmotic pressure on the cell approximately equal to that

created by blood plasma (isotonic solution). Na^+ is the main osmotically active ion in extracellular space. Sodium ion density in the blood stream is approximately 8 times as high ($132\text{--}150\text{ mole/m}^3$) as in erythrocytes ($17\text{--}20\text{ mole/m}^3$). Therefore as NaCl content in the solution decreases, a concentration gradient appears between the NaCl and the cell, and water begins to penetrate the membrane into the erythrocyte.

Solutions of most pure chemical agents have a very unstable pH. Therefore, when work has to be done within certain pH intervals, special buffer solutions are used whose pH changes very insignificantly. To stabilize erythrocyte membranes, we added hypoosmotic suspension environments with Na_2HPO_4 in combination with NaH_2PO_4 in the final concentration of 0.01M with $\text{pH}=7.4$, to take more precise measurements of dependence of erythrocyte size on osmolality of the solution.

Washed erythrocytes were placed in hypoosmotic solutions (with a concentration of under 0.85%) made by diluting the original isotonic solution.

II. LASER DIFFRACTOMETRY METHOD

The laser diffractometry method is based on the phenomenon of diffraction of laser radiation on individual and multiple biological micro-objects, it is characterized by high precision, sensitiveness, speed, minimum effect on the test object, possibility of simultaneous registration of a large number of small particles. The parameters of the diffraction pattern are unambiguously related to the parameters of the micro-objects, hence their size, shape and internal structure can be determined.

With diffraction on an aggregate of erythrocytes, the diffraction

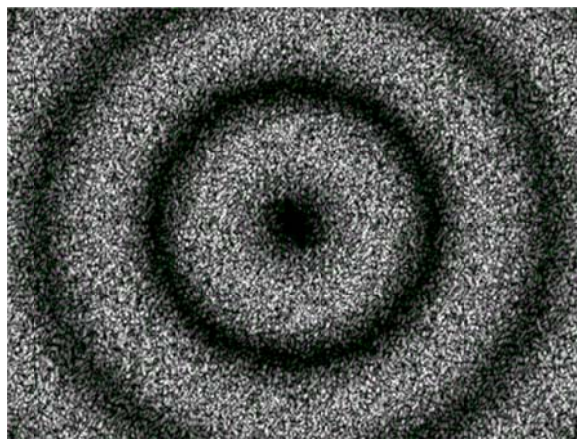


Fig. 3. *Distribution of intensity in a diffraction pattern*

pattern looks like a system of concentric rings (Fig. 3). The distance

between the rings is related to the average erythrocyte size characterizing this aggregate. That relation is inversely proportional, i.e. the smaller the measured object D the larger the diameter of diffraction ring l . Relative change in average erythrocyte size equals in absolute value the relative change in the diffraction ring diameter:

$$\frac{\Delta D}{D} = -\frac{\Delta l}{l},$$

where ΔD and Δl are changes in the average erythrocyte size and the diffraction ring diameter, correspondingly.

The experimental assembly used for studying erythrocyte deformability includes an LGN 215 He-Ne-laser ($\lambda = 0.63 \text{ mcm}$); an optical attenuator to measure laser power concentration; a bench for the sample; a lens for the Fourier transform; a photodetector; a personal computer (Fig. 4).

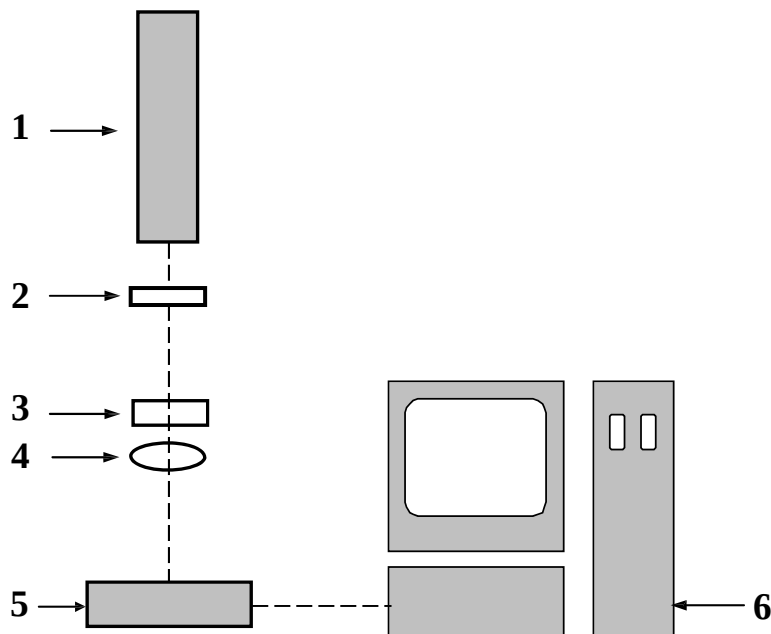


Fig. 4. *Experimental assembly layout: 1 – He-Ne-laser ($\lambda=0.63 \text{ mcm}$), 2 – optical attenuator, 3 – sample, 4 – lens, 5 – photodetector, 6 – PC*

To study the erythrocytes suspended in solutions of various osmolarity by laser diffraction method, the sample should be prepared so that the thickness of the layer is small, which is required to ensure a single scattering (Fig. 5). The erythrocytes in the treated sample should be evenly distributed on the surface of a Goryaev's chamber, there must be no overlapping (erythrocytes must make up a monolayer), which lets us disregard

the input from re-radiation of overlapping regions, and concentration should be sufficient to observe cell deformation of high intensity.

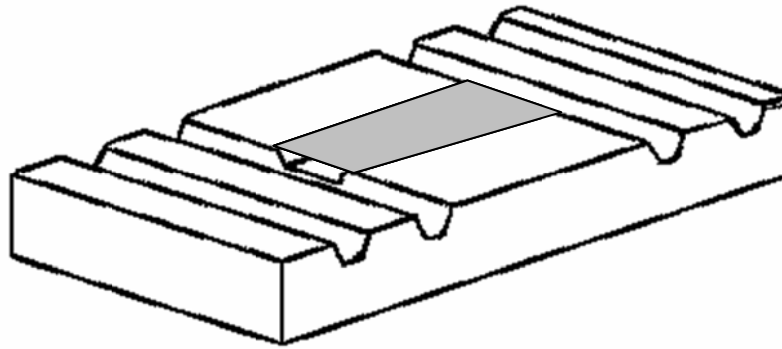


Fig. 5. *Goryaev's chamber*

These conditions are met when there is a certain ratio between the volume of erythrocytes and that of the solution.

With a concentration of 0.03-0.04 ml, erythrocytes make up a sufficiently even monolayer on the surface of the Goryaev's chamber, which produces good cell deformation close to the ideally round shape and sufficient intensity.

Experimental studies of the effect of exposure time of erythrocytes in solutions, during which osmotic equilibrium occurs, revealed that optimum time to study osmotic fragility and stiffness of erythrocyte membranes is two hours after the suspensions are made.

The following blood sample preparation technique for experimental studies is proposed: erythrocytes stabilized with sodium citrate, thrice washed for 10 minutes at 5,000 rpm, taken from the bottom of the tube in the amount of $0.03 \div 0.04$ ml per 2 ml of hypotonic solution are held for 2 hours and then placed in a Goryaev's chamber to observe a diffraction pattern and record the radii of the first and second minimums.

Relative change in the average diameter of the erythrocyte aggregate was determined by the change in the linear size of the diffraction rings in the laser diffractometer.

