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

**AIRES 分形共振器對人體血液中紅血球狀態影響之研究**

Paper Supervisor 論文指導教授

V.A. Tarlykov

St. Petersburg 聖彼得堡

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## 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.

**本論文的目標是探討 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（標記為 Sh3\_16ort\_clon3），製作於**

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 矽板上。分形共振器的主要功能元件是一種圖案。拓撲圖案線的寬度為 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廣泛存在的血液系統惡性疾病，其發病率持續上升。此疾病以多樣的型態和變異、極為多變的症狀著稱，且其成因不僅是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酶。**

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 歲以下患者佔 2%至**

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

**3%，而 80 歲者的發病率是 50 歲者的 10 倍。中位生存期約為 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診所的資料，多發性骨髓瘤患者的平均壽命在過去 25 年中持續增加，現今約為 5 年[1]。**

## I. OSMOTIC FRAGILITY 一、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).

從流變學角度來看，血液可視為含有不同形狀、大小及性質顆粒的液態介質。血液中大多數的細胞是紅血球，因此它們在血液流變性質的變化中扮演主要角色。描述血液最重要性質的參數包括黏度、聚集性及紅血球變形能力（ED）。

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

由於對生物體內發生的變化高度敏感，紅血球成為評估生物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.

細胞的一項重要生命參數是其對細胞膜外部影響的重組能力，這些影響來自外部及內部環境。紅血球變形指數（ED）在某種程度上可反映血流中紅血球的活力。

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）可用作評估紅血球變形指數的工具。其中一種影響是紅血球的滲透性腫脹。滲透脆性指的是紅血球對低滲溶液溶血作用的抵抗程度。

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.

低滲腫脹曲線的關鍵參數包括最低點的座標——球形化點；以及腫脹過程中紅血球半徑相對變化的振幅。這兩個參數描述了紅血球膜的彈性特性及其變形能力。

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].

根據本研究，隨著多發性骨髓瘤患者接受治療，血液流變學參數的變化呈現出正向趨勢。比較患者治療前後紅血球滲透性腫脹曲線，發現其在質與量上均有所改變[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.

根據先前研究[2-6]，治療過程中的正向趨勢與球形化點向較小低滲腫脹值的移動相符：即紅血球在腫脹過程中半徑相對變異值的增加。

#### 1.1. Change of erythrocyte radius in hypoosmotic solutions

##### 1.1. 低滲溶液中紅血球半徑的變化

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

讓我們考察紅血球在不同滲透壓溶液（即不同 NaCl 鹽含量）中的行為模型。

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，紅血球在相互碰撞及與血管壁接觸時會呈現各種形狀。在等滲溶液中（含有 0.85% 的 NaCl，這是對生物體正常的濃度），若無外部機械衝擊，紅血球的平衡形狀為雙凹圓盤狀，意即紅血球為圓盤細胞。當未受壓力的雙凹圓盤細胞膨脹成球形時，細胞膜承受的伸展應變非常小，但表面曲率變化很大。紅血球圓盤的中央區域變形為球體的極區，膜的擴張極少。較大的擴張主要發生在雙凹圓盤赤道區的周邊區域。

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

隨著溶液滲透壓的變化，紅血球會發生以下轉變：

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

當滲透壓升高（高滲溶液）時，紅血球會縮小。

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，紅血球的體積因水分進入而分兩階段增加（見圖 1）：

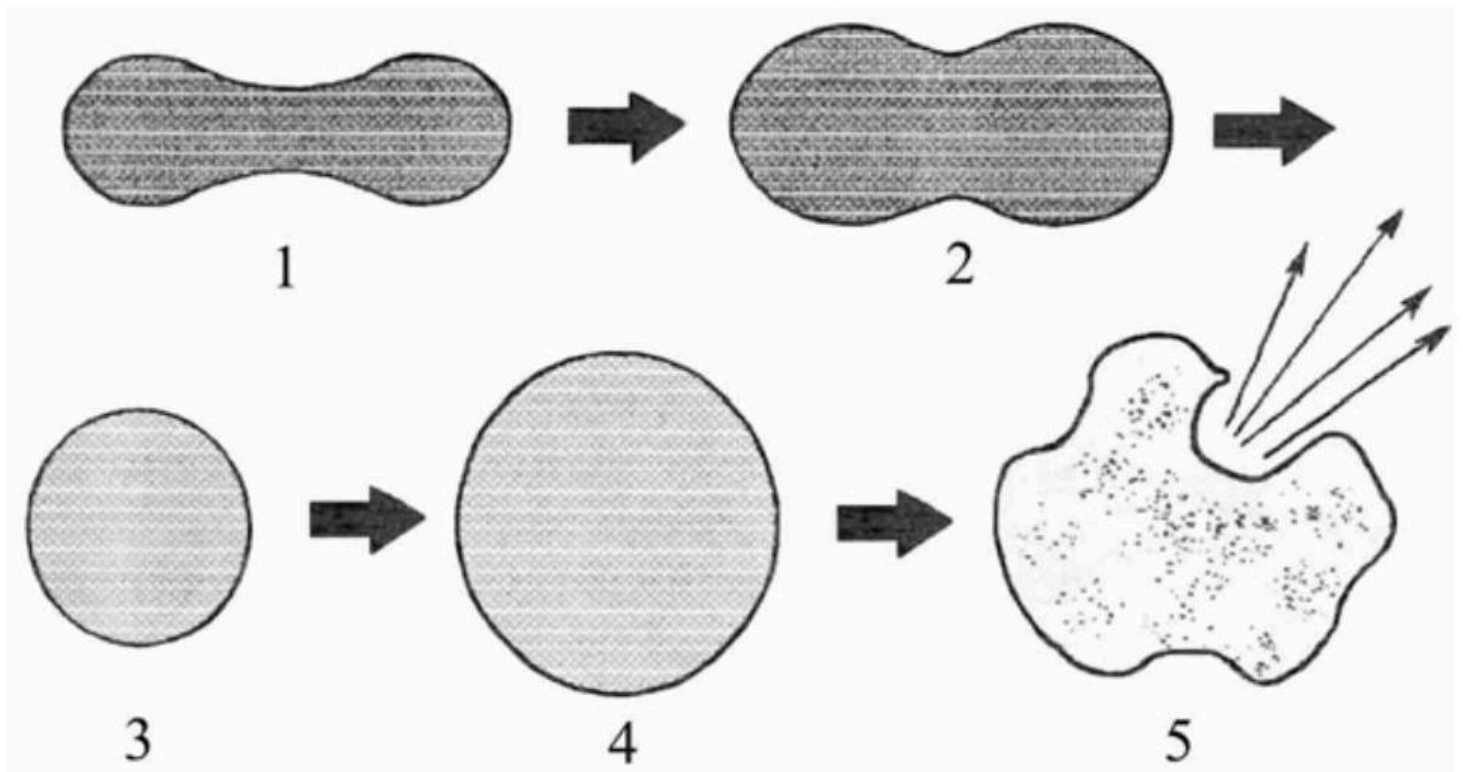


Fig. 1. Erythrocyte transformation in hypoosmotic swelling:

圖 1. 紅血球在低滲脹大過程中的轉變：

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

1 - 圓盤狀紅血球；2 - 圓盤狀紅血球膨脹；3 - 紅血球轉變為球狀紅血球；4 - 球狀紅血球膨脹；5 - 溶血

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

-a) 紅血球轉變為球體，膜表面積保持不變；

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) 紅血球球面積因細胞膨脹而增加，直到溶血（紅血球膜破裂）為止。

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  $\Delta V_0$  the

following equation should be true:

讓我們考慮紅血球在低滲脹中的行為[7]。在第一階段，細胞內壓力較低，可以幾乎無誤地說紅血球內外的滲透壓相同。基於上述情況，當在等滲環境中引入體積為  $\Delta V_0$  的蒸餾水以創造低滲條件時，以下方程式應成立：

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

where  $V_e$  is the erythrocyte volume in isotonic environments,  $V_0$  is the original volume of the isotonic environment,  $\Delta V_e, \Delta V_0$  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.

其中  $V_e$  為紅血球在等滲環境中的體積， $V_0$  為等滲環境的原始體積， $\Delta V_e, \Delta V_0$  分別為紅血球本身及外部環境的體積增量。因此，當引入蒸餾水時，紅血球體積的相對變化恰好與懸浮液體積的總相對變化相符。若懸浮液中含有多個紅血球，誤差也不大，情況同理。

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  $\mu\text{m}$ .

隨著引入懸浮液中的蒸餾水體積達到某一數值，紅血球形狀會變成球形。為建立紅血球在低滲溶液中的理論行為模型，取紅血球的平均直徑為 7.7 微米。

To determine the erythrocyte spherulation point on the theoretical

為了確定紅血球在理論上的球化點，...

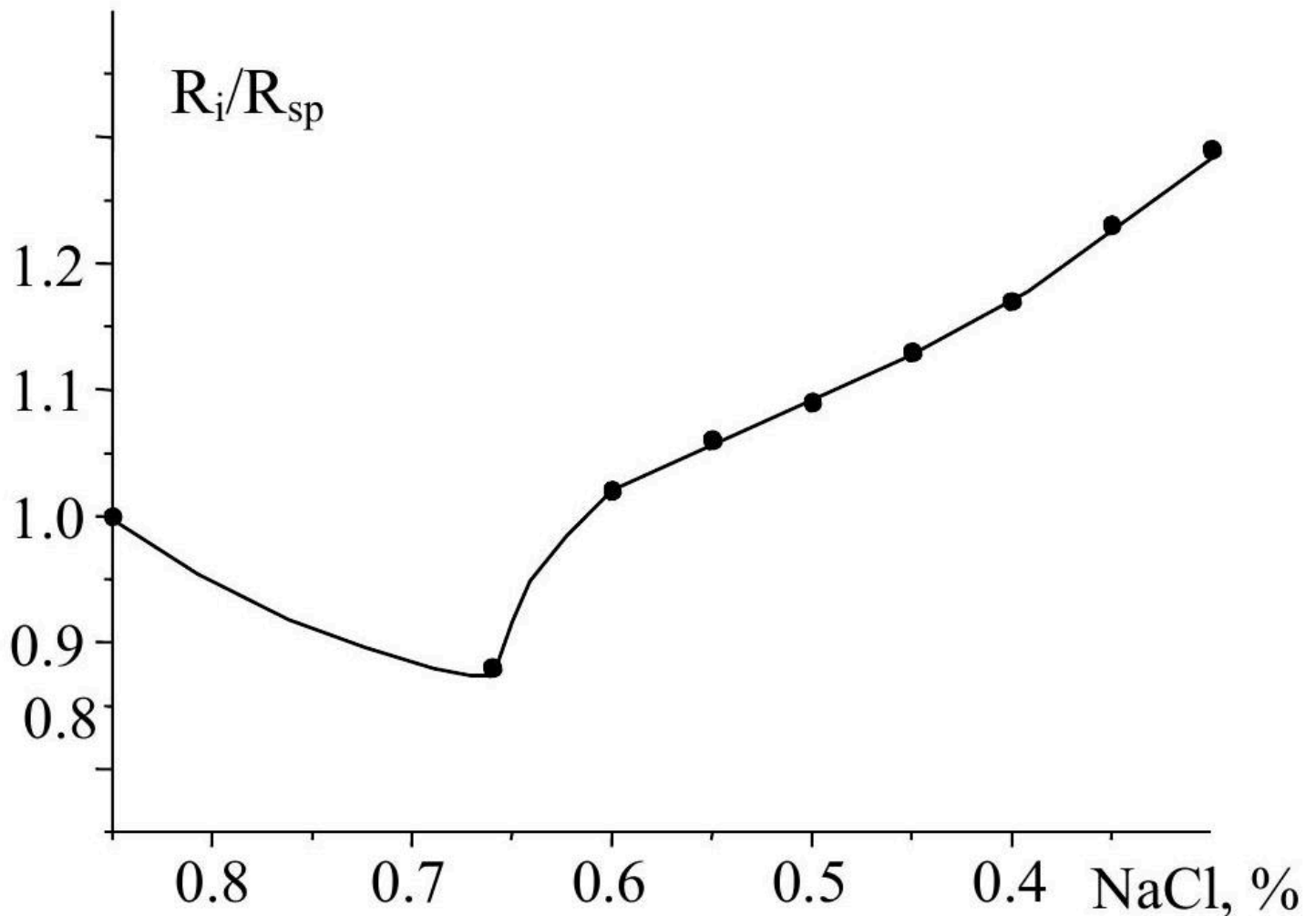


Fig. 2. Hypoosmotic curve of erythrocyte swelling:  $R_i$  - current erythrocyte radius,  $R_{sp}$  - erythrocyte radius corresponding to the spherulation point.

圖 2. 紅血球低滲脹曲線： $R_i$  - 當前紅血球半徑， $R_{sp}$  - 對應球形化點的紅血球半徑。

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，我們將紅血球體積的相對變化等同於懸浮液體積的相對變化，從而找出球形化點。

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

紅血球半徑對 NaCl 含量的依賴性呈現一個極小值，該極小值與球形化點相符（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.

為了進行血液檢測，必須使血液穩定，也就是要防止血液在 3 到 6 分鐘內自然凝固。靜脈血通常使用 3.8% 濃度的檸檬酸鈉溶液進行穩定處理，9 : 1。為了避免血漿干擾紅血球的檢測，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.

血液是從人體的正中肘靜脈以注射針抽取至乾燥的離心管中，抽取時第一滴血會滴在棉球上，以防止穿刺瞬間逸出的組織因子進入試管。管中加入 0.2 毫升的檸檬酸鈉以避免血液凝固。

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

為了避免紅血球暴露於血漿中的物質，血液經過離心處理。

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.

