INFLUENCE OF ANTICOAGULANTS ON ERYTHROCYTES SEDIMENTATION AS DETERMINED BY HE-NE LASER

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ABSTRACT

The effects of various anticoagulants on the sedimentation tendency of erythrocytes have been determined by observing their sedimentation profiles at various points along the height and width of a glass chamber by variation of the transmitted intensity of laser light. In the initial phase, the effects of heparin and sodium citrate are alike, but with the increase of sedimentation duration the erthyrocytes in the sample get mixed with heparin, at various levels below the plasma layer and move slower than that of sodium citrate. After prolonged duration of sedimentation for 22 hr, the effects of sodium citrate and heparin are comparable, whereas for ACD sample, the cellular movement is slower and forms distinct sedimentation profiles compared to others. The role of various mechanisms associated during sedimentation with different anticoagulants are discussed.

INTRODUCTION

THE erythrocytes sedimentation rate (ESR) is generally measured by the formation of the cell-free layer of plasma in the blood sample, kept in a vertical tube, during a fixed interval of time. These measurements depend on the clinical status of the blood and the type of anticoagulant used[1,2], which interacts with the coagulation mechanism of blood to prevent its clotting. Generally, these studies have been confined to the formation of the upper layer, and the details of the sedimentation behaviour of the erythrocytes in the presence of the various anticoagulants are not known. This analysis can play an important role to differentiate the interaction mechansim of these anticoagulants with various types of blood samples.

Recently we have devised a new technique to study the erythrocytes sedimentation profiles (ESP), which provides poin-to-point variation of the erythrocyte concentrations below the cell-free plasma layer[3], and have used it to compare the sedimentation behaviour of the erythrocytes with that of solid spheres[4], and to determine the influence of erythrocytes shape[5] and the influence of various diseases[6] on the erythrocyte sedimentation. As the mechanism of action of various anticoagulants on blood clotting differs from each other [7,10], the aim of the present work is to determine the ESP of normal blood mixed with various anticoagulants.

METHOD

The fresh blood samples from healthy subjects, on three consecutive days, were obtained by venepuncture, in the test tubes containing the anticoagulants. On the first day, 5 ml of blood were drawn in sodium citrate (3.8%) in the proportion of 5:1, on the second day in heparin (4 IU/ml), and on the third day in acid-citrate-dextrose (ACD) solution (10:1.3). The slight adjustment in the hematocrit for sodium citrate and ACD were made by allowing the blood to settle and then by removing a part of plasma from the sample. All hematocrit values were adjusted at 33%.

Each blood sample was filled in a sample holder, made of optically flat glass plates, upto a height of 30 mm (chamber dimensions: height: 30 mm; 16 mm; depth: 1.5 mm). The focussed laser radiations from a 2 mW He-Ne laser were used to scan the blood sample, mounted on the specially designed platform of an optical bench, and was scanned horizontally (from $x = 0$ to $x = 16$ mm) and vertically (from $h = 0$ to $h = 30$ mm (bottom of the chamber) at various intervals of time. The changes in the transmitted intensity (TI), depending on the cellular concentrations at various points in the blood column, were recorded by a photodetector assembly. From these values the ESP at various heights and widths were determined. The details of this technique are given elsewhere[3].

RESULTS

Figure 1 shows the ESP of blood samples mixed with heparin at various intervals of time. The blood cells tend to settle faster with increase of time. The distinctness of the profiles along the width observed at $t = 0$ hr changes with the increase of duration. Similar pattern of sedimentation was also observed with sodium citrate.

The ESP of blood sample mixed with ACD (figure 2) are significantly different from that of other two. The erythrocytes tend to make distinct sedimentation profiles with the increase of sedimentation duration

which is in contrast with that of heparinized blood.

As shown in these figures, the erythrocytes with ACD are still in a process to sediment along the height and are slower compared to that of heparin sample. The distribution of the erythrocytes along the height (at $x = 8$ mm) for these anticoagulants after 22 hr is shown in figure 3. These shows that the separation of plasma and erythrocytes has been completed for heparin and sodium citrate, whereas for ACD blood the erythrocytes are still in a process to settle down.