2.1. Experimental technique

The blood sample preparation technique for research is a rather labor-intensive process stretched over time and includes the following (Fig. 6):

- blood draw in a clinic;
- delivery of the blood to the lab for testing;
- blood centrifugation;
- preparation of working solutions;
- letting the blood settle (stabilization);

- experiment;
- processing of experimental findings.

The experiment was conducted in two versions:

- the first is a consistent version providing for minimum effect of probing laser radiation (the sample is subjected to radiation initially and after a certain amount of exposure time);

- in the second version, the same sample was subjected to radiation sequentially: initially, in 10, 20, 30 and 40 minutes accordingly, which caused a dose of radiation to accumulate and could entail uncontrollable effect with decreasing time of experiment.

The influence of the fractal resonator was achieved by placing the Goryaev's chamber with the blood samples on a fractal resonator on the side of the pattern.

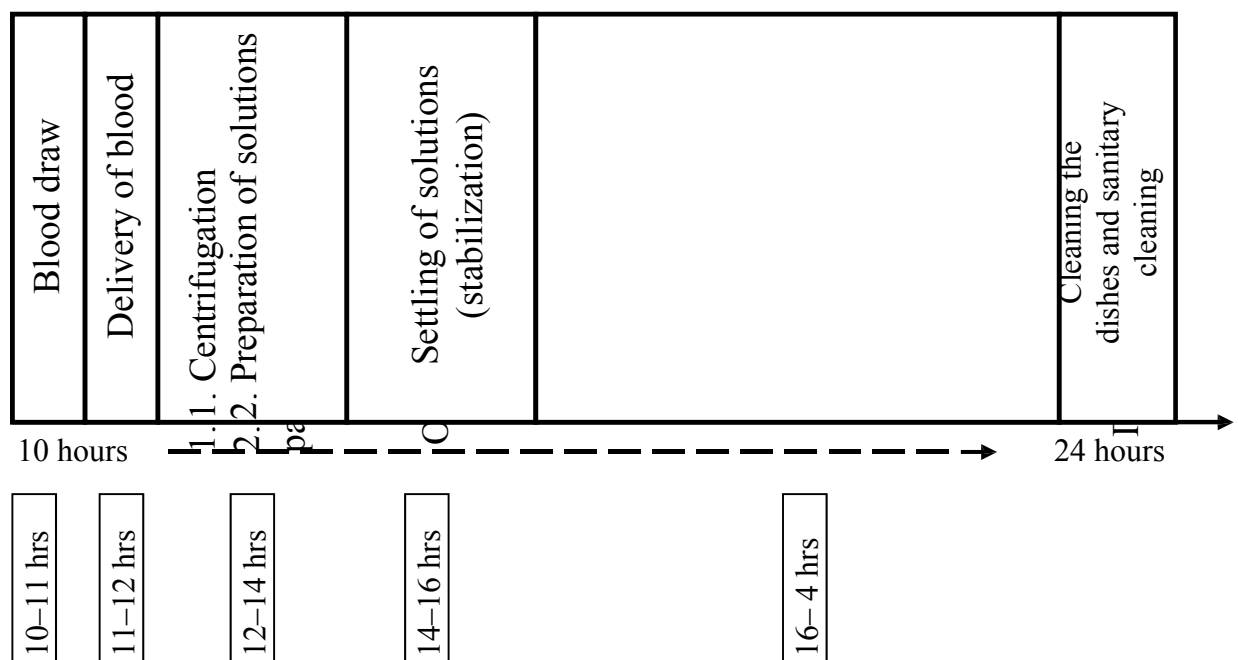


Fig. 6. *Experiment timeline for one blood sample*

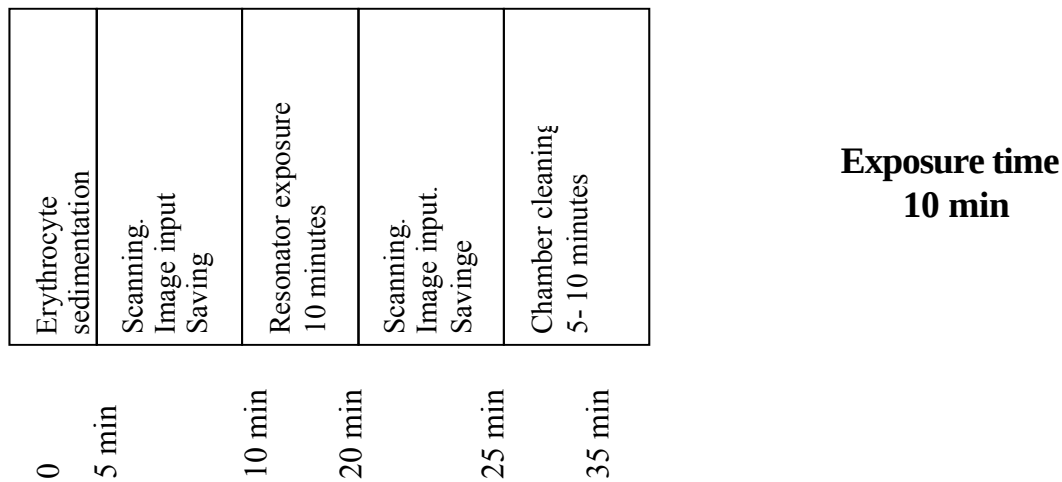


Fig. 7. *Experiment timeline for one NaCl concentration*

The total time of experiment with 10 minutes' exposure is 35 minutes for one concentration (Fig. 7).

The experiment to plot one dependency is carried out for 8 points. Obtaining data to plot one hypotonic curve takes about 5 hours.

Obtaining data to plot one hypotonic curve for 20 minutes' exposure time takes about 6 hours.

Obtaining data to plot one hypotonic curve for 40 minutes' exposure time takes about 8 hours.

In the experiment with one blood sample, 8 NaCl concentrations are tested: 0.85; 0.7; 0.65; 0.6; 0.55; 0.5; 0.45; 0.4.

Thus the total time of experiment with one blood sample, even with several operations running simultaneously, is about 14 hours.

The second process phase of the study is to process the obtained results in order to find quantitative data to plot hypoosmotic swelling curves.

III. EXPERIMENTAL FINDINGS

3.1. Study of the effect of a fractal resonator on the rheological parameters of erythrocytes (exposure time up to 15 minutes)

Preliminary analysis of the findings shows that test results heavily depend on the patient's initial state and the treatment they received.

24 December 2004. (Fig. 8)

Exposure to the resonator during 5 minutes and 10 minutes has a positive nature.

After 5 minutes' exposure at the initial stage of spherulation, the amplitude excursion increased relative to the change

in the erythrocyte radius; the nature of swelling began to match the typical case (occurrence of a jump and subsequent swelling).

After 10 minutes' exposure, the spherulation point shifted to the region of smaller osmotic pressure values, which corresponds to the positive trend of external influence; the nature of swelling also became expressly spasmodic.

28 December 2004. (Fig. 9)

The positive effect is very feebly pronounced. After 5 minutes the amplitude of the first jump increases slightly. After 10 minutes the amplitude of the first jump increases slightly.

18 January 2005. (Fig. 10)

The positive effect is very feebly pronounced. After 5 minutes the amplitude of the first jump increases slightly. After 10 minutes, no significant developments are observed.

21 January 2005. (Fig. 11)

The general trend is positive.

After 5 minutes the result of hypoosmotic swelling — the result of exposure — corresponds to regression: the amplitude of erythrocyte radius change drops, the spherulation point shifts towards larger osmotic pressure values (the elastic properties of the erythrocyte membrane decrease noticeably).

After 10 minutes, the trend is positive: the amplitude of erythrocyte radius change during swelling rose, the spherulation point shifted towards smaller hypoosmotic pressure values.