所得的沉澱物被置於含約 0.85% 濃度 NaCl 的生理鹽水中，並在相同條件下再次離心，以更徹底地清洗紅血球上的血漿成分。

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

通常使用約含有 0.85%NaCl 的生理食鹽水來處理洗滌過的紅血球，這會在細胞上產生約等同於血漿所造成的滲透壓（等滲溶液）。

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

血漿中  $\text{Na}^+$  是細胞外空間中主要的滲透活性離子。血液中鈉離子的濃度約為紅血球中 (  $17 - 20 \text{ mole/m}^3$  ) 的 8 倍 (  $132 - 150 \text{ mole/m}^3$  )。因此，當溶液中的 NaCl 含量降低時，NaCl 與細胞之間會產生濃度梯度，水分開始穿透膜進入紅血球。

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  $\text{Na}_2\text{HPO}_4$  in combination with  $\text{NaH}_2\text{PO}_4$  in the final concentration of 0.01 M with  $\text{pH} = 7.4$ , 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 pH 非常不穩定。因此，當必須在特定 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含有  $\text{Na}_2\text{HPO}_4$  且最終濃度為 0.01 M 的  $\text{NaH}_2\text{PO}_4$  與  $\text{pH} = 7.4$ ，以更精確測量紅血球大小與溶液滲透壓的依賴關係。



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

洗滌過的紅血球被置於低滲溶液中（濃度低於 0.85% ），該溶液是由原始等滲溶液稀釋而成。

## II. LASER DIFFRACTOMETRY METHOD

### II. 雷射繞射法

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.

雷射繞射法是基於雷射輻射在單一及多重生物微小物體上的繞射現象，其特點為高精度、高靈敏度、速度快，對測試物體影響最小，且能同時記錄大量微小顆粒。繞射圖樣的參數與微小物體的參數有明確對應關係，因此可用以判定其大小、形狀及內部結構。

With diffraction on an aggregate of erythrocytes, the diffraction

當雷射繞射作用於紅血球聚集體時，繞射

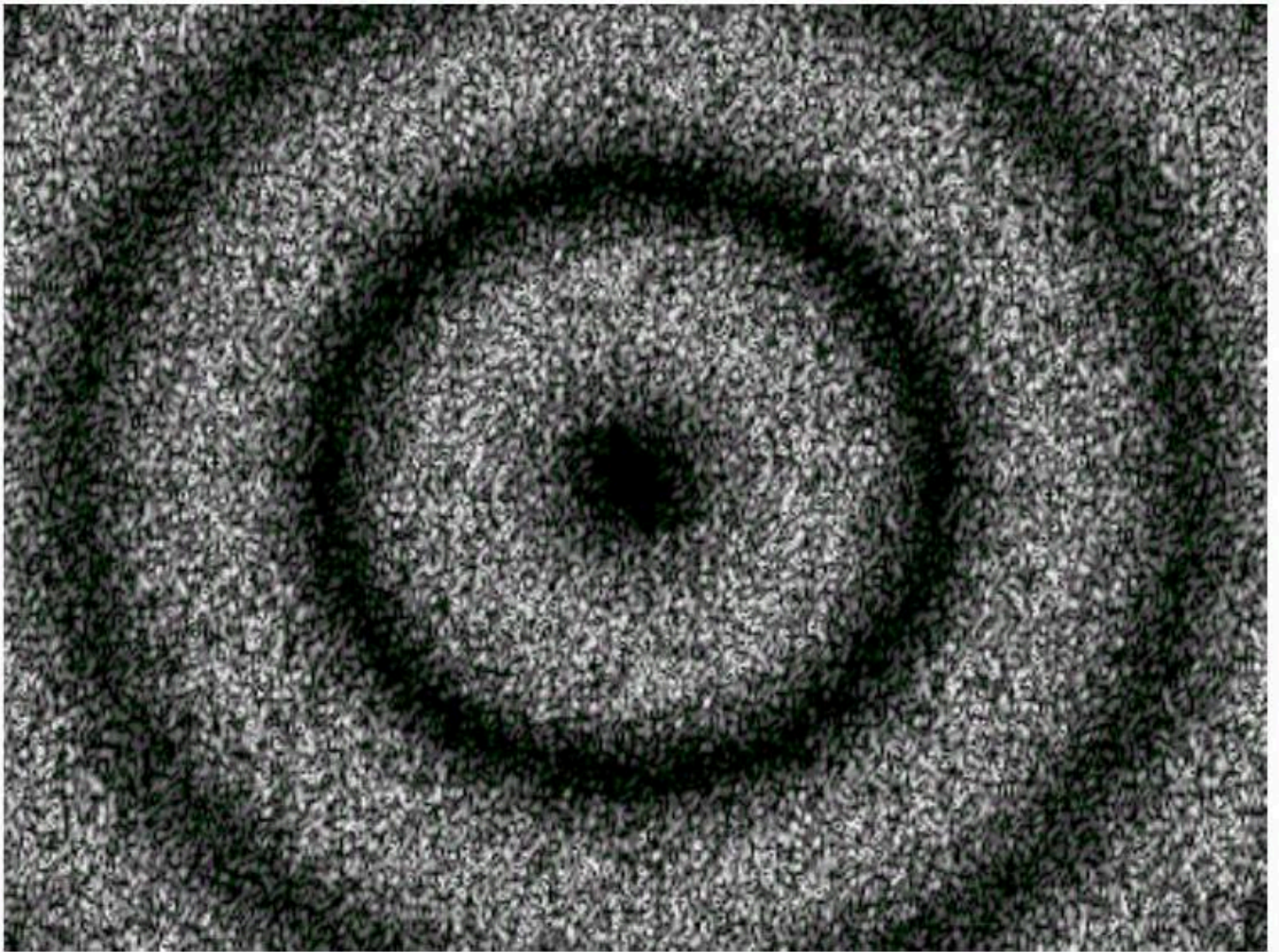


Fig. 3. Distribution of intensity in a diffraction pattern

### 圖 3. 繞射圖樣中強度的分布

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

該圖案看起來像是一組同心圓環系統（圖 3）。圓環之間的距離

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:

與表徵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  $\Delta D$  and  $\Delta l$  are changes in the average erythrocyte size and the diffraction ring diameter, correspondingly.

其中  $\Delta D$  和  $\Delta l$  分別是平均紅血球大小和繞射環直徑的變化。

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).

( $\lambda = 0.63\text{mcm}$ ) ；用於測量雷射功率濃度的光學衰減器；樣品台；傅立葉轉換用透鏡；光電偵測器；個人電腦（圖 4）。

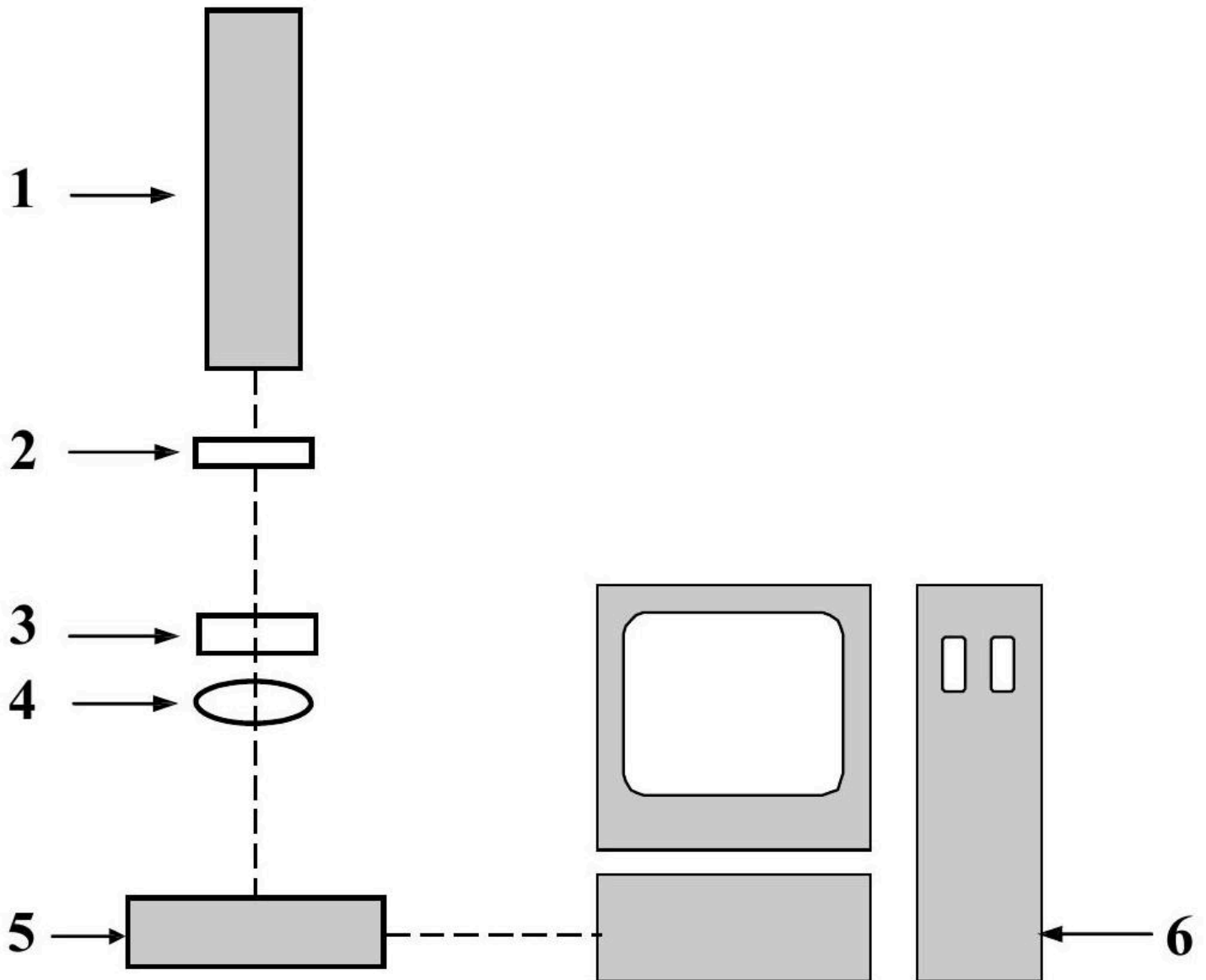


Fig. 4. Experimental assembly layout: 1— He-Ne-laser ( $\lambda = 0.63\text{mcm}$ ), 2 - optical attenuator, 3 - sample, 4-lens, 5- photodetector, 6-PC

圖 4. 實驗組裝佈局：1— He-Ne 雷射 ( $\lambda = 0.63\text{mcm}$ )，2 - 光學衰減器，3 - 樣品，4 - 透鏡，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

為了利用雷射繞射法研究懸浮於不同滲透壓溶液中的紅血球，樣品應準備成層厚度較薄，以確保單次散射（圖 5）。處理後的樣品中紅血球應均勻分布於 Goryaev 室表面，且不得重疊（紅血球必須形成單層），這使我們能忽略

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

重疊區域的再輻射輸入，且濃度應足夠以觀察高強度的細胞變形。

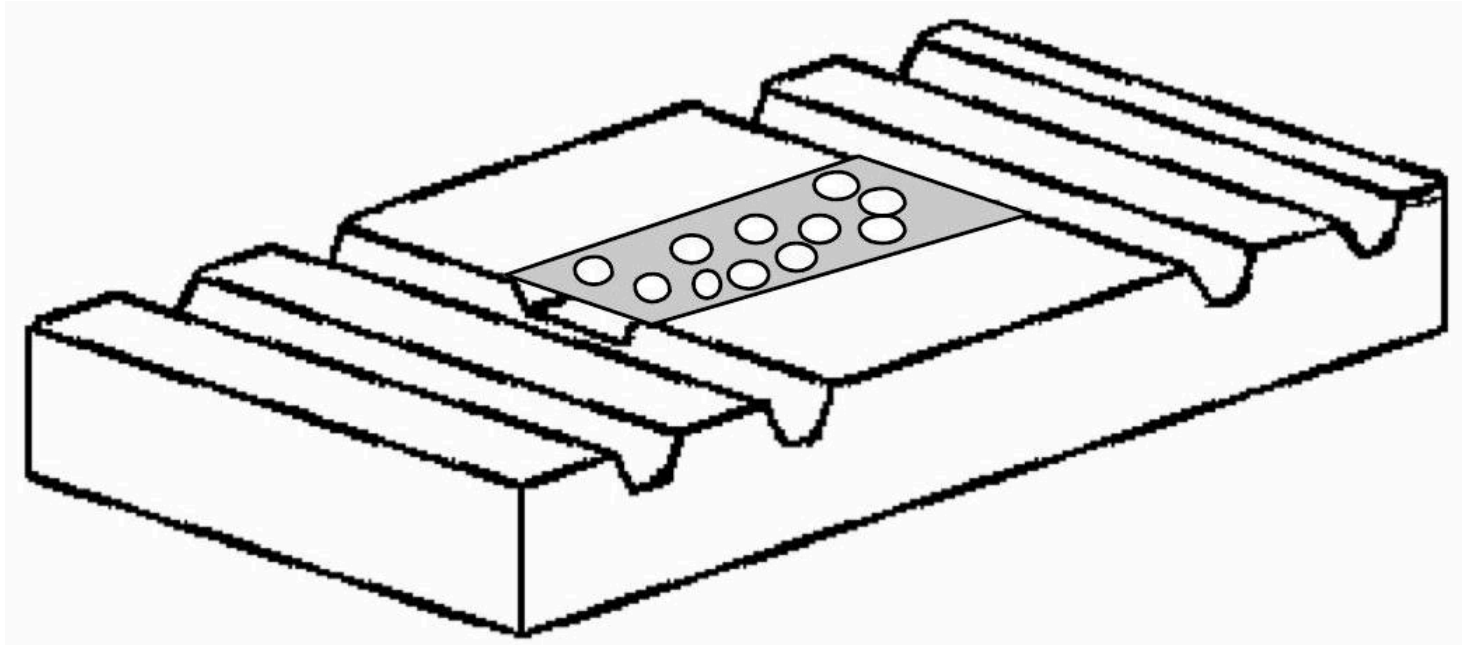


Fig. 5. Goryaev's chamber

#### 圖 5. Goryaev 腔室

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存在一定比例時，即符合此條件。

With a concentration of 0.03 – 0.04ml, 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.

在 0.03 – 0.04ml 的濃度下，紅血球在 Goryaev 腔室表面形成足夠均勻的單層，產生接近理想圓形的良好細胞變形及足夠的強度。

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.