The results presented here are the mean values of the three observations made with blood samples of the same subject (coefficient of variation less than 10%).

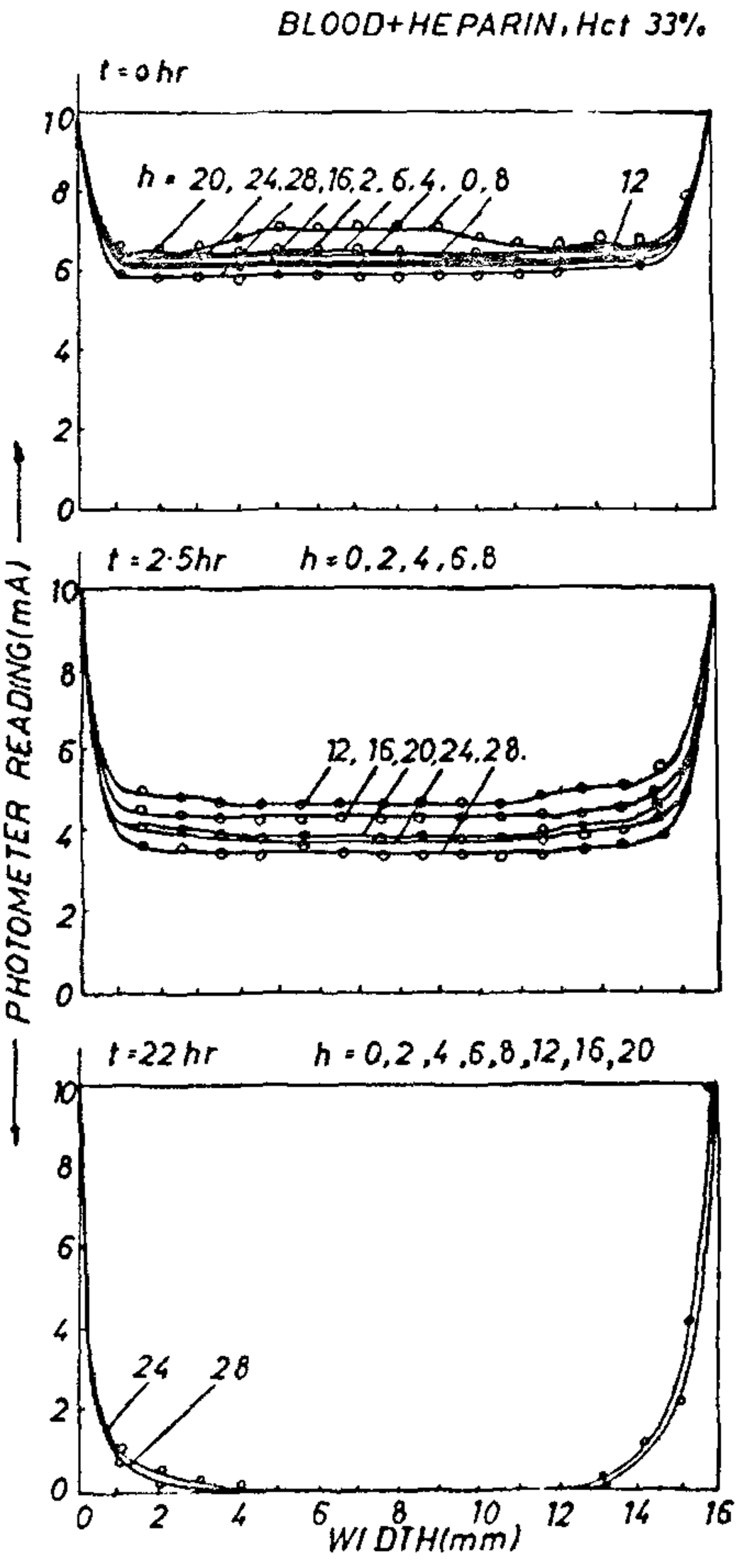

Figure 1. Photometer reading versus width of the sample holder at various heights of the blood column mixed with heparin at sedimentation durations 0, 2.5 and 22 hr.

