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聖彼得堡資訊技術、力學與光學國立大學

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

AIRES 分形諧振器對人體血液中紅血球狀態影響之研究

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TABLE OF CONTENCTS **目錄**

INTRODUCTION 引言

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.

本論文的目的是確定 AIRES 分形共振器對人體血液中紅血球功能狀態的影響性質。

The agent of influence was the AIRES fractal resonator, which is a fractal-matrix topological layout (designated as Sh3_16ort_clon3) made on a

影響因子為 AIRES 分形共振器，這是一種分形矩陣拓撲佈局（標示為 Sh3_16ort_clon3），製作於一個

7.7 × 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 .

7.7 × 7.7 矽板。分形共振器的主要功能元件是一種圖樣。拓樸圖樣線的寬度為 1 微米。

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.

研究對象為診斷為多發性骨髓瘤患者的血液。多發性骨髓瘤是一種常見的血液系統惡性疾病，其發病率持續上升。此疾病以型態與變異多樣著稱，臨床症狀極為多樣化，其成因不僅包括骨髓功能衰竭和骨骼異常，還包括腫瘤產生特定的單克隆免疫球蛋白或其酵素。

Multiple myeloma is known as a “disease of advanced age” (patients’ average age is 62 years); patients under 40 make up 2 to

多發性骨髓瘤被稱為「老年疾病」（患者平均年齡為 62 歲）；40 歲以下的患者占 2%到

3%, and 80 -year-olds get sick 10 times as often as 50 -year-olds. The median survival is approximately 50 months.

3% ，而且 80 歲者的發病率是 50 歲者的 10 倍。中位存活期約為 50 個月。

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].

根據俄羅斯血液學與輸血研究所的臨床資料，多發性骨髓瘤患者的平均存活期在過去 25 年間持續增加，現今大約為 5 年[1]。

I. OSMOTIC FRAGILITY **I. 滲透脆性**

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).

在流變學上，血液可視為含有不同形狀、大小和特性顆粒的液態介質。血球大宗為紅血球，因此它們在血液流變性質變化中扮演主要角色。描述血液最重要性質的參數包含黏度、凝集以及紅血球變形能力（ED）。

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

由於對體內變化極為敏感，紅血球是評估機體生理狀態的便利對象。

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.

細胞的一個重要參數是其對作用於細胞膜的外在衝擊（來自外部或內部環境）所產生的重新配置。可說 ED 在某種程度上反映了循環於血流中的紅血球的活力。

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.

對不同類型影響的抵抗力（穩定程度）可用作紅血球變形指數（ED）評估工具之一。其中一種影響類型是紅血球的滲透性膨脹。滲透脆性被理解為它們對低滲溶液溶血作用的抗性程度。

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.

低滲脹胀曲線的關鍵參數為最小值的座標——球化點；以及脹胀過程中紅血球相對半徑變化的振幅。這兩個參數描述了紅血球膜的彈性特性及其可變形能力。

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].

根據這項研究，隨著多發性骨髓瘤患者接受治療，血液流變參數的變化出現了正向趨勢。比較患者治療前後時，紅血球滲透性腫脹曲線在質與量上均發生了變化 [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.

治療過程的正向趨勢，根據先前的研究[2-6]，與球化點朝向較小低滲脹大值的移動一致：紅血球在脹大過程中半徑相對變化值的增加。

1.1. Change of erythrocyte radius in hypoosmotic solutions

1.1. 在低滲溶液中紅血球半徑的變化

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

讓我們考察在不同滲透壓溶液中（亦即含有不同 NaCl 鹽含量）紅血球的行為模型。

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.

在血液循環中，紅血球在彼此碰撞和與血管壁摩擦時會呈現各種形狀。在等張溶液中（含有 NaCl 濃度為 0.85% ，這對有機體而言是正常的）且沒有外來機械衝擊時，平衡形狀會是雙凹圓盤，意即紅血球為圓盤狀細胞。當未受張力的雙凹圓盤狀紅血球膨脹成球體時，細胞膜承受的是非常小的伸長應變，但表面曲率卻發生劇烈變化。紅血球圓盤的中央區域被變形為球體的極區，幾乎沒有膜的擴展；而大量的擴展主要發生在雙凹圓盤赤道區的周邊地帶。

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

當溶液滲透壓改变時，紅血球會轉變如下：

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

當滲透壓增加（高滲溶液）時，紅血球會收縮。

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

當滲透壓降低（低滲溶液）時，紅血球的體積因水進入而分兩個階段增大（見圖 1）：

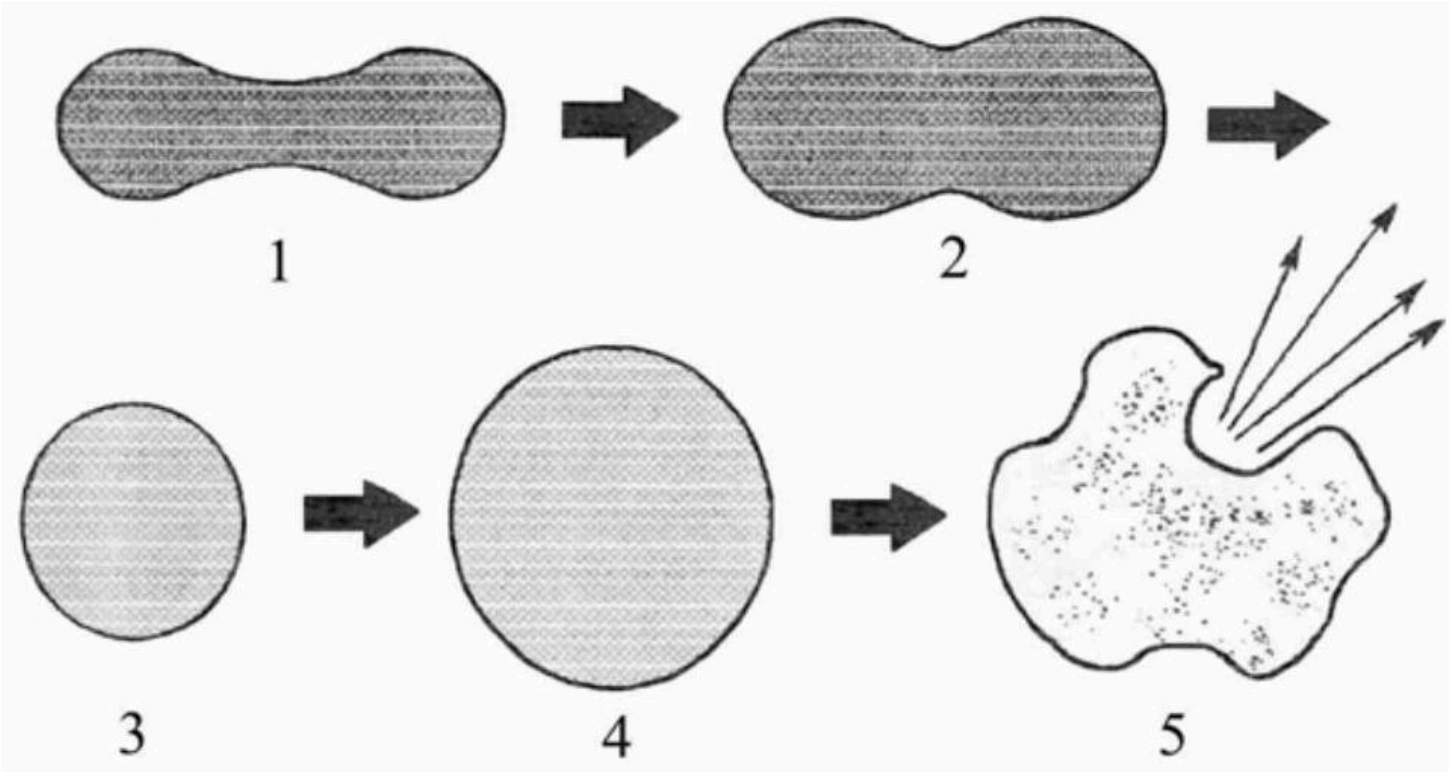


Fig. 1. Erythrocyte transformation in hypoosmotic swelling:

圖 1. 低滲脹裂中紅血球的形變：

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

1 - 圓盤狀紅血球；2 - 圓盤狀紅血球腫脹；3 - 紅血球轉變為球狀體；4 - 球形紅血球腫脹；5 - 溶血

-a) erythrocyte transforms into a sphere with its membrane surface area unchanged;

-a) 紅血球在膜表面積不變的情況下轉變為球體；

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).

b) 紅血球球形表面積因細胞膨脹而增加，直到發生溶血（紅血球膜破裂）。

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:

讓我們考慮低滲性膨脹下的紅血球行為 [7]。在第一階段，細胞內壓力偏低，而且可以在誤差很小的情況下說明紅血球內外的滲透壓相同。基於上述，當在等滲環境中通入體積為 ΔV_0 的蒸餾水以製造低滲條件時，應該符合下列方程式：

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

where $_e$ is the erythrocyte volume in isotonic environments, V_o is the original volume of the isotonic environment, $\Delta V_e, \Delta V_o$ 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.

其中 $_e$ 為等張環境下的紅血球體積， V_o 為等張環境的原始體積， $\Delta V_e, \Delta V_o$ 分別為紅血球自身與外部環境的體積增量。因此當加入蒸餾水時，紅血球體積的相對變化恰好與懸浮液總體積的相對變化相符。當懸浮液中含有大量紅血球時，誤差也不大，亦可適用相同結論。

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 mcm .

當加入懸浮液中的蒸餾水體積達到某一數值時，紅血球形狀會變為球形。為了建立紅血球在低滲溶液中行為的理論模型，取紅血球的平均直徑為 7.7 μm。

To determine the erythrocyte spherulation point on the theoretical

為了確定理論上紅血球球化點

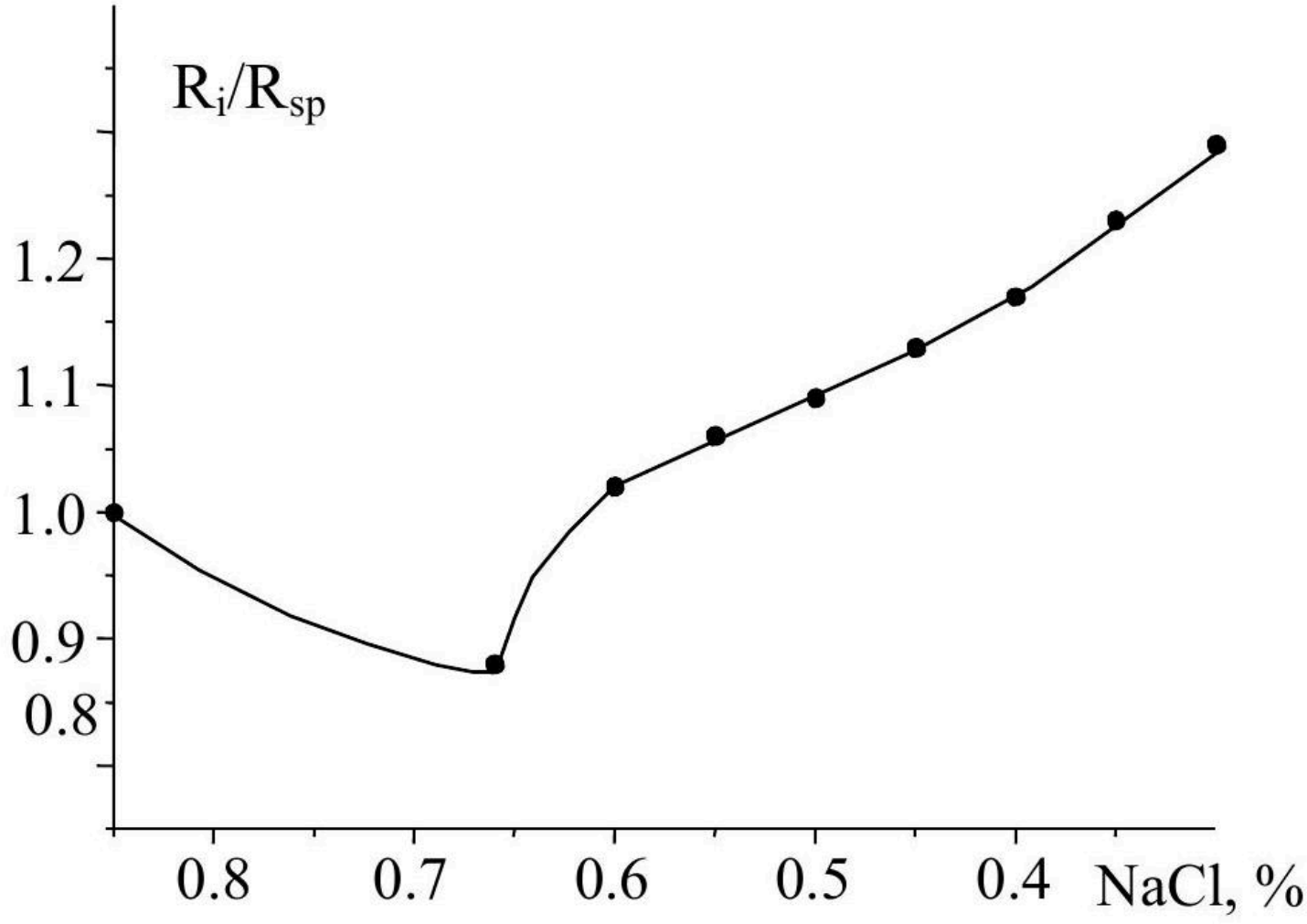


Fig. 2. Hypoosmotic curve of erythrocyte swelling: R_i - current erythrocyte radius, R_{sp} - erythrocyte radius corresponding to the spherulation point.

圖 2. 紅血球膨脹的低滲曲線：R_i - 當前紅血球半徑，R_{sp} - 對應球化點的紅血球半徑。

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.

描述紅血球在低滲溶液中行為的曲線時，我們將紅血球體積的相對變化視為懸浮液體積的相對變化，從而找出球化點。

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

紅血球半徑對 NaCl 濃度的依賴性存在一個極小值，該極小值與球化點相吻合（圖 2）。

1.2. Blood sample preparation technique

1.2. 血液樣本製備技術

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.

為進行血液檢測，必須使血液穩定化，即要防止通常在 3 到 6 分鐘後發生的血液凝固。靜脈血以 3.8% 的檸檬酸鈉鹼溶液穩定化，$9:1$ 。為避免血漿對紅血球檢測的干擾，應透過離心將紅血球與血漿及白膜層分離。

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.

血液以注射針從人體肘中位靜脈抽取到乾燥的離心管中，抽取時先讓幾滴血滴在紗布上以防止穿刺瞬間逸出的組織因子進入試管。於試管中加入 0.2 毫升無菌檸檬酸鈉以避免凝血。

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

為避免紅血球暴露於血漿中的物質，對血液進行離心分離。

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.