對紅血球在溶液中暴露時間的實驗研究顯示，在滲透平衡發生期間，研究紅血球膜的滲透脆性和剛度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 5,000rpm, taken from the bottom of the tube in the amount of 0.03 ÷ 0.04ml 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：以檸檬酸鈉穩定紅血球，於 5,000rpm 下洗滌三次，每次 10 分鐘，取自試管底部的紅血球，量為每 2 毫升低滲溶液中 0.03 ÷ 0.04ml，靜置 2 小時，然後置於 Goryaev 室中觀察繞射圖樣並記錄第一及第二極小值的半徑。

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，是通過激光繞射儀中繞射環線性尺寸的變化來確定的。

## 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):

血液樣本的製備技術是一個相當費時費力的過程，時間跨度較長，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;

製備工作溶液；

letting the blood settle (stabilization);

讓血液靜置（穩定化）；

experiment; 實驗；

processing of experimental findings.

實驗結果的處理。

The experiment was conducted in two versions:

實驗分為兩個版本進行：

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);

第一種是持續版本，提供最小的探測雷射輻射影響（樣本在初始時及經過一定曝露時間後接受輻射）；

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.

第二種版本中，同一樣本依序接受輻射：分別在初始、10、20、30 及 40 分鐘時接受輻射，導致輻射劑量累積，並可能因實驗時間縮短而產生無法控制的效應。

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.

分形共振器的影響是透過將含血液樣本的 Goryaev 腔室置於分形共振器側邊的圖案上來實現的。

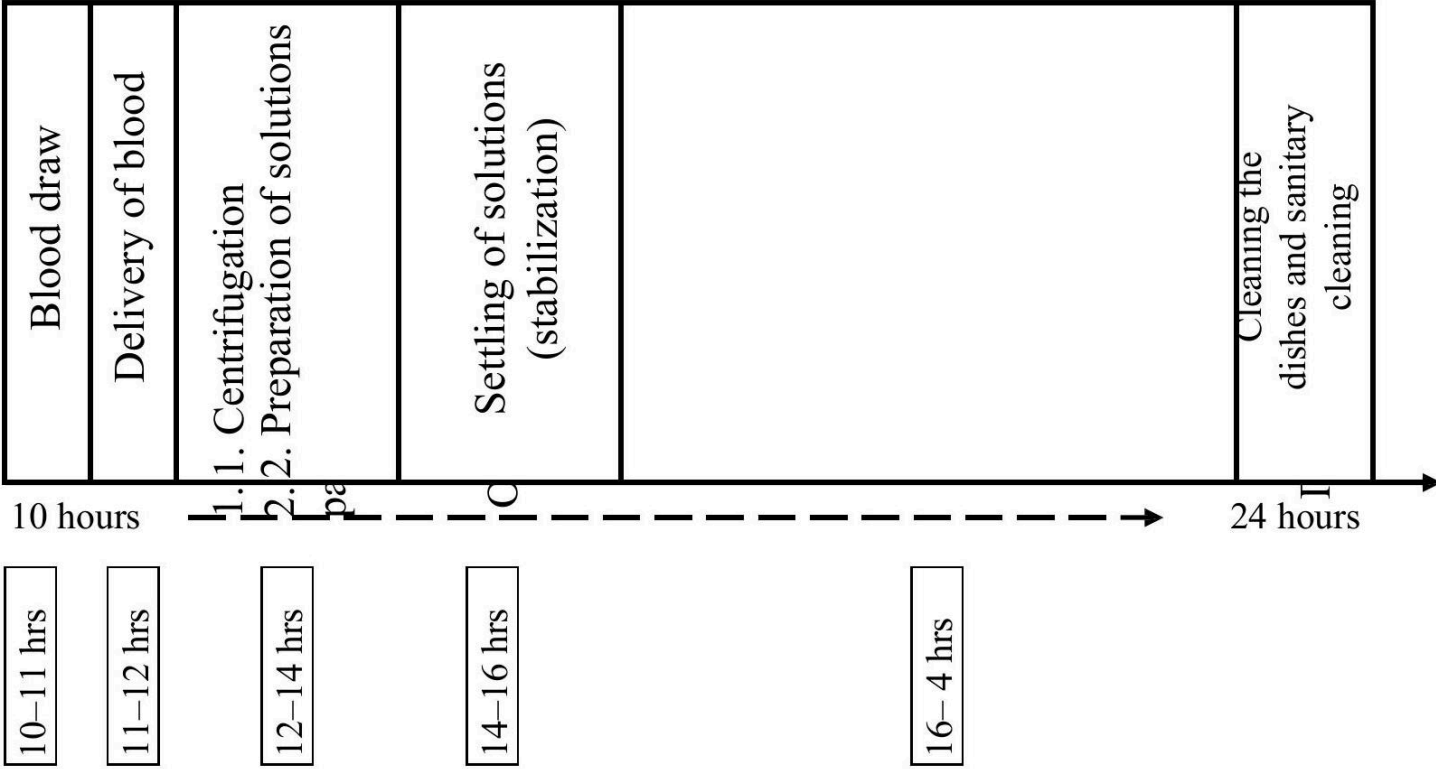


Fig. 6. Experiment timeline for one blood sample

圖 6. 單一血液樣本的實驗時間軸

Erythrocyte sed- imentation 紅血球沉降	Scanning, Image input Saving 掃描。影像 輸入 保存		Resonator expo- sure 10 minutes 共振器曝露 10 分鐘		Scanning, Image input. Savige 掃描。影像 輸入。保存		Chamber clean- ing 5-10 minutes 腔室清潔 5- 10 分鐘	
o 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

暴露時間 10 分鐘

Fig. 7. Experiment timeline for one NaCl concentration

圖 7. 單一 NaCl 濃度的實驗時間線

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

對於一個濃度，含 10 分鐘暴露時間的實驗總時長為 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.

繪製一個依賴關係的實驗共進行 8 個點。獲取繪製一條低滲曲線的數據約需 5 小時。

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

獲取繪製一條暴露時間為 20 分鐘的低滲曲線數據約需 6 小時。

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

獲取繪製一條暴露時間為 40 分鐘的低滲曲線數據約需 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間約為 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.

研究的第二階段是處理所獲得的結果，以尋找定量數據來繪製低滲脹曲線。

### III. EXPERIMENTAL FINDINGS

#### 三、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. 分形共振器對紅血球流變參數影響的研究（暴露時間最多至 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 分鐘呈現正面效應。

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).

在球形化初期階段暴露 5 分鐘後，振幅變動相較於變化有所增加

在紅血球半徑中；腫脹的性質開始符合典型情況（出現跳躍及隨後的腫脹）。

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.

經過 10 分鐘的曝露後，球狀化點移動到較小滲透壓值的區域，這對應於外部影響的正向趨勢；腫脹的性質也明顯變得痙攣性。

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.

正面效應表現得非常微弱。5 分鐘後，第一次跳躍的振幅略有增加。10 分鐘後，第一次跳躍的振幅略有增加。

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。5 分鐘後，第一次跳躍的振幅略有增加。10 分鐘後，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.

整體趨勢呈現正向。

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).

經過 5 分鐘後，低滲脹大結果——暴露的結果——顯示退化：紅血球半徑變化的振幅下降，球形化點向較高的滲透壓值移動（紅血球膜的彈性特性明顯降低）。

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.

經過 10 分鐘後，趨勢轉為正向：紅血球在脹大過程中半徑變化的振幅上升，球形化點向較低的低滲透壓值移動。

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

經過 15 分鐘後，紅血球振幅擺動進一步增加，低滲脹大曲線上出現跳躍。

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。對於所有曝露時間（ 5, 10, 15 分鐘），紅血球在低滲脹大幅度的半徑均超過初始幅度，且跳躍現象更為明顯。

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 15 分鐘曝露時間下，呈現向較低低滲壓力移動的趨勢。紅血球膜的彈性特性在所有曝露時間均呈現正向趨勢：紅血球脹大的速度增加。

### 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普遍顯示出共振器外部影響所產生的正向趨勢。

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 日及 28 日）顯示隨著暴露時間延長，共振器影響的改善趨勢更加明顯。

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.

紅血球的流變參數（聚集性、變形能力、內在黏度）取決於多種因素。特別是在多發性骨髓瘤（MM）患者中，這些參數與患者血液中總蛋白和副蛋白的水平密切相關，而在副蛋白血症型的疾病中，這些蛋白質水平總是升高。同時，我們研究了患有不同類型疾病的患者血液，包括患有 **Bence Jones** 骨髓瘤和非分泌型骨髓瘤的患者，後者血液中不分泌副蛋白。慢性腎功能不全和貧血在患者中常見，這些因素同樣不可忽視。此外，研究還包括了新入院且未接受過治療的患者血液，以及接受過化療方案的患者血液。細胞毒性藥物對紅血球膜的彈性有負面影響。同時，在化療過程中，腫瘤細胞被破壞，這導致與血液流變密切相關的凝血系統發生嚴重變化。毫無疑問，患者的年齡也應被考慮在內。因此，研究涵蓋了相當多樣化的患者群體。

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，需具備以下條件：

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

實驗應持續進行，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

共振器效應的分析應考慮疾病的版本與階段，並分別針對新入院患者（最初於開始前）進行

of specific therapy) and patients who underwent intensive chemotherapy;

接受特定療法的患者以及接受密集化療的患者；

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

建議研究延長共振器暴露時間（最多至 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. 第二階段研究：延長共振器暴露時間（最多至 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。暴露 40 分鐘後，對共振器的反應為負面。然而，此結果也可能與環境的負面影響有關，例如隨著暴露時間增加，樣本乾燥等問題。

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。最顯著的效果同樣出現在暴露 30 分鐘後。暴露 40 分鐘後，對共振器的反應為負向。

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四倍）。

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

整體趨勢呈現正向。最顯著的效果也出現在暴露 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。相同樣本連續暴露（因雷射輻射劑量增加四倍）。整體趨勢呈現正向。

### **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).

根據所進行的實驗，似乎無法確定最佳曝露時間（實驗次數較少；需要對長時間置於 Goryaev 腔室中的樣本可能變化進行更詳細的分析）。

### **CONCLUSION 結論**

10 blood samples from patients with multiple myeloma were tested.

對 10 位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.

發現分形共振器對紅血球流變參數的外部影響呈現正向趨勢。

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，由於血液樣本數量有限，且來自極為多樣化的多發性骨髓瘤患者，且未記錄所施治療（化療）的影響，因此需要進一步收集資料，以便後續編制同質性患者群組（包括血液中有副蛋白型與無副蛋白型的患者、新入院患者及接受化療方案的患者，並具備同名血紅素及肌酸酐參數）。