After 15 minutes there was further increase in the amplitude excursion of erythrocytes, a jump appeared on the hypoosmotic swelling curve.

25 January 2005. (Fig. 12)

The general trend is positive. For all exposure times (5, 10, 15 minutes), the erythrocyte radius amplitude of hypoosmotic swelling for the specified exposure times exceeds the initial amplitude, and the jump is more pronounced.

28 January 2005. (Fig. 13)

The general trend is positive. The spherulation point is more pronounced, and with 15 minutes' exposure time it showed a tendency to shift towards lower hypoosmotic pressure. The elastic properties of erythrocyte membrane for all exposure times show a positive trend: the speed of erythrocyte swelling grows.

Conclusions

The completed preliminary research of six blood samples generally showed a positive trend produced by external influence from the resonator.

Three blood samples (24 December 2004, 25 and 28 January 2005) show a clear trend for greater improvement of resonator influence as exposure time extends.

The rheological parameters of erythrocytes (aggregation, deformability, intrinsic viscosity) depend on a whole range of factors. Namely with multiple myeloma (MM), they are closely connected with the level of total protein and paraprotein in patients' blood, which is always elevated in paraproteinemic versions of the disease. Meanwhile, we studied blood from patients with different versions of the disease including patients suffering from Bence Jones myeloma and nonsecretory myeloma in which no paraprotein is secreted in the blood stream. By no means unimportant are chronic renal insufficiency and anaemia often developing in patients. Moreover, the study included blood of newly-admitted patients who had not been treated previously, and blood of patients who had been on chemotherapy regimens. Cytostatic drugs have a negative impact on elasticity of the erythrocyte membrane. At the same time, during chemotherapy tumor cells are destroyed, which causes serious shifts in the coagulation system closely connected with blood rheology. Undoubtedly, patients' age should be taken into account. Thus, rather diverse patients were studied.

Therefore discussion of the obtained result brought us to the conclusion that in order to receive sufficiently reliable data, the following is required:

1. the experiment should be continued to study the effect of the resonator on the state of erythrocytes;
2. analysis of the effect of the resonator should be performed with consideration of the version and stage of the disease, separately for newly admitted patients (initially before the start of specific therapy) and patients who underwent intensive chemotherapy;
3. a suggestion was made to study the effect of increasing resonator exposure time (up to 40 minutes).

3.2. The second phase of the study with extended resonator exposure time (up to 40 minutes)

4 February 2005. (Fig. 14)

The general trend is positive. The most significant effect is observed after 30 minutes. After 40 minutes' exposure reaction to the resonator is negative. However this result may also be connected to negative impact of the environment as exposure time increases, for example drying up of the preparation.

8 February 2005. (Fig. 15)

The general trend is positive. The most significant effect is also observed after 30 minutes' exposure. After 40 minutes' exposure reaction to the resonator is negative.

11 February 2005. (Fig. 16)

The experiment design is modified. The same sample was exposed consecutively (as the laser radiation dose quadruples).

The general trend is positive. The most significant effect is also observed after 30 minutes' exposure.

11 February 2005. (Fig. 17)

The experiment design is unmodified. The same sample was exposed consecutively (as the laser radiation dose quadruples). The general trend is positive.

Conclusions

The second experiment phase showed that external influence from the resonator displays a positive trend.

It does not seem possible to determine optimal exposure time based on the conducted experiments (the number of conducted experiments is small; a more detailed analysis of possible change of a sample kept in a Goryaev's chamber for a long time is required).

C O N C L U S I O N

10 blood samples from patients with multiple myeloma were tested.

A positive trend was detected in external influence of a fractal resonator on rheological parameters of erythrocytes.

However, the small selection of blood samples from extremely diverse multiple myeloma patients, no record of the effect of administered treatment (chemotherapy) require further gathering of materials in order to subsequently compile homogeneous groups of patients (blood from patients with paraproteinemic versions and those with no paraprotein in the blood stream, newly admitted patients and those who had been on a chemotherapy regimen, with cognominal haemoglobin and creatinine parameters).

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APPENDIX

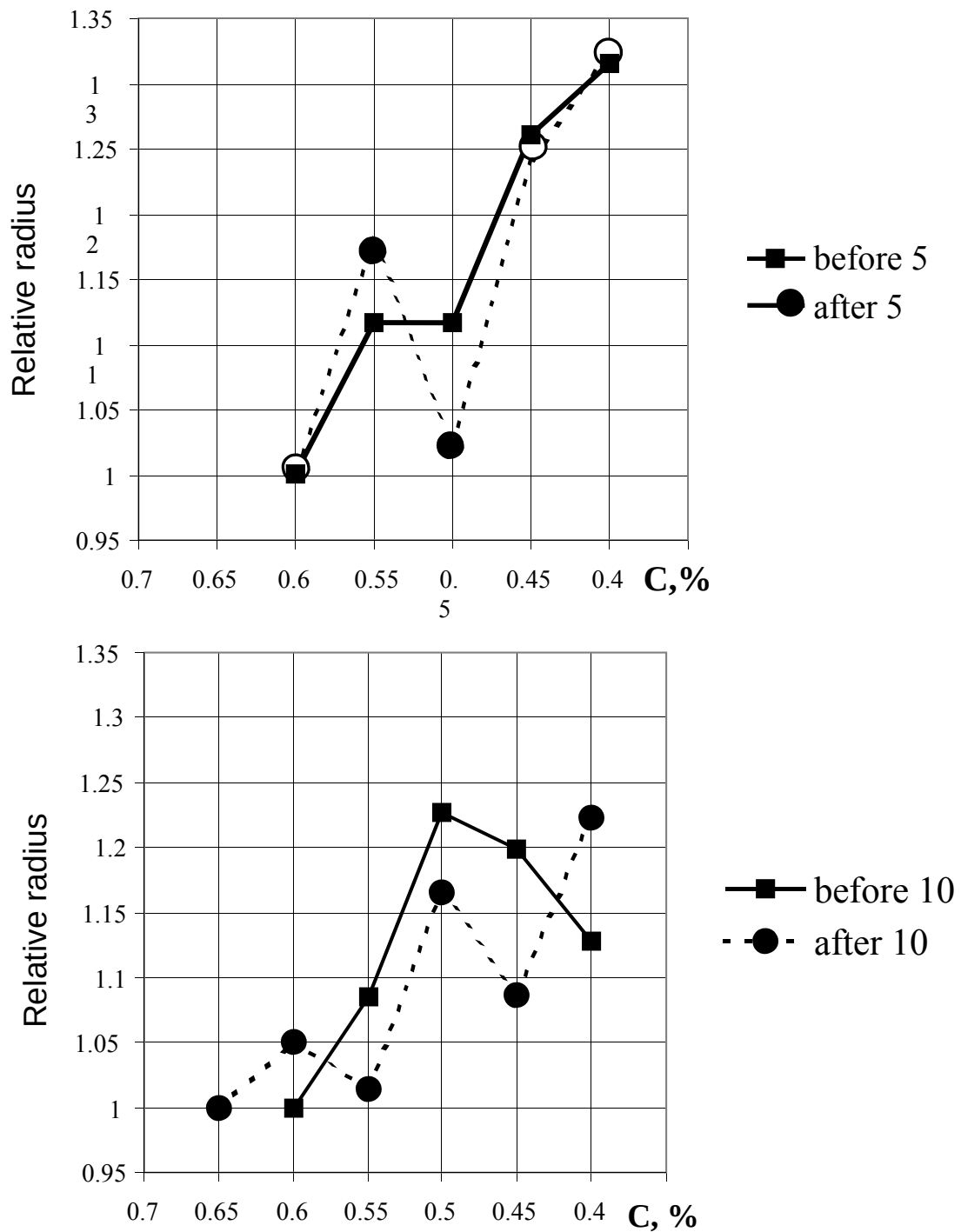


Fig. 8

24 December 2004 Patient, male, 56 years old.

G myeloma, stage III A, anaemia (78 g/l)

High level of total protein and paraprotein in the blood stream (130 and 54 g/l, correspondingly). The patient had already received several rounds of chemotherapy.

