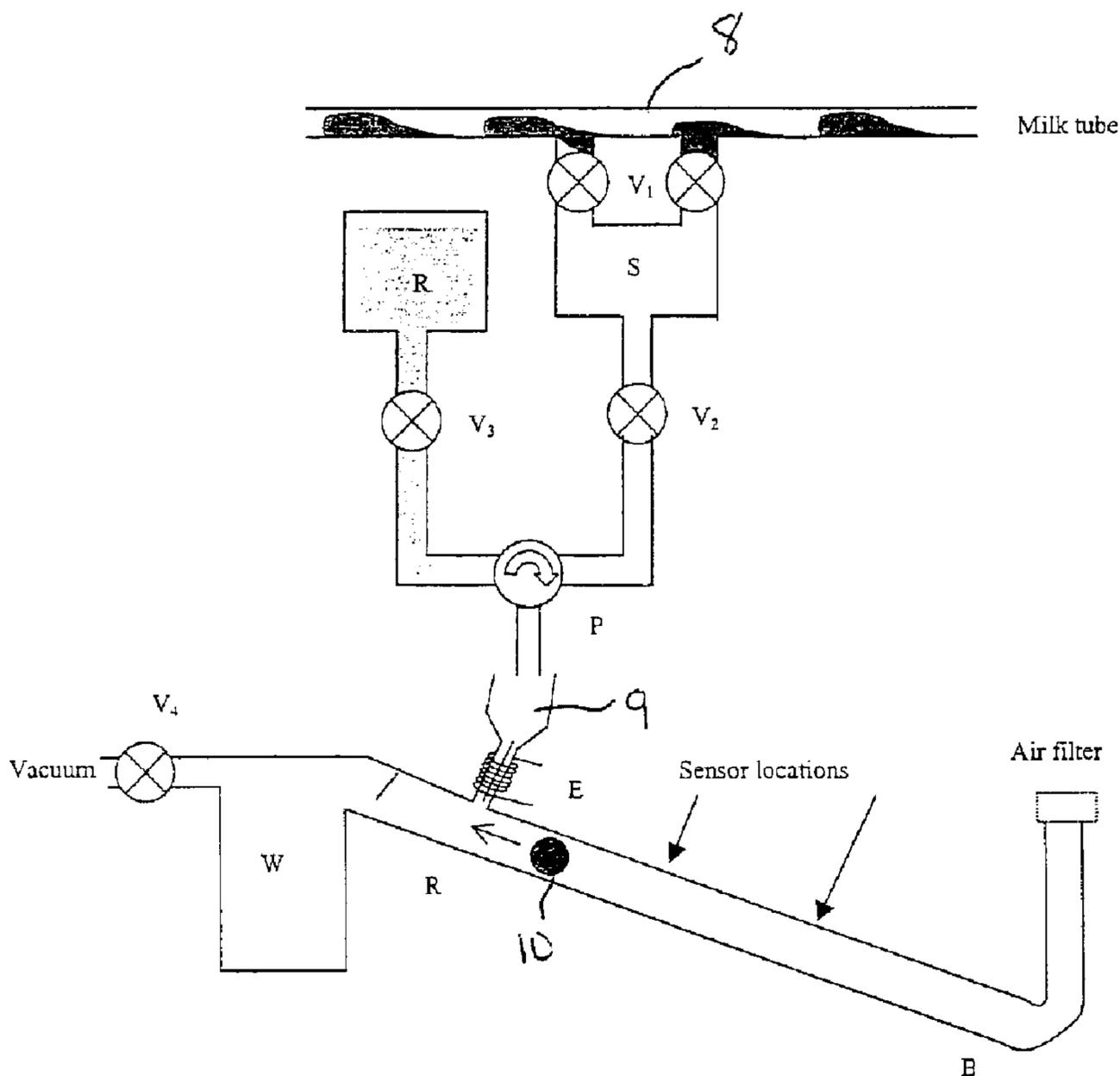




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 (54) Title: MASTITIS DETECTION



(57) Abrégé/Abstract:

Testing procedures for milking animals whereby a milk flow has a sample flow diverted therefrom which is then tested for somatic cell count indicative of mastitis. Such testing of the diverted flow is automatic and involves a measure of viscosity change in the

(57) **Abrégé(suite)/Abstract(continued):**

sample after the addition of an anionic detergent which causes gelling to an extent determined by the somatic cell count. As a consequence calibratable data from each milking animal can be generated and used as a comparative tool between animals and/or to monitor changes in the somatic cell count (SCC) of individual animals between milkings.

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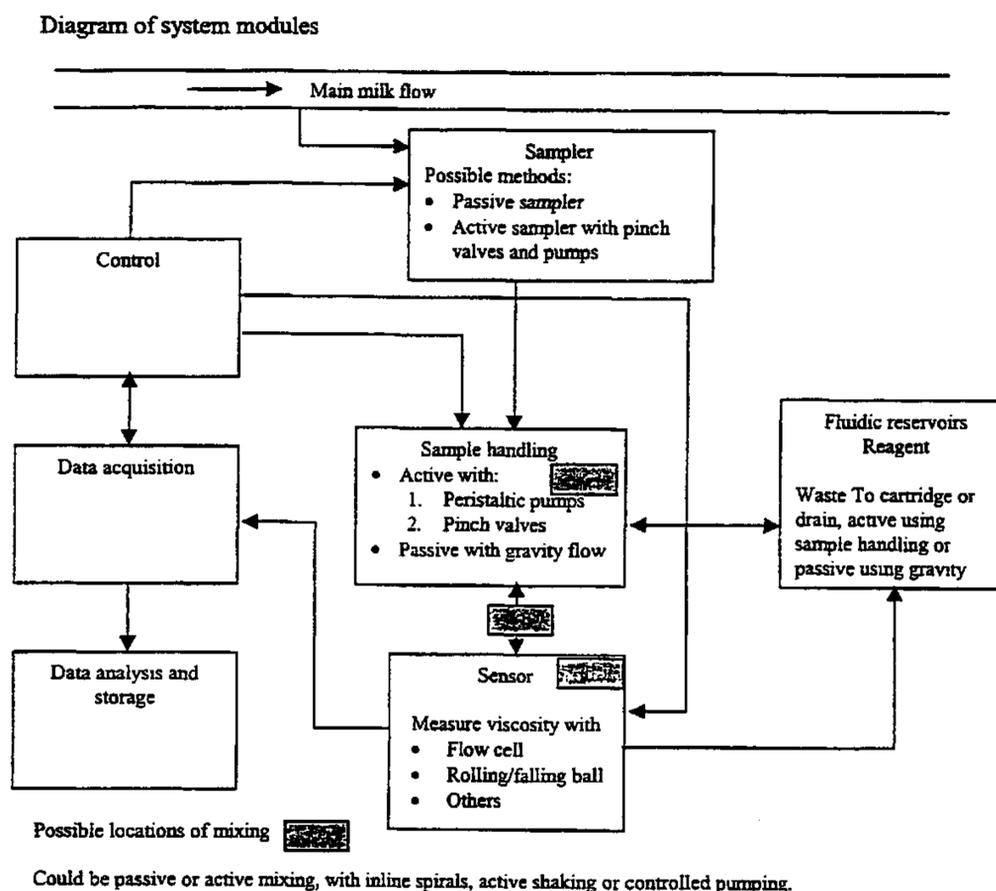
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(54) Title: MASTITIS DETECTION



(57) Abstract: Testing procedures for milking animals whereby a milk flow has a sample flow diverted therefrom which is then tested for somatic cell count indicative of mastitis. Such testing of the diverted flow is automatic and involves a measure of viscosity change in the sample after the addition of an anionic detergent which causes gelling to an extent determined by the somatic cell count. As a consequence calibratable data from each milking animal can be generated and used as a comparative tool between animals and/or to monitor changes in the somatic cell count (SCC) of individual animals between milkings.

WO 01/35728 A1

**WO 01/35728 A1**



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*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

## MASTITIS DETECTION

### TECHNICAL BACKGROUND

The present invention relates to mastitis detection and to related apparatus, methods and  
5 procedures.

The present invention recognises that an advantage is obtained for a farmer where there  
is the prospect of on-line monitoring of the somatic cell count indicative of mastitis infection  
in individual animals. Such monitoring either allows a treatment regime to be instituted for the  
particular animal or animals and/or for the isolation of or recriminations, if appropriate, or in  
10 respect of any such affected milk (whether at the milking parlour, farm, factory or elsewhere).

### BACKGROUND ART

Procedures exist whereby a non isolated flow of milk might directly be tested for  
mastitis. Such procedures have tended to find favour but at the cost of reduced accuracy over  
isolated milk testing procedures.

15 *Journal of Dairy Research* (1998) 65 187-198 "Changes in Electrical Conductivity and  
Somatic Cell Count Between Milk Fractions From Quarters Subclinically Infected with  
Particular Mastitis Pathogens" *M W Woolford et al.* discloses that intramammary infection  
(which seriously reduces milk yields) is frequently associated with increases in electrical  
conductivity in milk owing to increased levels of sodium and chloride ions in the milk. The  
20 monitoring of electrical conductivity can therefore be a means of automatically tracking udder  
health. Yet *Woolford et al* states that factors such as temperature, fat concentration, milk solids,  
bacterial type, and milk fraction have variously been found to influence the measure of  
electrical conductivity. It is stated that such factors are important since the increase in  
electrical conductivity induced by intramammary infection is typically in the range of 15% to  
25 50% only whereas the increase in somatic cell count (SCC) is usually at least 1000% increase.  
Moreover *Woolford et al* states there are substantial physiological variations in the normal  
electrical conductivity levels that preclude comparisons of absolute milk electrical conductivity  
levels amongst cows for the purposes of identifying infection.

Examples of procedures and related apparatus that have been developed reliant on  
30 electrical conductivity to test milk flows include EP0748156 of Gasgoine-Melotte B.V. and  
PCT/SE97/00671 (published as WO97/40374) of Alfa Laval Agri AB.

The full content of such *Woolford et al* publication and specifications EP0748156 and  
WO97/40374 are hereby here included by way of reference.

-2-

*Gasgoine-Melotte BV* in their EPO 748156 comment on two sampling procedures so that still milk can be tested for conductance. They refer *inter alia* to EP-B-137367.

The *Woolford et al* publication in addition makes reference to somatic cell count (SCC) as a diagnostic criteria by indicating that, for example, greater than 500,000 cells/ml might be indicative of infection whilst less than 500,000 cells/ml may be indicative of no infection.

**Table 1 (from *Woolford et al*) Diagnostic criteria for electrical conductivity, within-cow electrical conductivity ratio and somatic cell count used for prediction of the infection status of individual quarters.**

10

Predicted quarters status	Required diagnostic criteria	
	Conductivity	Somatic cell count, cells/ml
Infected	ECR > 1.15	> 500000
	or EC > 7000 $\mu$ S	
Uninfected	ECR < 1.5	<500000

15

where EC is electrical conductivity and ECR is electrical conductivity ratio

*Woolford et al* indicates that reliance on somatic cell count (SCC) using a criteria based on 500,000 cells/ml provided greater sensitivity than did the electrical conductivity measures.

20 Test procedures for mastitis for manually isolated milk are known where the milk that is tested is not subsequently returned to the main volume of the milk.