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APPENDIX 附錄

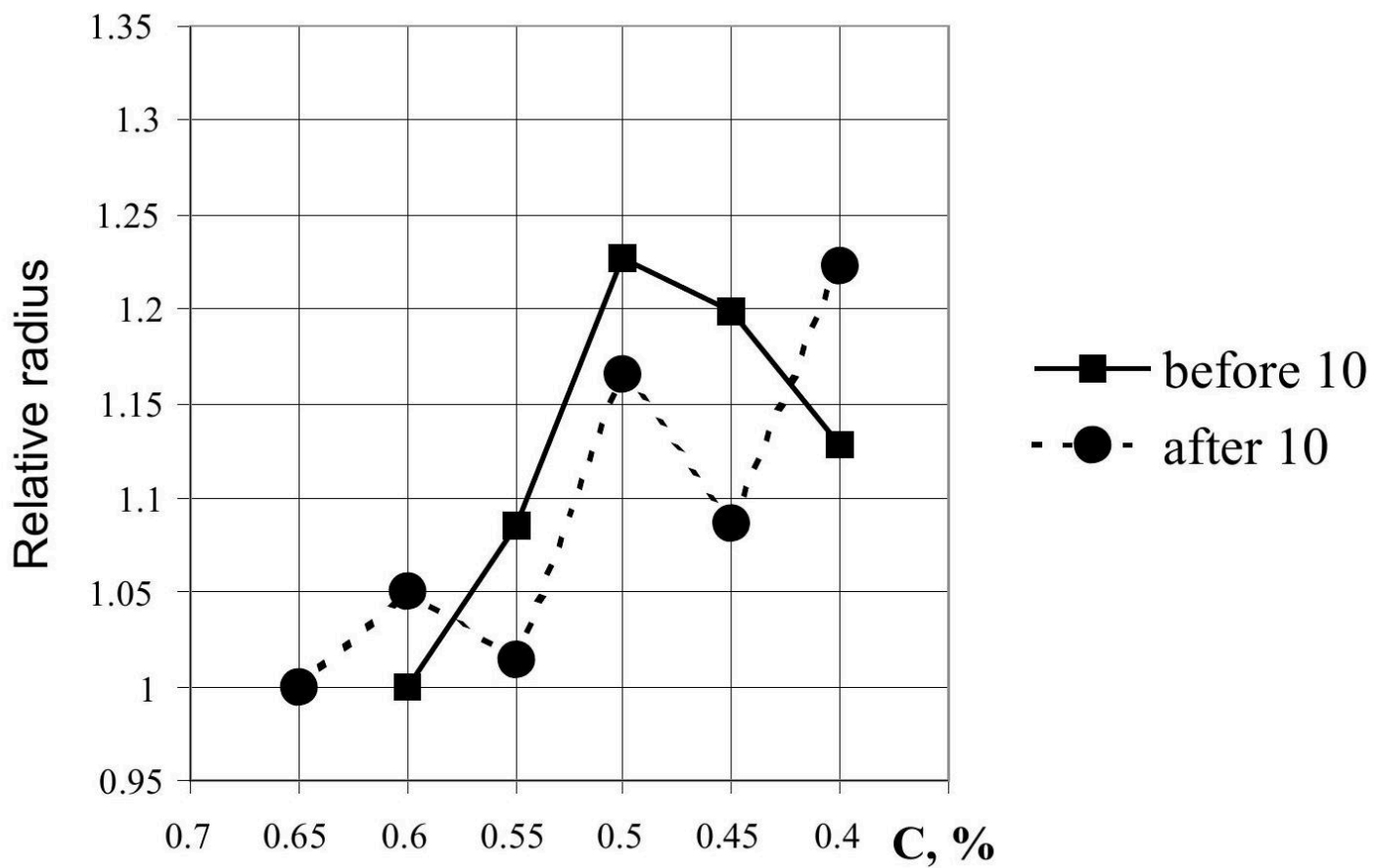
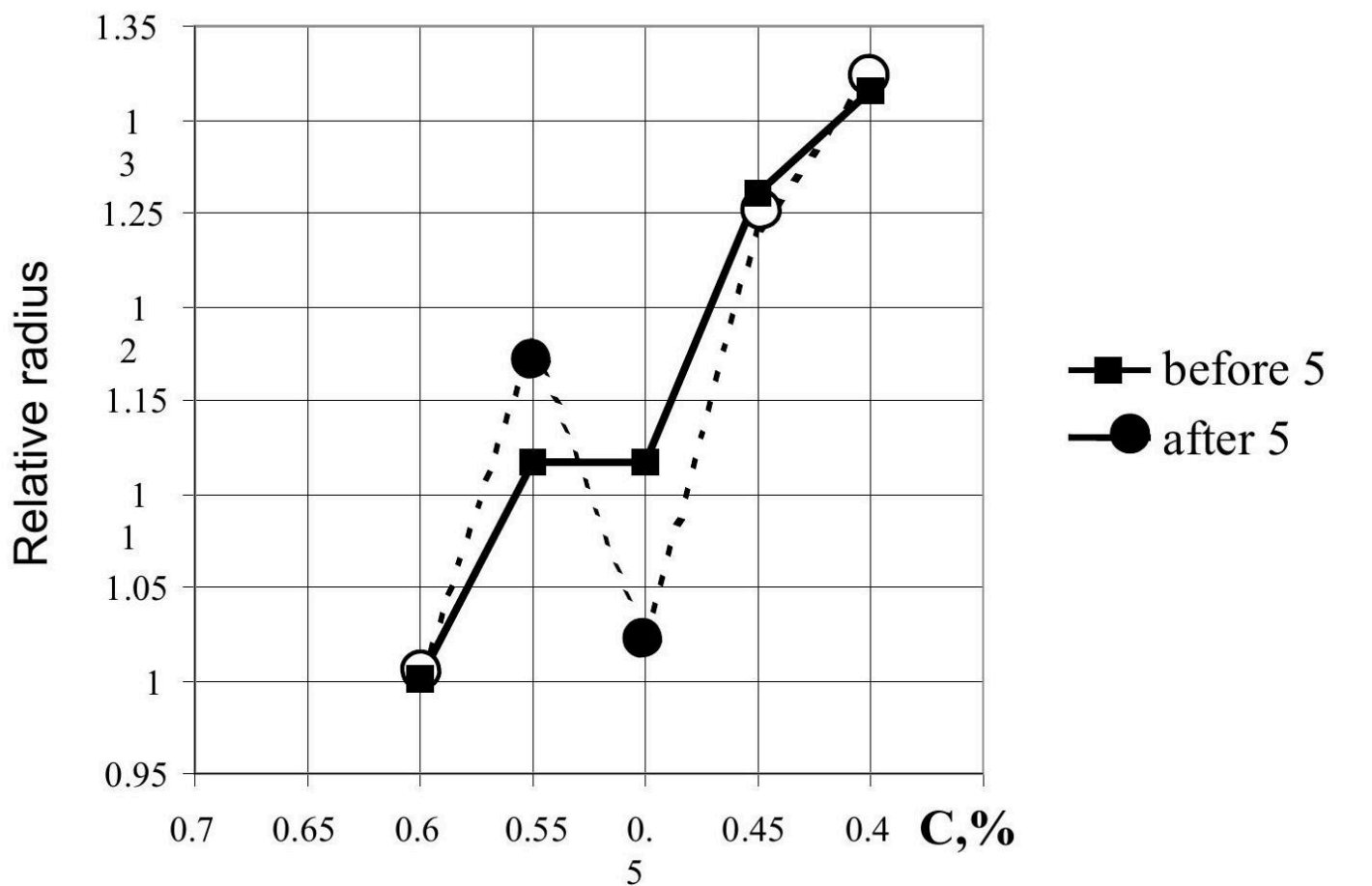


Fig. 8 圖 8

24 December 2004 Patient, male, 56 years old.

2004 年 12 月 24 日，患者，男性，56 歲。

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

**多發性骨髓瘤，第三期 A，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.

**血液中總蛋白和副蛋白水平均偏高（分別為 130 和 54 g/l ）。**患者已接受多次化療。



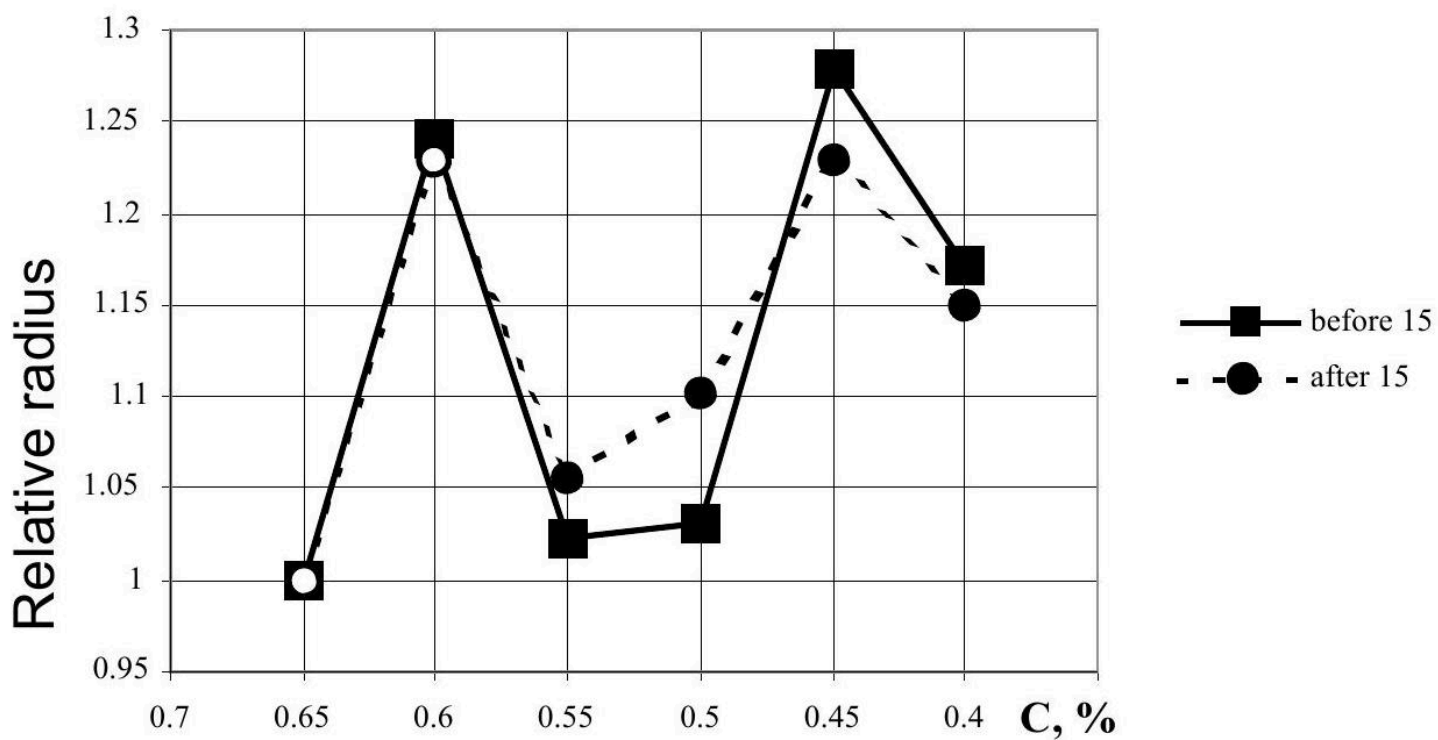
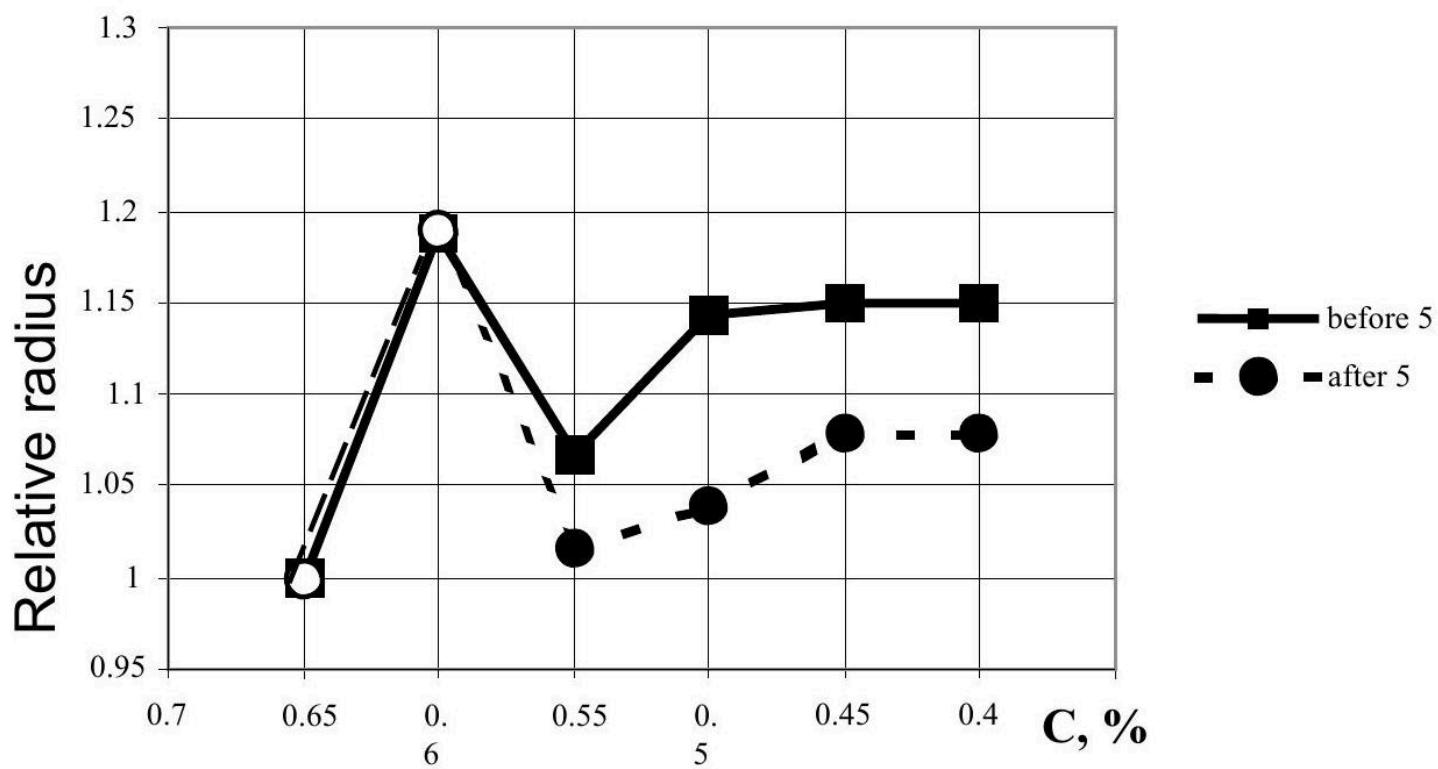


Fig. 9 圖 9

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

2004 年 12 月 28 日。患者，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).