將所得沉渣置於含約 0.85% NaCl 的生理鹽水中，並在相同條件下離心，以更充分地洗去血漿中的成分。

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

通常處理被洗滌的紅血球時會使用含約 $0.85\%\mathrm{NaCl}$ 的生理鹽水，該溶液對細胞造成的大致滲透壓與……相當。

created by blood plasma (isotonic solution). $\mathrm{Na}+$ is the main osmotically active ion in extracellular space. Sodium ion density in the blood stream is approximately 8 times as high ($132-150\ \mathrm{mole/m^3}$) as in erythrocytes ($17-20\ \mathrm{mole/m^3}$). 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.

由血漿（等滲溶液）所造成。$\mathrm{Na}+$ 是細胞外間隙中主要的滲透活性離子。血流中鈉離子的密度約為紅血球內的 8 倍（$132-150\ \mathrm{mole/m^3}$ 比 $17-20\ \mathrm{mole/m^3}$）。因此隨著溶液中 NaCl 含量的降低，NaCl 與細胞之間會出現濃度梯度，水開始穿透膜流入紅血球。

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 $\mathrm{Na_2HPO_4}$ in combination with $\mathrm{NaH_2PO_4}$ in the final concentration of 0.01 M with $\mathrm{pH}=7.4$, to take more precise measurements of dependence of erythrocyte size on osmolality of the solution.

大多數純化學試劑的溶液其 pH 非常不穩定。因此，當必須在某些 pH 範圍內進行實驗時，會使用 pH 變化極小的特殊緩衝溶液。為了穩定紅血球膜，我們在低滲懸浮環境中加入了 $\mathrm{Na_2HPO_4}$ ，並與 $\mathrm{NaH_2PO_4}$ 以最終濃度 0.01 M 以及 $\mathrm{pH}=7.4$ 一起混合，以便更精確地測量紅血球大小對溶液滲透度的依賴性。

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

洗滌後的紅血球被置於由稀釋原始等滲溶液所製成的低滲溶液中（濃度低於 0.85% ）。

II. LASER DIFFRACTOMETRY METHOD

II. 雷射繞射法（LASER DIFFRACTOMETRY）

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.

激光繞射法是基於激光輻射在單個或多個生物微小物體上發生繞射的現象，其特點是精度高、靈敏度高、速度快、對被測物影響最小，且能同時登記大量小顆粒。繞射圖樣的參數與微小物體的參數有明確對應關係，因此可以確定它們的大小、形狀和內部結構。

With diffraction on an aggregate of erythrocytes, the diffraction

在紅血球聚集體上的繞射，繞射

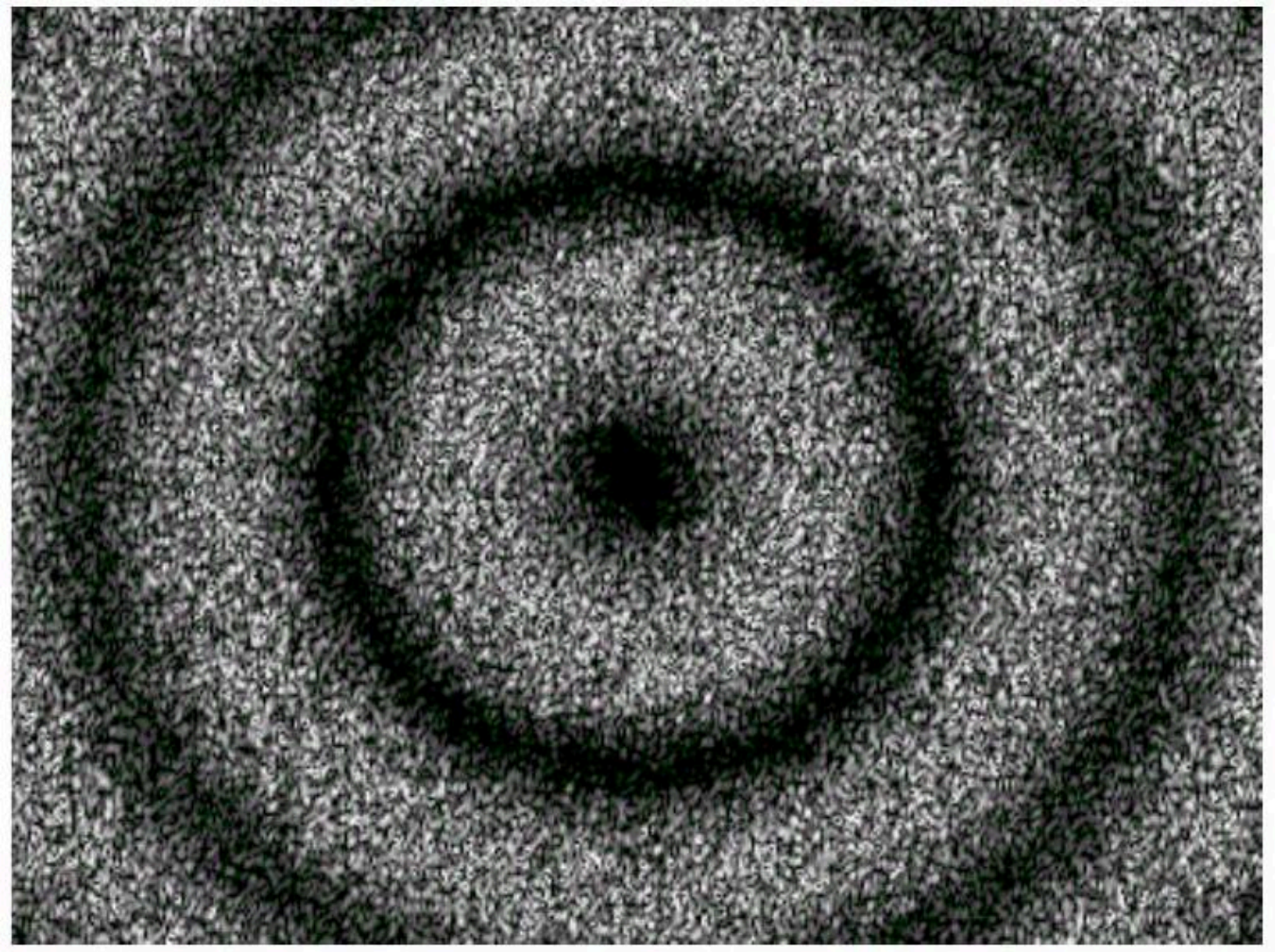


Fig. 3. Distribution of intensity in a diffraction pattern

圖 3. 繞射圖樣中強度的分佈

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

圖樣呈現為同心圓環系統（圖 3）。環間距離...

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 1. Relative change in average

erythrocyte size equals in absolute value the relative change in the diffraction ring diameter:

同心環之間的距離與描述此聚集體的平均紅血球大小有關。這種關係是反比的，也就是說，測量物體越小 D ，繞射環 1 的直徑越大。平均紅血球大小的相對變化在絕對值上等於繞射環直徑的相對變化：

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

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

其中 ΔD 與 Δl 分別是平均紅血球大小和繞射環直徑的變化量。

The experimental assembly used for studying erythrocyte deformability includes an LGN 215 He-Ne-laser

用於研究紅血球變形能力的實驗裝置包括一台 LGN 215 He-Ne 雷射

$(\lambda = 0.63\text{mcm})$; 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).

$(\lambda = 0.63\text{mcm})$ **；一個用於測量雷射功率強度的光學衰減器；樣品工作臺；傅立葉變換用的透鏡；光電偵測器；以及一台個人電腦（圖 4）。**

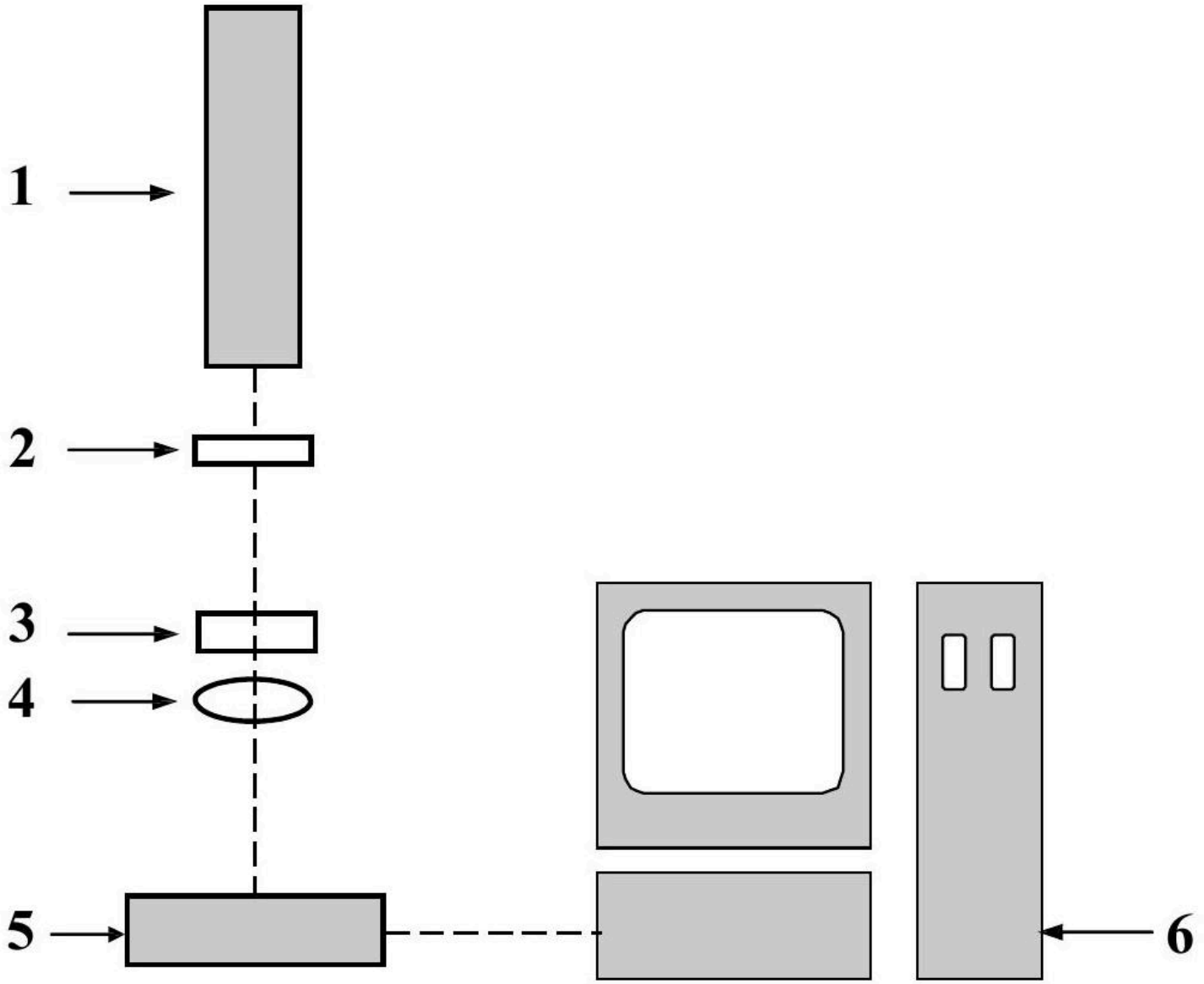


Fig. 4. Experimental assembly layout: $1-$ He-Ne-laser $(\lambda = 0.63\text{mcm})$, 2 - optical attenuator, 3 - sample, 4-lens, 5-photodetector, 6-PC

圖 4. 實驗組裝佈局： $1-$ He-Ne 雷射 $(\lambda = 0.63\text{mcm})$ 、2 - 光衰減器、3 - 樣品、4 - 鏡片、5 - 光電偵測器、6 - 電腦

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

為了用雷射繞射法研究懸浮於不同滲透壓溶液中的紅血球，樣品應製備成薄層，以確保單次散射（見圖 5）。處理後的樣品中紅血球應均勻分布於 Goryaev 鏡室的表面，不可重疊（紅血球必須形成單層），如此可忽略

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

來自重疊區域再輻射的影響，且濃度應足以觀察高強度的細胞變形。

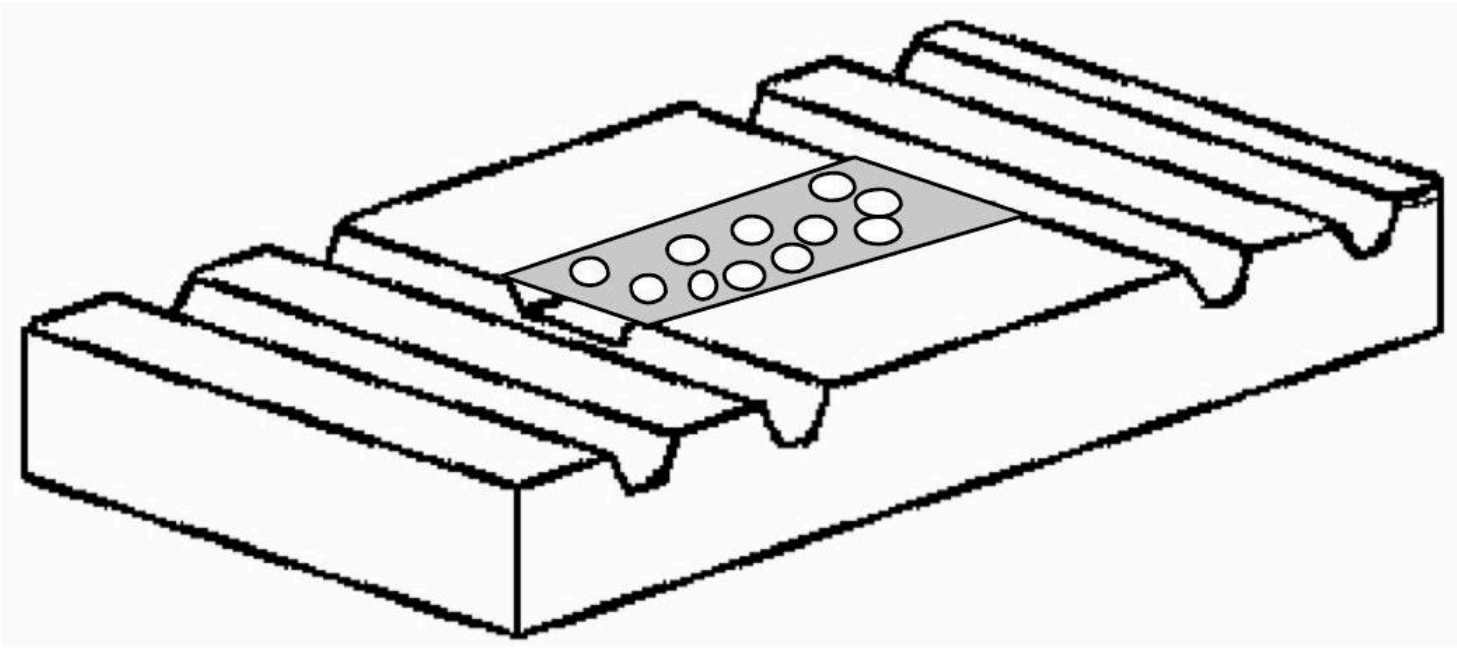


Fig. 5. Goryaev's chamber

圖 5. Goryaev 鏡室

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

當紅血球體積與溶液體積之間符合某一比例時，即滿足這些條件。

With a concentration of $0.03 - 0.04\text{ml}$, 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.