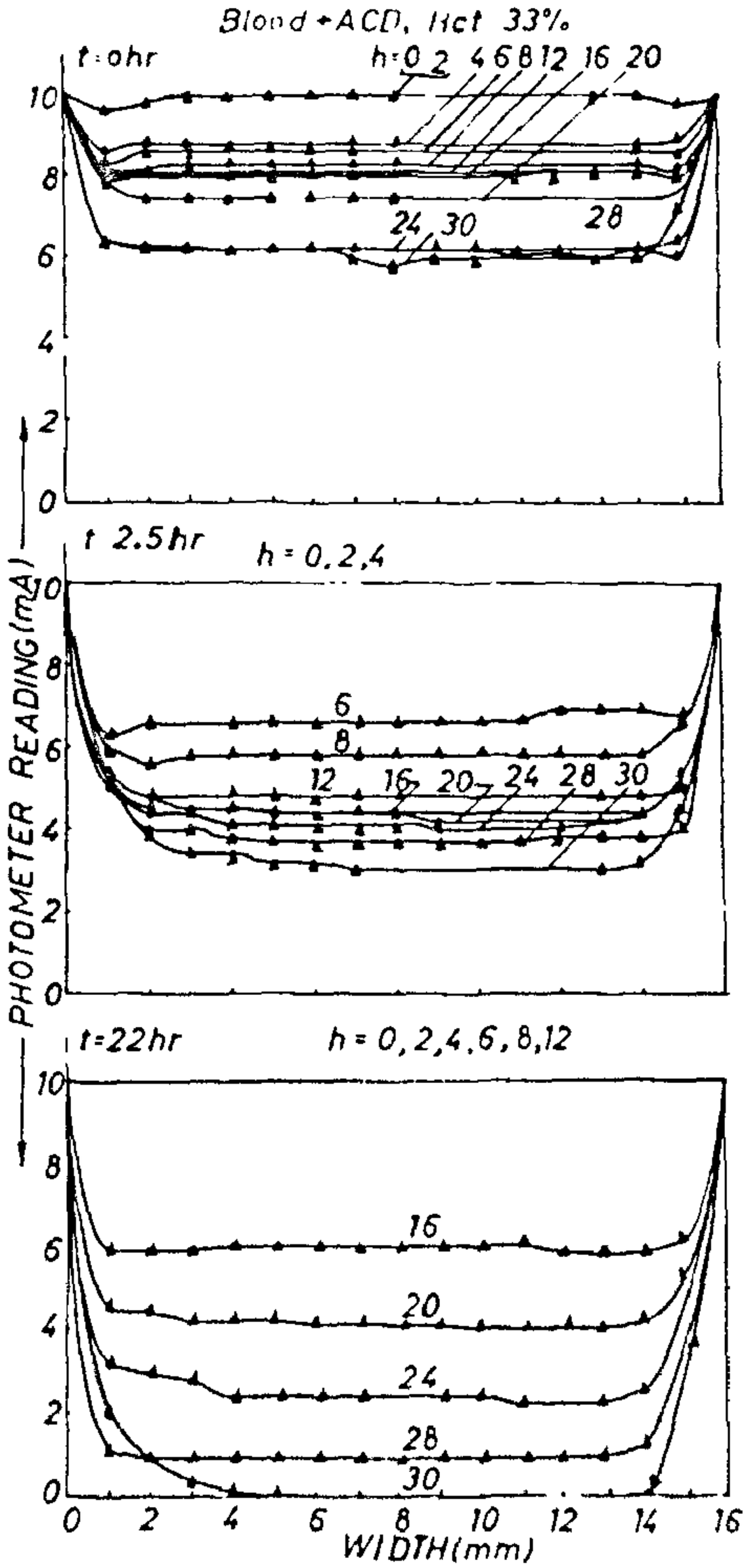

Figure 2. Photometer reading versus width of the sample holder at various heights of the blood column mixed with ACD at sedimentation durations 0, 2.5 and 22 hr.