新入院患者，先前未接受治療。Bence Jones 骨髓瘤，第三期 B，血液中肌酸酐中度升高，尿液中檢出副蛋白，但血液中未檢出 (o)。

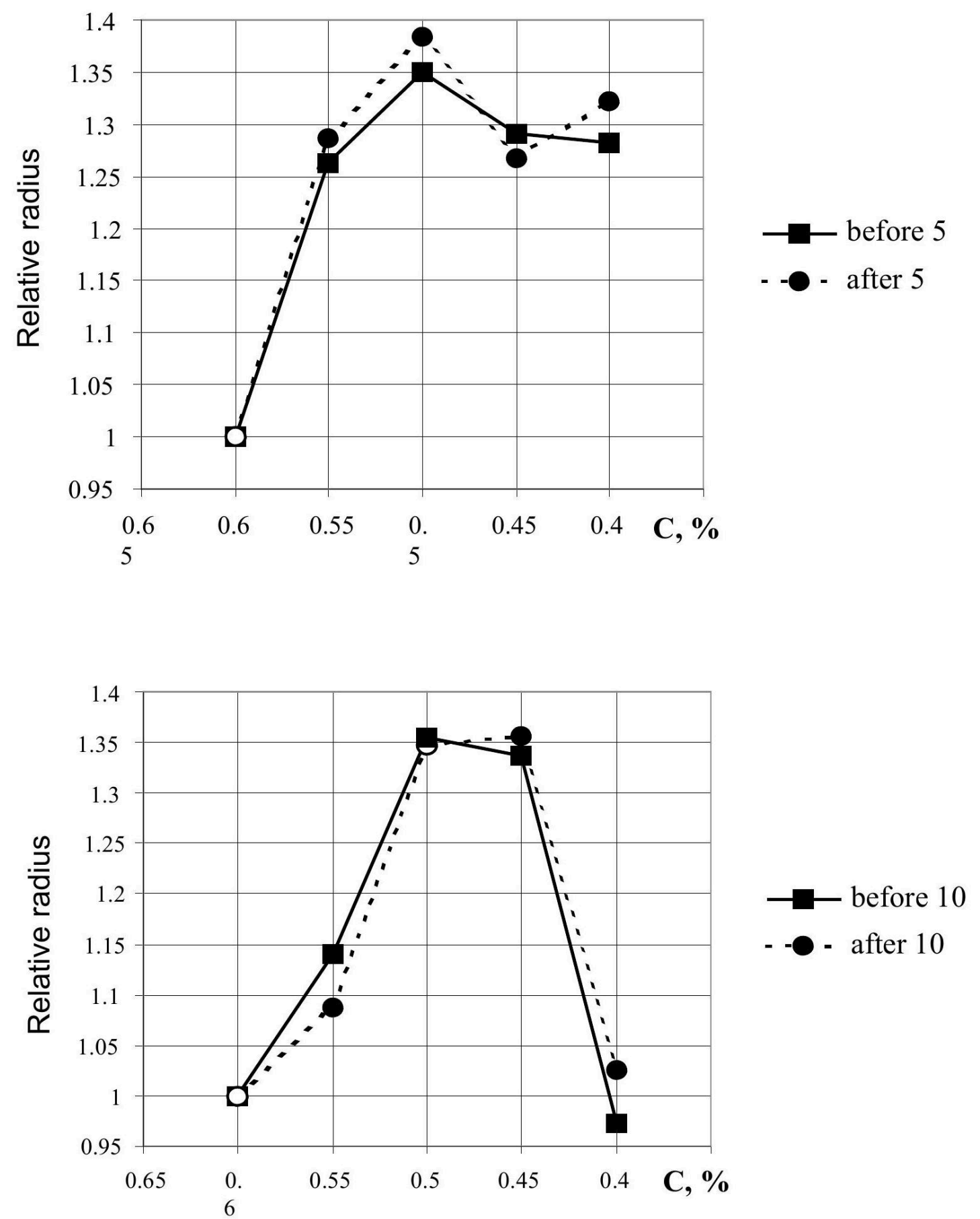


Fig. 10 圖 10

18 January 2005 Patient, female 81 years old.

**2005 年 1 月 18 日。患者，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).

**多發性骨髓瘤，第三期 A，新入院患者，先前未接受治療。貧血，血液中總蛋白和單克隆蛋白水平均偏高（分別為 108 和 41 g/l）。**

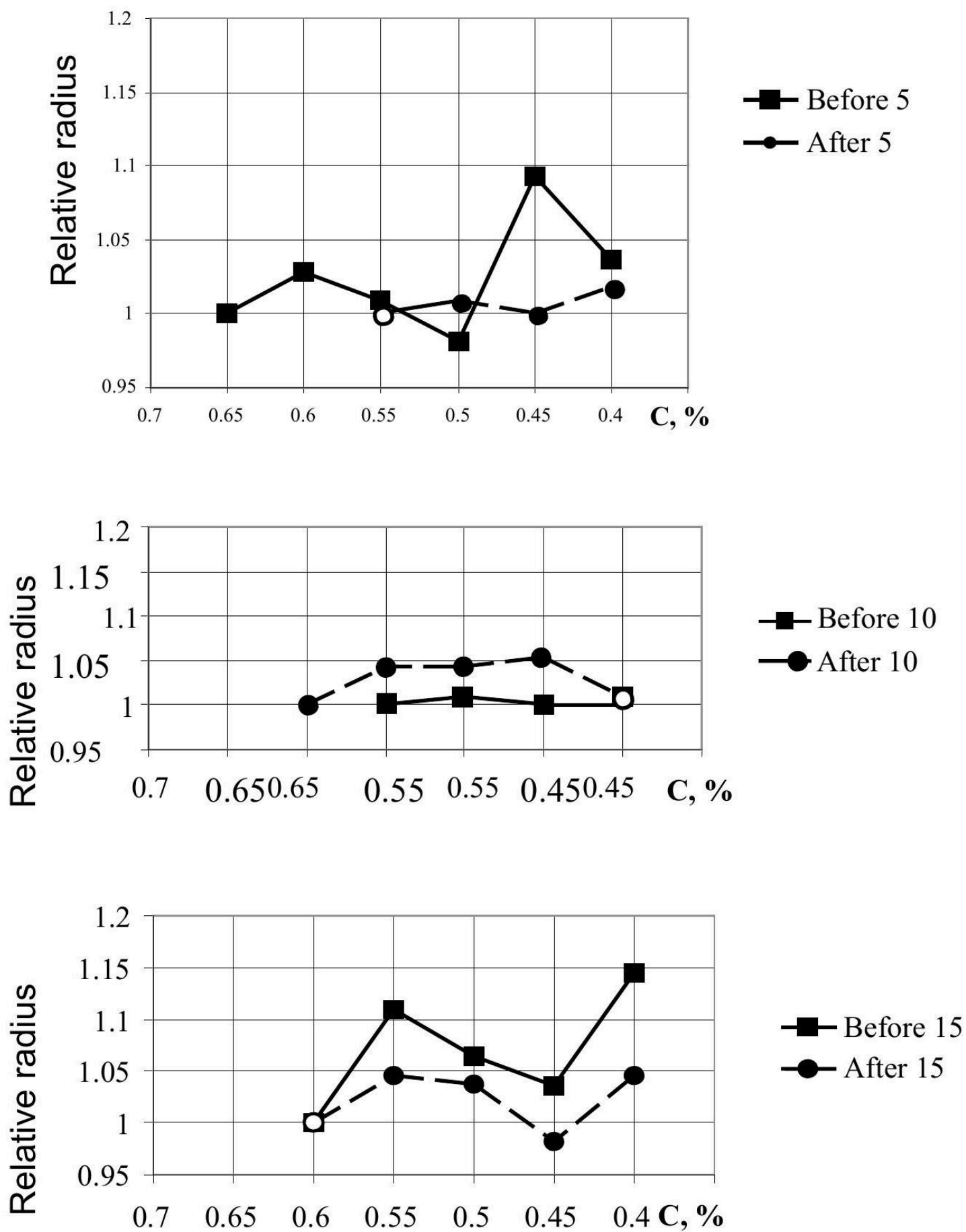


Fig. 11 圖 11

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 (o). However, concentration of fibrinogen in the blood stream (related to chemotherapy and

disintegration of cells) is high, 5.5 g/l. High aggregation.

接受多輪化療，處於臨床血液學緩解期。血液中蛋白質水平正常，未檢測到單克隆蛋白（o）。然而，血液中纖維蛋白原濃度偏高（與化療及細胞解體有關），為 5.5 g/l。高聚集狀態。

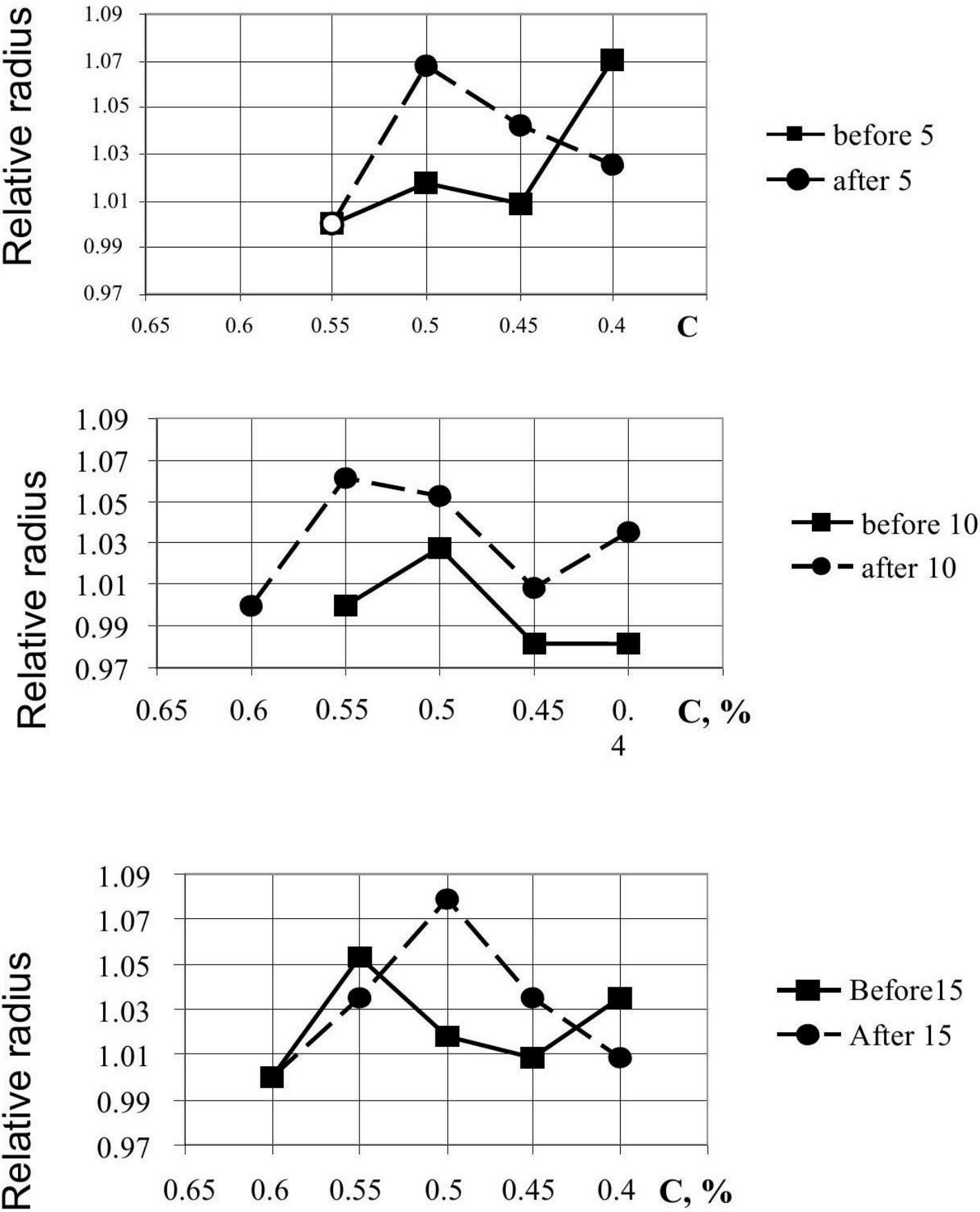


Fig. 12 圖 12



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 ).