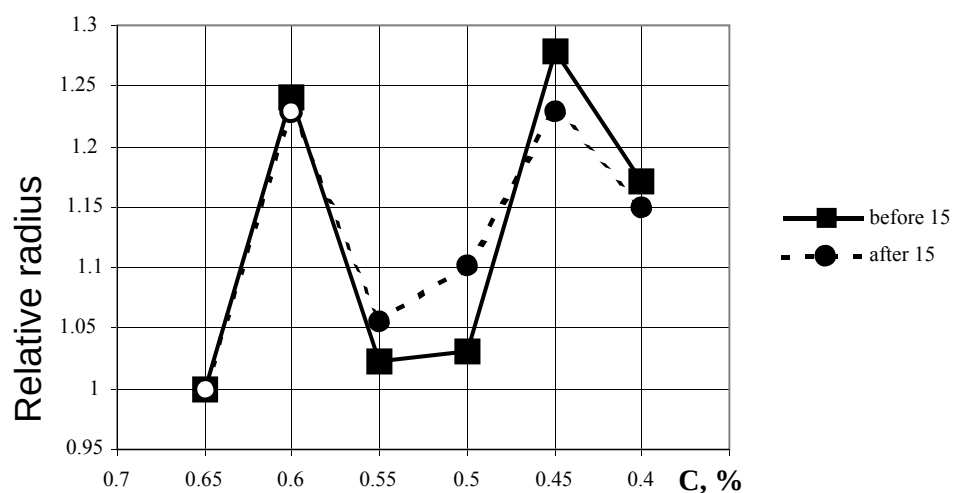
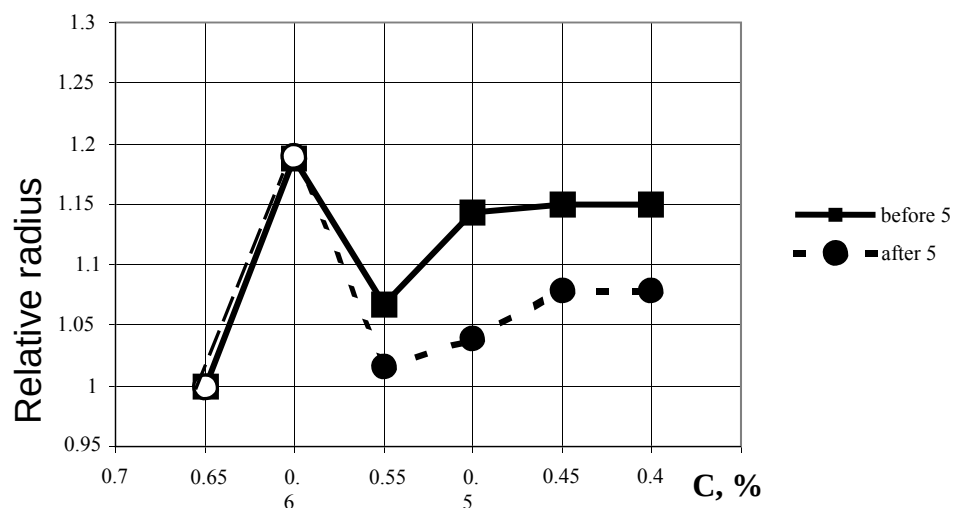


Fig. 9

28 December 2004. Patient, male, 80 years old.

Newly admitted patient, previously untreated. Bence Jones myeloma, stage III B, moderately elevated creatinine in the blood stream, paraprotein is detected in urine, but not present in the blood stream (0).

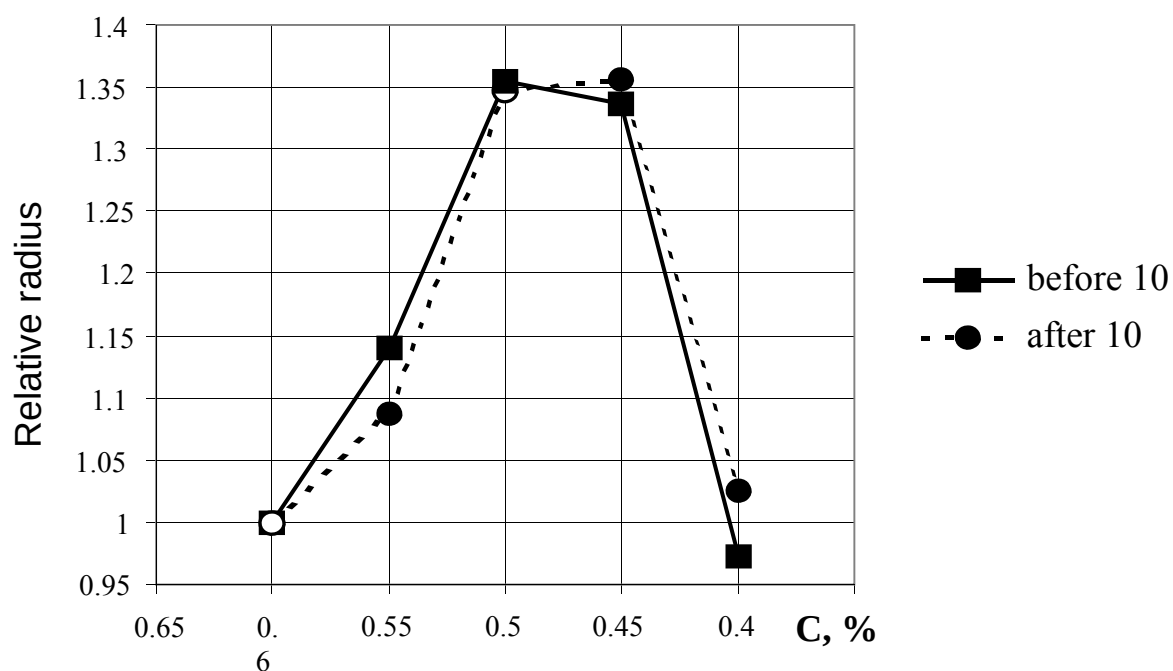
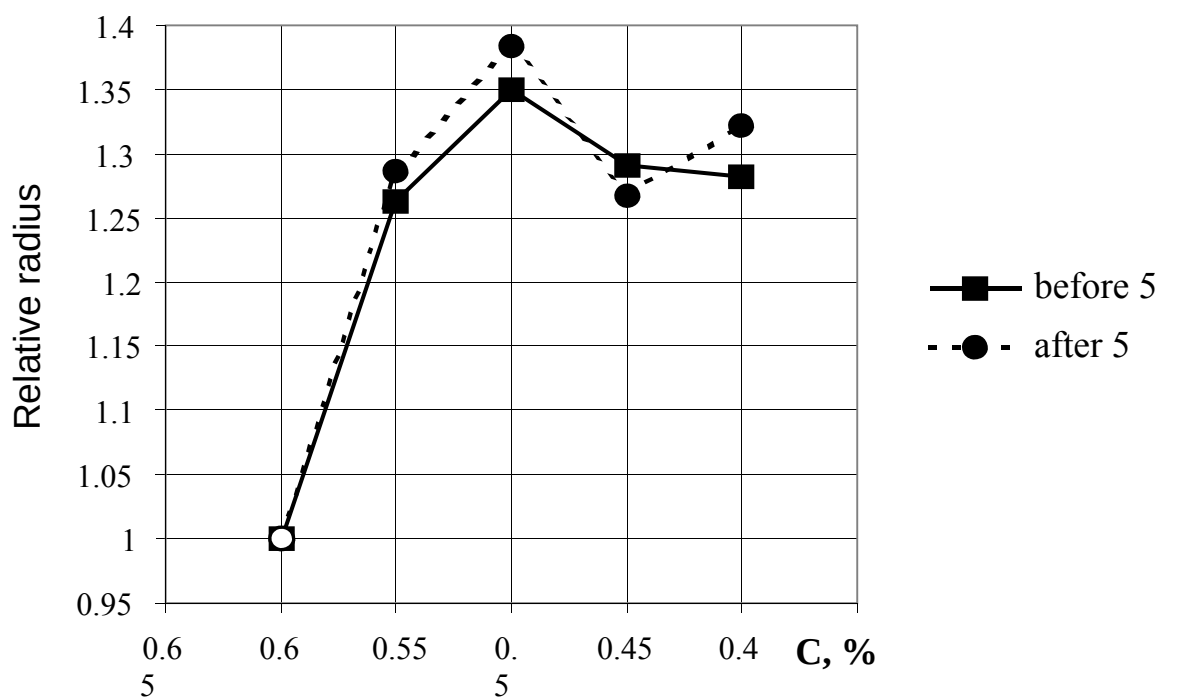