One type of such isolated milk testing regime is a cow-side gel forming mastitis test procedure. This is typified by the Rapid Mastitis Test (RMT) [or California Mastitis Test (CMT)]. See "*Journal of American Veterinary Medical Association*, Vol 130, March 1, 1957 -  
25 No. 5 - "Experiments and Observations Leading to Development of the California Mastitis Test" of *O. W. Schalm and D. O. Noorlander*.

The CMT procedure utilises an anionic surfactant (e.g. a detergent such as, for example, sodium lauryl sulphate commonly marketed at TEEPOL™). The CMT procedure results in a precipitate or gel indicative of the degree of infection (i.e. the SCC).

30 *Australian Journal of Dairy Technology* 23, 129 (1968) *E A Kernohan* has shown that accuracy of somatic cell count (SCC) reliant upon the CMT procedure is dependant on the relative amounts of milk and reagent utilised. For best results preferably a near one to one

volume ratio of a suitable reagent to milk is used in equal volumes for best results [for example, 2% w/v sodium laurel sulphate (commonly marketed as TEEPOL™) in water used in equal volume with milk].

Historically the CMT procedure of *Schalm and Noorlander* was graded on a score  
5 outlined below.

This score corresponds to SCC (*Milchwissenschaft* 19, 65-69 (1964) Halbquantitative Ausarbeitung des Schalmtestes für wissenschaftliche Zwecke of *Keirmeier and Keis*). The measurement technique used an “eyeball” technique. Therefore some variation exists in interpretation:

- 10 • (-) negative, remains liquid,
- (T) trace, slight precipitate which tends to disappear with more movement, >116 000 cells/ml
- (+) weak positive, precipitate but not gel formation, >315 000 cells/ml
- (++) distinct positive, thickness immediately with some gel formation, >600 000  
15 cells/ml
- (+++) strong positive, distinct gel formation adherence to bottom of paddle and during swirling peaks forms >1 000 000 cells/ml

*The Journal of Milk and Food Technology* 27, 271-275 (1964) - “The Wisconsin Mastitis  
20 Test - An Indirect Estimation of Leucocytes in Milk” of *Thompson and Postle* discloses the Wisconsin Mastitis Test (WMT). In the WMT the milk and detergent were mixed in a tube. The tube was then inverted and a small hole in the top (3/64 inch diameter) allows the watery part to drain out. The height of the residual was then measured after at least a 1 min inversion. This is still in use in some small laboratories today with an active SCC range from 100 000 to  
25 1.2 million cells/ml.

This test was then investigated in the mid 1970s (*Milchwissenschaft* 27 (2) 1972 “A simple semi-automatic viscosimeter for the estimation of somatic cells in milk”, *Whittlestone et al.*, and *Milne et al.*, 1976) to try and automate the testing for use in the laboratory.

It was found that some ways of measuring the viscosity of the gel destroyed the gel.  
30 Ultimately a rolling ball viscometer where the time for a ball to roll through the gel was timed. This eliminated errors due to gel destruction. The active SCC ranges were 250 000 - 2 million cells/ml (*Whittlestone et al.*) and 100 000 - 1.3 million cells/ml (*Milne et al.*) respectively. This was also undertaken in Germany (*Kiermier and Keis* 1964).

See also the publications:

- *The Australian Journal of Dairy Technology*, 21, 138-139, (1966) "An Automatic Viscometer for the Measurement of the California Mastitis Reaction" *Whittlestone et al*;
- *Journal of Milk & Food Technology* 33, 35-354 (19...) "A Viscometric Method for the Estimation of Milk Cell count" *Whittlestone et al*;
- *Milchwissenschaft* 27, (2) 84-86 (1972) "A Simple Semi-automatic Visosimeter for the estimation of somatic cells in Milk" *Whittlestone et al* and
- *New Zealand Journal of Dairy Science and Technology*, 11, 21-23 (1976) *Milne et al* each discuss procedures for measuring the viscosity of the gels of such prior art detergent/milk SCC procedures with a view to determining a viable measurement regime.

Procedures disclosed include orifice or capillary viscometers (ie; moving gel) as well as falling ball or rolling ball viscometers (ie; stationary gel).

*Milne et al* standardised a *Whittlestone et al* type rolling ball viscometer to a tilt angle of 25° in preference to less accurate falling ball viscometers as even very gentle shear forces were found to cause a significant decrease in viscosity. *Milne et al* found about a 3.5 sec tilt time resulted at that 25° tilt angle when they used a ball of 4.7mm diameter in a tube of 5.5mm I.D in a gel made using 2% w/v TEEPOL 610™ (Shell Chemical Company) in water solution as the reagent in volume ratios of 10ml of the reagent to 5ml of milk.

The present invention recognises a particular accuracy and convenience is available for any such monitoring regime reliant upon the gelling of a milk sample using an appropriate non-ionic surfactant and thereafter to test the viscosity by appropriate means to thereby (by reference to some calibration of sample viscosity against the extent of mastitis infection and/or somatic cell count) to provide an indicator representation or record for the particular sample and thus the particular animal.

## DISCLOSURE OF THE INVENTION

It is therefore an object of the present invention to provide apparatus, methods and systems appropriate to the end of enabling on-line monitoring of milk from animals being milked or at least to allow the automatic or semi automatic testing of milk sampled from a milk volume (preferably a milk flow).

In a first aspect the present invention consists in a **method of testing a milk flow for mastitis**, said method comprising or including

diverting a sample flow from a said milk flow, and

thereafter automatically testing such diverted milk flow by measuring an attribute of any

somatic cells in at least part of the milk of that sample.

Preferably said attribute is of the DNA of the somatic cells.

Preferably the measuring is by reference to a gelling characteristic of the milk under the action of an anionic detergent, such gelling being dependent upon at least part of the DNA of  
5 somatic cells.

Preferably said measurement is by reliance upon the viscosity of at least part of the milk of that sample when in a predetermined admixture for a predetermined time with an appropriate predetermined anionic detergent such as to enable the viscosity indicator to be comparable with previous and/or subsequent testing to enable changes in somatic cell count  
10 to be determined between the milk flows to be compared.

Preferably said milk flow is from a single animal and an identifier of that animal and an indicator of viscosity and thus of the somatic cell count is coupled for comparative purposes.

In another aspect the present invention is **a method of determining whether or not there is a somatic cell count change between milkings in milk of an animal**, said method  
15 comprising or including

- (i) from one milking,
  - (a) diverting a sample flow from the milk flow from the animal, and
  - (b) automatically testing such diverted milk flow for somatic cells by measuring an attribute of any somatic cells in at least part of the milk  
20 of that sample or sample flow, and
- (ii) in another milking,
  - (a) diverting a similar sample flow from the milk flow from the animal, and
  - (b) similarly testing such diverted milk flow for somatic cells by measuring the same said attribute of any somatic cells in at least part of the milk  
25 of that sample or sample flow, and
- (iii) comparing data resulting from said testings to determine any somatic cell count change.

Preferably said attribute is of the DNA of the somatic cells.

Preferably the measuring is by reference to a gelling characteristic of the milk under  
30 the action of an anionic detergent, such gelling being dependent upon at least part of the DNA of somatic cells.

Preferably each said measurement is by reliance upon the viscosity of at least part of the milk of that sample when in a predetermined admixture for a predetermined time with an

appropriate predetermined anionic detergent such as to enable the viscosity indicated between the testings to be comparable thereby to enable the changes in somatic cell count to be qualitatively and/or quantitatively determined between the milkings.

Preferably said automatic testing is of stationary milk.

5 Alternatively said automatic testing is of a milk flow whilst the milk moves.

In still another aspect the invention is **a method of testing a milk flow for mastitis**, said method comprising or including

diverting a sample flow from a said milk flow,

10 providing an inflow of an anionic detergent and an inflow of said milk sample in predetermined proportions into a dwell zone from whence the detergent/milk admixture as a gelling and/or gelled product can outflow only at a lesser rate than said inflow(s),

feeding the outflow admixture product of said dwell zone to a viscosity testing zone, and testing in said viscosity testing zone the outflow admixture product for viscosity and generating an output signal indicative of such viscosity, and

15 if necessary, clearing (i) the viscosity testing zone or (ii) the viscosity testing zone and said dwell zone of said admixture without feedback into the milk flow from which the sample flow was diverted.

Preferably said inflows are not mixed prior to said dwell zone.

20 Preferably said milk flow is the milk from at least one teat of a known animal, such animal being identified by an output signal from an animal identifying sensor.

Preferably a receiver receives the output signal indicative of viscosity and associates such output signal with the animal by reference to either an input signal or a said animal identifying sensor output signal.

25 Preferably the data received by said receiver is stored for comparative purposes with subsequent data received at subsequent milkings from the same animal.

Preferably said sample flow is drawn off a milk flow during milking, such draw off being substantially identical for each milking having regard to

(ii) commencement of milking generated milk flow,

(iii) duration or volume of draw off, and/or

30 (iv) elapsed milk flow.

Preferably said inflow of anionic detergent is a pumped inflow.

Preferably said inflow of said milk sample is one of

(v) an inflow under gravity (metered or unmetered)

-7-

(vi) a pumped inflow (metered or unmetered), or

(vii) both (i) and (ii).

Preferably said inflow of said milk flow and said inflow of said anionic detergent are pumped inflows.

5 Preferably the testing in said viscosity testing zone of the outflow admixture product for viscosity is whilst the at least partly gelled product is stationary.

Preferably a rolling ball test for viscosity is utilised which generates said output signal indicative of such viscosity, such output signal being an elapsed time or a function of an elapsed time.

10 In another preferred form said viscosity testing zone involves an outflow dependent viscosity testing device without moving parts.

Preferably said dwell zone and said viscosity testing zone are a common chamber of apparatus into which said inflows are provided.

Preferably the clearing is self clearing during and/or after the viscosity testing.

15 Preferably there is a clearing step and the clearing involves a valved outflow.

Preferably there is a clearing step and the clearing involves a pumped outflow.

Preferably there is a clearing step and the clearing involves an application of a vacuum source to at least the viscosity testing zone.

Preferably no flushing fluid other than drawn in air is utilised in said clearing step.

20 In yet another aspect the invention is **a method of testing a milk flow for mastitis**, said method comprising or including (and in any workable order)

diverting a sample from a said milk flow from an animal,

providing an animal identifier input or signal capable of identifying said animal to data acquisition, analysis and storage means;

25 providing an inflow of an anionic detergent and an inflow of said milk sample in predetermined proportions into a dwell zone to allow at least partial gelling of the mixture, and

providing an outflow of the at least partially gelled mixture of said dwell zone to and/or through and/or using a viscosity testing zone and (immediately or subsequently) generating an output signal to said data acquisition, analysis and storage means indicative of the viscosity of

30 a predetermined part of the mixture that has been subject to a predetermined gel forming dwell time post mixing (whether prior to, during or post said inflow(s)), and

and, if necessary, clearing (i) the viscosity testing zone or (ii) the viscosity testing zone and said dwell zone of said mixture without feedback into the milk flow from which the sample

was diverted.

Preferably said inflows are not mixed prior to said dwell zone.

