

[54] **METHOD FOR COUNTING BLOOD
PLATELETS**[75] Inventor: **Thomas Mauthner**, Livonia, Mich.[73] Assignee: **Cambridge Chemical Products, Inc.**,
Detroit, Mich.[22] Filed: **June 14, 1973**[21] Appl. No.: **369,827**[52] **U.S. Cl.** **356/39; 252/408; 356/36**[51] **Int. Cl.** **G01n 33/16; G01n 1/00**[58] **Field of Search**..... **356/36, 38, 39**[56] **References Cited****UNITED STATES PATENTS**

3,503,684	3/1970	Preston, Jr. et al.....	356/39
3,733,548	5/1973	Coulter et al.....	356/39

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[57] **ABSTRACT**

To facilitate the direct observation and counting of platelets in whole blood serum, a blood sample is mixed with a solution which hemolyzes the red and white cells in a short time without destroying any of the platelets. The platelets can then be observed and counted through a microscope without being masked by the larger cells. The composition includes a dye, preservatives and fixatives and a surfactant which lowers the surface tension of the solution to a controlled level which hemolyzes the red and white cells while leaving the higher surface tension platelets undisturbed.

2 Claims, No Drawings

METHOD FOR COUNTING BLOOD PLATELETS

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to methods of treating whole blood serum so as to hemolyze the red and white cells without disturbing the platelets to facilitate microscopic examination and counting of the platelets and to compositions for use in such treatment.

2. Prior Art

The count of platelets per unit volume of whole blood serum is an important diagnostic indicator, particularly in connection with disorders of blood coagulation. Electronic devices such as the Coulter counter are available for counting the platelets in a blood sample, but with high accuracy are expensive and are often not available in a modest clinical laboratory. Microscopic techniques for counting blood platelets are handicapped by the fact that the platelets are much smaller than the accompanying red and white blood cells and these larger cells mask some of the platelets making it extremely difficult to obtain an accurate platelet count.

The problem of counting platelets is further complicated by the fact that the platelets are colorless and the tendency of the platelets to agglutinate, disintegrate readily, and react with airborne bacteria. Previous platelet stain solutions have been prepared using formaldehyde, sodium citrate and brilliant cresyl blue dye. These solutions have been useful in making rough estimates of the platelet count but no microscopic method has been previously regarded as satisfactory.

The present invention is directed to compositions for staining the platelets, preserving them against agglutination, and most importantly eliminating the masking red and white cells, and to a method of counting platelets employing the composition.

SUMMARY OF THE INVENTION

The present invention is broadly directed to a composition having a surface tension sufficiently low so that when a blood sample is dissolved in the composition the large, relatively low surface tension red and white cells are quickly hemolyzed, and yet sufficiently high so that the smaller, higher surface tension platelets are not destroyed by the composition over relatively larger periods of time. Dissolving a blood sample in this composition allows platelets to be counted easily and with a high degree of accuracy with conventional microscopes, phase microscopes or electron microscopes.

The present invention is based on my discovery that when whole blood serum is dissolved in a composition having a surface tension, measured at 22° C in distilled water of between 31.0 and 29.8 dynes per centimeter, the red and white blood cells in the serum will be hemolyzed within a relatively few seconds, but the platelets will not be hemolyzed in long periods. These surface tension limitations are relatively sharp and when a compound having a surface tension of more than 31.5 dynes per centimeter is employed the red and white cells are not fully hemolyzed within a matter of a few minutes. When a composition having a surface tension of less than 29.8 dynes per centimeter is employed some of the platelets are also quickly hemolyzed. The method of the present invention therefore comprises dissolving a blood sample in a composition having a surface tension within these critical limits and then microscopically studying the dissolved sample after about

30 seconds to count the platelets which are readily observable in the absence of the red and white cells.

The action of the nonionic surfactants in hemolyzing the red and white cells is believed to be solely through a reduction of Vanderwaal's forces cause a "bursting" of the cells. The effect of the composition on the platelets is not only a function of the surface tension of the composition but also of the percentage of surfactant in the composition. While the ability of the surfactant to lower the surface tension of the composition stabilizes after a certain percentage of the surfactant in the composition has been reached increasing percentages of surfactant above the plateau percentage will tend to increase the rate which the platelets are hemolyzed. For example, a composition containing 0.05 percent of a given surfactant may have a resulting surface tension of 30.0 dynes per centimeter. Concentration of 0.25 percent of the same surfactant may only lower the surface tension to 29.8 dynes per centimeter but will still greatly accelerate the rate at which platelets in the blood serum are hemolyzed. When the percentage of surfactant is so high that any platelets are hemolyzed in less than about 5 minutes the composition becomes extremely difficult to use in connection with the method of the present invention. Accordingly, compositions used in connection with the preferred embodiment to the invention should not contain surfactants in concentrations greater than about 0.1 percent.