Fig. 10

18 January 2005 Patient, female 81 years old.

A-myeloma, stage III A, newly admitted patient, previously untreated. Anemia, high level of total protein and paraprotein in the blood stream (108 and 41 g/l, correspondingly).

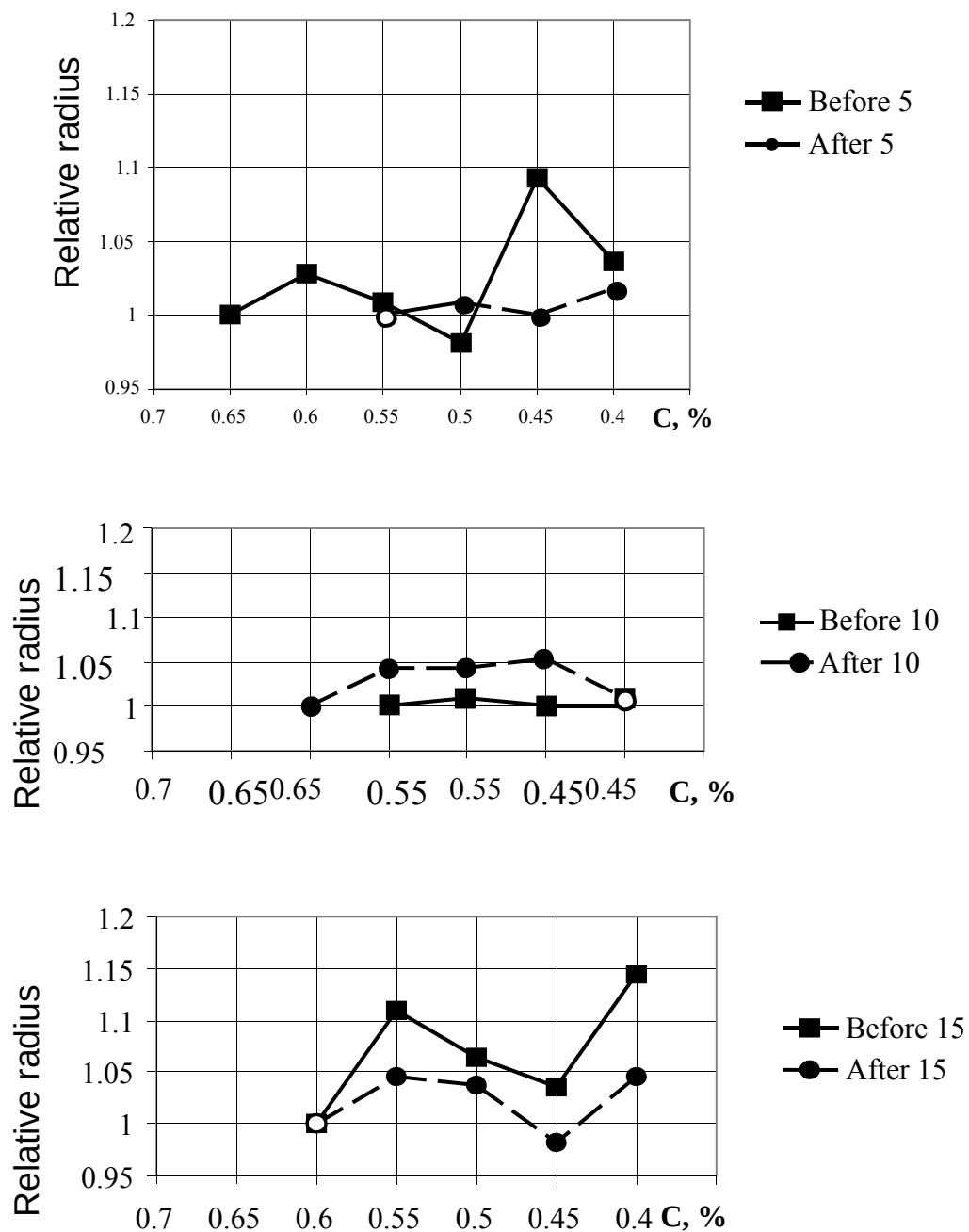


Fig. 11

21 January 2005. Patient, male, 56 years old.

Received several rounds of chemotherapy, in clinical hematologic remission. Protein level in the blood stream is normal, paraprotein is not detected (0). However, concentration of fibrinogen in the blood stream (related to chemotherapy and disintegration of cells) is high, 5.5 g/l. High aggregation.