Preferably the data received by said acquisition, analysis and storage means is stored for comparative purposes with subsequent data received at subsequent milkings from the same  
5 animal.

Preferably said milk flow is a milking caused milk flow.

Preferably said sample flow is drawn off a milk flow during milking, such draw off being substantially identical for each milking having regard to

- (i) commencement of milking generated milk flow,
- 10 (ii) duration or volume of draw off, and/or
- (iii) elapsed milk flow.

Preferably the testing in said viscosity testing zone of the outflow admixture product for viscosity is whilst the at least partly gelled product is stationary.

Preferably a rolling ball test for viscosity is utilised which generates said output signal  
15 indicative of such viscosity, such output signal being an elapsed time or a function of an elapsed time.

Preferably there is a vacuum "recocking" of the ball for a subsequent viscosity test along with the emptying of the already tested admixture product from said viscosity testing zone or said viscosity testing zone and said dwell zone.

20 Preferably said inflow of anionic detergent is a pumped inflow.

Preferably said inflow of said milk sample is one of

- (i) an inflow under gravity (metered or unmetered),
- (ii) a pumped inflow (metered or unmetered), or
- 25 (iii) both (i) and (ii).

Preferably said inflow of said milk flow and said inflow of said anionic detergent are a pumped inflows.

Preferably there is a clearing step and the clearing involves an application of a vacuum source to at least the viscosity testing zone.

Preferably no flushing fluid other than drawn in air is utilised to clear materials.

30 Preferably (despite the embodiment of the inventive method being used) said predetermined proportions are in the volume ratios of 5:1 to 1:5.

Preferably said predetermined proportions are in the range of from 2:1 to 1:2 inclusive.

Preferably said predetermined proportions are substantially 1:1.

Preferably said anionic detergent is a Gardinol Type Detergent as defined in the Merck Index.

Preferably said anionic detergent is an aqueous solution of about 2% w/v sodium laurel sulphate.

5 Preferably the procedure is fully automatic once initiated.

In another aspect the invention comprises, **in or associated with a milk flow path for milk of an individual animal from at least one cup of a milking claw to any accumulation reservoir or conduit for such flow, the provision of**

(I) means to divert part of the flow as a sample into apparatus forming at least part of  
10 a mastitis testing regime,

(II) such apparatus, and

(III) means to generate a signal or representation or record indicative of the test result insofar as mastitis or somatic cell count is concerned for the milk sample taken into said apparatus.

15 Preferably means is provided whereby, after initiation, steps (II) and (III) proceed automatically after step (I).

Preferably means is provided whereby all steps proceed automatically once initiated by an operator during preparation for milking or during milking.

20 Preferably said apparatus includes means to mix an appropriate surfactant with the milk sample thereby to generate a gel indicative of somatic cell count, means to test the gel thus generated for viscosity and means to cause or allow the gel to clear from the apparatus.

Preferably said apparatus is such that an identical testing regime is followed for each sample taken from the milk flow such that for an individual animal or as between individual animals, or both, there is a comparative basis.

25 Preferably there is provided data acquisition, analysis and storage means and there is provided means to provide an animal identifier input or signal capable of identifying an animal being milked to said data acquisition, analysis and storage means and said means to generate a signal or representation or record itself provides an output signal to said data acquisition, analysis and storage means indicative of the viscosity of the milk sample gel in the apparatus  
30 for tying to the animal identified.

Preferably said apparatus includes a rolling ball type viscosity tester capable of generating an output signal reliant on a elapsed time or a function of elapsed time.

In yet another aspect the invention is **apparatus for testing a milk flow for mastitis,**

-10-

said apparatus comprising or including

means to divert a sample flow from a said milk flow, and

means thereafter automatically to test such diverted milk flow by measuring an attribute of any somatic cells in at least part of the milk of that sample.

5 Preferably said means automatically to test is reliant upon the mixing of an anionic detergent with the milk sample and thereafter obtaining some measure of the viscosity of any resultant gel.

In still another aspect the invention is **apparatus** for or suitable for performing a method of determining whether or not there is a somatic cell count change between milkings in milk  
10 of an animal, said method comprising or including

(i) from one milking,

(a) diverting a sample flow from the milk flow from the animal, and

(b) automatically testing such diverted milk flow for somatic cells by measuring an attribute of any somatic cells in at least part of the milk  
15 of that sample or sample flow, and

(ii) in another milking,

(a) diverting a similar sample flow from the milk flow from the animal, and

(b) similarly testing such diverted milk flow for somatic cells by measuring the same said attribute of any somatic cells in at least part of the milk  
20 of that sample or sample flow, and

(iii) comparing data resulting from said testings to determine any somatic cell count change,

**said apparatus** comprising or including

the same means useful for each of steps (i)(a) and (ii)(a),

25 the same means for steps (i)(b) and (ii)(b) (save for any expendables, e.g. anionic detergent), and

data acquisition, analysis and storage means to perform step (iii) by reference to an identifier it has for the animal.

30 Preferably said each of steps (i)(b) and (ii)(b) said attribute is a viscosity change response arising from the treatment of the milk sample with an anionic detergent.

Preferably there is means that uses an appropriate surfactant to generate a gel from the milk sample capable, means to test the gel thus generated for viscosity or some function of viscosity and means to cause or allow the gel to clear from the apparatus.

-11-

In yet another aspect the invention is **a method of testing an animal for mastitis by on-line testing of milk from such an individual animal as it is being milked**, such testing being reliant upon an automated surfactant gelling of an automatically diverted sample from the milk flow in a predetermined and reproducible manner and the automated measurement of the  
5 viscosity or some function of viscosity thereof thereby to generate data from whence somatic cell count can be assessed or compared.

In another aspect the invention is **apparatus** substantially as herein described with reference to any one, some or all of the accompanying drawings.

In another aspect the invention **a method of testing an animal, animals, a milk flow**  
10 **or milk** when performed substantially as herein described with reference to any one, some or all of the accompanying drawings.

In still a further aspect the present invention consists in **a milking shed** having provision for a testing regime in accordance with any of the earlier embodiments stated.

In another aspect the present invention consists in, **in a milk flow path for milk of an**  
15 **individual animal from at least one cup of a milking claw to any accumulation reservoir or conduit for such flow** (particularly where the individuality of the milk accumulation or flow is lost), **the provision of**

means to take part of the flow as a sample into apparatus forming at least part of a mastitis testing regime,

20 such apparatus having means that uses an appropriate surfactant to generate a gel from the milk sample capable, means to test the gel thus generated for viscosity and means to cause or allow the gel to clear from the apparatus, and

means to generate a signal or representation or record indicative of the test result for the milk sample taken into said apparatus.

25 Preferably such apparatus includes or comprises means adapted to merge or mix a non-anionic surfactant with the milk sample taken into the apparatus so as to generate a gel, and means thereafter, by reference to the viscosity of the gel, to generate an indicator dependent on the somatic cells in the sample.

Preferably said apparatus is capable of discharging the gel just tested.

30 Said apparatus may include self-flushing means.

In yet another aspect the present invention consists in, **in a milk flow path for milk of an individual animal from the cups of a milking claw, the provision of**

means to take part of the flow as a sample into apparatus forming at least part of a

-12-

mastitis testing regime, said apparatus having means that uses an appropriate surfactant to generate a gel from the milk sample capable, means to test the gel thus generated for viscosity and means to cause or allow the gel to clear from the apparatus, and

5 means to generate a signal, representation and/or record indicative of the presence of or level of somatic cells in the milk sample, such signal, representation or record having been derived from the apparatus provided measure of viscosity of the gel.

10 Preferably reference to "viscosity" and a measure thereof is with respect to some reproducible mechanical interaction with or some reproducible physical characteristic the gel or of the gel with a surface or surfaces, (e.g. the gel falling through a conduit and being timed in its fall, a mass falling through the gel and being timed in its fall, etc.) capable of providing between samples of different somatic cell count a difference in measure data that either corresponds to such difference or can be calibrated (eg; to a plot or some equation) to provide appropriate relativity.

15 Preferably said signal representation or record is preferably associated with an identifier for the animal involved. In this respect preferably means is provided to scan an animal being milked to thereafter correlate such information to the test result for the milk sample to be taken from the milk flow of that animal.

In a further aspect the present invention consists in **a method of testing for mastitis in an animal** which comprises the use of a regime herein set forth.

20 In still a further aspect the present invention consists in **a method of on-line testing of milk from individual animals in a milking parlour for somatic cells thereby to determine the extent of mastitis in such animals by arranging for an indexed machine testing of a milk sample from each indexed animal**, such testing being reliant upon a (preferably automated) surfactant gelling of the sample and the automated measurement of the viscosity thereof.

25 Preferably such samples are diverted from the milk flow.

In still a further aspect the present invention consists in **apparatus for or suitable for association with the milk line of a milking machine to test milk of an animal being milked for somatic cells (and thus testing the animal being milked for mastitis)**, said apparatus comprising or including

means defining a liquid chamber having an ability to receive two liquid feeds,

one inlet feed to be an inflow of a sample flow of milk from the milk line, and the other inlet feed to be an inflow of an anionic detergent of a kind that will

-13-

cause a viscosity increase as a result of at least partial gelling of the resultant milk/detergent fluid, and

means to provide a measure of and to generate a data output indicative of the viscosity or a function of viscosity of at least part of the resultant milk/detergent fluid calibratable to the somatic cell count of the milk inflow,

**and wherein** there is provision whereby, after said means to provide a measure of and to generate a data output has at least taken a reading indicative of viscosity or a function of viscosity, said milk/detergent fluid can be cleared from the apparatus without contamination of milk in the milk line in order to allow a subsequent milk flow sample to be likewise tested.

In yet a further aspect the present invention consists in **apparatus for or suitable for association with the milk line of a milking machine to test milk of an animal being milked for somatic cells (and thus testing the animal being milked for mastitis)**, said apparatus comprising or including

means defining a first chamber having an ability to receive two liquid feeds,

one inlet feed to be an inflow of a sample flow of milk from the milk line, and the other inlet feed to be an inflow of an anionic detergent of a kind that will cause a viscosity increase as a result of at least partial gelling of the resultant milk/detergent fluid,

means defining a second chamber to receive at least part of the resultant milk/detergent fluid from said first chamber,

means to provide a measure indicative of viscosity or a function of viscosity of the resultant milk/detergent fluid in said second chamber calibratable to the somatic cell count of the said sample flow of milk, and

means to generate a data output indicative of said measure.

Preferably said apparatus includes a reservoir for an anionic detergent.

Preferably said apparatus has means in use adapted to reproducibly control the two inlet feeds.

Preferably there is provision in said means to provide a measure whereby said milk/detergent fluid can be cleared from the apparatus without contamination of milk in the milk line in order to allow a subsequent milk flow sample to be likewise tested.

In one embodiment said means to provide a second chamber is or includes the barrel of a rolling ball viscometer.

Preferably said means to provide a measure are spaced sensors (eg; optical sensors) of

a rolling ball viscometer.

Alternatively said means defining a first chamber and said means defining a second chamber are interconnected such that a chamber is defined into which there is adapted to be a greater infeed of fluids than outfeed therefrom thereby in use ensuring, for at least part of the milk/detergent fluid, a reproducible gelling time at which the fluid is measured by said means to provide a measure.