The preferred composition of the present invention consists of a solution, in water and alcohol of a surfactant that produces the desired surface tension in the desired concentrations and certain known preservatives as well as unique preservatives and a unique stain. The surfactant is preferably present in a solution in concentrations of between 0.001 and 0.1 percent. The surfactant is preferably chosen from the group consisting of nonionic, cationic and anionic surfactants which are capable of producing the desired surface tension in the percentages indicated. Surfactants which would produce the desired surface tension in concentrations lower than 0.001 percent but would produce lower surface tensions in higher concentrations are undesirable since small variations in concentration of these surfactants may produce appreciable variations on the surface tension of the composition. Nonionic surfactants producing the desired surface tension in the desired concentration are preferable to those anionic and cationic surfactants which will produce the surface tension since the later tend to chemically react with various of the blood constituents to produce products which complicate the counting process.

Various nonionic, anionic, cationic surfactants may be used in connection with the compositions of the present invention. While ionic surfactants have the capability of producing the desired range of surface tension in low concentrations and provide the advantage of a clear background for counting the platelets, they have two disadvantages. First they react with protein in the blood forming complex salts which may obscure the counting process and secondly they have a tendency to hemolyze the platelets at relatively high surface tensions relative to the nonionic surfactants. This is probably due to chemical attack on the platelets by the polar groups of the ionic surfactants whereas the nonionic surfactants tend to hemolyze the red and white cells solely due to a reduction in Vanderwaal's forces.

A preferred composition also contains potassium fluoride to prevent the rapid destruction of the sample supported on a slide by exposure to airborne bacteria. Potassium fluoride, being a salt of a strong acid and a strong base does not have a PH effect on the solution. The fluoride ion provided by the potassium fluoride is the active component in the preservative action. The high solubility of the potassium fluoride is extremely useful in preserving the blood samples on the slide.

In addition to the potassium fluoride the preferred composition includes formaldehyde as a solution preservative and a chelating agent such as sodium citrate to stop coagulation.

The preferred solution further contains alphazurine 2G dye. This particular dye has not previously been used for the dying of platelets and I have found that it is more selective and provides a better contrast with the background products than brilliant cresyl blue which has previously been used as a stain for platelets. The preferred composition also contains an alkaline metal oxalate, and preferably potassium oxalate, which acts as an anticoagulant and a chelating agent to improve the clarity of observation of the stained platelets.

Sodium Citrate	2.2% by weight
Potassium Oxalate	0.7% by weight
Potassium Fluoride	1.8% by weight
Ethyl Alcohol 95%	8.0% by weight
Formaldehyde 37% volume	0.4% by weight
Alphazurine 2G dye	0.125% by weight
Distilled water and Surfactant	Balance

Varying percentages of surfactants to be tested between 0.002 and 0.01 percent were added to the base solution. Slides prepared with blood samples dissolved in the solution were observed to determine the time required to achieve complete hemolysis of the red and white cells. In those cases where the time of complete hemolysis was less than about 20 minutes, the count of the platelets were made using the solution and microscopic techniques and the results were compared to the platelet count as determined by a Coulter electronic counter to determine if any appreciable percentage of the platelets were destroyed. The surface tensions of the various solutions were also measured using a Fisher Du Nuoy Tensiometer, Model 21. Using this method, the following results were achieved:

Surfactant	Percentage Added to Formula	Time of Complete Red and White Cell Hemolysis	Microscopic Platelet Count % Relation to Count Made by Coulter Electronic Counter	Surface Tension At 22°C - Fisher DuNuoy Tensiometer Model 21
9-10 MOL				
Alkyl Phenoxy (polyethylene oxy)	.003%	over 120 min.	Not Performed	32.7
Ethanol	.005%	over 80 min.	-3.6%	31.6
	.008%	8 min.	-1.8%	30.6
	.009%	6 min.	-2.0%	30.5
	.01%	1.8 min.	-1.2%	30.2
Sodium Alkyl Ether Sulfate	.002%	over 200 min.	Not Performed	32.8
	.004%	>1 min.	Platelets Destroyed	29.7
	.008%	>1 min.	Platelets Destroyed	28.9
15-16 MOL				
Alkyl Phenoxy (polyethylene oxy)	.003%	<240 min.	Not Performed	34.4
Ethanol	.007%	<240 min.	Not Performed	33.5
Sodium Lauryl Sulphate	.015%	30 min.	Platelets Destroyed	33.5
	.003%	50 min.	-8.4%	32.0
	.005%	20 min.	-.8%	31.2
	.01%	>1 min.	Platelets Destroyed	29.2
Alkyl Dimethyl Benzyl Ammonium Chloride	.002%	80 min.	-4.2%	32.2
	.004%	2.5 min.	-2.1%	29.8
	.006%	>1 min.	Platelets Destroyed	26.2
Alkyl Aryl Sulfonate	.004%	<200 min.	Not Performed	33.1
	.008%	<200 min.	Not Performed	32.0
	.015%	>1 min.	Platelets Destroyed	29.4
Sodium Di-octyl Sulfo Succinate	.002%	over 200 min.	Not Performed	32.8
	.004%	>1 min.	Platelets Destroyed	27.6
	.006%	>1 min.	Platelets Destroyed	23.4
Substituted Imidazoline	.03%	<200 min.	Not Performed	34.6
	.01%	<200 min.	Not Performed	31.6
	.01%	>1 min.	Platelets Destroyed	31.2