當濃度為 $0.03 - 0.04\text{ml}$ 時，紅血球在 Goryaev 室的表面會形成足夠均勻的單層，這可使細胞變形接近理想的圓形並產生足夠的強度。

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.

對紅血球在發生滲透平衡之溶液中暴露時間影響的實驗研究顯示，研究紅血球滲透脆性與細胞膜剛性的最佳時間是在懸浮液製備後兩小時。

The following blood sample preparation technique for experimental studies is proposed: erythrocytes stabilized with sodium citrate, thrice washed for 10 minutes at $5,000\text{rpm}$, taken from the bottom of the tube in the amount of $0.03 \div 0.04\text{ml}$ 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.

提出以下用於實驗研究的血液樣本製備技術：以檸檬酸鈉穩定的紅血球，在 $5,000\text{rpm}$ 下以三次各 10 分鐘方式洗滌，從試管底部取出每 2 毫升低滲溶液添加 $0.03 \div 0.04\text{ml}$ 的量，靜置 2 小時，然後置於 Goryaev 室觀察繞射圖樣並記錄第一與第二極小值的半徑。

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.

紅血球聚集體平均直徑的相對變化，是由雷射繞射儀中繞射環線性尺寸的變化所決定的。

2.1. Experimental technique

2.1. 實驗技術

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

用於研究的血液樣本處理技術是一項相當耗時且分段進行的流程，包含以下步驟（圖 6）：

blood draw in a clinic;

在診所抽血；

delivery of the blood to the lab for testing;

將血液送至實驗室進行檢測；

blood cenrifugation; **血液離心；**
preparation of working solutions;

配製工作溶液；

letting the blood settle (stabilization);

讓血液沉澱（穩定化）；

experiment; **實驗；**
processing of experimental findings.

實驗結果的處理。

The experiment was conducted in two versions:

該實驗以兩種版本進行：

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);

第一種為一致性版本，旨在使探測雷射輻射的影響降至最低（樣本在最初以及經過一定暴露時間後接受輻射）；

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.

在第二個版本中，同一樣本依序接受輻射：最初分別為 10、20、30 及 40 分鐘，導致輻射劑量逐漸累積，並可能在實驗時間縮短時產生難以控制的影響。

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.

利用把裝有血液樣本的 Goryaev 室置於圖案一側的分形共振器上，以達到分形共振器的影響。

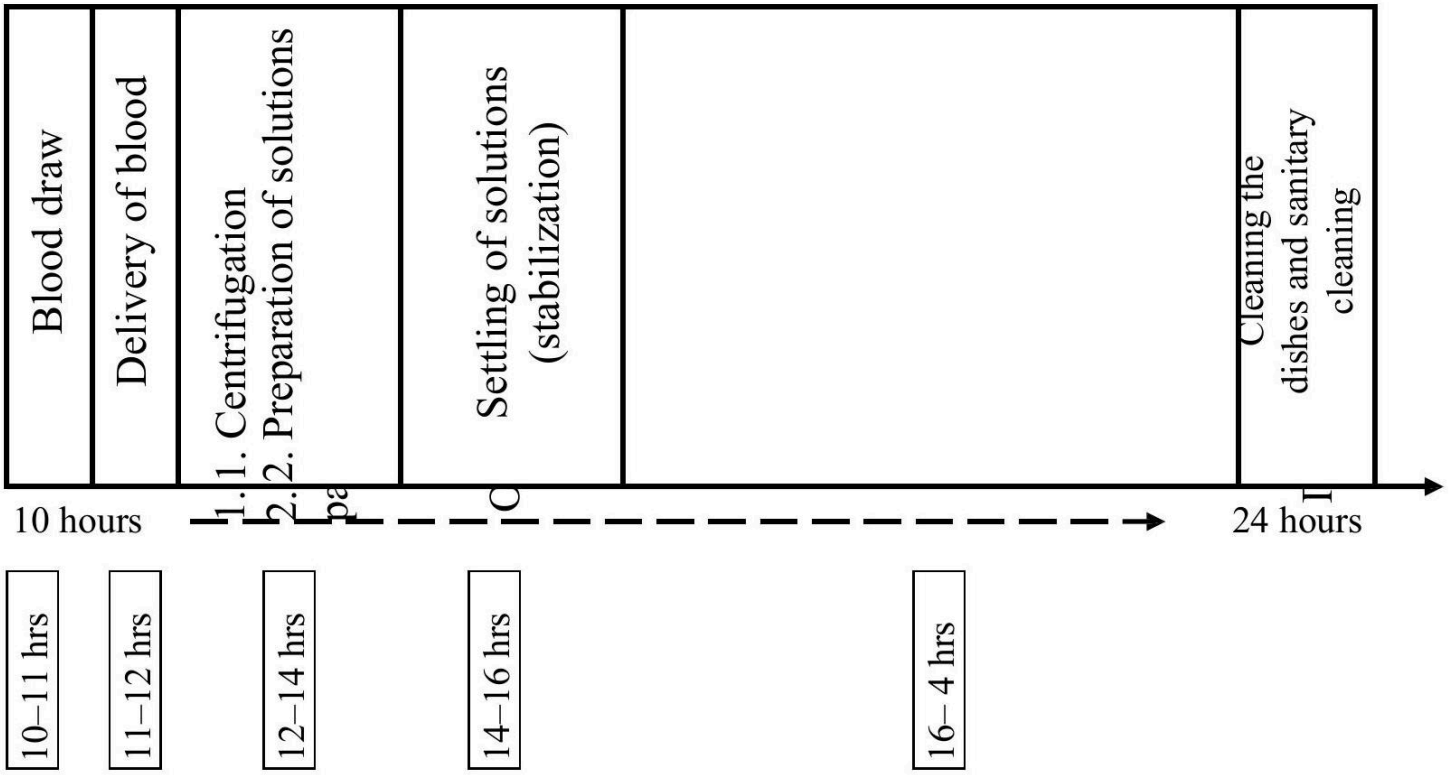


Fig. 6. Experiment timeline for one blood sample

圖 6. 單一血液樣本的實驗時間線

Erythrocyte sedimentation **紅血球沉降率**	Scanning. Image input Saving **掃描。影像輸入 正在儲存**		Resonator exposure 10 minutes **共振器曝光 10 分鐘**		Scanning. Image input. Savinge **掃描。影像輸入。正在儲存**		Chamber cleaning 5-10 minutes **腔室清潔 5-10 分鐘**	
0 5 min		10 min **10 分鐘**		20 min **20 分鐘**	25 min **25 分鐘**			35 min **35 分鐘**

Exposure time 10 min

曝露時間 10 分鐘

Fig. 7. Experiment timeline for one NaCl concentration

圖 7. 單一 NaCl 濃度的實驗時間表

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

對於單一濃度，含 10 分鐘曝露的整個實驗總時長為 35 分鐘（圖 7）。

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

繪製一條依存曲線的實驗需進行 8 個測點。獲得繪製一條低張曲線所需的資料大約需要 5 小時。

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

獲得繪製單條低滲曲線（暴露時間為 20 分鐘）所需的資料約需 6 小時。

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

獲得繪製單條低滲曲線（暴露時間為 40 分鐘）所需的資料約需 8 小時。

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.

在單一血液樣本的實驗中，測試了 8 種 NaCl 濃度：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.

因此，即使同時進行多項操作，單一血液樣本的實驗總時長仍約為 14 小時。

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

研究的第二個程序階段是處理取得的結果，以尋找可用於繪製低滲透壓腫脹曲線的定量資料。

III. EXPERIMENTAL FINDINGS

III. 實驗結果

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

3.1. 研究分形諧振器對紅血球流變參數的影響（暴露時間最多達 15 分鐘）

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

初步分析結果顯示，測試結果在很大程度上取決於患者的初始狀態及其所接受的治療。

24 December 2004. (Fig. 8)

2004 年 12 月 24 日。（圖 8）

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

暴露於共振器 5 分鐘及 10 分鐘具有正面效應。

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

在球化的初始階段，經過 5 分鐘暴露後，相較於紅血球半徑的變化，振幅偏移增加；

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

腫脹的性質開始符合典型情形（出現跳躍然後隨後腫脹）。

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.

在暴露 10 分鐘後，球形化點向較小滲透壓值區域移動，這對應於外界影響的正向趨勢；腫脹的性質也變得明顯呈痙攣狀。

28 December 2004. (Fig. 9)

2004 年 12 月 28 日。（圖 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.

正向效果微弱可見。經過 5 分鐘後，第一次跳變的振幅略微增加。經過 10 分鐘後，第一次跳變的振幅亦稍微增加。

18 January 2005. (Fig. 10)

2005 年 1 月 18 日。（圖 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.

正面效應表現得非常微弱。經過 5 分鐘後，第一個跳躍的振幅略微增加。經過 10 分鐘，則未觀察到顯著變化。

21 January 2005. (Fig. 11)

2005 年 1 月 21 日。（圖 11）

The general trend is positive.

整體趨勢為正面。

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).

5 分鐘後，低滲膨脹的結果——暴露後的反應——對應於退行：紅血球半徑變化的振幅下降，球化點向較大滲透壓值移動（紅血球膜的彈性特性顯著降低）。

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.

10 分鐘後，趨勢呈現正向：紅血球在膨脹期間半徑變化的振幅上升，球化點向較小的低滲透壓值移動。

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

15 分鐘後，紅血球的振幅偏移進一步增加，低滲膨脹曲線出現跳躍。

25 January 2005. (Fig. 12)

2005 年 1 月 25 日。（圖 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.

整體趨勢為正向。對於所有暴露時間（ $5, 10, 15$ 分鐘），所指定暴露時間下紅血球低滲脹大半徑振幅均超過初始振幅，且此跳增更為顯著。

28 January 2005. (Fig. 13)

2005 年 1 月 28 日。（圖 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.

整體趨勢為正向。球形化點更為明顯，且在暴露 15 分鐘時顯示出向較低低滲壓力移動的傾向。所有暴露時間的紅血球膜彈性性質皆顯示正向趨勢：紅血球膨脹的速率增加。

Conclusions 結論

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

完成的六份血液樣本初步研究整體顯示，來自共振器的外在影響普遍呈現正向趨勢。

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.

三份血液樣本（2004 年 12 月 24 日、2005 年 1 月 25 日與 1 月 28 日）顯示，隨著暴露時間延長，共振器的影響明顯有更大的改善趨勢。

The rheoloical 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.

紅血球的流變參數（聚集、變形能力、本體黏度）取決於一整套因素。特別是在多發性骨髓瘤（MM）患者中，這些參數與血中總蛋白及副蛋白（paraprotein）水平密切相關，而在有副蛋白血症的疾病類型中此類指標總是偏高。另一方面，我們研究了來自不同疾病類型患者的血液，包括罹患班斯—瓊斯（Bence Jones）骨髓瘤以及在血液中不分泌副蛋白的無分泌型骨髓瘤患者。慢性腎功能不全和貧血——這些在患者身上常見的情形——也絕非不重要。此外，本研究納入了新入院且未接受過治療患者的血液，以及正在接受化療療程患者的血液。細胞毒性藥物會對紅血球膜的彈性產生負面影響。與此同時，化療過程中腫瘤細胞被破壞，這會引起與血液流變密切相關的凝血系統重大變動。無疑地，應將患者年齡納入考量。因此，本研究涵蓋了相當多樣的患者群。

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

因此對所獲得結果的討論使我們得出結論：為了獲得足夠可靠的數據，需滿足下列條件：

the experiment should be continued to study the effect of the resonator on the state of erythrocytes;

應繼續進行實驗以研究共振器對紅血球狀態的影響；

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

在分析共振器影響時，應考慮疾病的類型與分期，並分別對新入院的病人（在開始專門治療之前）以及接受過強力化療的病人進行研究；

of specific therapy) and patients who underwent intensive chemotherapy;

在具體療法開始前（初始階段）應單獨評估新入院患者，並對已接受強化化療的患者另行分析

a suggestion was made to study the effect of increasing resonator exposure time (up to 40 minutes).

提出建議研究延長共振器曝露時間（最多達 40 分鐘）的影響。

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

3.2. 研究的第二階段：延長共振器曝露時間（最多達 40 分鐘）

4 February 2005. (Fig. 14)

2005 年 2 月 4 日。（圖 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.

整體趨勢為正向。最顯著的效果出現在 30 分鐘後。曝露 40 分鐘後對共振器的反應呈負向。然而此結果也可能與隨著曝露時間增加而產生的環境負面影響有關，例如樣本乾燥等。

8 February 2005. (Fig. 15)

2005 年 2 月 8 日。（圖 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.

整體趨勢為正向。最顯著的效果也在暴露 30 分鐘後觀察到。暴露 40 分鐘後對共振器的反應則為負向。

11 February 2005. (Fig. 16)

2005 年 2 月 11 日。（圖 16）

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

實驗設計有所修改。相同樣本連續暴露（因而雷射輻射劑量增加為原來的四倍）。

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

整體趨勢是正向的。最顯著的效應也同樣出現在曝露 30 分鐘後。

11 February 2005. (Fig. 17)

2005 年 2 月 11 日。（圖 17）

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

實驗設計未作修改。相同的樣本被連續暴露（隨著雷射輻射劑量增加為四倍）。總體趨勢為正向。

Conclusions **結論**

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

第二階段實驗顯示，來自共振器的外部影響呈現正向趨勢。

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).

根據已進行的實驗似乎無法決定最佳暴露時間（進行的實驗數量較少；需要更詳細分析長時間置於 Goryaev 腔中樣本的可能變化）。

CONCLUSION **結論**

10 blood samples from patients with multiple myeloma were tested.

對 10 份來自多發性骨髓瘤患者的血液樣本進行了測試。

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

檢測到分形共振器對紅血球流變參數有外在影響的正向趨勢。

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).