In still a further aspect the present invention consists in **somatic cell count data and/or comparisons thereof** generated using a method or apparatus of the present invention.

In another aspect the invention is **a method of testing an animal, animals, a milk flow or milk** when performed substantially as herein described with reference to any one, some or all of the accompanying drawings.

In still a further aspect the present invention consists in **a milking shed** having provision for a testing regime in accordance with any of the earlier embodiments stated.

Reference herein to diversion of or the taking of a sample of milk includes (where the context might allow) milk from one or more teats of the same animal or bulk milk, ie: milk from a number of animals.

SCC data can be used for comparative purposes of for an absolute decision (eg; greater or less than 500 000 cells/ml) for an individual teat, for an animal or for a grouping of animals.

## **DETAILED DESCRIPTION OF THE INVENTION**

Preferred forms of the present invention will now be described with reference to the accompanying drawing in which;

**Figure 1** (Figure 1A and 1B) shows for the same herd (hereafter referred to as "DRC" herd) the time changes in somatic cell count (SCC) measured in thousands of cells per millilitre with the peaks showing the actual somatic cell count, **Figure 1A** shows for a July through April period the upper 50% of the DRC herd ranked in order of average SCC, the peaks being against a scale ranging from 0 to 5 million cells/mL, whilst correspondingly **Figure 1B** shows the remainder of the herd likewise ranked for the same period but over a range of somatic cell count depicted on an axis running to 300,000 cells/mL only, the diagram thus showing the significant variation per animal over the period and the extent and localised nature of infection in a herd,

**Figure 2** is a diagrammatic view of apparatus which shows a method whereby a diverted flow can be despatched into a container and thence from a machine controlled valve down a tube or the like (eg; a Gilmont™ Viscometer available from Cole-Parmer International, USA)

with the viscosity of the gel formed in the container by appropriate means (not shown) being determined by the time of passage between two zones, (eg; measuring the gel flow commencement interface and gel flow completion interface using a light emitter and detector).

**Figure 3** shows the effect of sodium laurel sulphate concentration in respect of  
5 TEEPOL™ (14% w/v) of Shell Chemical Company using stock solution of bovine blood leucocytes,

**Figure 4** shows the effect of angle on a rolling ball type viscometer when compared with a Gilmont type falling ball viscometer, ie; which allows the ball to fall vertically (with respect to various aqueous concentrations of glycerine),

10 **Figure 5** shows the effect of ball size on the time the ball descends at various angles using 50% glycerine in water as the fluid, the slope of the resultant lines if plotted through the data points being approximately equal,

**Figure 6** shows the effect of changing ratio of milk to anionic detergent in water reagent (the milk being held to 1),

15 **Figure 7** shows the effect of SCC on viscosity using a 1:1 milk ratio,

**Figure 8** shows the effect of SCC on viscosity using a 2:1 milk ratio,

**Figure 9** shows a simplified plot of time for a rolling ball fall against SCC thereby showing a dynamic range of preference within which any testing regime should preferably operate, such dynamic range being described hereafter,

20 **Figure 10** shows a cross section of a rolling ball tube of rolling ball type viscometer of a kind in respect of which results for a quarter inch and five sixteenth inch balls are plotted in Figure 5,

**Figure 11** is a plot (*White and Rattray, 1965*) showing for a particular cow and its four teats the changes in SCC during an after milking, the rise in cell count during milking as they  
25 have indicated often being a factor or 10 or more and with any decline in cell count between milkings arising from dilution effects in the lower cisternal region of the animal and which decline may be subject to daily fluctuations in yield,

**Figure 12** is a prototype set up of apparatus to which the drawing sequence 12a to 12g refers,

30 **Figures 12A through 12G** show in respect of a milk tube line or the like apparatus in accordance with the present invention showing diagrammatically various stages of a mastitis testing regime,

**Figure 13** is a block diagram showing preferred flow parameters and options, and

**Figure 14** is a block flow diagram of electronic systems useful in apparatus and methods of the present invention.

Our invention provides a system on-line preferably in the milking parlour (eg; whether conventional or robotic) preferably using a gel type test. The viscosity of the gel can be measured by standard viscosity measurements (Cole Parmer 1999) [eg;. time of air bubble travel, time of draining from container, or other].

In Figure 2 a container 1 leads to a drainage tube 3 controlled by a valve 2. In the container 1 there can be a merging or other mixing of a milk sample and sufficient non-anionic detergent to allow a standard gel representative of somatic cell count to be generated. A light emitter 4 or 6 in conjunction with a light detector 5 and 7 respectively determines the commencement of gel flow down the tube 3 and its termination. Such light sensor system if desired can be used in the apparatus of Figures 12 to 12g.

Figure 3 - This figure was obtained by spinning down blood obtained from the Ruakura research abattoir, with heparin to stop blood clots. It was spun down so that the leukocytes could be extracted without red blood cells. This was then diluted with 0.15M NaCl to produce three solutions. The first solution was between 500 000–1 000 000 cells/ml. The second and third were  $\frac{1}{2}$  and  $\frac{1}{5}$ , of the first respectively.

Within 12 hours of blood being harvested it was tested. This was done by adding 4ml of reagent to 4ml cell solution in a 12ml centrifuge tube. When they were mixed a timer started, and the tube inverted once. When the timer reached 20 seconds the solution was poured into the Gilmont viscometer. Before the first sample the viscometer was rinsed once with water. Then the stainless steel ball was added and the cap screwed on. The time taken for the ball to pass between the marks on the tube (10cm apart) was timed. The viscometer was then inverted and the ball timed between the points again. This was then averaged.

The gel was then tipped out from the viscometer and it was rinsed once with water and the cycle started again.

Figure 4 - this figure was obtained by using viscosity standards made from glycerine mixed with water. These standards were 0%, 25%, 50% and 75% glycerine in water. These standards were run through the Gilmont viscometer. This result was compared to the viscometer made in the laboratory. This was a 8.4mm diameter ID tube with a 7.9mm stainless ball. One result was taken with the viscometer at 90 degrees (i.e. vertical) and the other at 15 degrees from the horizontal.

Figure 5 - this figure was obtained using the viscometer in the laboratory, using the 50%

-17-

glycerine standards. The angle was varied from the horizontal with two different diameter balls. The 6.4mm and 7.9mm balls were stainless and carbon steel respectively. See Figure 10.

Figure 10 shows a cross section of the tube. The tube T, has an internal diameter  $D_1$  and the ball B, has a diameter  $D_2$ . The difference between the two diameters determines the speed through the liquid. See Figure 5 for example. As the ball diameters increases it slows the movement down. However if the ball diameter becomes too large the non-homogenous nature of the gel stops the ball from rolling even though it could roll through a standard gel solution. (i.e. glycerine mixed with water).

Figure 6 - this figure was taken with raw milk from the DRC herd within four hours of harvesting. 14% Teepol™ was used as the reagent. It was mixed with the same milk sample at different ratios using the same procedure as Figure 3. It was measured in the Gilmont viscometer in the same way as Figure 3.

Figure 7 - this figure was created by mixing reagent, (14% Teepol™), 1:1 with milk before two hours had elapsed since harvest. The tests were done between 15-25 degrees centigrade. Both the mixing and measuring procedure was the same as Figure 3.

Figure 8 - this is exactly same as Figure 7 except the ratio reagent:milk was 1:2.

Figure 12A through 12G depict diagrammatically a preferred system in accordance with the present invention (eg; as shown in Figure 12) with the explanations as to the system modules and electronic systems being shown by way of flow diagrams Figures 13 and 14 respectively. In Figures 12A through 12G are shown the following

- milk tube or line 8 showing the pulsing movement of milk there along,
- a reagent reservoir "R",
- milk line flow diversion valves  $V_1$ ,
- a sample reservoir "S",
- reagent valve  $V_3$ ,
- milk sample flow control valve  $V_2$ ,
- a flow control pump P,
- a dwell zone 9 with inflow greater than outflow,
- cell solenoid E to control a ball 10 retention pin to hold the ball until released in its upper cot condition as shown in Figure 12B,
- sensor locations 11 and 12,
- an air filter 13,
- a vacuum valve  $V_4$ , and

- a waste reservoir W.

The operation of the apparatus can best be described by reference to the cycle description in Table 2.

**Description of Cycle: Table 2**

Step in Cycle	Description	Components Used
<b>Figure 12A</b>	<b>Evacuation and reset</b>	
a.1.1	Turn on valve to evacuate the chamber and move ball up	V <sub>4</sub> On
a.1.2	Move pin up to allow ball to pass	E (electro magnet) On
a.1.3	Turn on valve and pump to remove any previous sample	V <sub>2</sub> and P On
a.2	Time delay	
a.3.1	Pin moved down	E Off
a.3.2	Sample valve and pump stopped	V <sub>2</sub> and P Off
a.3.3	Time delay	
a.3.4	Vacuum switched off	V <sub>4</sub> Off
<b>Figure 12B</b>	<b>Sampling milk sample</b>	
b.1	Valve open	V <sub>1</sub> On
b.2	Time delay	
b.3	Valve closed	V <sub>1</sub> Off
<b>Figure 12C</b>	<b>Mixing and sample into tube</b>	
c.1.1	Sample valve open	V <sub>2</sub> On
c.1.2	Reagent valve open	V <sub>3</sub> On (may alternate with V <sub>2</sub> )
c.1.3	Pump on	P On
c.1.4	Time delay	
c.2.1	Pump Off	P Off
c.2.2	Sample valve closed	V <sub>2</sub> Off
c.2.3	Reagent valve closed	V <sub>3</sub> Off
<b>Figure 12D</b>	<b>Settling time</b>	
d.1	Time for ball to pass sensors	
<b>Figure 12E</b>	<b>Ball release and measure</b>	
e.1	Pin moved up	E On
e.2	Time ball to pass to predetermined places in measured	
<b>Figure 12F</b>	<b>Ball reaches bottom</b>	
<b>Figure 12G</b>	<b>Reset and evacuation</b>	
g.1.1	Turn on valve to empty the chamber and move ball up	V <sub>4</sub> On
g.1.2	Move pin up to allow ball to pass	E (electro magnet) On
g.1.3	Turn on valve and pump to remove any previous sample	V <sub>2</sub> and P On
g.2	Time delay	
g.3.1	Pin moved down	E Off

Step in Cycle	Description	Components Used
g.3.2	Sample valve and pump stopped	V <sub>2</sub> and P Off
g.3.3	Time delay	
g.3.4	Vacuum switched off	V <sub>4</sub> Off

5

The aforementioned cycle in respect of Figures 12A through 12G shows one method whereby apparatus could be worked. Another arrangement could be typified by the following tabulated procedure in **Table 3**.

Block Diagram of Rolling Ball Sensor (**Table 3**).

10

Method of getting sample into tube. This could include pumps, gravity

15

Tube with ball in it. Tube diameter could be variable, and ball diameter viable.

20

Way to hold ball at top system. Include Pin actuated by electromagnet or electromagnet holding ball directly

Method to get ball up, this could include air flow/vacuum evacuation, gravity or electric field.

System for measuring ball, this could include light beam(s) being blocked, electromagnetic detection.

25

Method to evacuate the tube, this could include gravity, pumping or vacuum.