In the preferred practice of the present invention a small quantity of blood sample is introduced into approximately 100 times that volume of the solution. The two are thoroughly mixed and spread on a slide. In about one minute after the slide preparation it is viewed in a microscope and the stained platelets are fully visible without interference from the red and white cells which have been hemolyzed.

It is therefore seen that my invention greatly simplifies and increases the accuracy of microscopic counting of blood platelets using a relatively low cost and highly stable solution.

To determine the utility of various surfactants in connection with the present invention, the following base composition was prepared:

The following preferred examples of my invention are intended to be illustrative only and not to limit the scope of the invention:

EXAMPLE 1

Distilled water	86.765%	by weight
Sodium Citrate	2.2%	by weight
Potassium Oxalate	0.7%	by weight
Potassium Fluoride	1.8%	by weight
Ethyl Alcohol 95%	8.0%	by weight
Formaldehyde 37% volume	0.4%	by weight
Alphazurine 2G dye	0.125%	by weight
Alkyl Phenoxy (polyethylene oxy) Ethanol	0.01%	by weight
TOTAL:	100%	

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This composition has a surface tension 30.2 dyn/cm. As set forth above, the red and white blood cells are completely hemolyzed within 2 minutes of dissolving a blood sample in the solution. A blood sample dissolved in this preferred composition and then analyzed by microscope tested out to have a platelet count within 1.4 percent of the same count achieved using a Coulter electronic counter.

EXAMPLE 2

This composition is the same composition as Example 1 but the nonionic Alkyl Phenoxy (polyethylene oxy) Ethanol is replaced with an anionic Sodium Lauryl Sulfate in the amount of 0.006 percent by weight of the composition. The distilled water content is increased to provide the balance. This composition has a surface tension of 30.0 dyn/cm. the red and white blood cells are completely hemolyzed in approximately 5 minutes. The microscopic platelet count using this solution was within 3 percent of the count produced by the Coulter method.

EXAMPLE 3

In this example the basic formula of Example 1 is used but the nonionic surfactant was replaced with a cationic surfactant, alkyl dimethyl benzyl ammonium chloride in the amount of 0.004 percent by weight of the solution. The solution produced a surface tension of 29.8 dyn/cm., the red and white cells were completely hemolyzed within 2½ minutes and the microscopic count of platelets in the solution was within 2.4 percent of the count as analyzed by a Coulter counter.

EXAMPLE 4

Various combinations of ionic and nonionic surfactants may also be employed with the compositions of the present invention. When ionic surfactants are

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added to the composition containing nonionic surfactants a clearer background is attained.

One such composition involves the base composition of Example 1 but employing 0.006 percent by weight of the nonionic alkyl phenoxy (polyethylene oxy) ethanol and 0.002 percent by weight of anionic Sodium Lauryl Sulfate. This composition produced a surface tension of 30.1 dyn/cm., completely hemolyzed the red and white blood cells in less than four minutes and produced a platelet count under microscope of within 1.6 percent of that achieved with the Coulter counter.

EXAMPLE 5

In this example 0.005 percent of the nonionic surfactant of Example 1 was combined with a 0.002 percent of the cationic surfactant of Example 3. The resultant composition had a surface tension of 30.1 dyn/cm., completely hemolyzed the red and white cells within four minutes and provided a count under microscopic analysis of within 2 percent of the count achieved with the Coulter counter.

Having thus described by invention, I claim:

1. The method of counting platelets in a specimen of whole blood serum comprising dissolving the specimen in a solution having a surface tension in distilled water, measured at 22°C of between 29.8 and 31.0 dynes/cm. and observing and counting the platelets through a microscope after the red and white cells have been hemolyzed.

2. The method of counting the blood platelets in a sample of whole blood serum comprising dissolving the sample in a solution containing a surfactant, the solution having a surface tension in distilled water, measured at 22° C. of between 29.8 and 31.0 dynes/cm. to hemolyze the red and white cells, and observing and counting the platelets as viewed with a microscope.

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