然而，由於血液樣本數量少且來自極為多樣的多發性骨髓瘤患者，且未記錄所施治療（化療）的影響，因此需要進一步蒐集資料，以便之後編成同質性的患者群組（區分血液中有副蛋白者與無副蛋白者、新入院患者與已接受化療療程者，並配合相同姓名的血紅素與肌酐參數）。

BIBLIOGRAPHY **參考文獻**

Бессмельцев С.С., Абдукадыров К.М. Множественная миелома [Multiple myeloma]. - Saint Petersburg: «Издательство «Диалект», 2004, - 448 с.
Бессмельцев С.С., Скворцова Ю.А., Тарлыков В.А., Александрова Л.А. Влияние лечебного плазмафереза на трансформацию эритроцитов больных с множественной миеломой в условиях гипоосмотического гемолиза (метод лазерной дифрактометрии) [Influence of Therapeutic Plasmapheresis on Transformation of Erythrocytes of Multiple Myeloma Patients under the Hypoosmotic Hemolysis (Laser diffractometry method)]. Эфферентная терапия [Efferent therapy], 1999. - Т. 5. - № 2. - С. 24-28.
Bessmeltsev S.S., Lendiaev A.V., Skvortsova Y.A., Tarlykov V.A. Laser diffractometry of erythrocyte deformation under the hypoosmotic hemolysis//Proceedings SPIE. - 2000. - V. 4316. - P. 83-88.

4 Бессмельцев С.С., Лендяев А.В., Скворцова Ю.А., Тарлыков В.А. Особенность трансформации сферулированного эритроцита при осмотическом набухании в норме и патологии (метод лазерной дифрактометрии) [Particularity of the transformation of a spherulated erythrocyte during osmotic swelling under normal and pathological conditions]. Сборн. докл. 6 -й российской НТ конф. “Электромагнитная совместимость технических средств и биологических объектов” ЭМС-2000 [Book of abstracts of the 6th Russian Science-and-Technology Conference “Electromagnetic Compatibility of Technological Tools and Biological Objects” EMC-2000], Saint Petersburg, 2000. С. 569-572.

5 Бессмельцев С.С., Скворцова Ю.А., Тарлыков В.А, Исследование жесткости мембраны эритроцитов у больных множественной миеломой на фоне терапии, включающей лечебный плазмаферез [Study of Erythrocyte Membrane Stiffness]. Эфферентная терапия [Efferent Therapy], 2000 - Т. 6. - № 1. - С. 36-41.

6 Бессмельцев С.С., Лендяев А.В., Скворцова Ю.А., Тарлыков В.А. Лазерная дифрактометрияоптических и механических свойств эритроцитов [Laser Diffractometry of Optical and Mechanical Properties of erythrocytes]. Оптический журнал [Optical Journal]. - 2000. - Т. 67. - № 4. - С. 47-51.
7 Петренко Ю.М., Владимиров Ю.А. Изменение размеров эритроцитов при набухании в гипоосмотических средах [Change in Erythrocyte Size during Swelling in Hypoosmotic Environments]. Биофизика [Biophysics], 1987, т. 32, №3, с. 448.

APPENDIX **附錄**

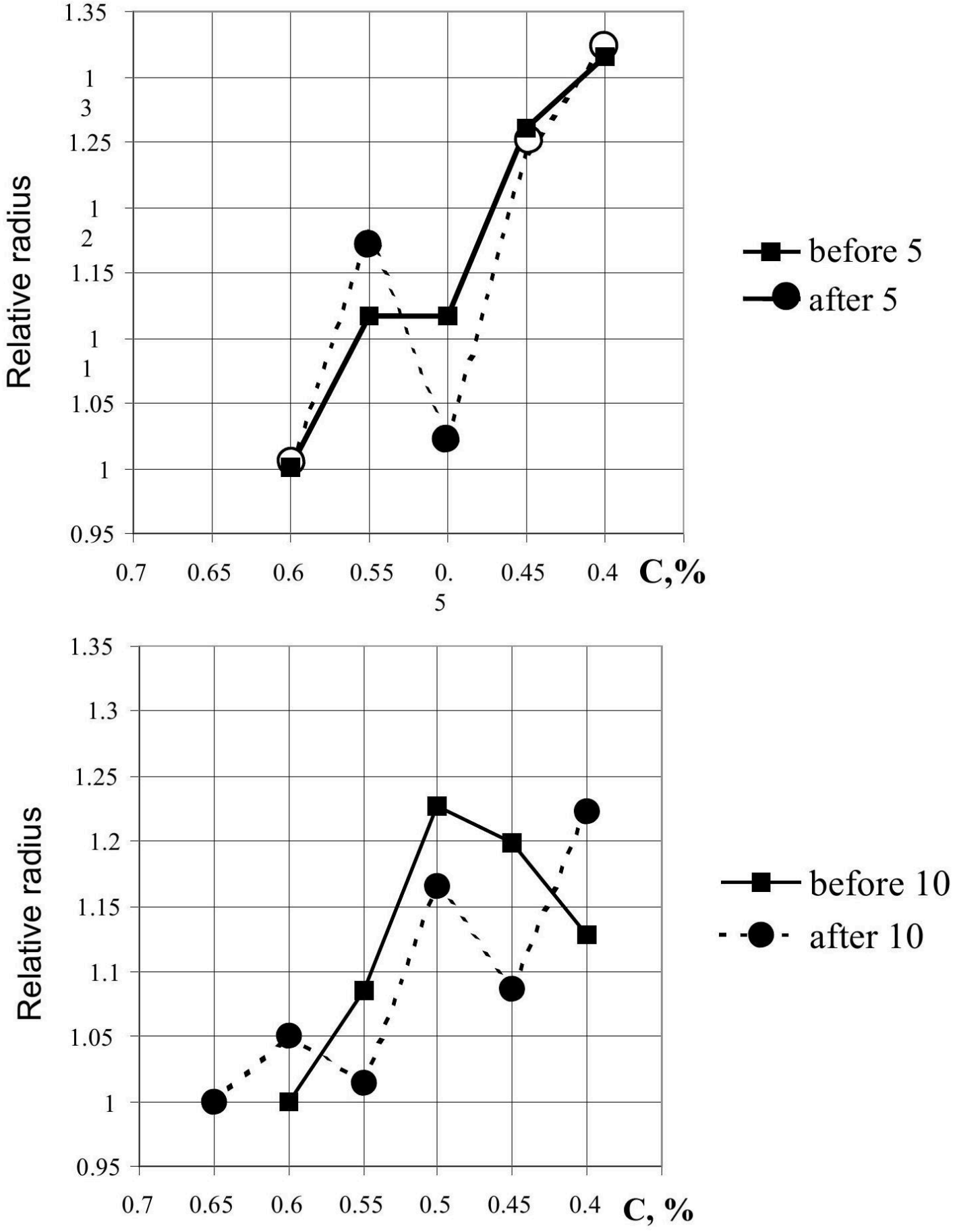


Fig. 8 **圖 8**
24 December 2004 Patient, male, 56 years old.

2004 年 12 月 24 日 病患，男性，56 歲。

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

多發性骨髓瘤，IIIA 期，貧血（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.

血液中總蛋白與旁蛋白水準偏高（分別為 130 與 $54\ g/l$ ）。病患已接受過數輪化療。

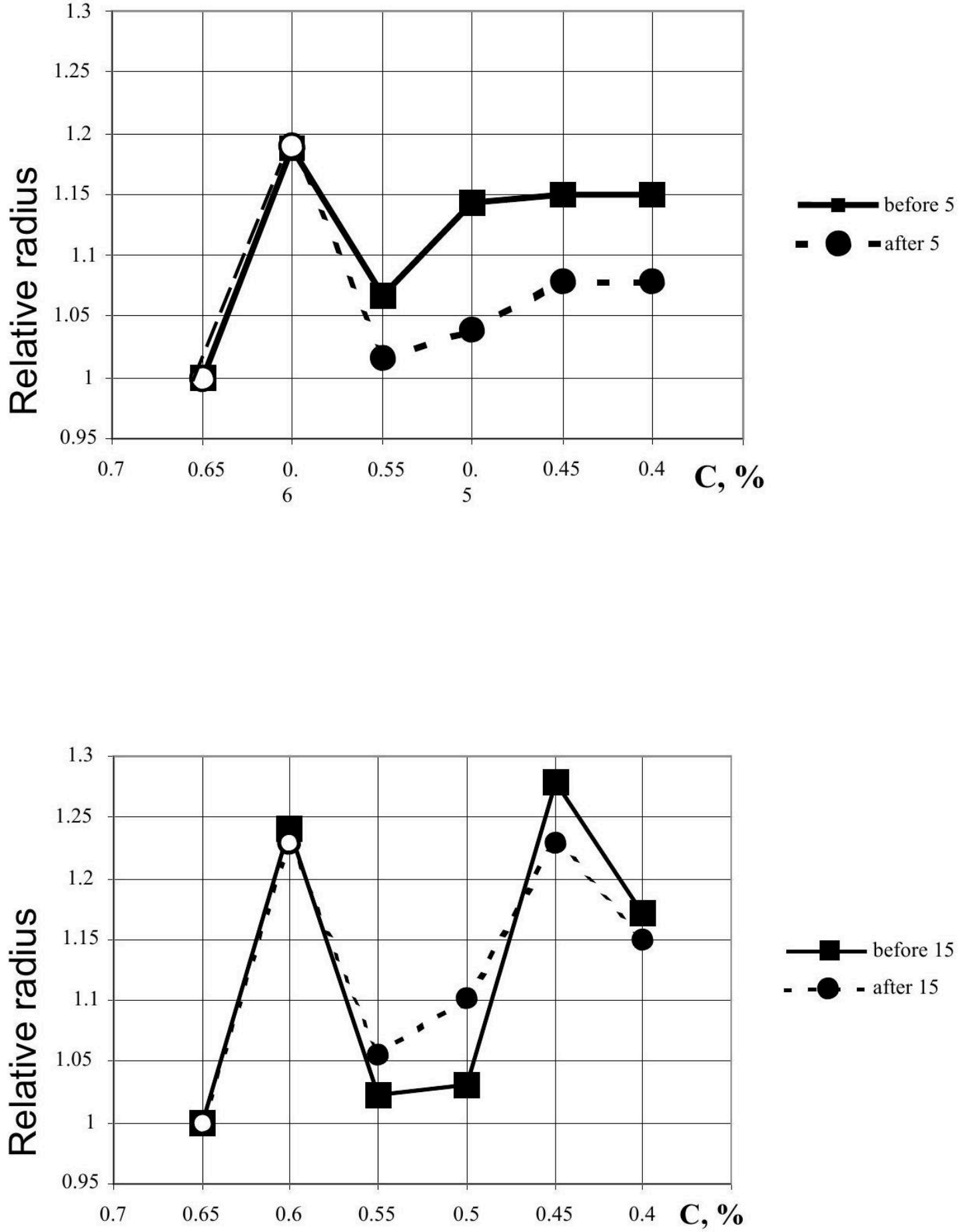


Fig. 9 **圖 9**
28 December 2004.Patient, male, 80 years old.

2004 年 12 月 28 日。病患，男性，80 歲。

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 (o).

新近收治病患，先前未接受治療。Bence Jones 型骨髓瘤，III B 期，血液中肌酸酐中度升高，尿中可檢出異常蛋白，但血液中未見（o）。

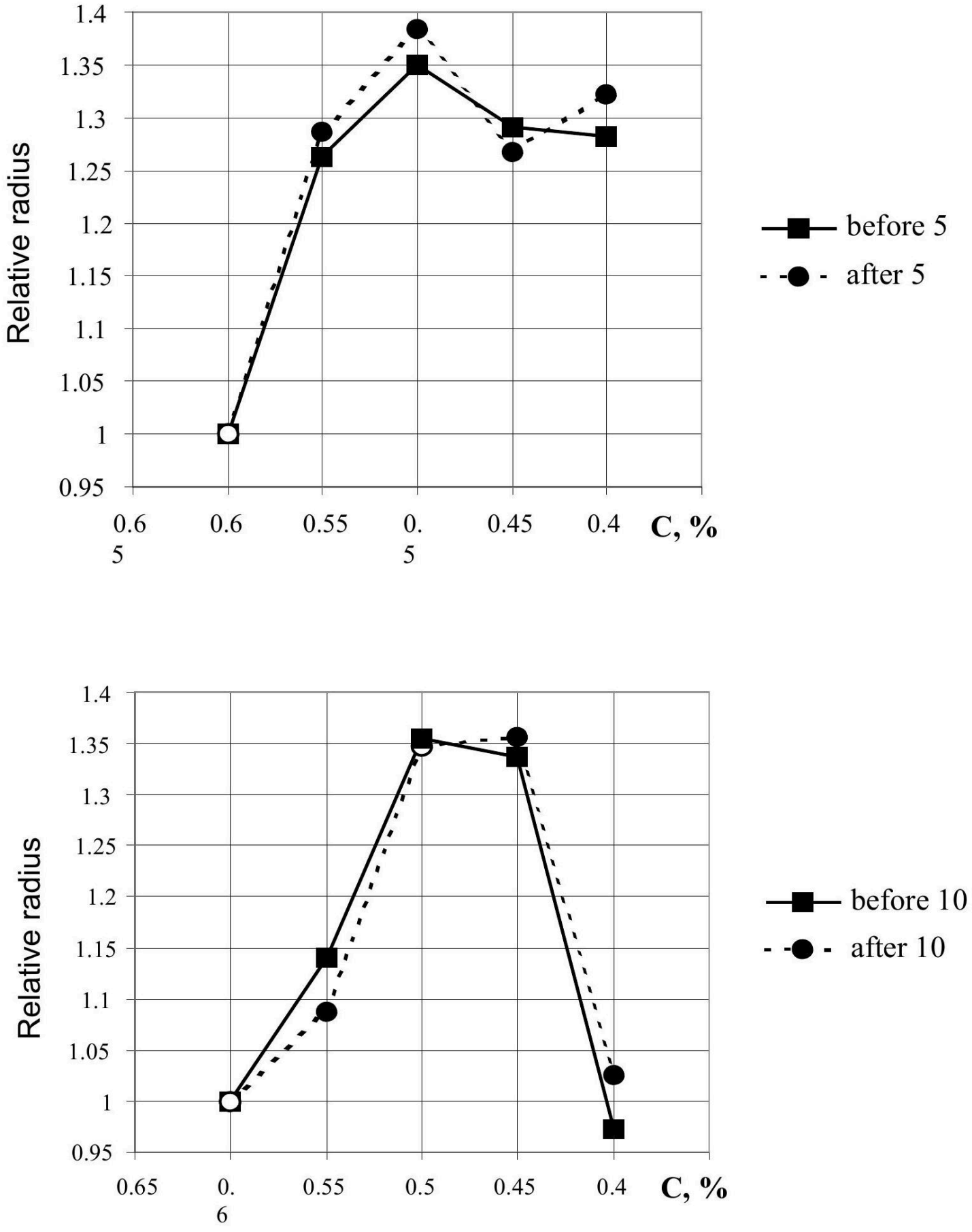


Fig. 10 **圖 10**

18 January 2005 Patient, female 81 years old.

2005 年 1 月 18 日 病患，女性，81 歲。

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).