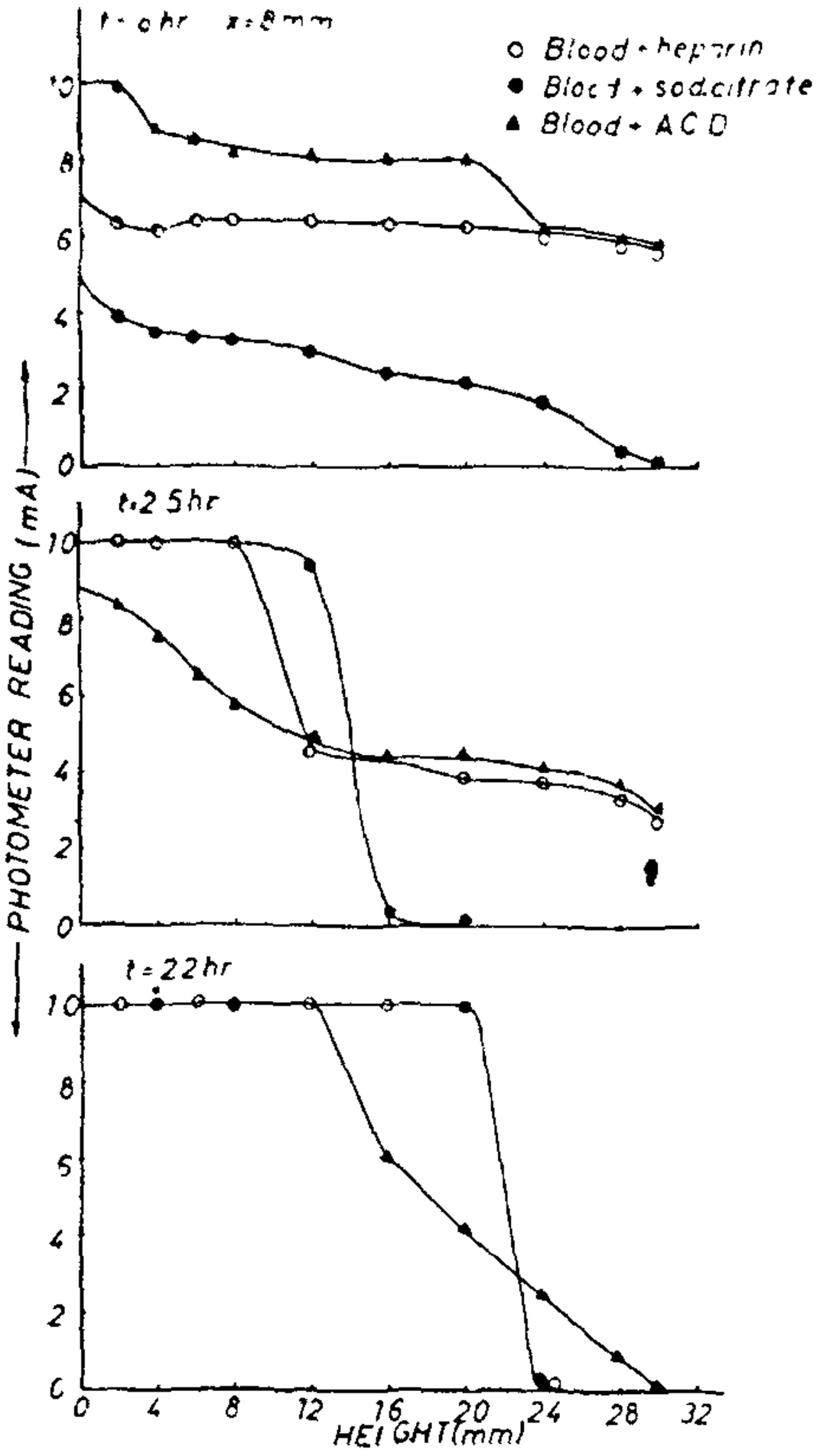

Figure 3. Photometer reading versus height of the sample holder for blood samples treated with various anticoagulants, at $x = 8$ mm, at different time intervals.

Discussion

The interactions of the anticoagulants with the clotting mechanism differ from each other. Sodium citrate interact with the calcium content of the blood, whereas ACD, which also interacts with the calcium content, helps to maintain the normal functioning of the erythrocytes for a longer duration by the presence of glucose. On the other hand, heparin inhibit the coagulation by a different mechanism—by acting at the several sites in the coagulation sequence: (i) inhibition of factor XIa; (ii) inhibition of thrombin; (iii) inhibition of factor Xa; (iv) inhibition of factor XIIa[11].