Still a further arrangement could be that depicted by Table 4.

**Table 4**

5	Method of getting sample into measurement system, this could include pumps, gravity
	Measurement system.
10	Method to evaluate and clean measurement system. Could include gravity, pumping or vacuum.

The apparatus is preferably automated online but can be, if desired, provided with a command feature whereby a farmer can run the testing regime for a particular cow or stall or during particular milkings.

#### **Placement in Herringbone, rotary and robot**

In an herringbone milking system the SCC measuring device preferably will be placed such that it will analyse milk flowing between the claw and the milk line. It will be attached to the milk line and have the milk from the dropper tubes flow through it.

In a rotary it preferably will be again placed such that it will analyse milk flowing between the claw and the milk line. It will be attached to the rotary platform or milk line.

In a robotic milker preferably there will be one device per quarter or one device for the composite milk. It will be placed between the cups and milk collection vessel. Or after the collection vessel to test the composite milk.

#### **Calibration equations**

Figure 9 shows an example of a calibration curve; this is obtained by measuring a sample with a known amount of SCC and recording the change in time ( $\Delta t$ ). These results are then transformed using some linear transformation technique. The results are then plotted with  $\Delta t$  versus SCC.

Over the dynamic range the  $\Delta t$  is related to the SCC by the following equation where  $b_0$

-21-

and  $b_1$  are the coefficients of linear regression. These are calculated from least squares analysis of the data.

$$\Delta t = b_0 + b_1 \text{SCC}$$

The SCC can then be determined by solving for SCC, which yields the following  
5 equation. Therefore by measuring the  $\Delta t$ , it is possible to determine the SCC.

$$\text{SCC} = \frac{\Delta t - b_0}{b_1}$$

From the foregoing therefore it is believed that standard surfactant amounts coupled with standard milk sample amounts in substantially similar ambient conditions will provide sufficient consistency to allow worthwhile data to be generated from the detectors and to be processed to provide effective information to a dairy farmer.

10

### **Changes over milking, and over day to day**

*Woolford et al* 1998 shows that SCC can be used to determine mastitis,. If a threshold of 500000 cells/ml (see Table 1) is used the disease status can be determined. If the first milk is used (fore milk) the SCC only gives 5% false positives. If main milk is used then less false  
15 positives are obtained but more infections are missed. If the last milk (strippings) are used 9% of cows are false positives but more are detected (see tables 4 & 5 in *Woolford et al*).

As shown in attached Figure 11 the SCC changes over a milking. *White and Rattray* (1965) reported changes in cell count levels during and after milking, the rise in cell count during milking often being by a factor of ten or more.

20 Figure 11 shows individual quarter variations in cell count over a 70 minute period prior to, during, and after milking.

Therefore it is important to analyse the milk at a predetermined time after the start of milking every day. *Schalm et al* 1957 also stated that the SCC rose between the start of milking (foremilk) and the end (strippings).

25 *Woolford et al* (1998) also showed the measurement of milk every day is important to determine the health of the cow due to high day-to-day variation. In this respect see Figures 1A and 1B which are Figures 2 & 3 from *Woolford et al*.

-22-

The present invention envisages preferably any desired same time consistency during milking eg; tied to one or more of

- (i) commencement of milking generated milk flow,
- (ii) duration or volume of draw off, and/or
- 5 (iii) elapsed milk flow.

10

1. A method of testing a milk flow for mastitis, said method comprising:
  - diverting a sample flow from a said milk flow;
  - providing an inflow of an anionic detergent and an inflow of said milk sample in predetermined proportions into a dwell zone from whence the detergent/milk admixture outflow is at a lesser rate than said inflow regardless of a viscosity increase of the detergent/milk admixture, the difference between the inflow and outflow rates providing a dwell time for the admixture in the dwell zone;
  - feeding the outflow admixture product of said dwell zone to a viscosity testing zone;
  - testing in said viscosity testing zone the outflow admixture product for viscosity and generating an output signal indicative of such viscosity; and if necessary, clearing the viscosity testing zone and said dwell zone of said admixture without feedback into the milk flow from which the sample flow was diverted.
2. A method of determining whether or not there is a somatic cell count change between milkings in milk of an animal by applying the method as claimed in claim 1 to separate milkings, and comparing data resulting from said testings to determine any somatic cell count change.
3. A method claimed in either claim 1 or claim 2, wherein said attribute is of the DNA of the somatic cells.
4. A method as claimed in any one of claims 1 to 3, wherein said milk flow is from a single animal and an identifier of that animal and an indicator of viscosity and thus of the somatic cell count is coupled for comparative purposes.
5. A method as claimed in any one of claims 1 to 4, wherein said inflows are not mixed prior to said dwell zone.
6. A method as claimed in any one of claims 1 to 5, wherein said milk flow is the milk from at least one teat of a known animal, such animal being identified by an output signal from an animal identifying sensor.

7. A method as claimed in any one of claims 1 to 6, wherein a receiver receives the output signal indicative of viscosity and associates such output signal with the animal by reference to either an input signal or a said animal identifying sensor output signal.
8. A method as claimed in claim 7, wherein the data received by said receiver is stored for comparative purposes with subsequent data received at subsequent milkings from the same animal.
9. A method as claimed in any one of claims 1 to 8, wherein said sample flow is drawn off a milk flow during milking, said drawing off being substantially identical for each milking having regard to any one or more of:
  - (i) commencement of milking generated milk flow,
  - (ii) duration or volume of draw off, and
  - (iii) elapsed milk flow.
10. A method as claimed in any one of claims 1 to 9, wherein the viscosity testing zone is a flowcell.
11. A method as claimed in any one of claims 1 to 10, wherein said dwell zone and said viscosity testing zone are a common chamber of apparatus into which said inflows are provided.
12. A method as claimed in any one of claims 1 to 11, wherein the inflows of milk sample and anionic detergent to the dwell zone are tangential.
13. A method as claimed in any one of claims 1 to 12, wherein said predetermined proportions are in the range of from 2:1 to 1:2 inclusive.
14. A method as claimed in any one of claims 1 to 12, wherein said predetermined proportions are substantially 1:1.
15. A method as claimed in any one of claims 1 to 14, wherein said anionic detergent is a gardinol type detergent.

- 25 -

16. A method as claimed in any one of claims 15, wherein said anionic detergent is an aqueous solution of about 2% weight/volume sodium laurel sulphate.

17. A method of testing a milk flow for mastitis as claimed in any one of claims 1 to 16, wherein there is also provided an animal identifier input or signal capable of identifying said animal to data acquisition, analysis and storage means.

18. A method of testing a milk flow for mastitis as claimed in any one of claims 1 to 17, wherein there is also provided a means of generating an output signal to said data acquisition, analysis and storage means indicative of the viscosity of a predetermined part of the mixture that has been subject to a predetermined gel forming dwell time post mixing.

19. A method as claimed in any one of claims 1 to 19 used for testing an animal for mastitis by on-line testing from such an animal as it is being milked.

20. An apparatus for association with the milk line of a milking machine to test milk of an animal being milked for somatic cells, and therefore mastitis, the apparatus including:

means defining a liquid chamber having an ability to receive two inlet feed; one inlet feed to be an inflow of a sample flow of milk from the milk line;

the other inlet feed to be an inflow of an anionic detergent of a kind that will cause a viscosity increase as a result of at least partial gelling of the resultant milk/detergent fluid;

means to provide a measure of and to generate data output indicative of the viscosity of at least part of the resultant milk/detergent fluid calibratable to the somatic cell count of the milk inflow;

means defining a second chamber to receive at least part of the resultant milk/detergent fluid from said liquid chamber; and

means to clear the milk/detergent fluid from the apparatus without contamination of milk in the milk line in order to allow a subsequent milk flow sample to be likewise tested,

wherein said means defining a liquid chamber and said means defining a

second chamber are interconnected such that the liquid chamber has a greater infeed of fluids than outfeed therefrom, irrespective of a viscosity increase, the difference between the inflow and outflow rates providing a dwell time for the admixture in the dwell zone.

21. An apparatus as claimed in claim 20, wherein said apparatus is such that an identical testing regime is followed for each sample taken from the milk flow such that for an individual animal or as between individual animals, or both, there is a comparative basis.

22. An apparatus as claimed in either claim 20 or 21, wherein there is provided data acquisition, analysis and storage means and means to provide an animal identifier input or signal capable of identifying an animal being milked to said data acquisition, analysis and storage means and said means to generate a signal or representation or record itself provides an output signal to said data acquisition, analysis and storage means indicative of the viscosity of the milk sample gel in the apparatus for tying to the animal identified.

23. An apparatus as claimed in any one of claims 20 to 22, configured to test milk of an animal being milked for somatic cells and thus testing the animal being milked for mastitis.

24. An apparatus as claimed in any one of claims 20 to 23 used for determining whether or not there is a somatic cell count change between milkings in milk of an animal by comparing data resulting from said respective milkings to determine any somatic cell count change.

25. An apparatus as claimed in claim 24 including means for generating a gel from the milk sample by use of an appropriate surfactant, means for testing the gel thus generated for viscosity or some function of viscosity, and means for causing or allowing the gel to clear from the apparatus.

26. An apparatus as claimed in any one of claims 20 to 25, including means adapted in use to reproducibly control the two inlet feeds.

- 27 -

27. An apparatus as claimed in any one of claims 20 to 26, wherein the two inlet feeds are positioned tangentially to the first liquid chamber.

28. An apparatus as claimed in any one of claims 20 to 27, wherein the means to provide a measure indicative of the viscosity is a flow cell.

29. An apparatus as claimed in any one of claims 20 to 28, wherein no moving parts of the apparatus can contact the milk sample, anionic detergent, or admixture thereof.