曾接受多次化療，目前復發，進行多輪強化化療。血液中高濃度的副蛋白（ 48 g/l ）、纖維蛋白原（ > 5 g/l ）。

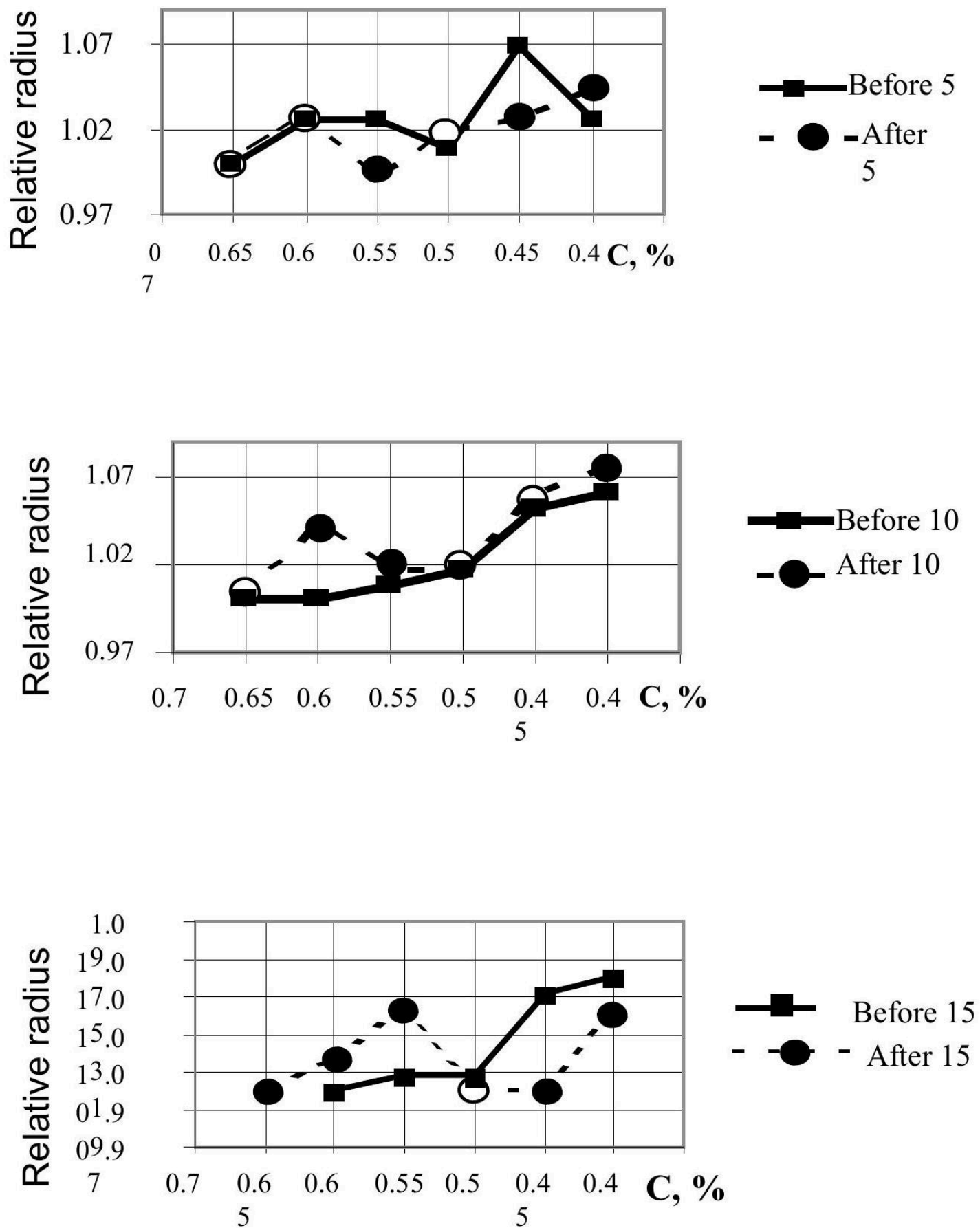


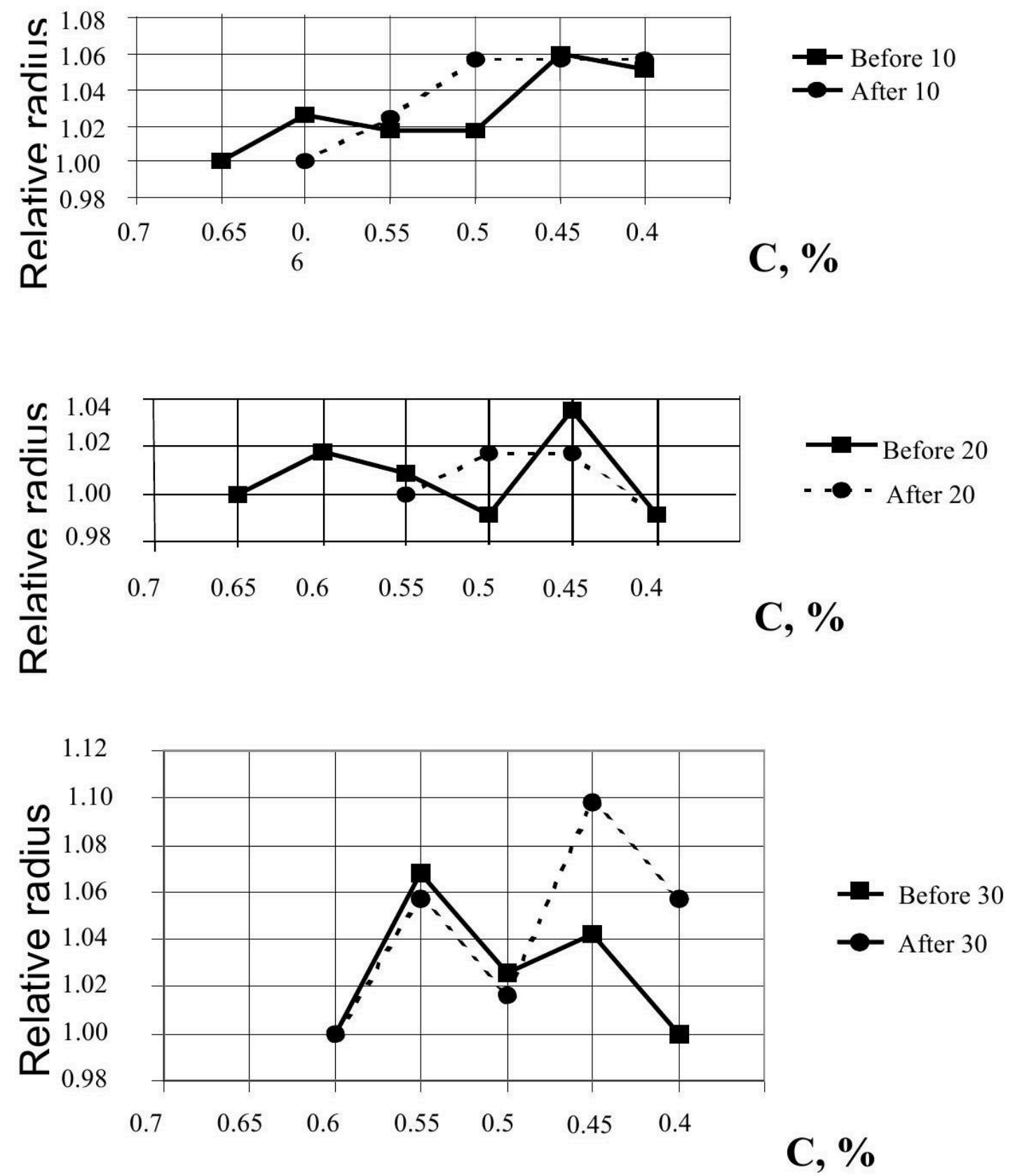
Fig. 13 圖 13

28 January. Patient, female, 76 years old.

1月28日。患者，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).

新入院患者。先前未接受治療。A 型骨髓瘤，II A 期，中度貧血，血液中總蛋白及副蛋白水平均偏高（分別為 109 及 48 g/l）。



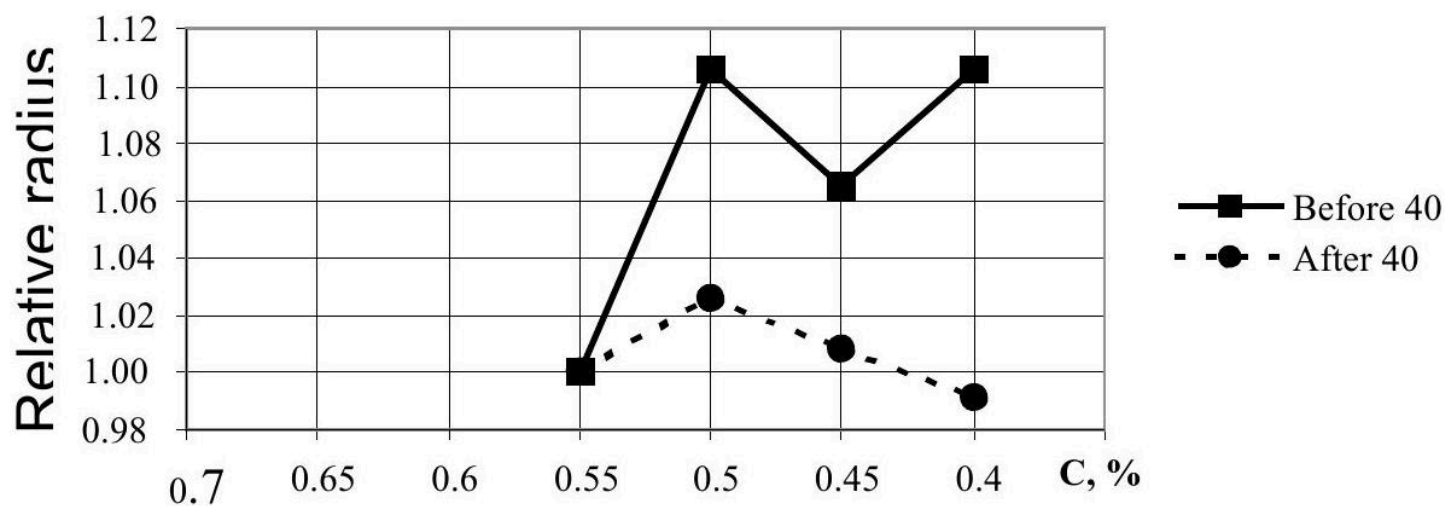


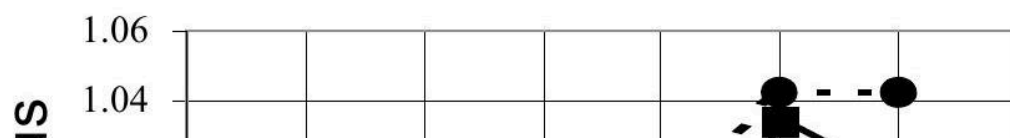
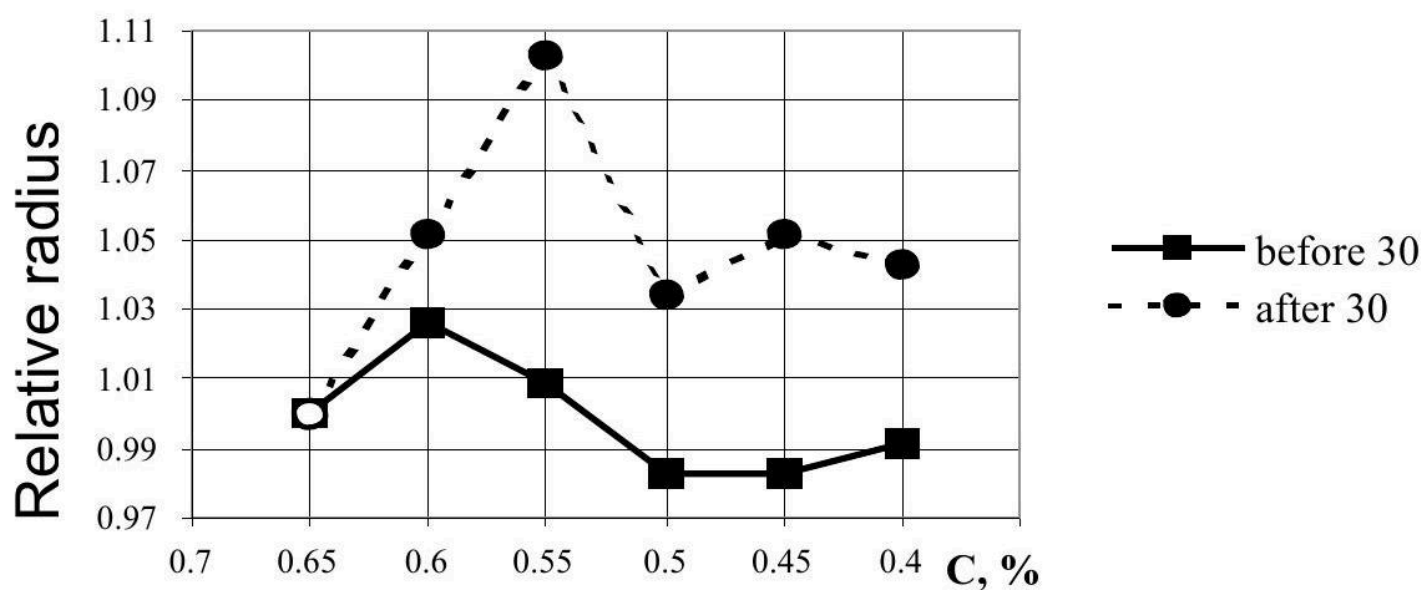
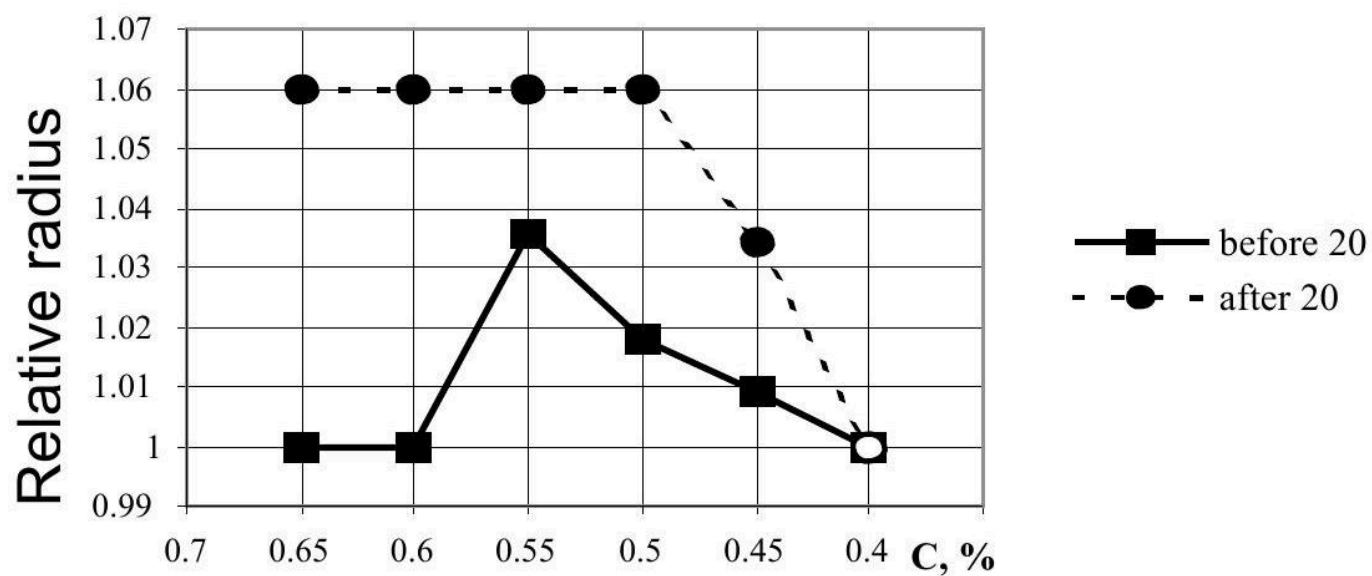
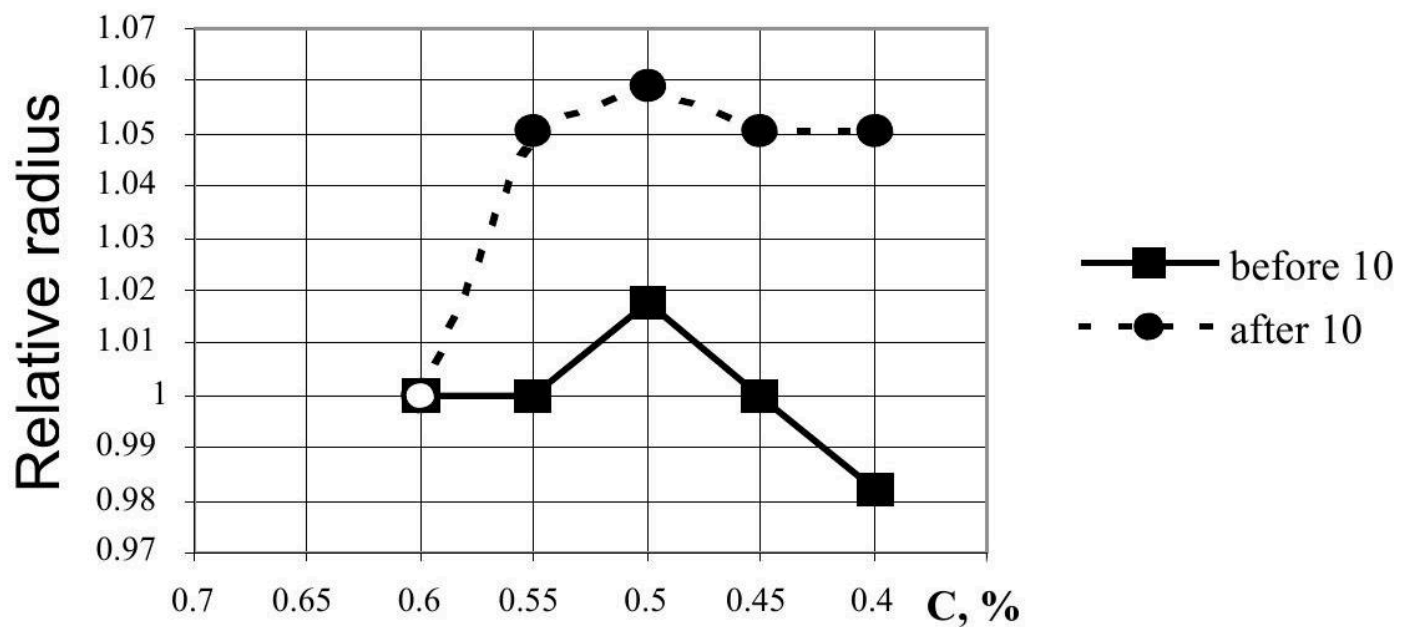
Fig. 14 圖 14