A-骨髓瘤，第三期 A，剛入院患者，先前未接受治療。貧血，血中總蛋白與副蛋白水準偏高（分別為 108 與 41 g/l **）** **。**

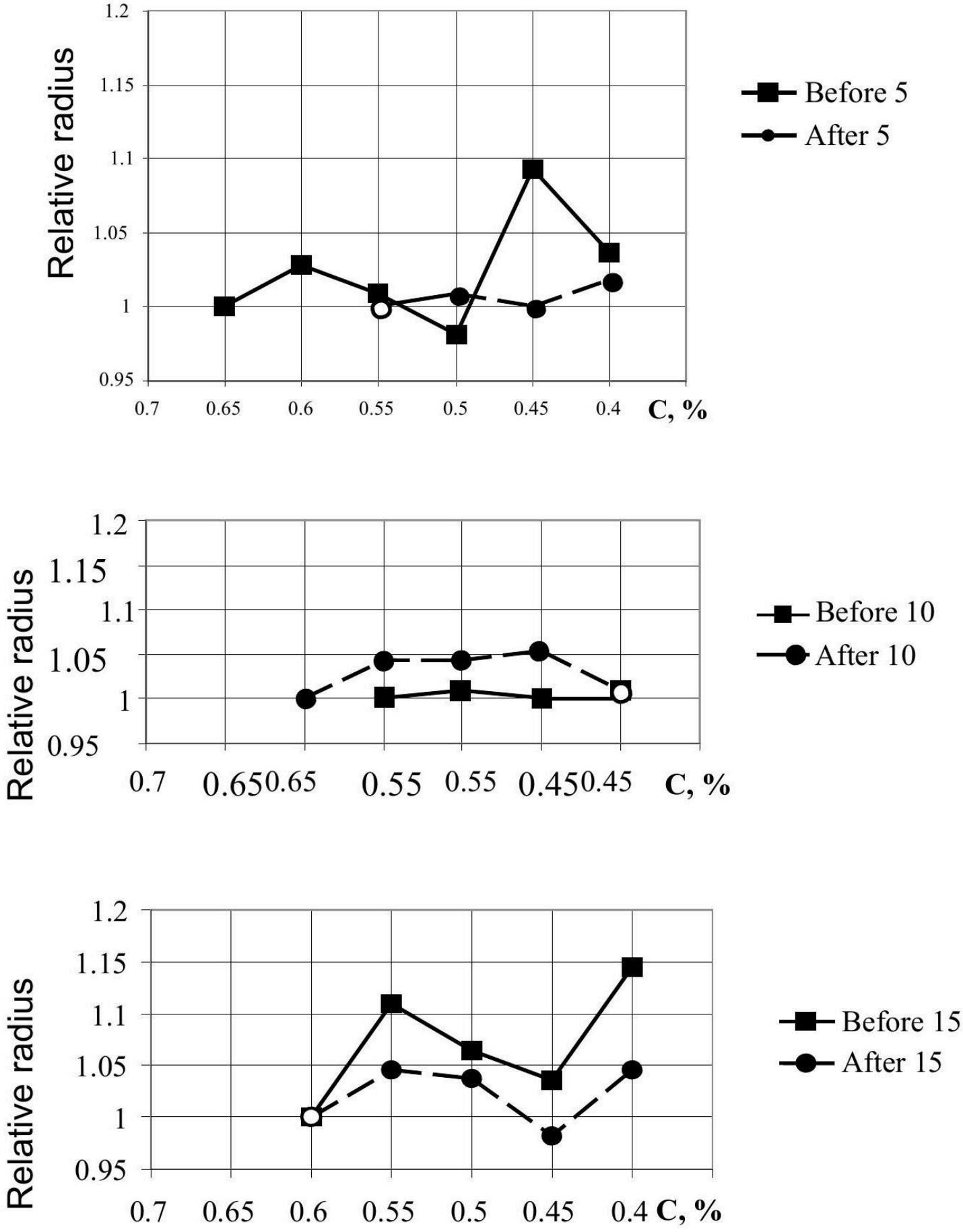


Fig. 11
21 January 2005. Patient, male, 56 years old.

2005 年 1 月 21 日。患者，男性，56 歲。

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.

曾接受多次化療，目前臨床血液學緩解。血中蛋白含量正常，未檢出副蛋白（o）。但血中纖維蛋白原濃度（與化療及細胞崩解相關）偏高，$5.5\ g/l$ 。高聚集。

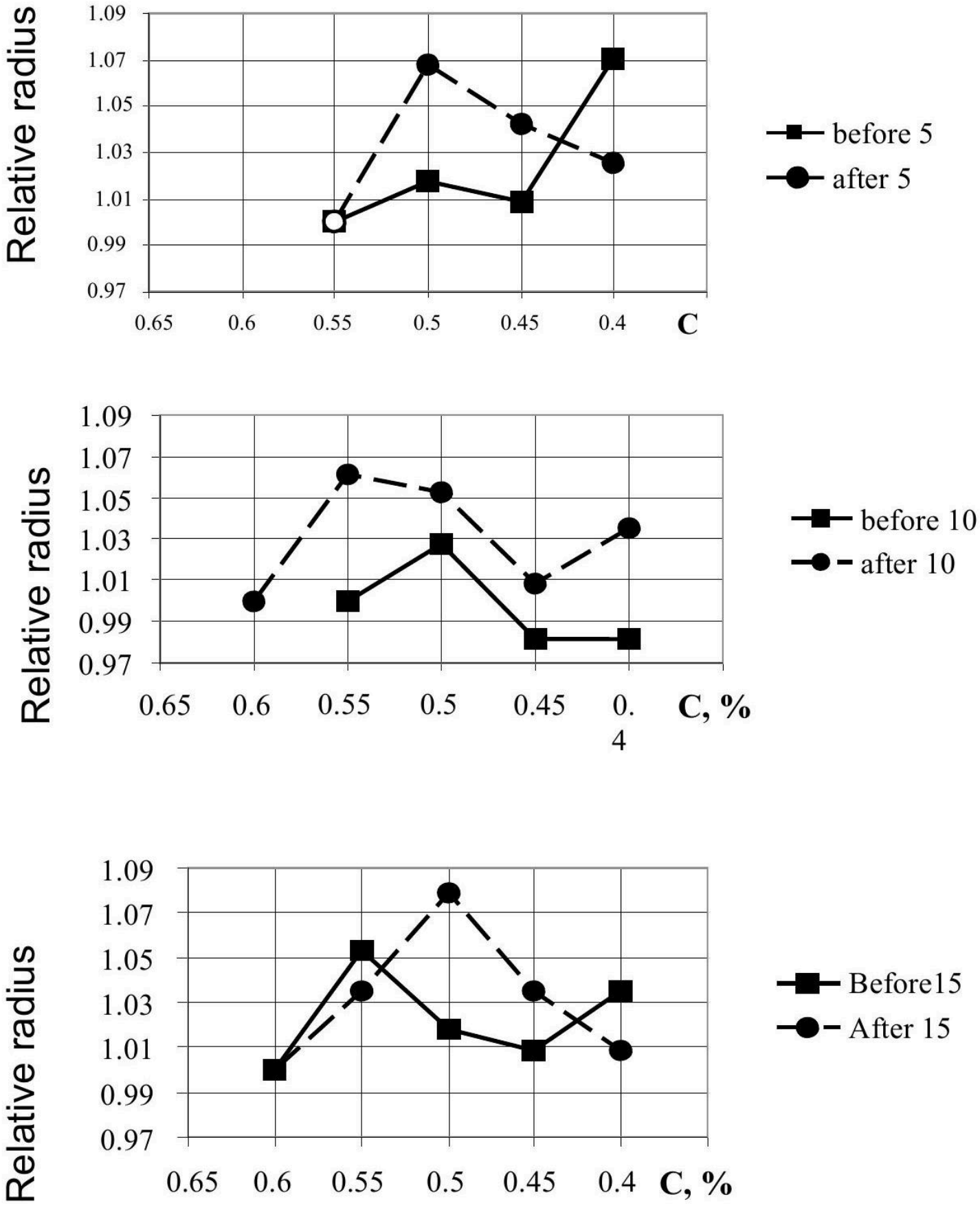


Fig. 12 **圖 12**

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

2005 年 1 月 25 日。患者，女性，69 歲。

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 ($>5\ g/l$).

已接受多次化學療法，目前復發，正在進行密集化療。血液中有高濃度的副蛋白（$48\ g/l$）、纖維蛋白原（$>5\ g/l$）。

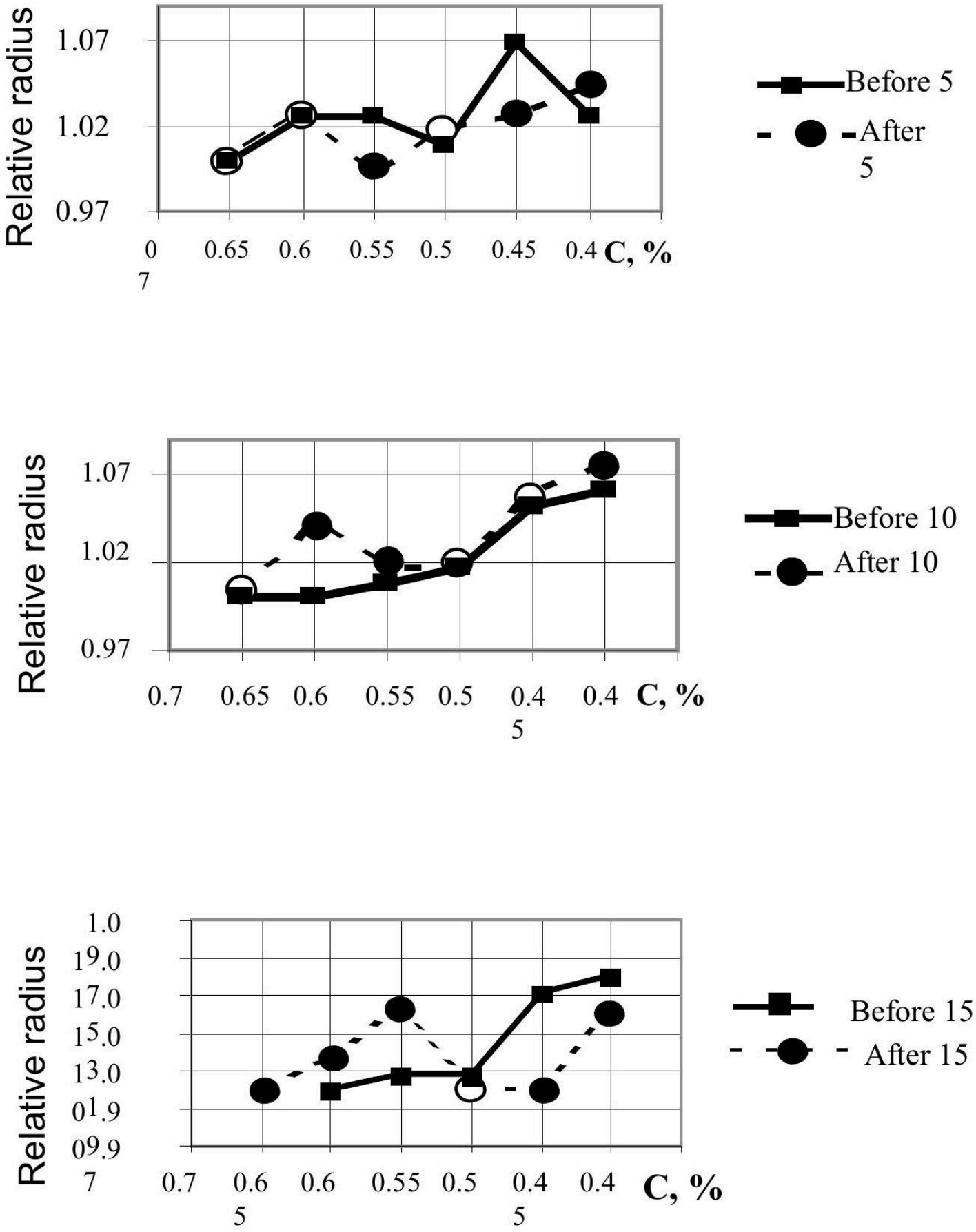


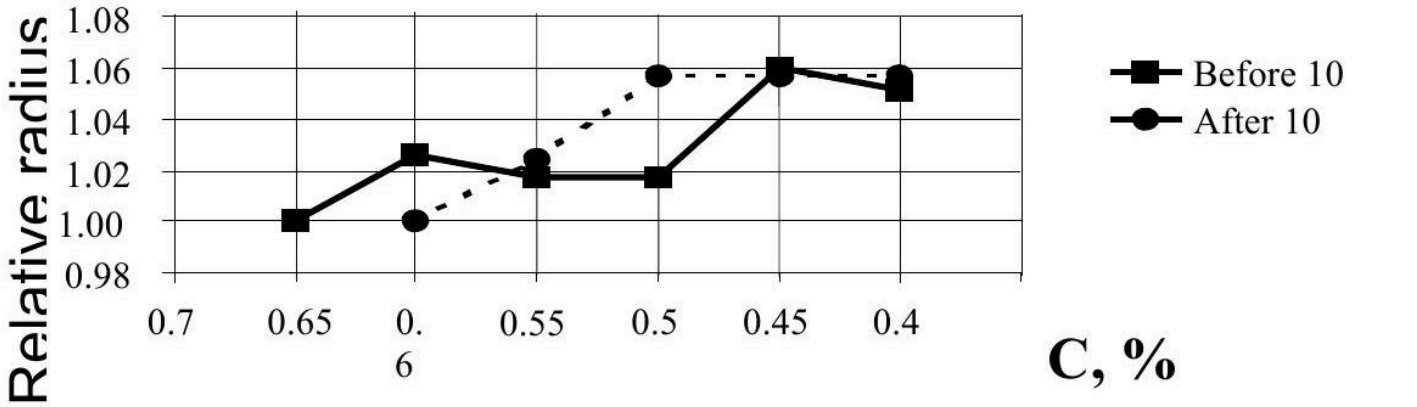
Fig. 13 **圖 13**

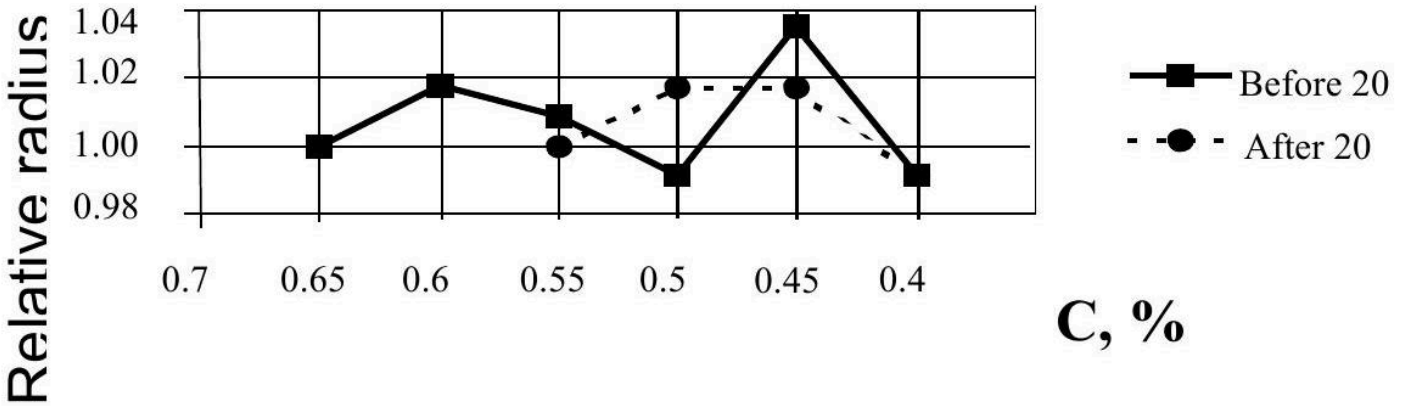
28 January. Patient, female, 76 years old.