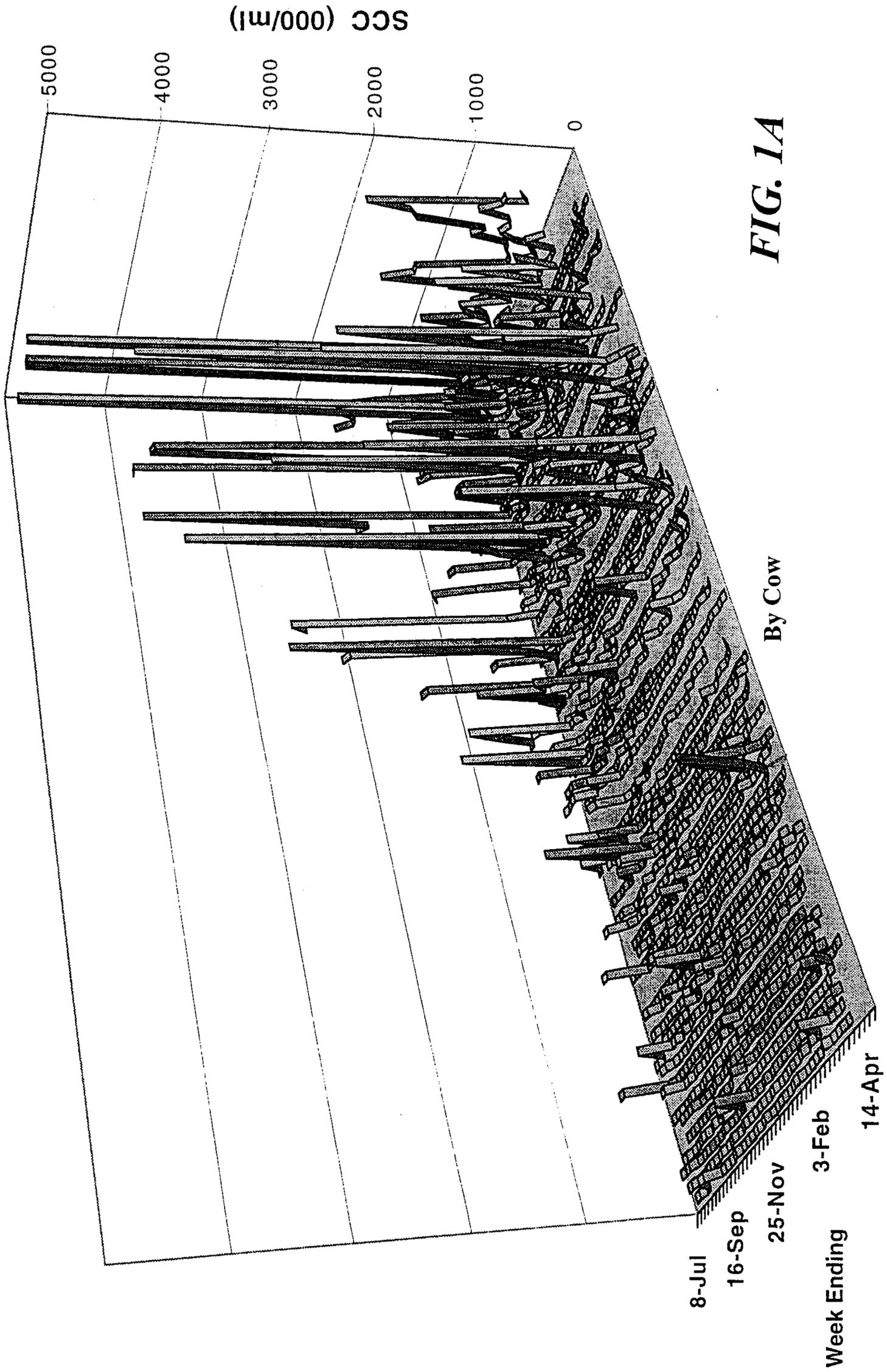


FIG. 1A

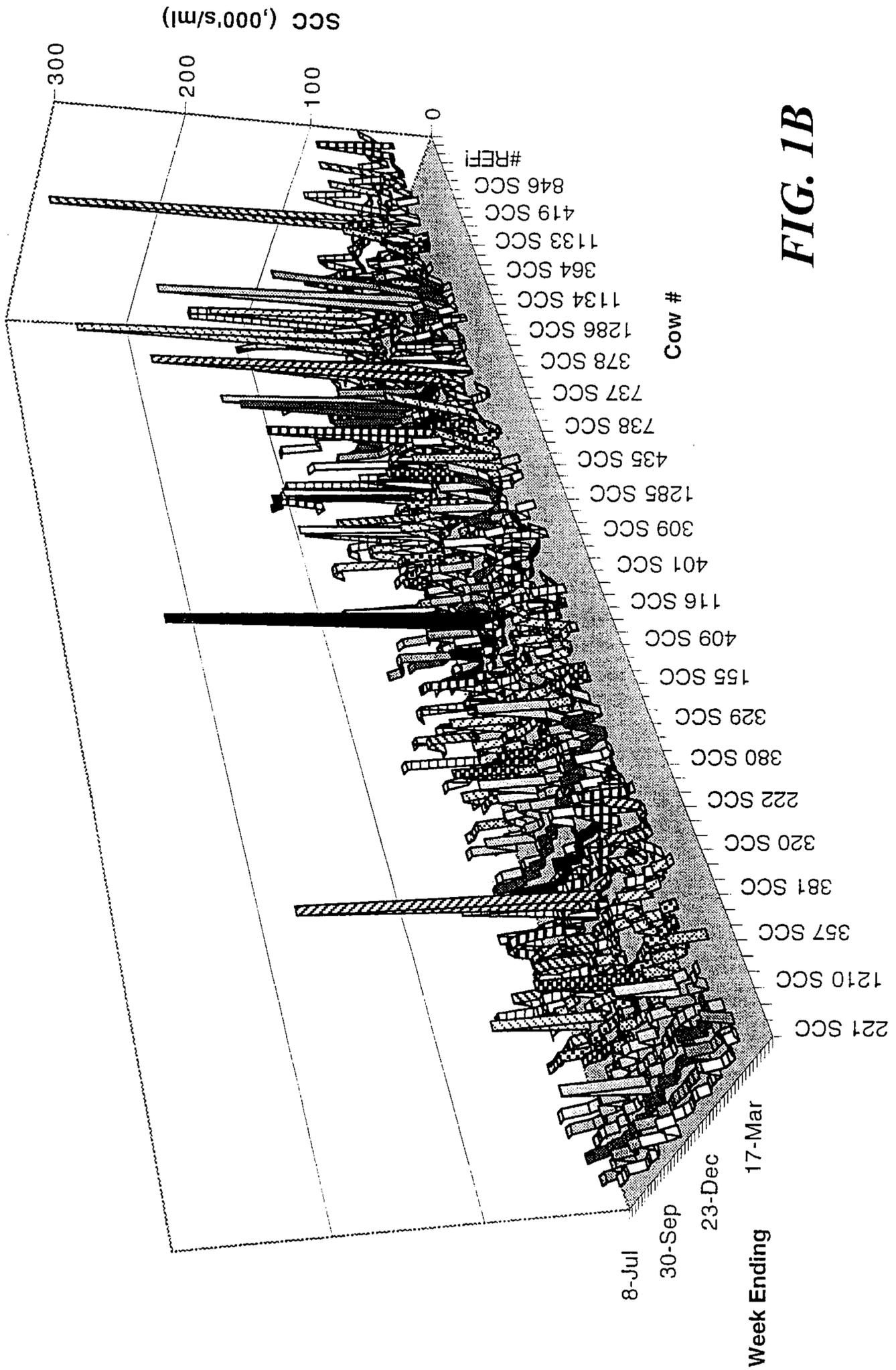
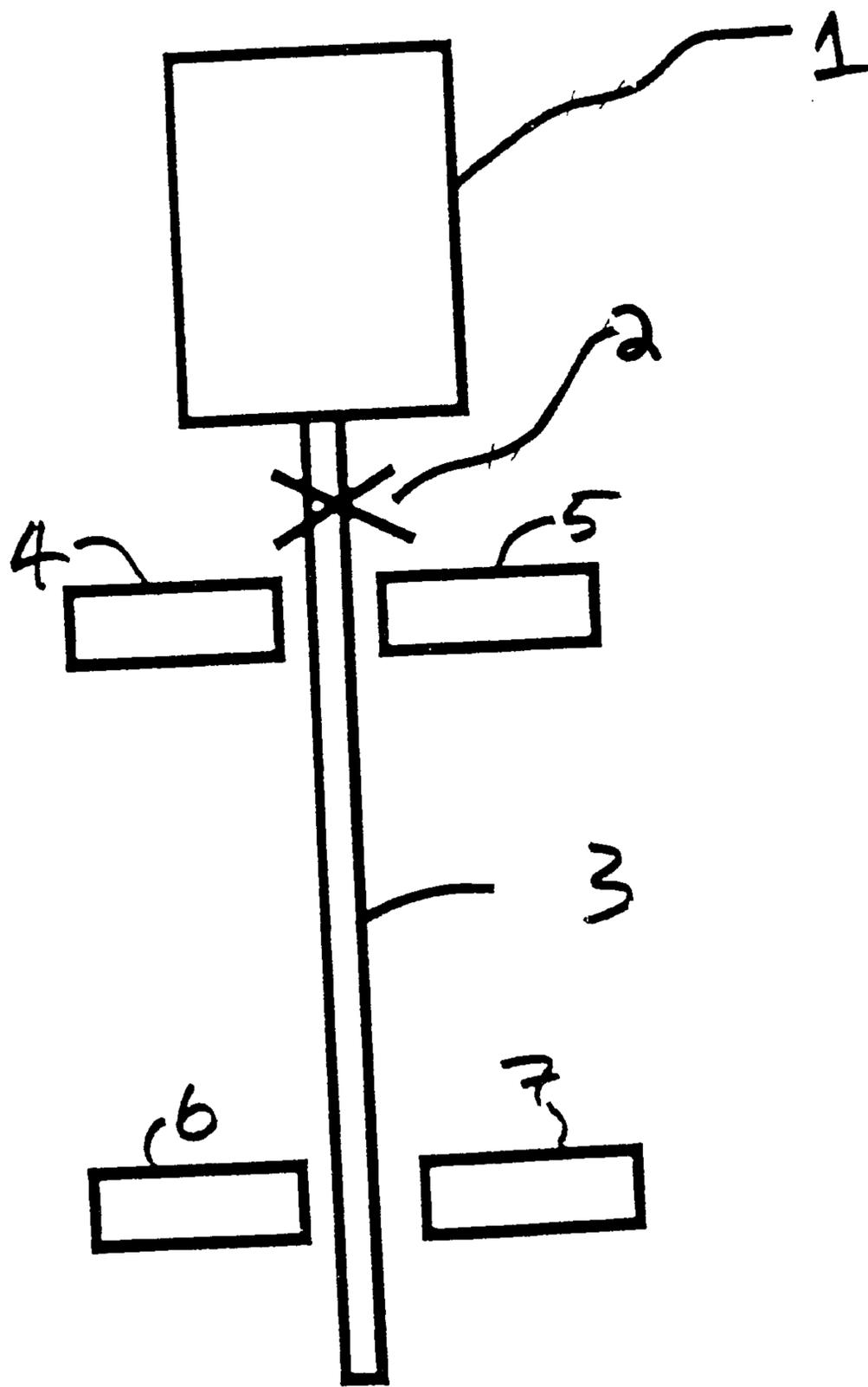
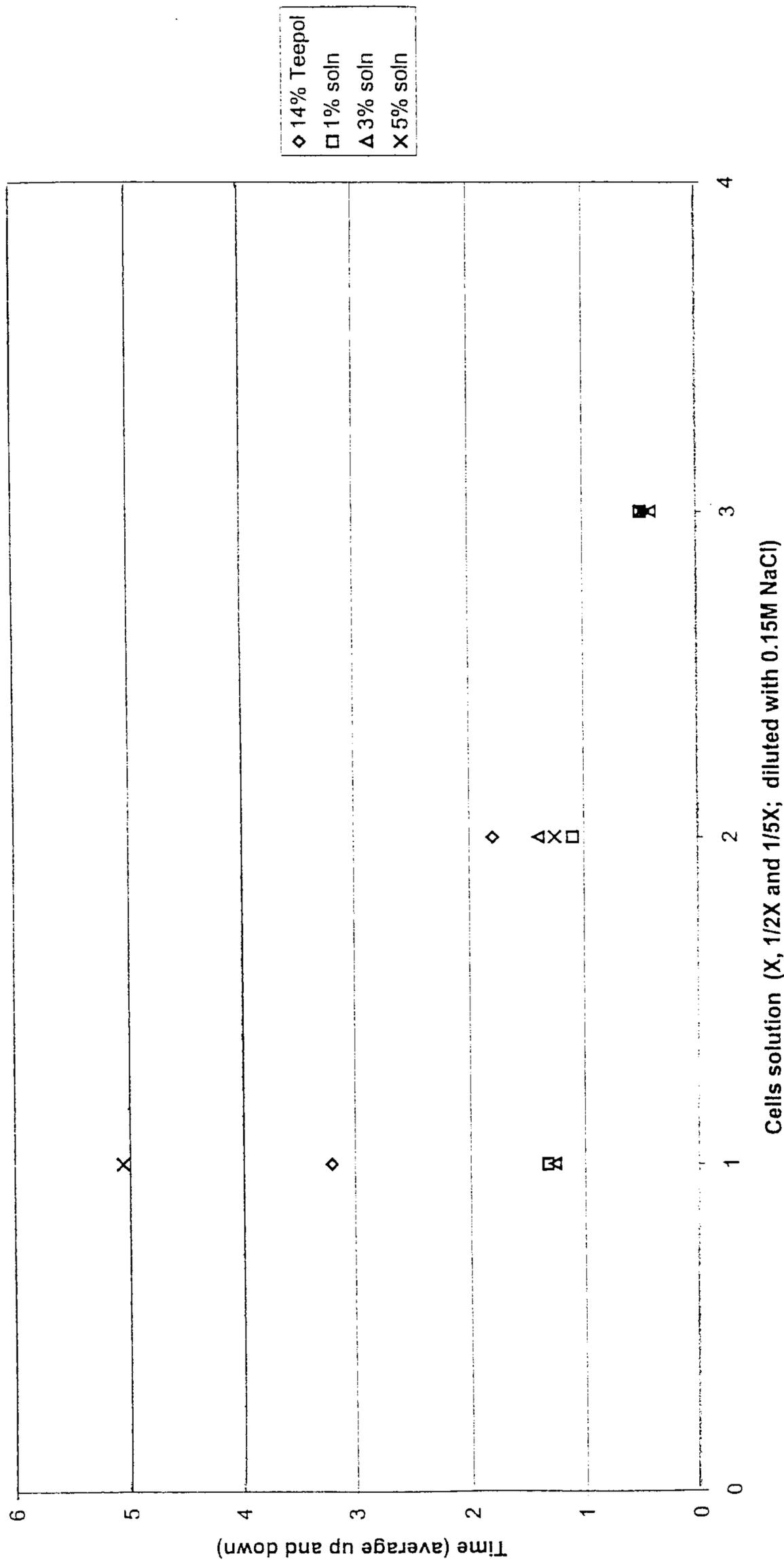