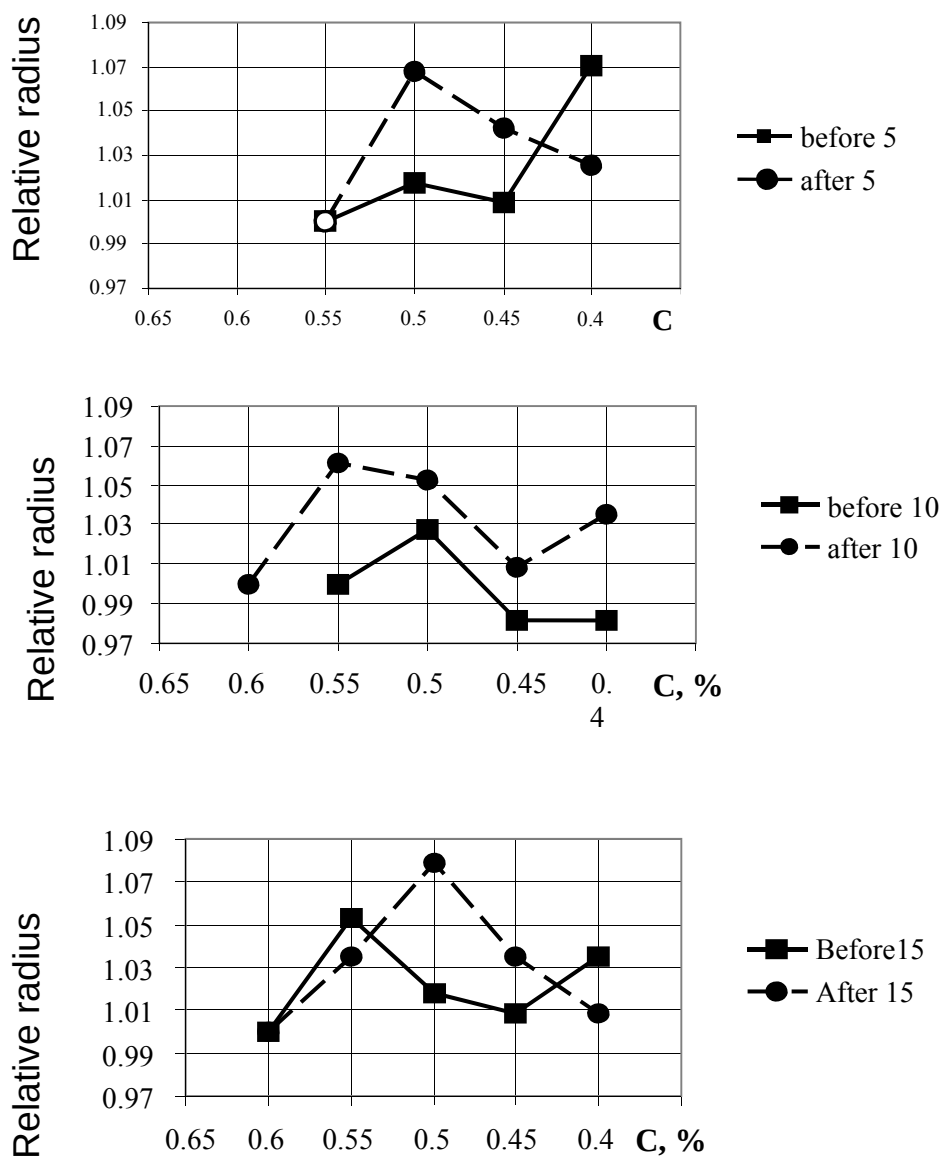


Fig. 12

25 January 2005. Patient, female, 69 years old.

Had received multiple rounds of chemotherapy, currently in relapse, rounds of intensive chemotherapy. High level of paraprotein in the blood stream (48 g/l), fibrinogen (>5 g/l).

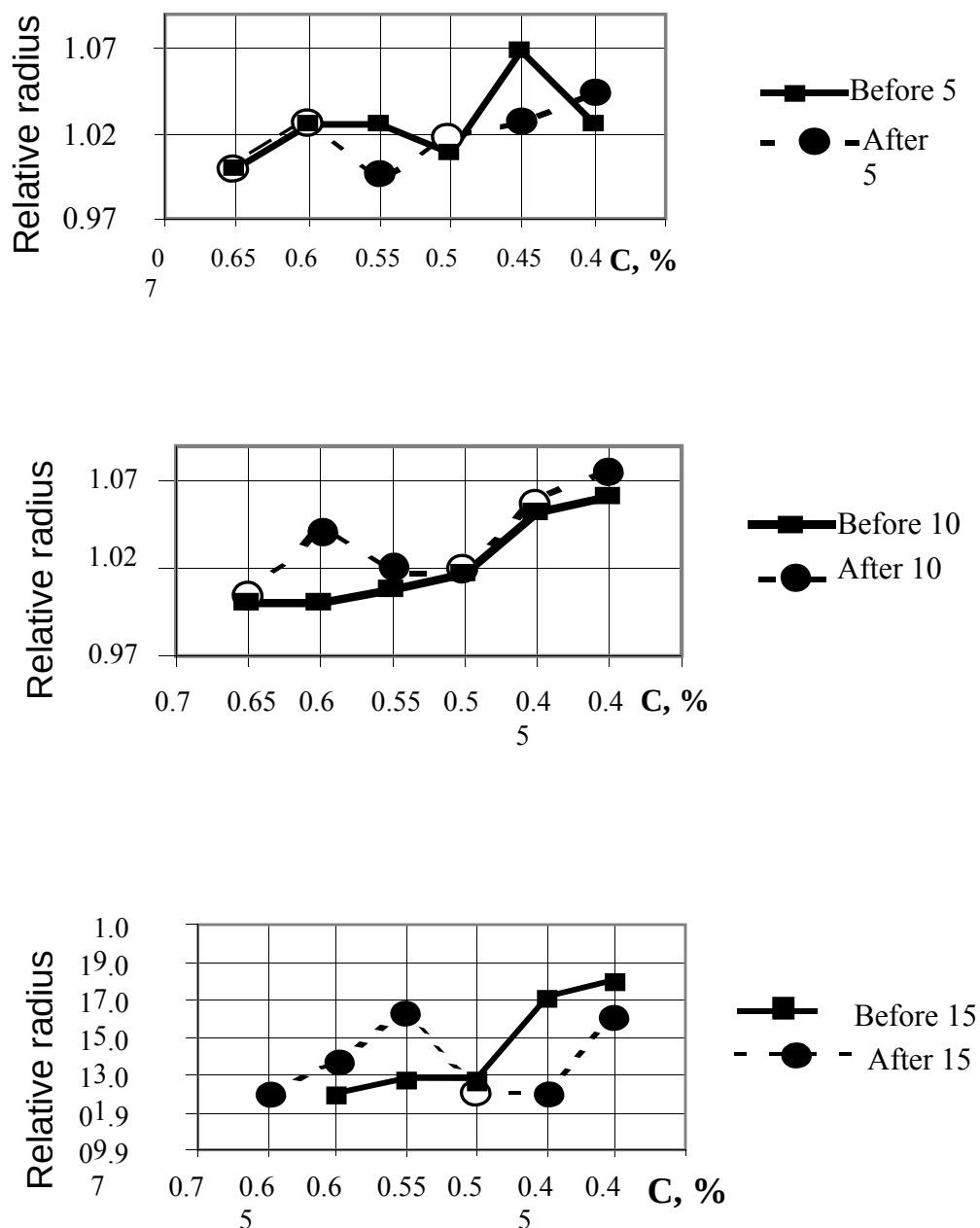


Fig. 13

**28 January. Patient, female,
76 years old.**

Newly admitted patient. Previously untreated. A-myeloma, stage II A, moderate anemia, high level of total protein and paraprotein in the blood stream (109 and 48 g/l, correspondingly).