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

2005 年 2 月 4 日。患者，女性，66 歲。

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

A-骨髓瘤，多發局部型，3A 期，經多次治療，貧血（血紅素  $73 \text{ g/l}$ ），總蛋白及副蛋白水平均偏高（分別為  $104.5$  及  $50.5 \text{ g/l}$ ）。





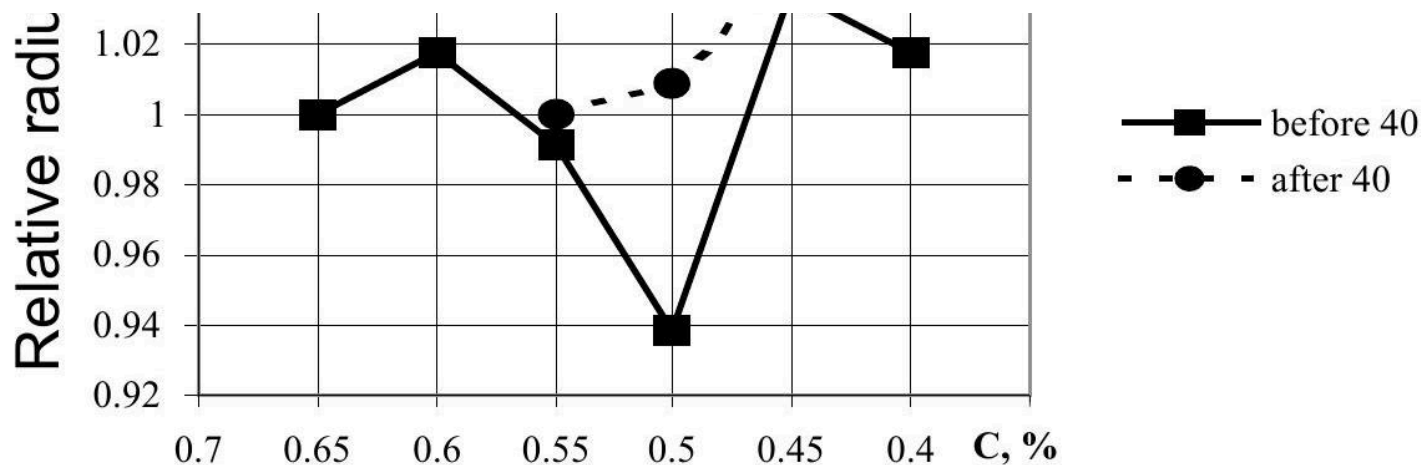


Fig. 15 圖 15

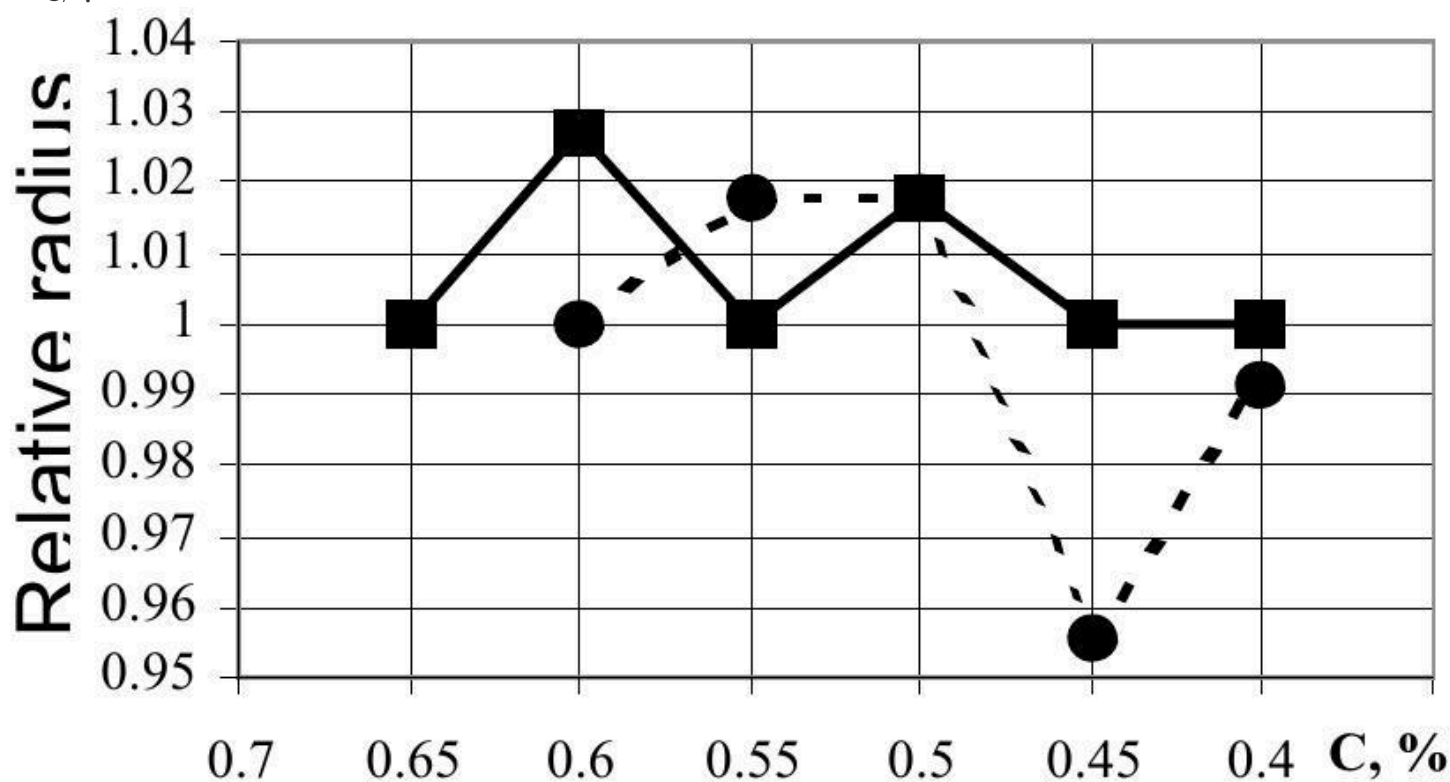
8 February 2005. Patient, male, 52 years old.

2005 年 2 月 8 日。患者，男性，52 歲。

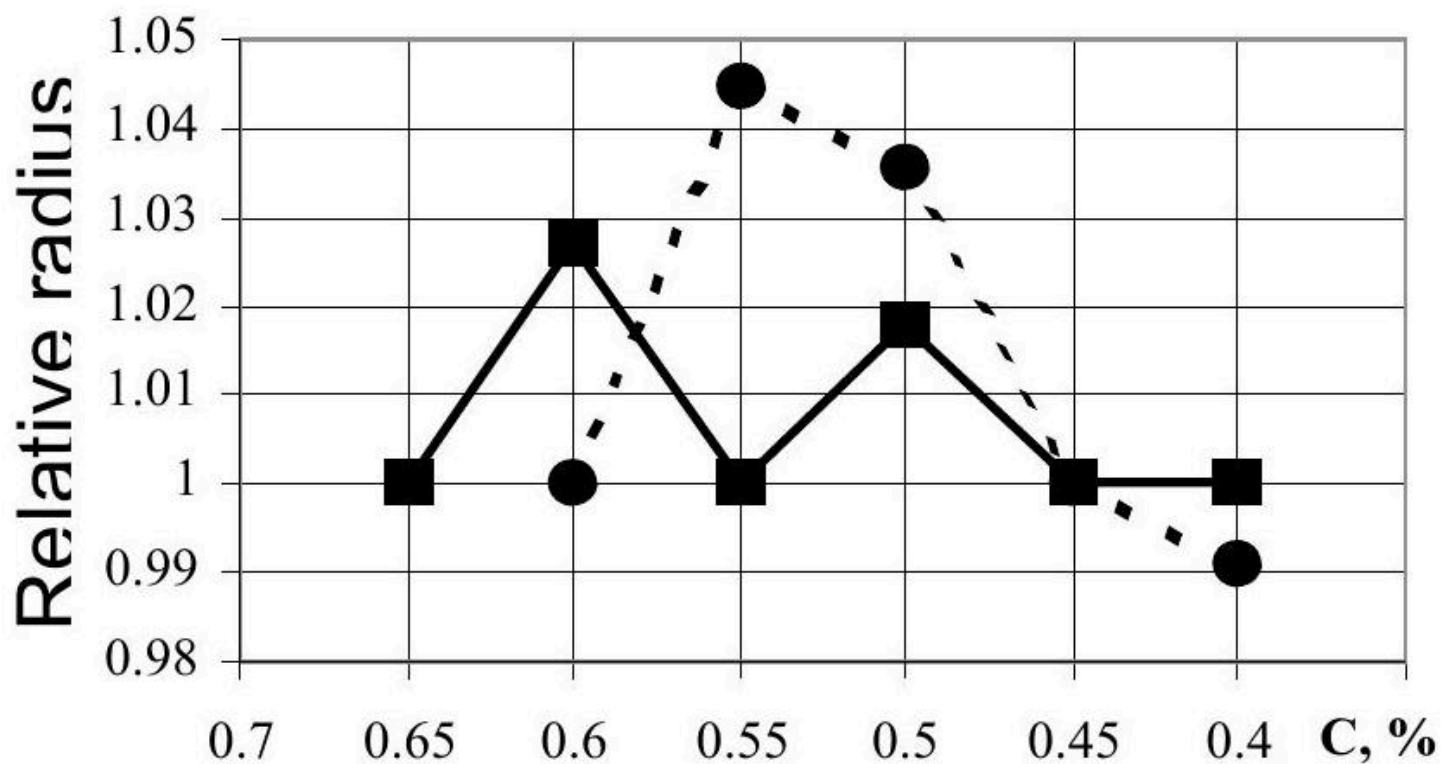
Bence Jones myeloma, multiple local form, stage 3B, relapse, for which the patient has already received 3 rounds of chemotherapy. Total protein 92, paraprotein 0, elevated fibrinogen  $3.4 \text{ g/l}$ , and moderately elevated creatinine in the blood stream,

Bence Jones 骨髓瘤，多發局部型，3B 期，復發，患者已接受 3 輪化療。總蛋白 92，副蛋白 0，纖維蛋白原  $3.4 \text{ g/l}$  升高，血液中肌酸酐中度升高，

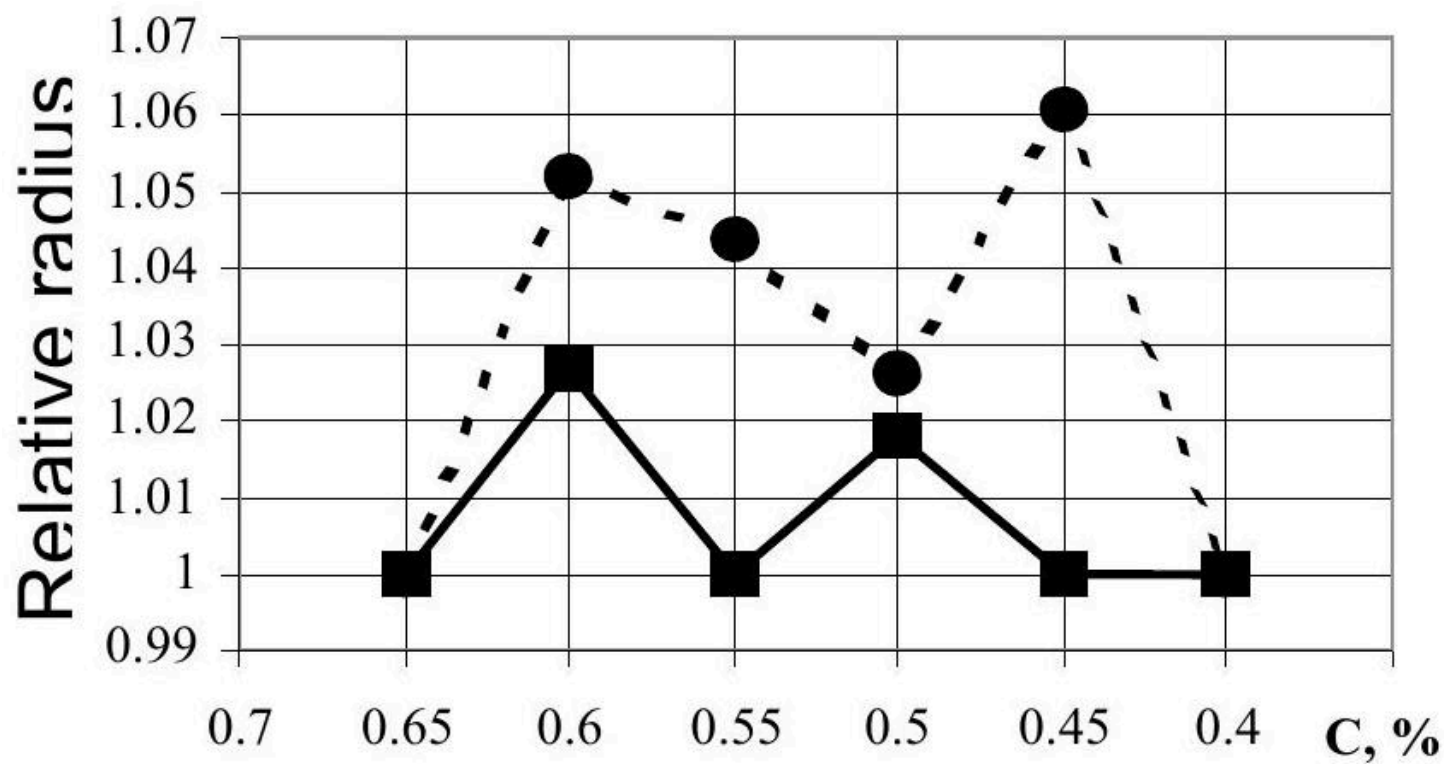
$114 \text{ g/l}$ .