FIG. 1B

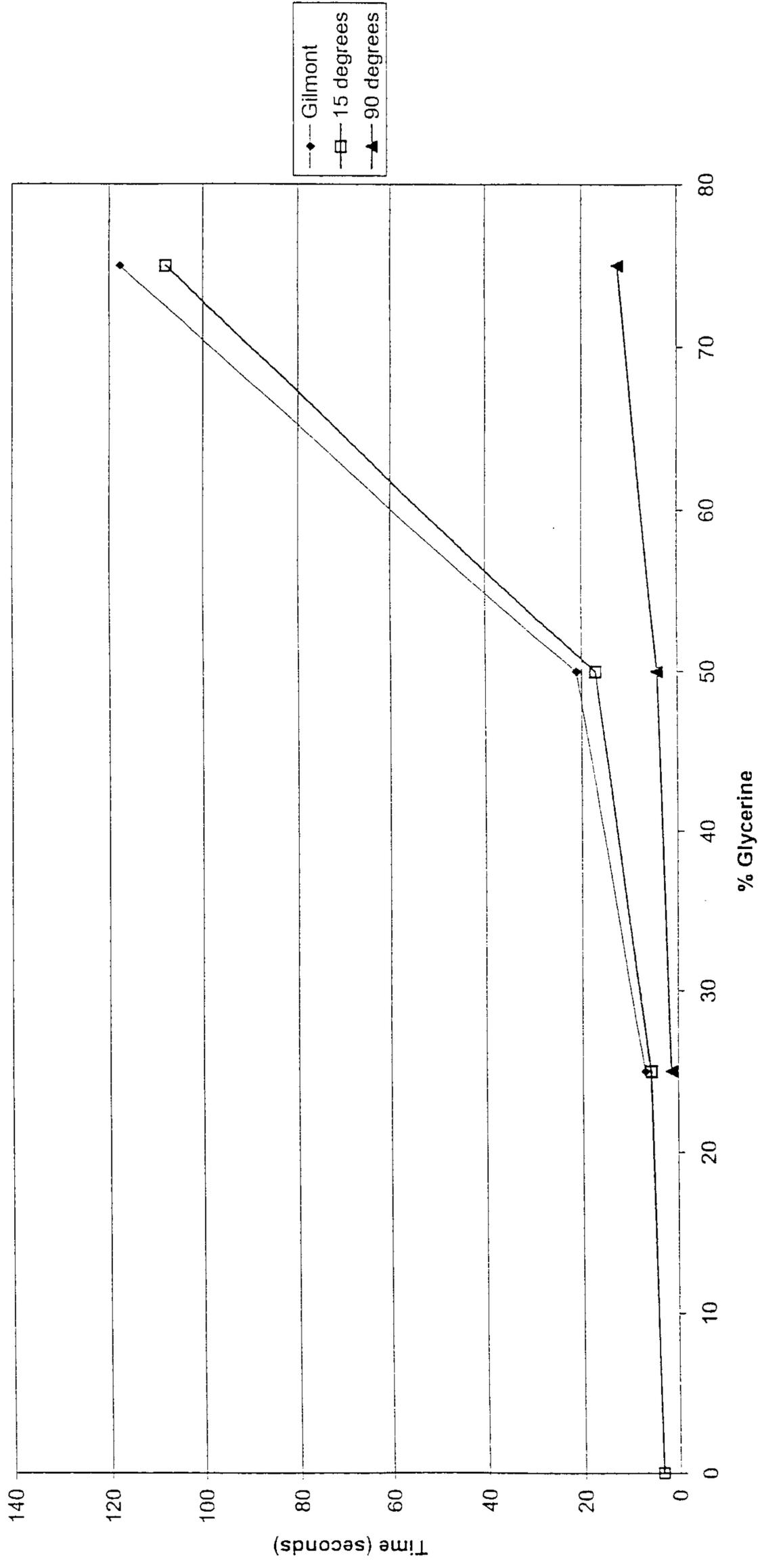
FIG. 2



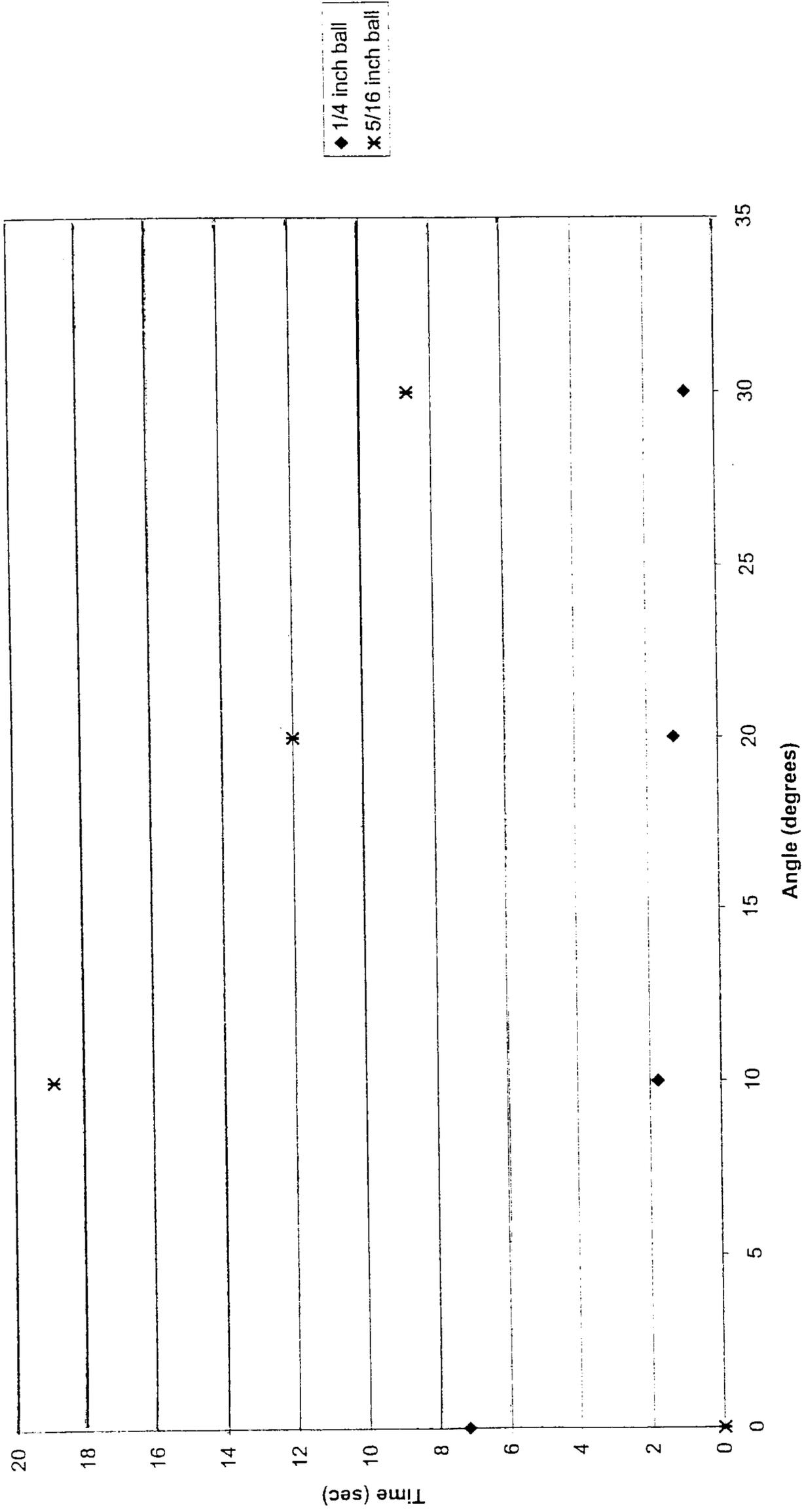
**FIG. 3** Effect of Sodium Lauryl Sulphate concentration verses Teepol using stock solutions of bovine blood leucocytes.