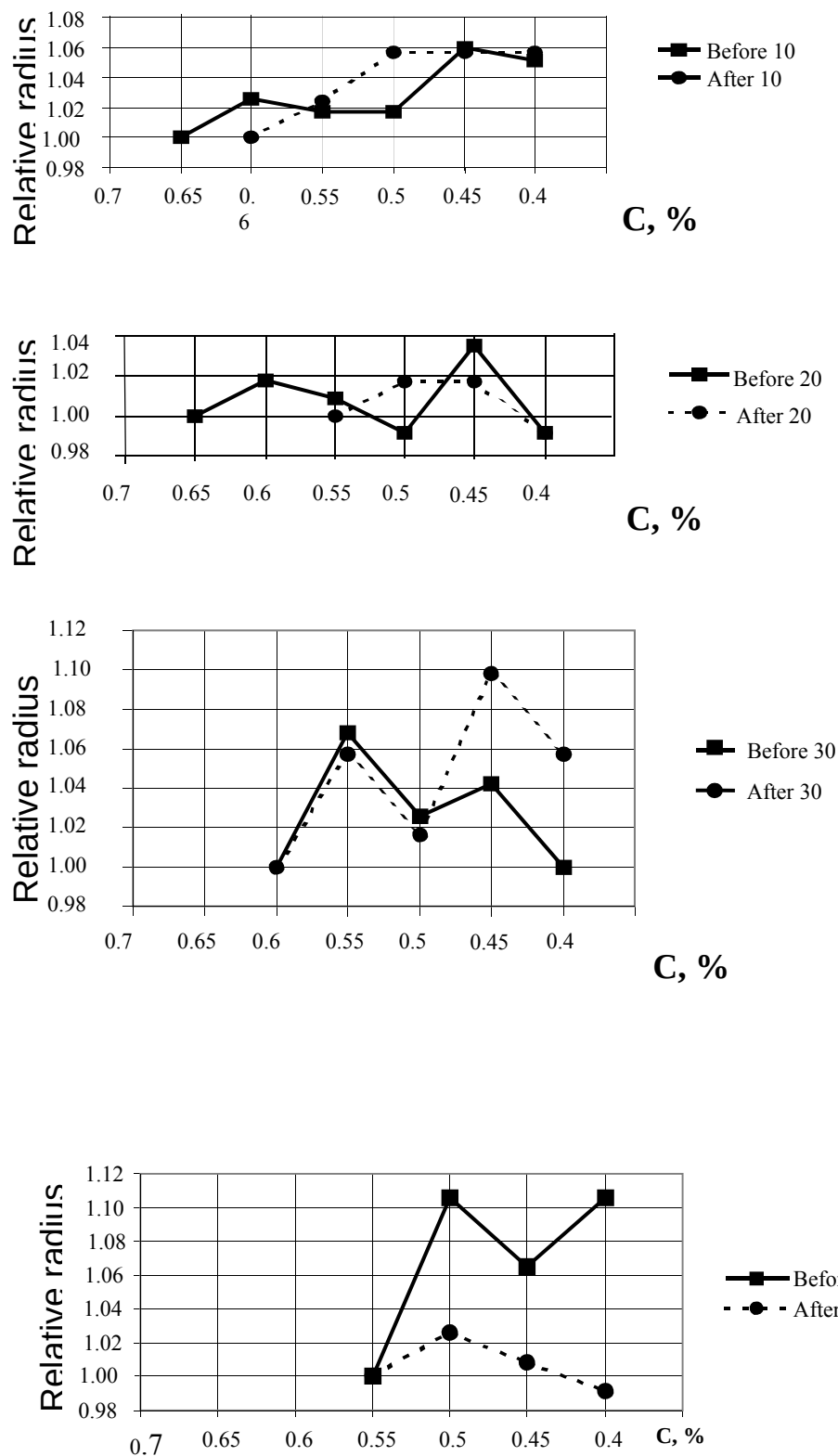


Fig. 14

4 February 2005. Patient, female, 66 years old.

A-myeloma, multiple local form, stage 3A, treated many times, anemia (haemoglobin 73 g/l), high level of total protein and paraprotein (104.5 and 50.5 g/l, correspondingly).

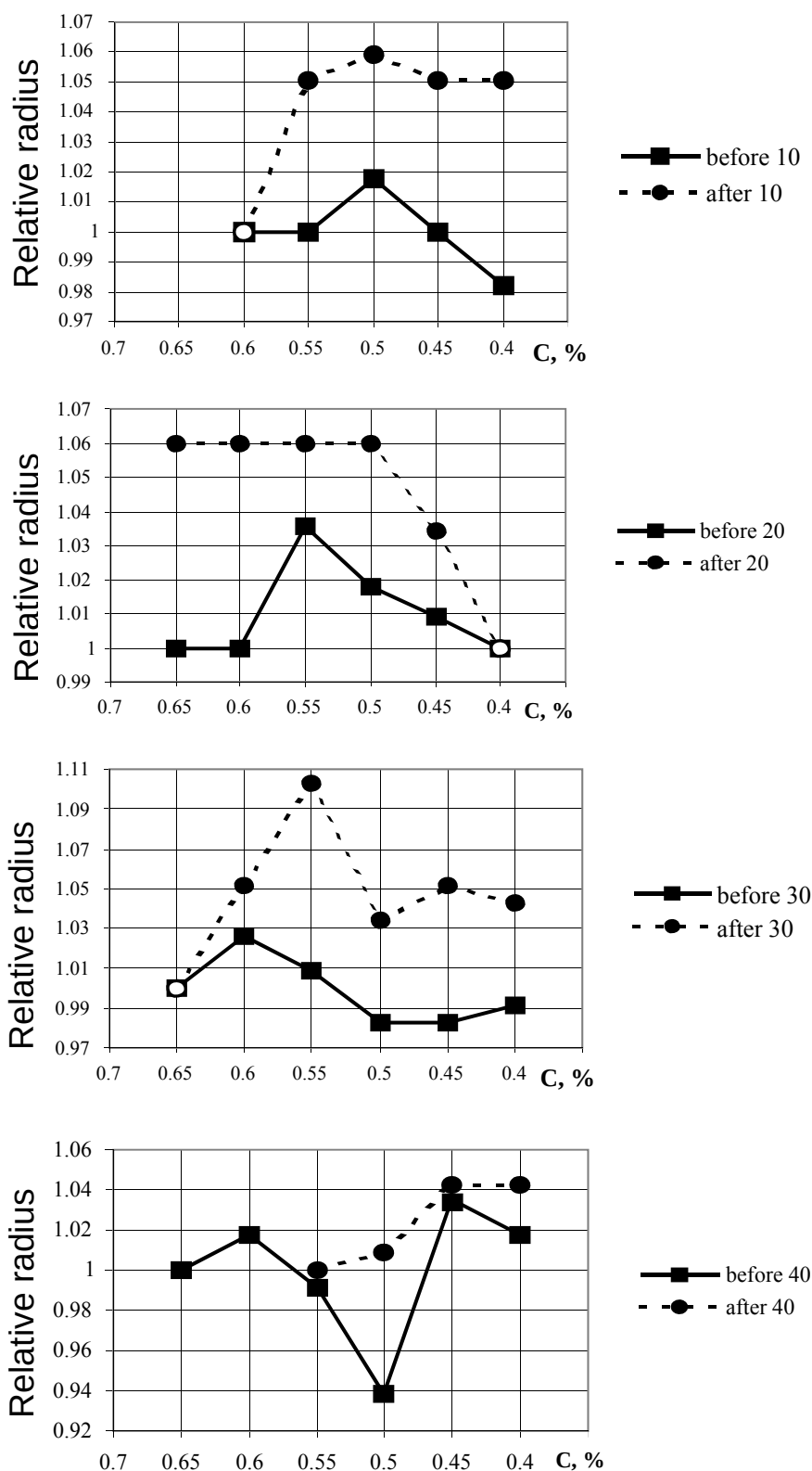


Fig. 15
8 February 2005. Patient, male, 52 years
old.

Bence Jones myeloma, multiple local form, stage 3B, relapse, for which the patient has already received 3 rounds of chemotherapy. Total protein 92, paraprotein 0, elevated fibrinogen 3.4 g/l, and moderately elevated creatinine in the blood stream, 114 g/l.