The distribution of the erythrocytes at various points in the blood column depends on the rouleaux formation tendency of erythrocytes[12], plasma viscosity[13], and the interaction with the various constituents of plasma and finally their packing towards the bottom[6]. Our observations show that the distributions of the erythrocytes at all levels, even for the same blood sample, at constant haematocrit, are affected by the anticoagulants. Initially (at $t \simeq 0$ hr), due to the decrease in plasma viscosity by sodium citrate the erythrocytes tend to settle faster than that of the others. The distribution of the erythrocytes after 2.5 hr is shown by the gradual increase in the packing of the erythrocytes towards the bottom. After 22 hr, the packing (as observed by the present experimental technique) of erythrocytes for ACD is not complete, whereas for heparin and sodium citrate the packing has attained its maximum.

The three-stage sedimentation process, as proposed by Cutler[14], explains the salient features of the ESR studies, whereas in the case of ESP, specially for slow moving rouleaux chains as in ACD solution, the sedimentation process may be described in the following four stages:

(1) Formation of the rouleaux.
(2) Sinking of rouleaux downward to form a cell-free layer of plasma and their interaction with plasma constituents.
(3) The existence of a transition zone and the formation of distinct sedimentation profiles.
(4) Uniform packing at the bottom and a clear cell-free layer.

In conclusion, the application of the sodium citrate as an anticoagulant is ideal for short-term applications as the time required for the initial sedimentation process is considerably less. To elaborate the role of various blood constituent, requiring longer time durations, it is preferable to use ACD due to its anticoagulant and nutritive characteristics.

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Fellows elected at the Annual General Meeting held on 1st October 1982: (1) Dr. S. B. Bhatia, Chandigarh, (2) Dr. M. S. Chadha, Bombay, (3) Dr. D. Chakravorty, Kanpur, (4) Dr. D. K. Dastur, Bombay (5) Dr. A. S. Divatia, Calcutta, (6) Dr. V. C. Dumir, Chandigarh, (9) Dr. Akhtar Husain, Lucknow, (10) Dr. D. J. Jussawalla, Bombay, (11) Dr. R. N. Kapil, Delhi, (12) Dr. S. S. Katiyar, Kanpur, (13) Dr. C. G. Khatri, Ahmedabad, (14) Dr. P. M. Mathews, Madras, (15) Dr. G. Mehta, Hyderabad, (16) Dr. A. S. Mukherjee, Calcutta, (17) Dr. S. Ramanujam, New Delhi, (18) Dr. N. S. Subba Rao, New Delhi, (19) Dr. P. V. S. Rao, Bombay, (20) Dr. S. R. V. Rao, Delhi, (21) Dr. A. K. Saha, Calcutta, (22) Dr. Satya Prakash, Ahmedabad, (23) Dr. S. P. Sen, Nadia, (24) Dr. V. P. Sharma, New Delhi, (25) Dr. H. N. Siddiquie, Goa, (26) Dr. D. V. Singh, Roorkee, (27) Dr. S. K. Sinha, New Delhi, (28) Dr. B. L. K. Somayajulu, Ahmedabad, (29) Dr. S. Varadarajan, New Delhi, (30) Dr. M. A. Viswamitra, Bangalore.

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NOBEL PRIZE IN PHYSICS, CHEMISTRY AND MEDICINE

Nobel Prize in Physics for 1982 has been awarded to Professor Kenneth Wilson of Cornell University, Ithaca, USA, for his work on "Waynmatter changes under different conditions."

The Nobel Prize for Chemistry has been awarded to Prof. Aaron King of South Africa for his work on "Crystallographic electron microscopy and on important nucleic acid protein substances".

The 1982 Nobel Prize for Medicine has been awarded jointly to Prof. John R. Vane (UK) and Dr. Sune Bergstrom, Dr. Bengt Samuelsson (Sweden) for their discoveries chiefly prostaglandins and related biologically active substances.