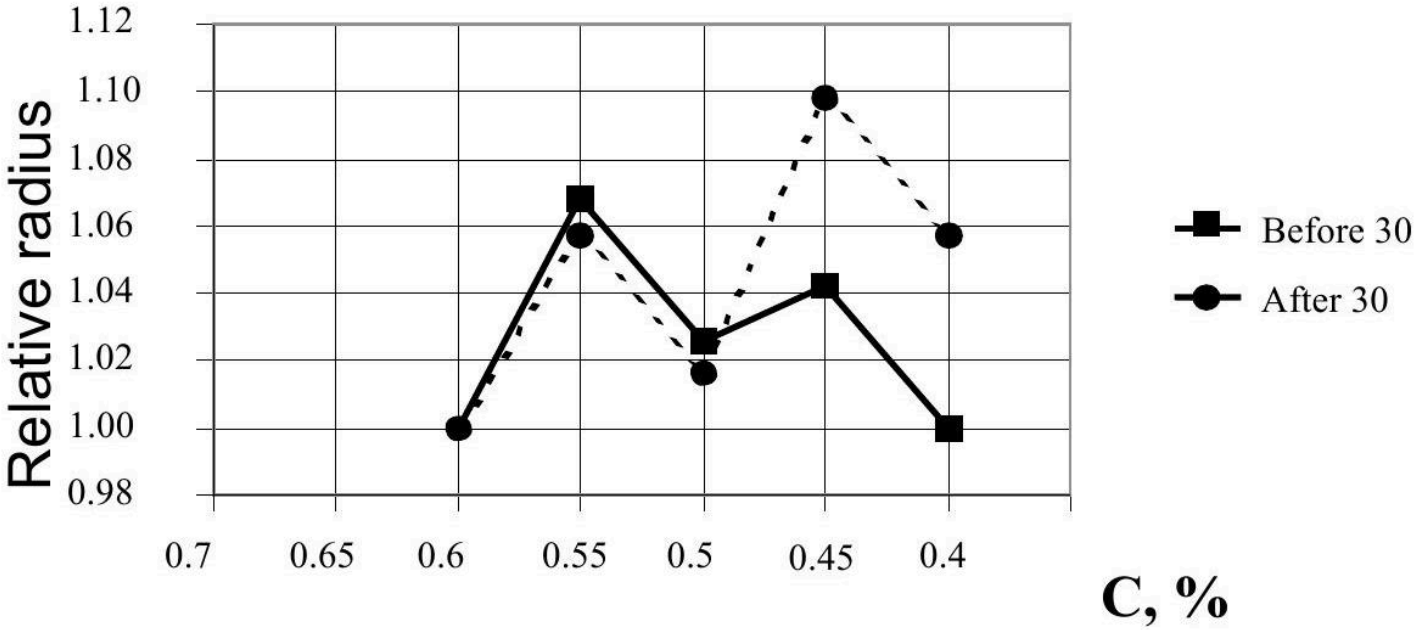
1 月 28 日。病患，女性，76 歲。

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).

新入院病患。先前未接受治療。A 型多發性骨髓瘤，II A 期，中度貧血，血液中總蛋白與旁蛋白濃度偏高（分別為 109 與 48 g/l）。







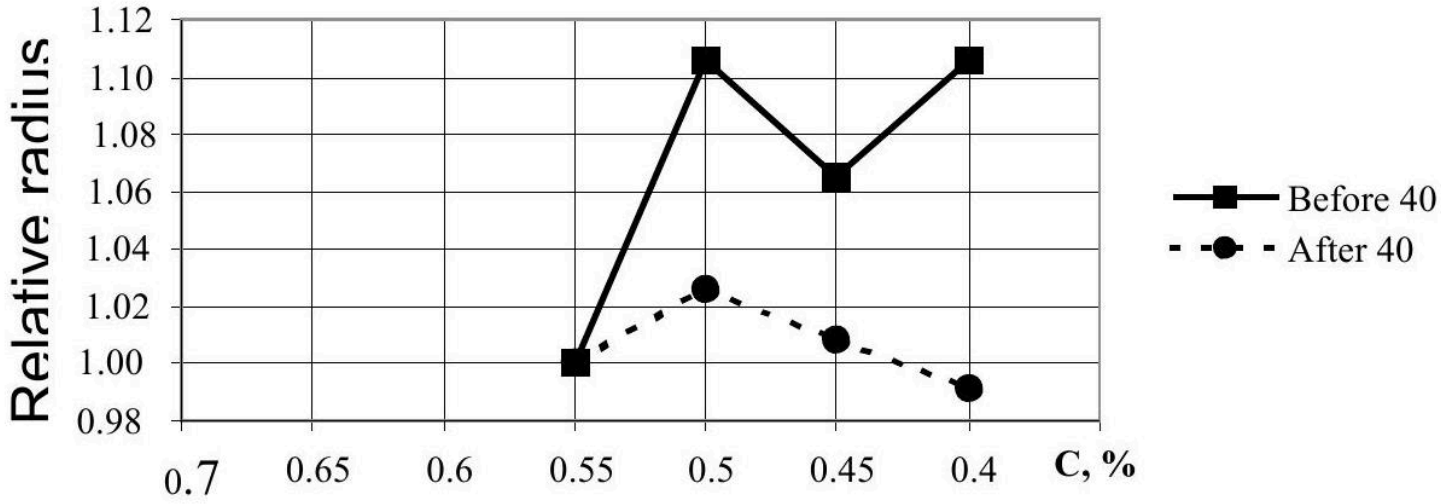


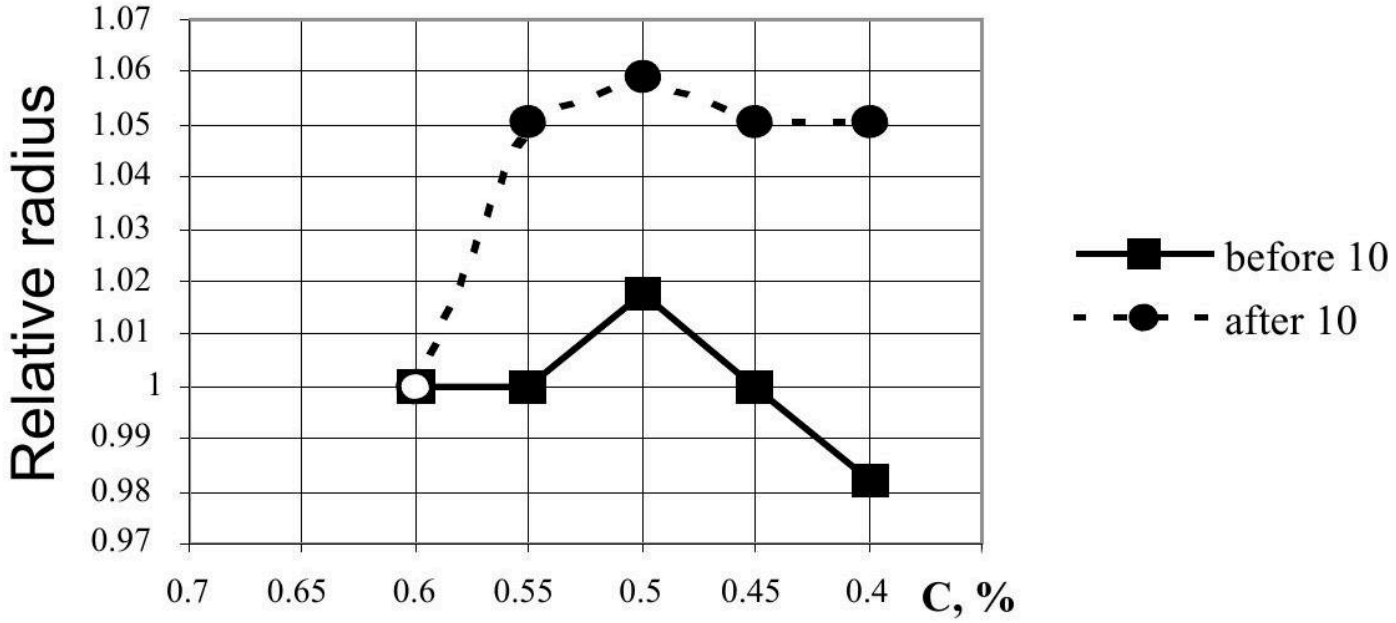
Fig. 14 **圖 14**

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

2005 年 2 月 4 日。病患，女性，66 歲。

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).

A-骨髓腫，多發局部型，第三期 A 級，曾多次治療，貧血（血紅素 $73\ g/l$），總蛋白及單克隆蛋白水準偏高（分別為 104.5 及 $50.5\ g/l$）。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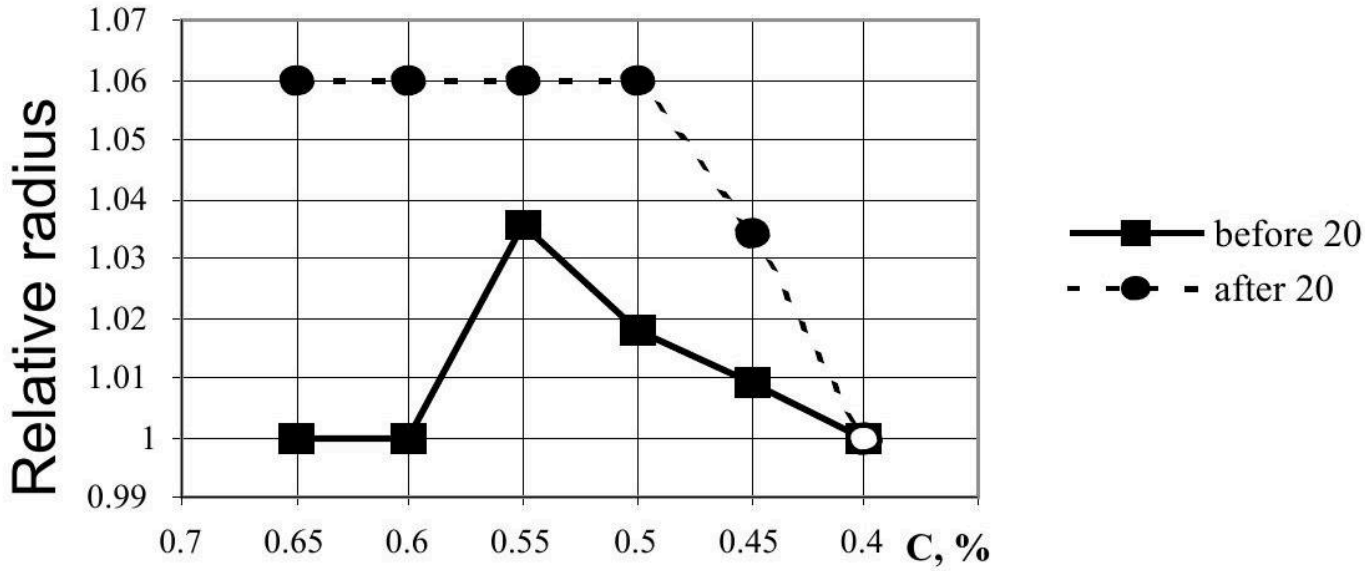
C, %
before 10
after 10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C, %
before 20
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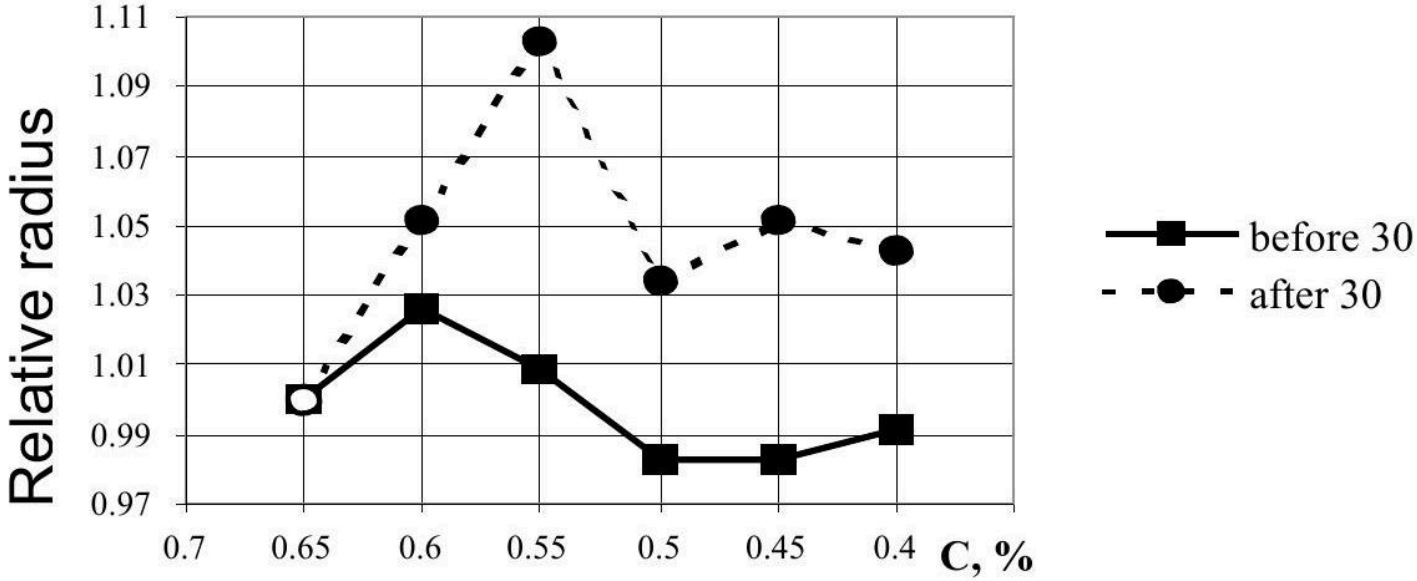
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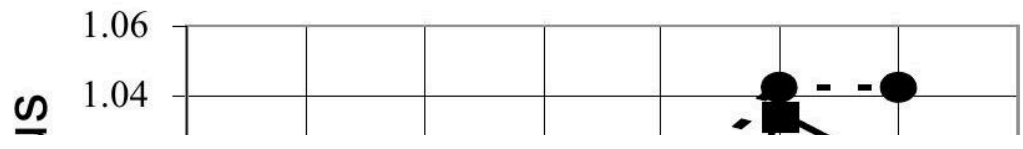
C, %
before 30
after 30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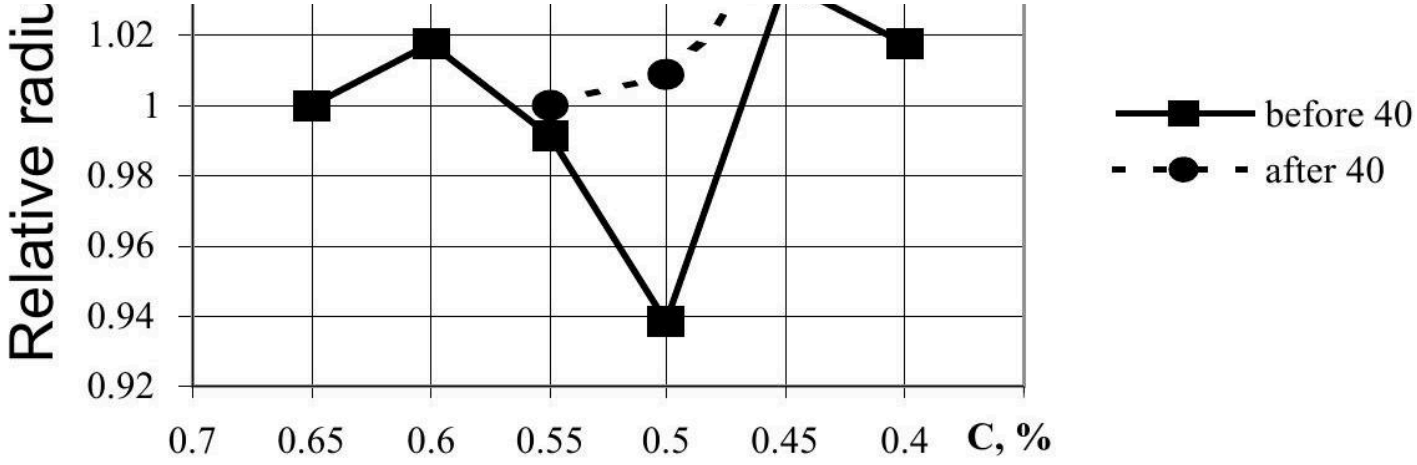