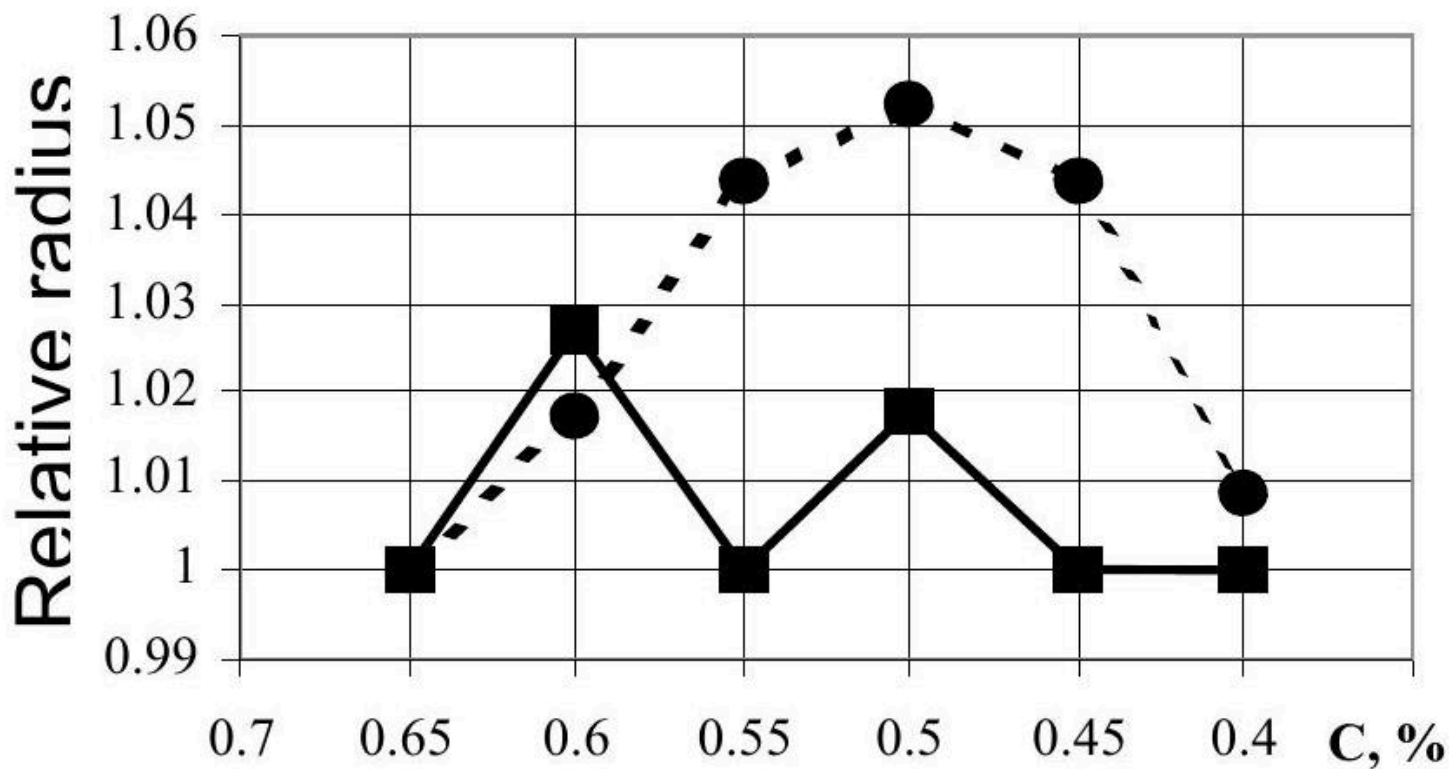
before 之前



before 之前



before 之前



—→ before —→ 之前

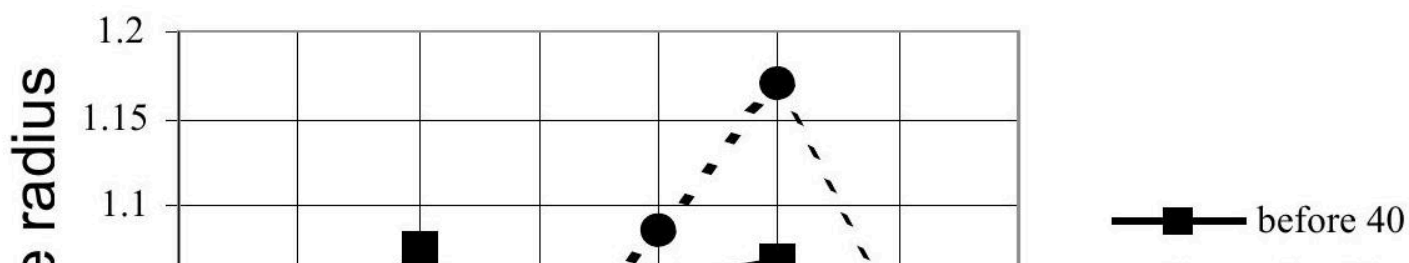
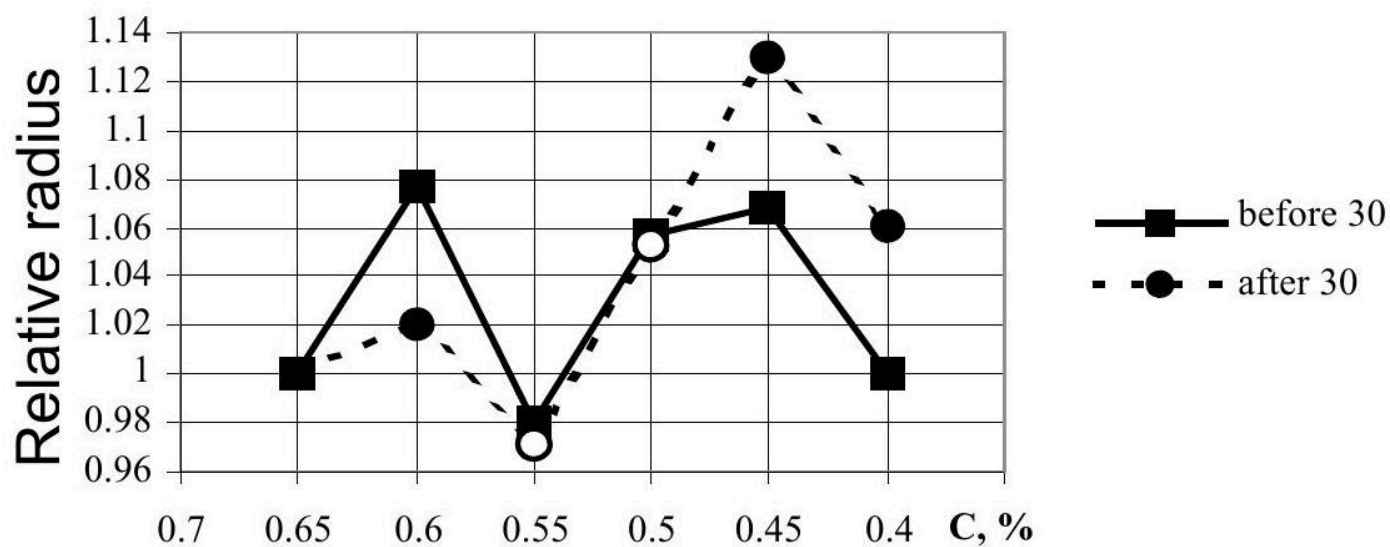
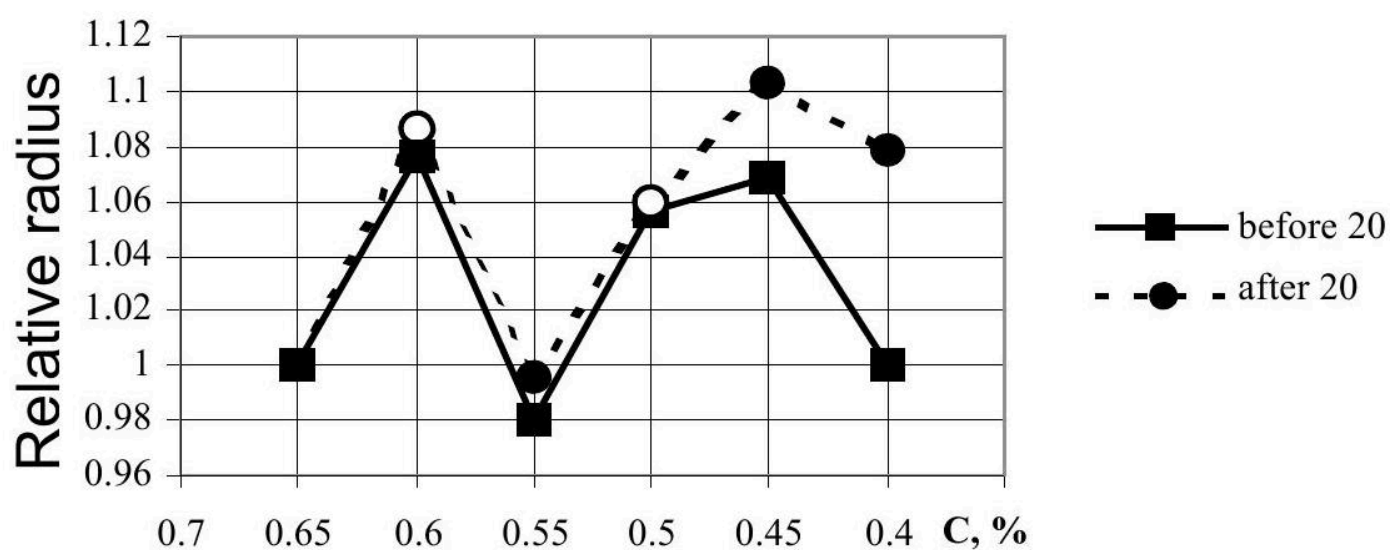
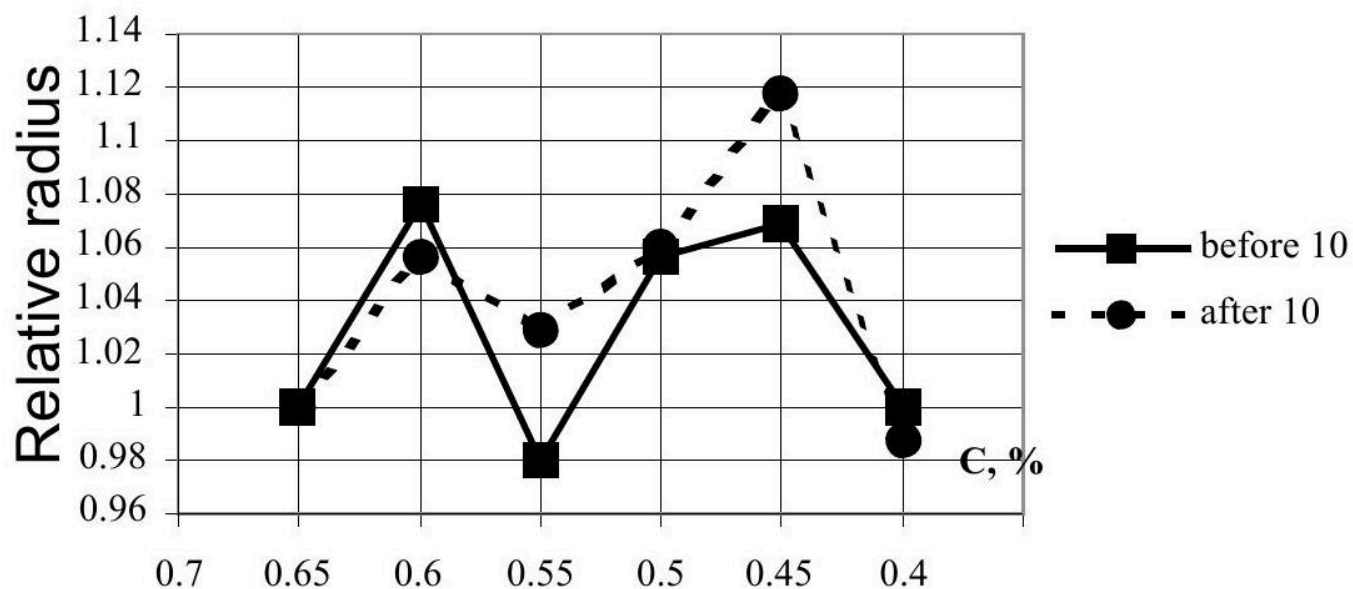
Fig. 16 圖 16

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

2005 年 2 月 11 日。患者，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/l and 50 g/l, correspondingly).

多發性骨髓瘤，局部多發型，3A 期。病情嚴重，經多次治療，貧血（ 67 g/l），總蛋白及副蛋白偏高（分別為 138.4 g/l 和 50 g/l）。



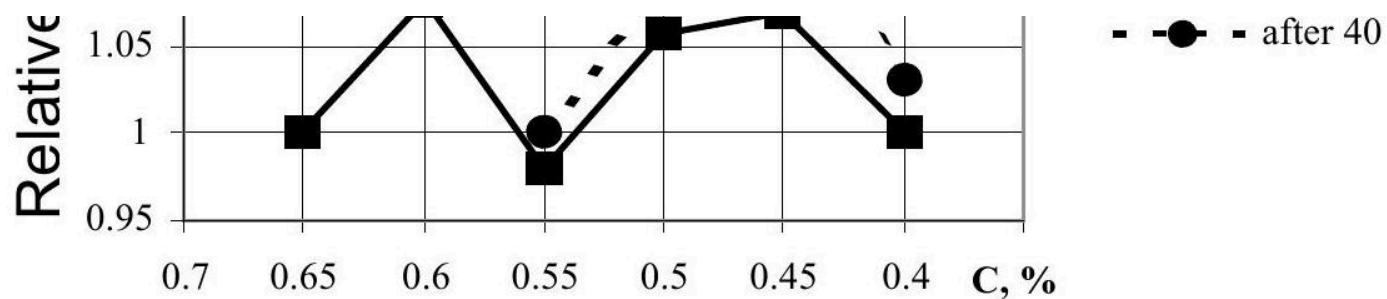


Fig. 17 圖 17

15 February 2005. Patient, male, 66 years old

2005 年 2 月 15 日。患者，男性，66 歲

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

非分泌型骨髓瘤，檢查無變化，副蛋白 o 。病程數年，經近期多次治療後，病情有所改善。