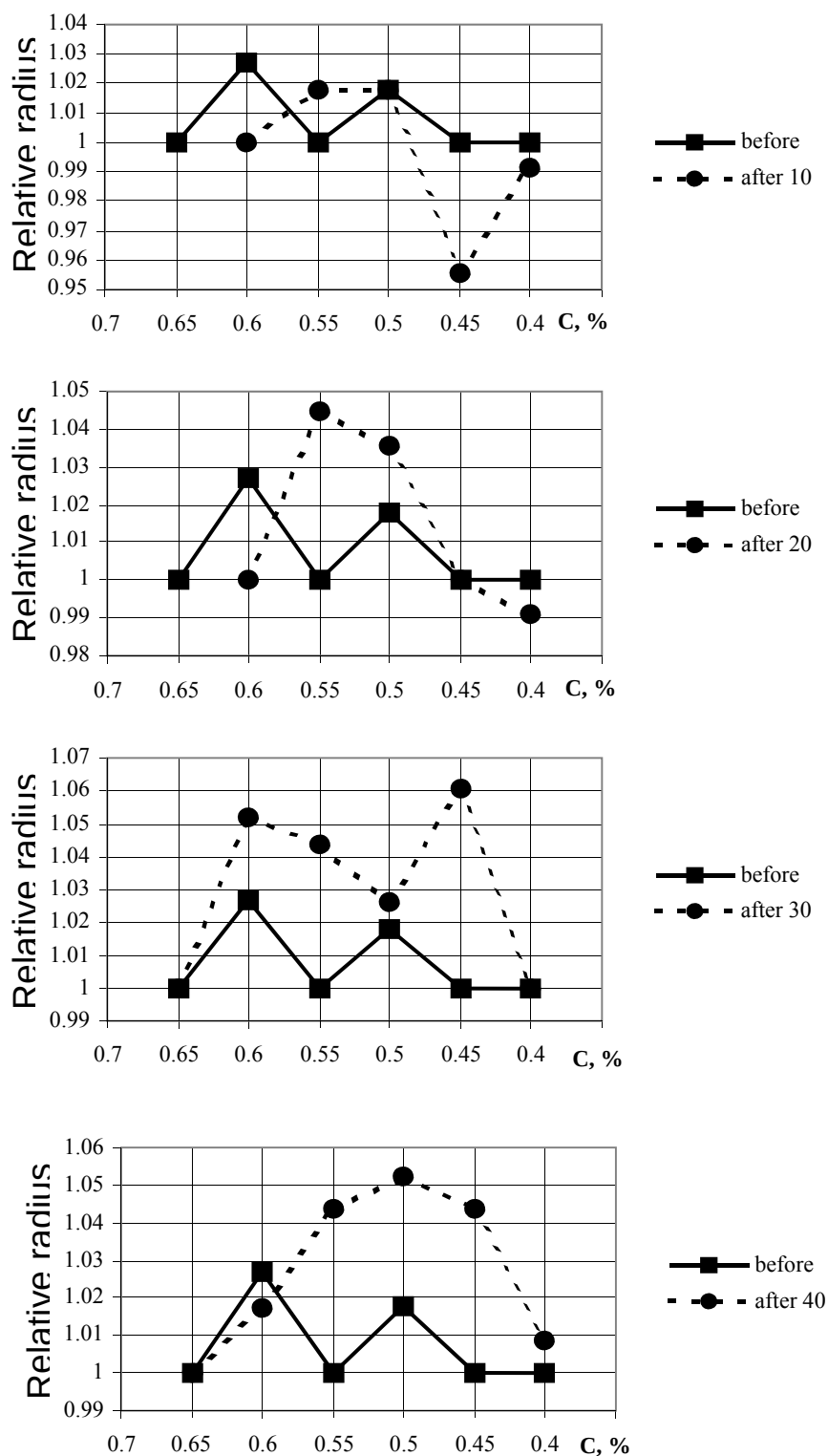


Fig. 16

11 February 2005. Patient, female, 73 years old.

G myeloma, multiple local form, stage 3A. Serious patient, treated many times, anaemia (67 g/l), high total protein and paraprotein (138.4 g/l and 50 g/l, correspondingly).

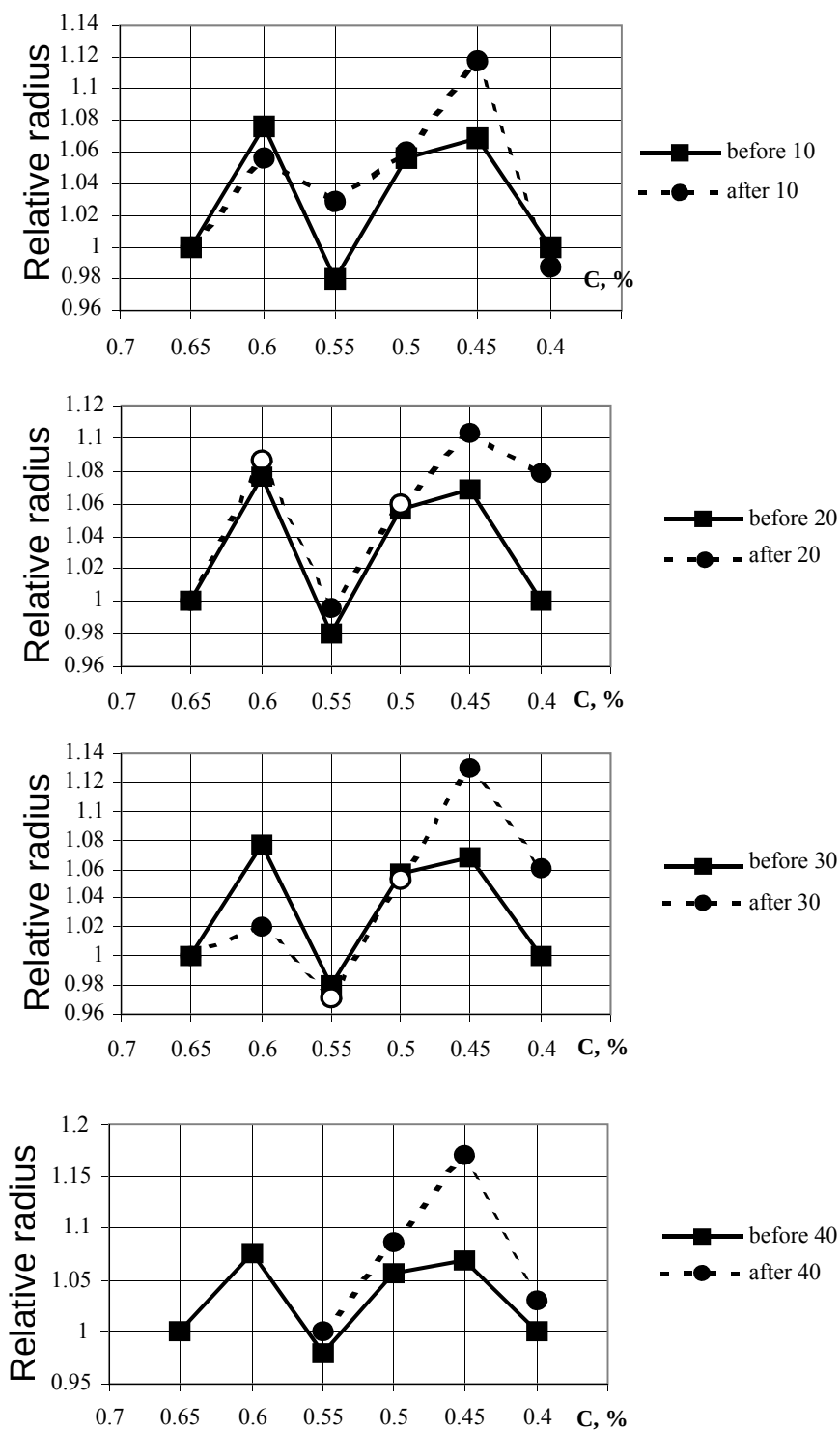


Fig. 17

15 February 2005. Patient, male, 66 years old

Nonsecretory myeloma, no change in tests, paraprotein 0. Sick for several years, after recent rounds of treatment, improvement is detected.