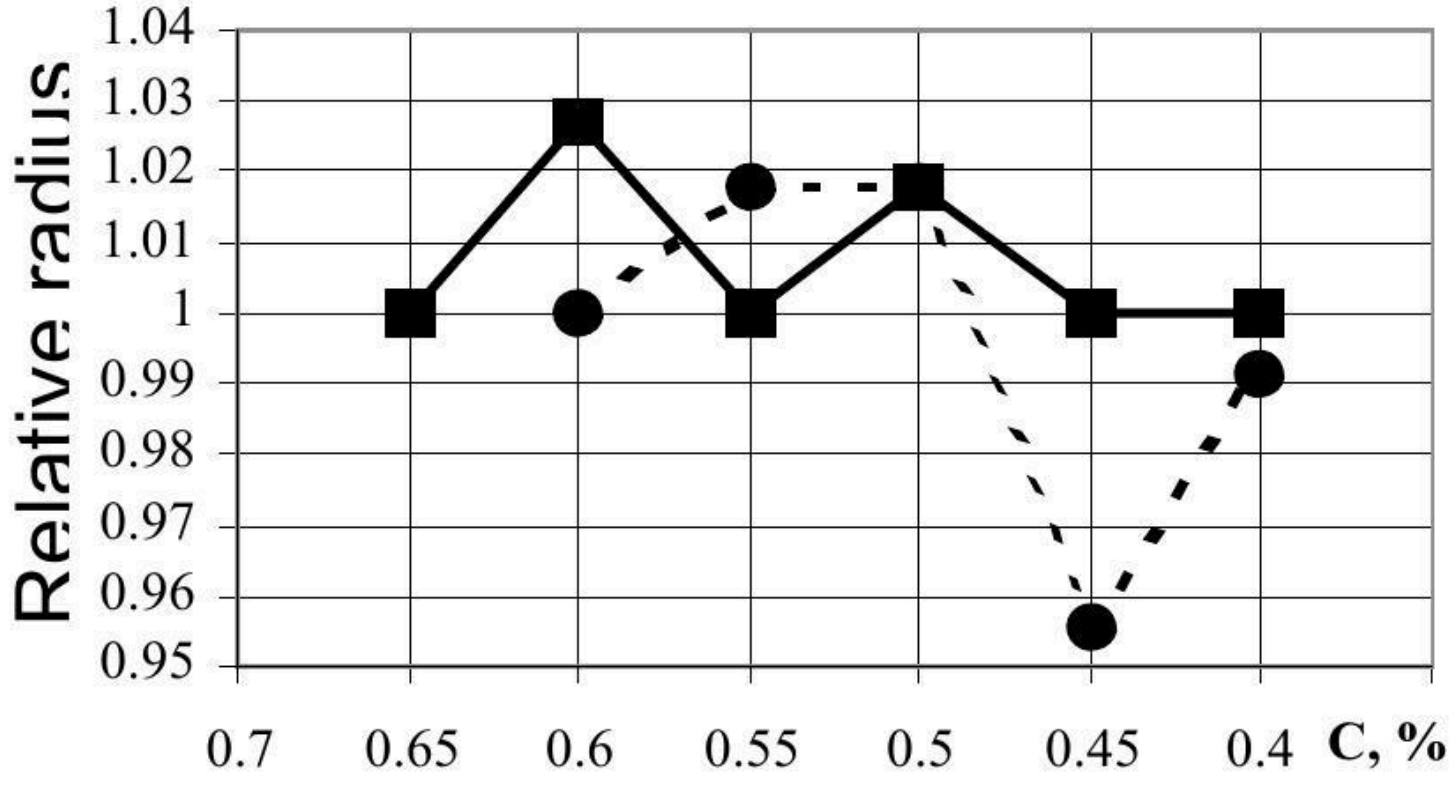
Fig. 15 **圖 15**
8 February 2005. Patient, male, 52 years old.

2005 年 2 月 8 日。病患，男性，52 歲。

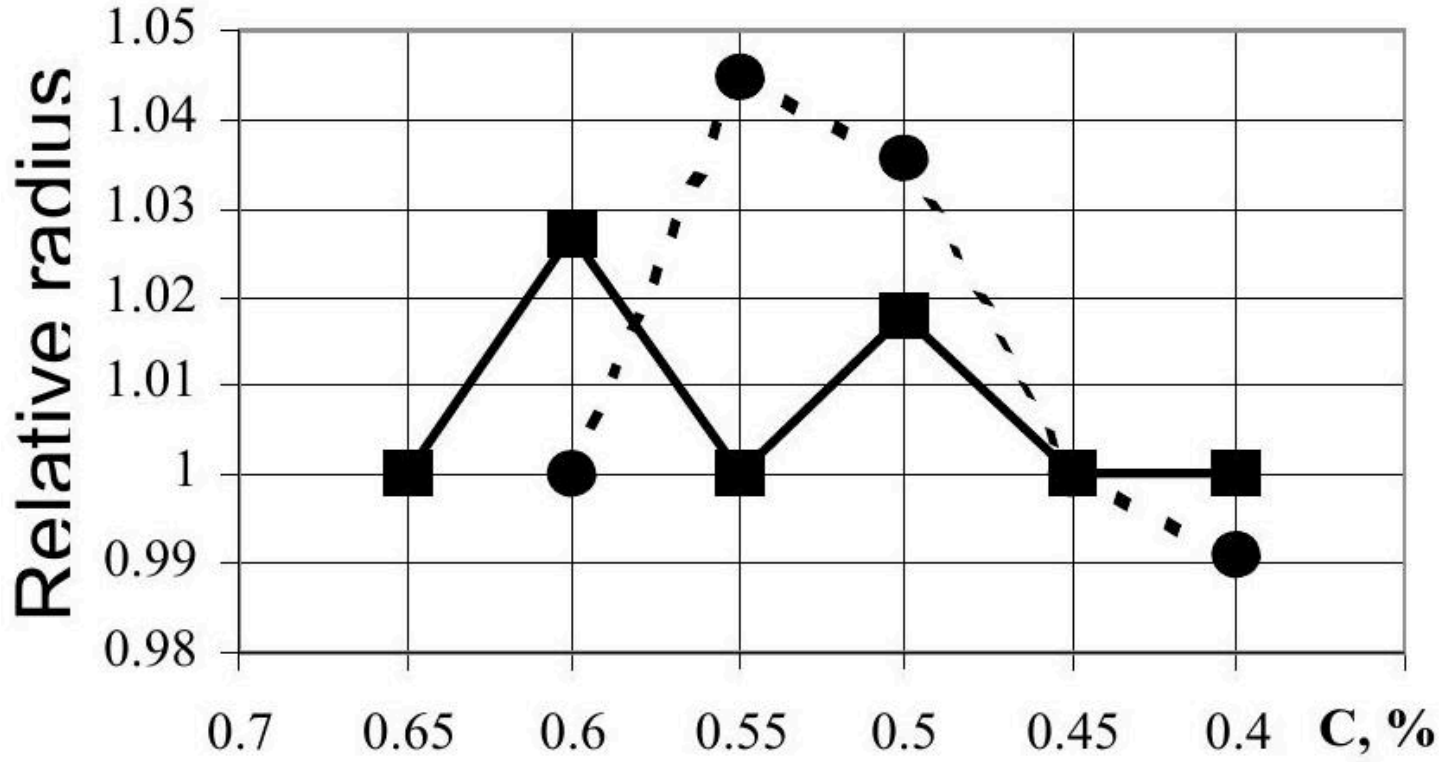
Bence Jones myeloma, multiple local form, stage 3B, relapse, for which the patient has already received 3 rounds of chemotherapy. Total protein 92, paraprotein 0 , elevated fibrinogen $3.4\ g/1$, and moderately elevated creatinine in the blood stream,

Bence Jones 型骨髓腫，多發局部型，第三期 B 級，復發，病人已接受 3 期化療。總蛋白 92，單克隆蛋白 0，纖維蛋白原升高 $3.4\ g/1$ **，血液中肌酐中度升高。**

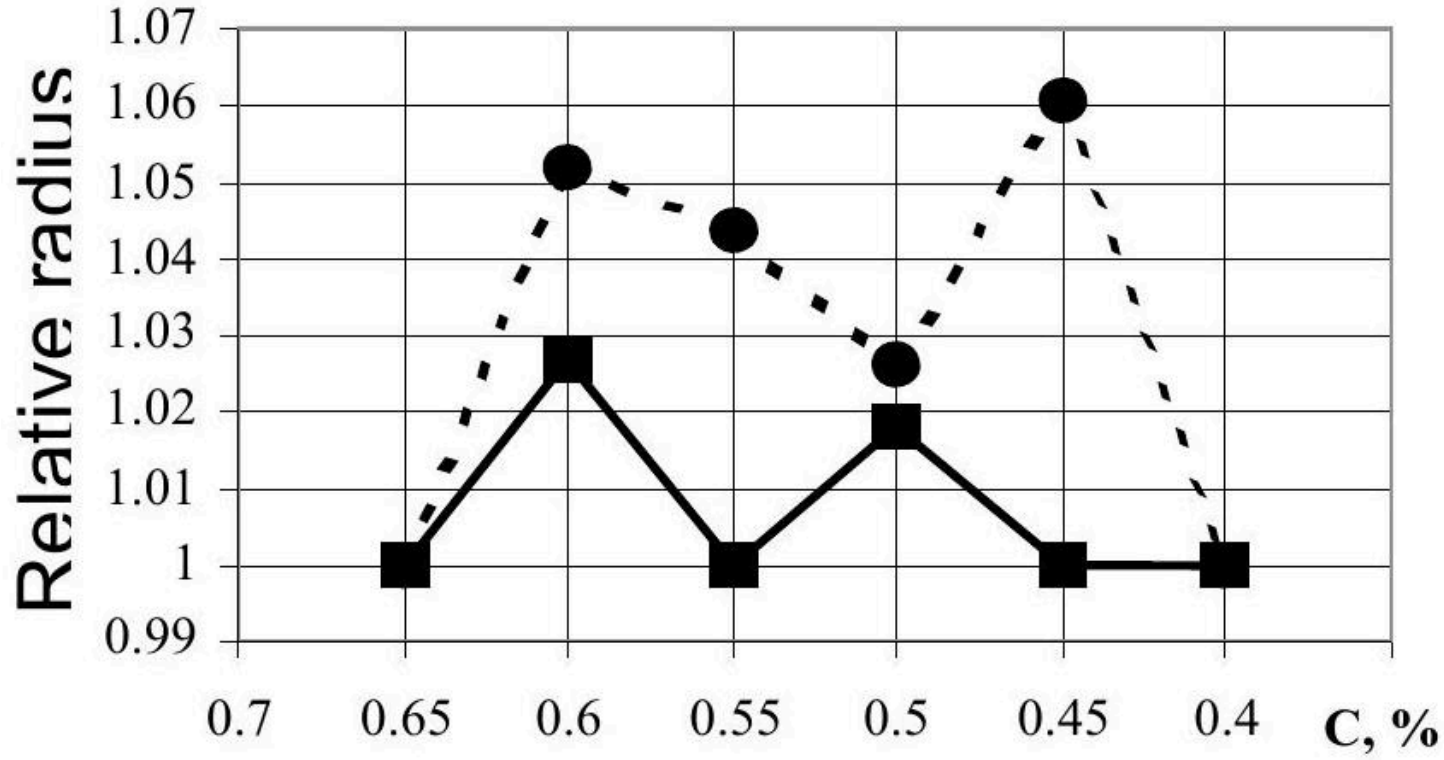
114 g/l.



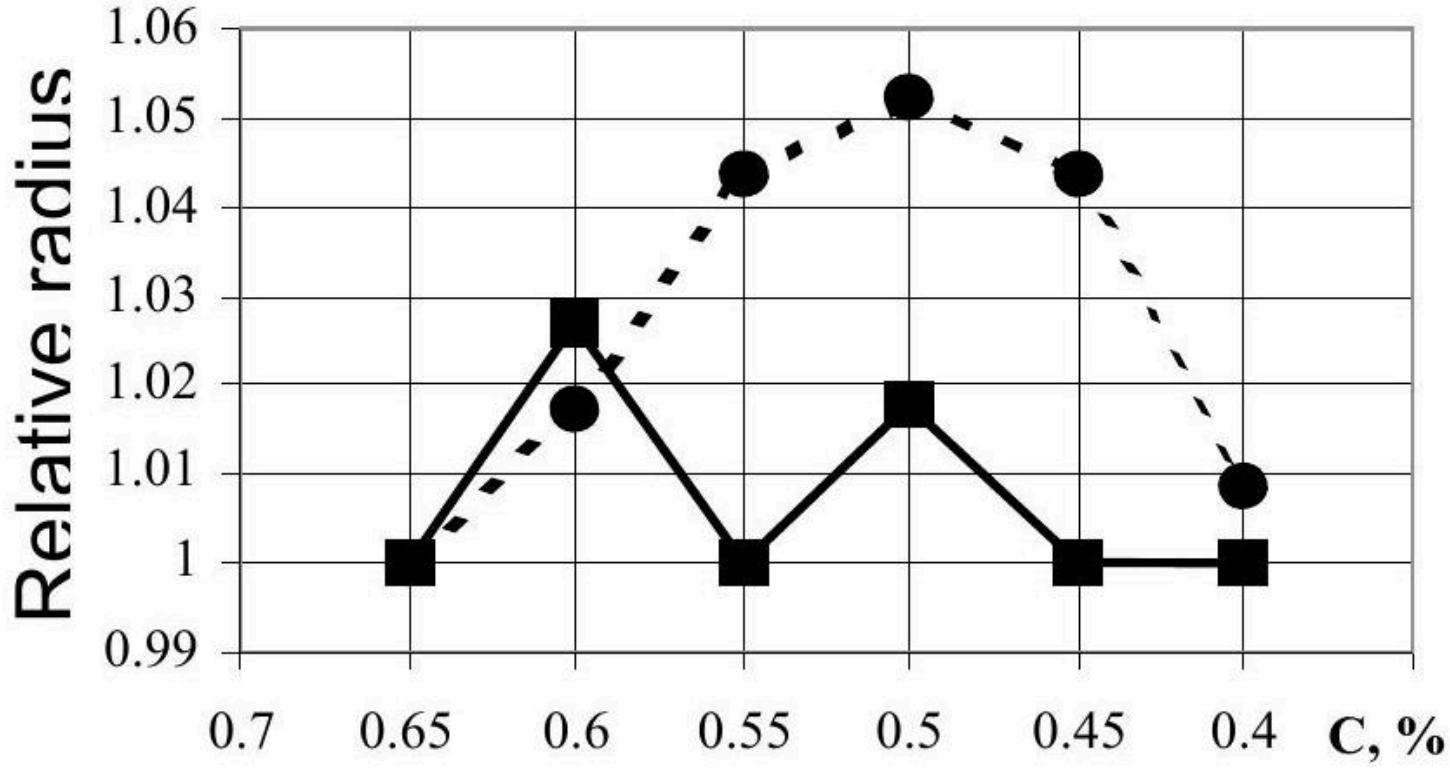
before **之前**



before 之前



before 之前



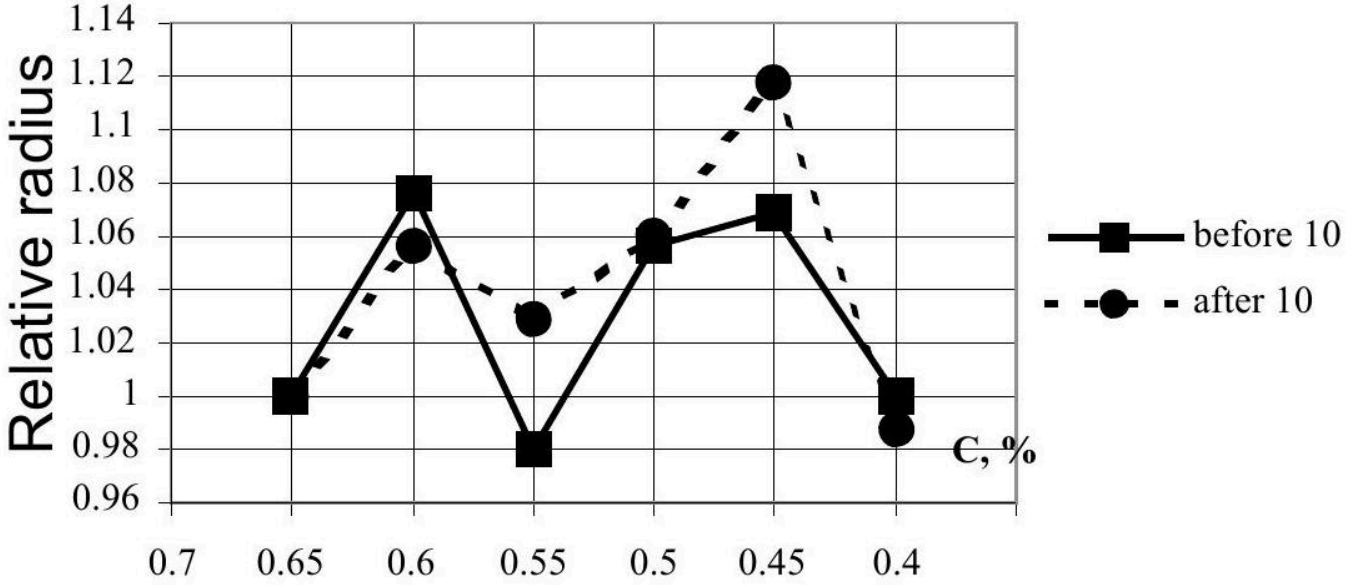
⟶ before ⟶ **之前**

Fig. 16 **圖 16**
11 February 2005. Patient, female, 73 years old.

2005 年 2 月 11 日。病患，女性，73 歲。

G myeloma, multiple local form, stage 3A. Serious patient, treated many times, anaemia (^{67} g/l), high total protein and paraprotein ($^{138.4}$ g/1 and ^{50} g/1, correspondingly).

G 型骨髓瘤，多發局限型，3A 期。病情嚴重，經多次治療，貧血（ ^{67} g/l **），總蛋白與副蛋白偏高（分別為** 138.4 g/1 **與** 50 g/1 **）。**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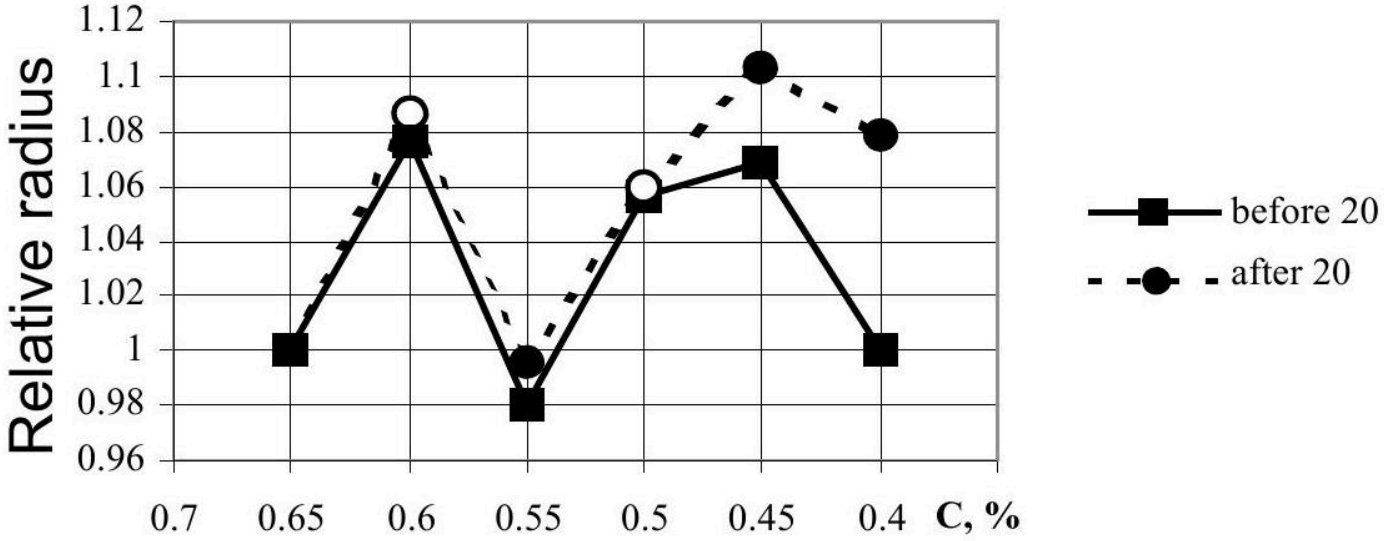
0.45
0.4
C, %
before 10
after 10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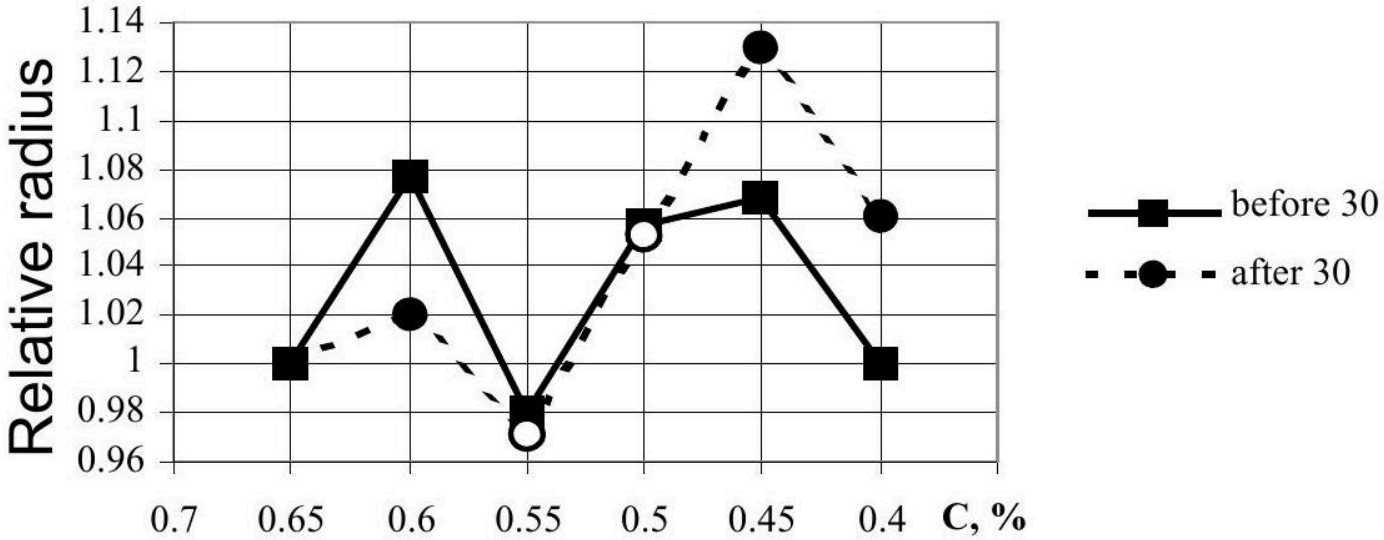
0.45
0.4
C, %
before 20
after 20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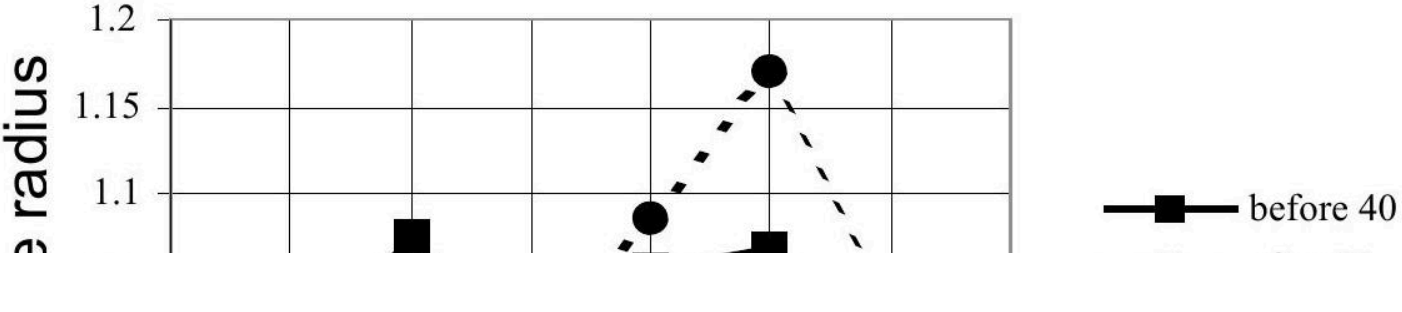
C, %
before 30
after 30


1.2
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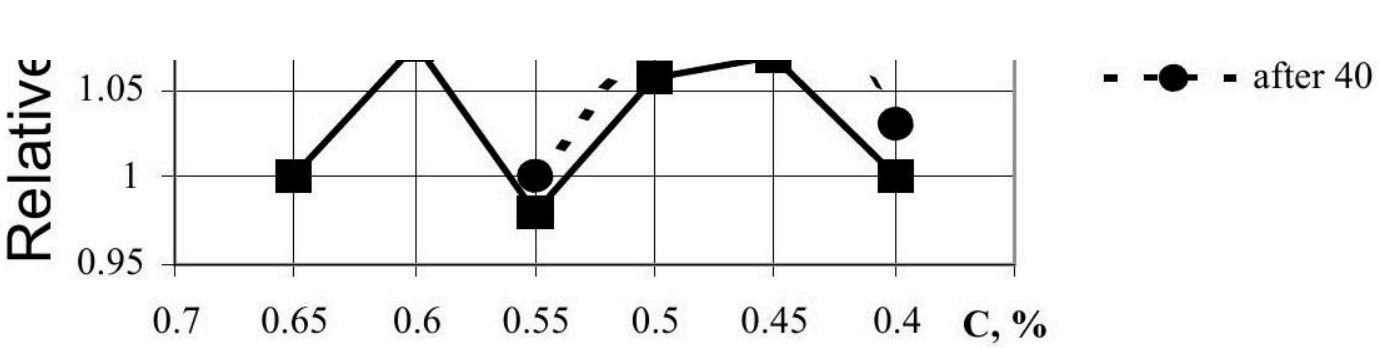

Fig. 17 **圖 17**

15 February 2005. Patient, male, 66 years old

2005 年 2 月 15 日。病患，男性，66 歲

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

不分泌型骨髓瘤，檢查無變化，副蛋白為 0。病程數年，經最近幾輪治療後，偵測到改善。