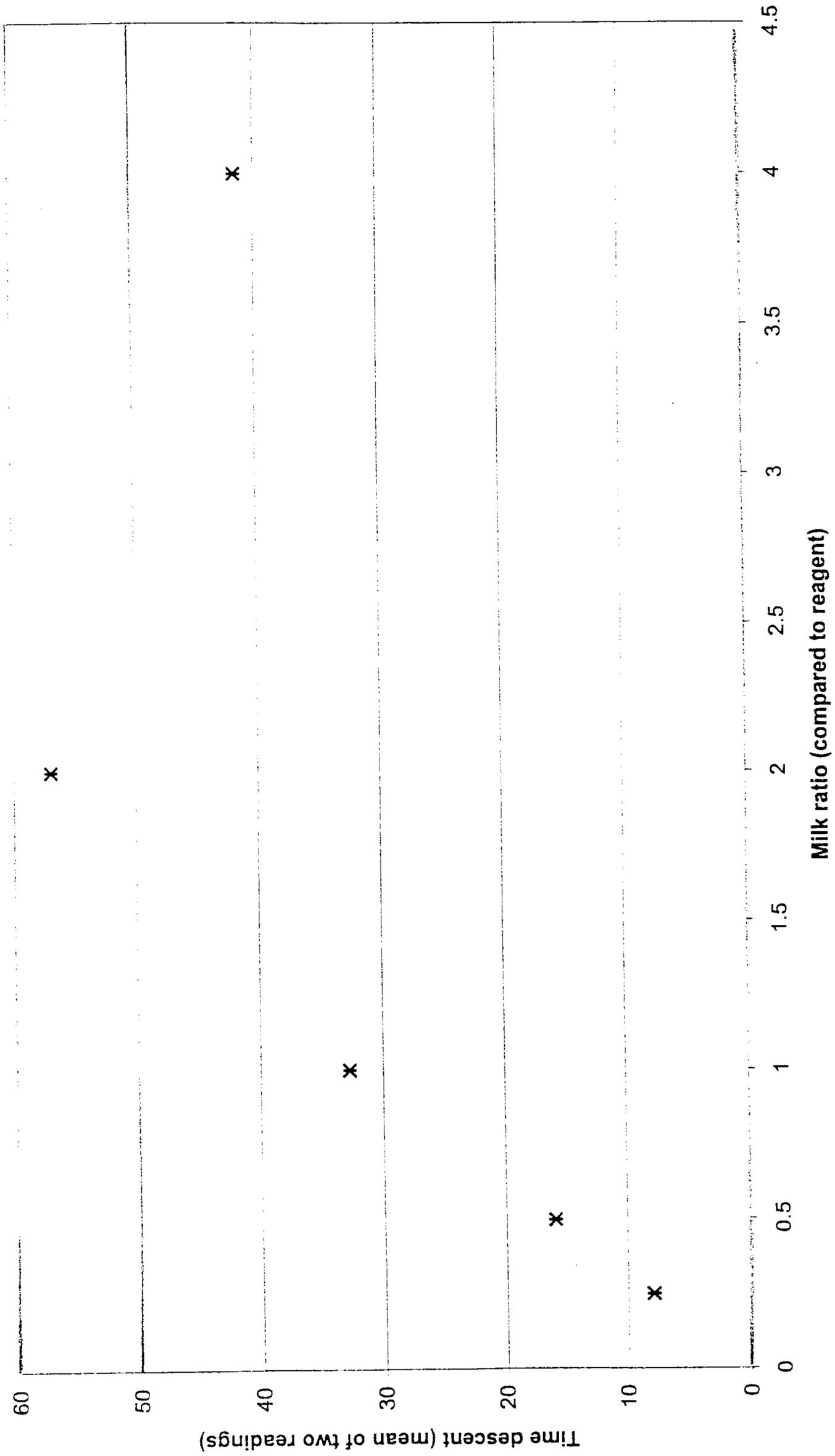
**FIG. 4** Effect of angle on prototype viscometer, compared with Gilmont viscometer at 90 degrees.



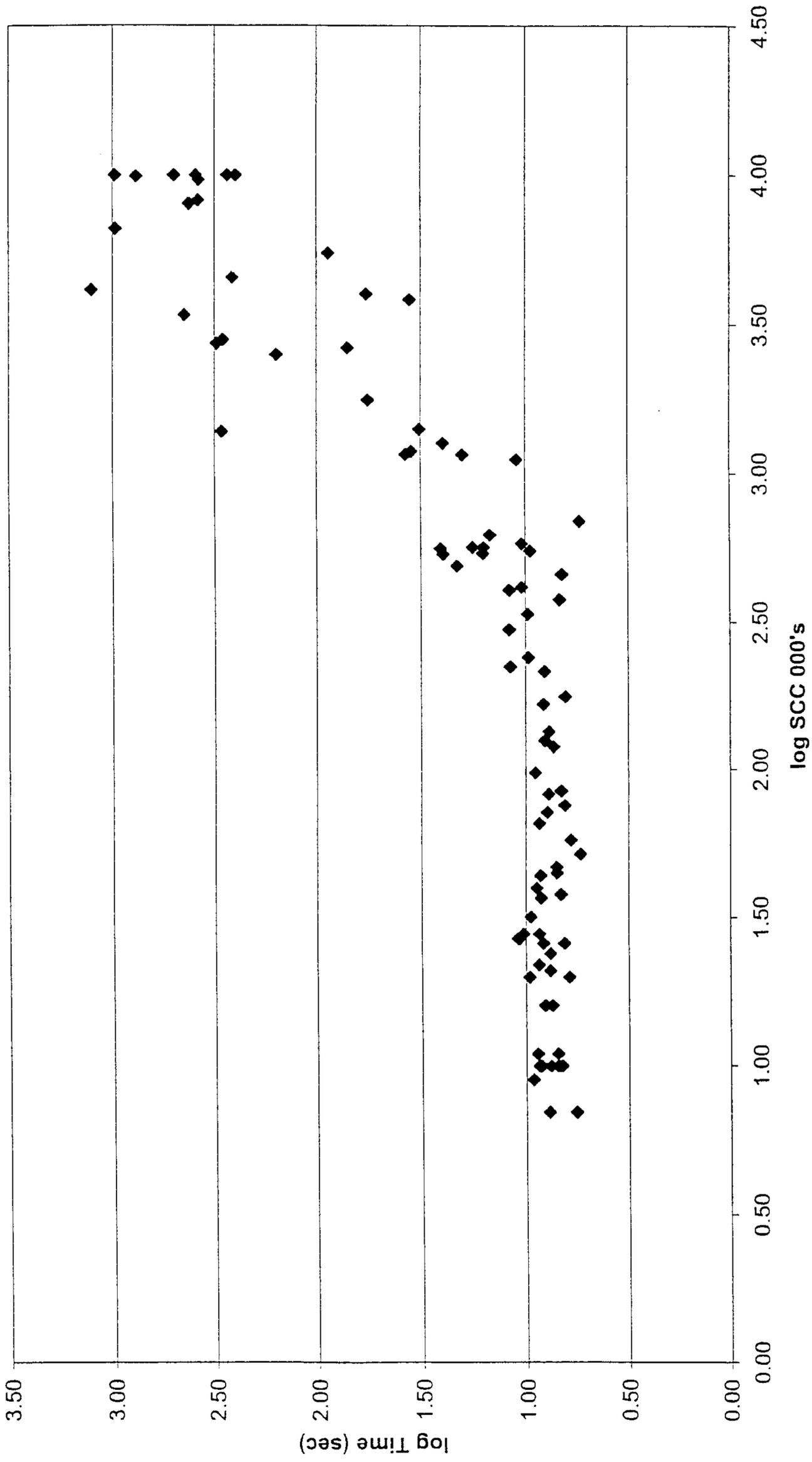
**FIG. 5** Effect of ball size on time ball descent at varying angles.  
Used 50% glycerine as fluid, slope of the two lines approximately equal



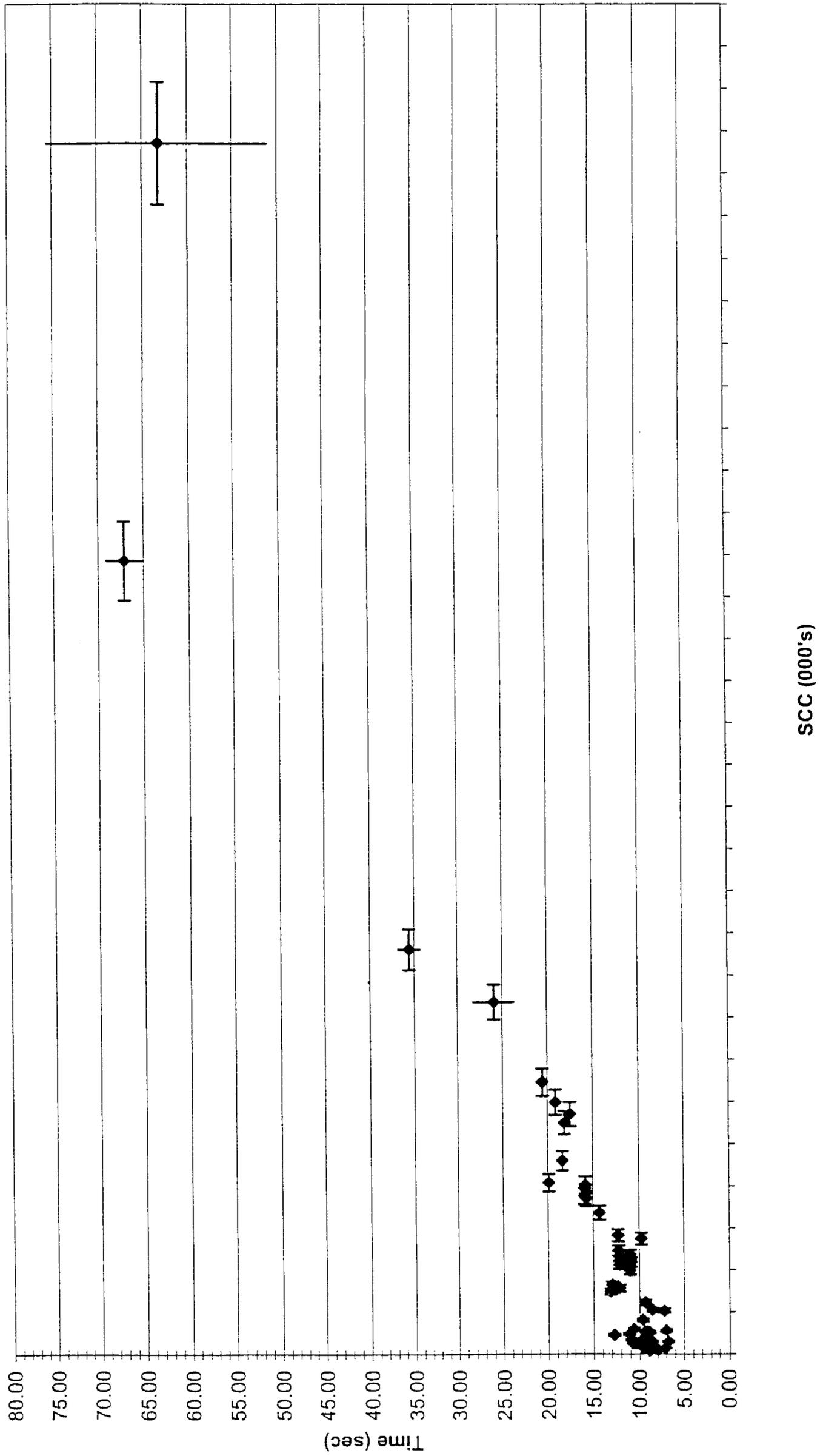
**FIG. 6** Effect of changing ratio of Milk:Reagent (milk held to one).

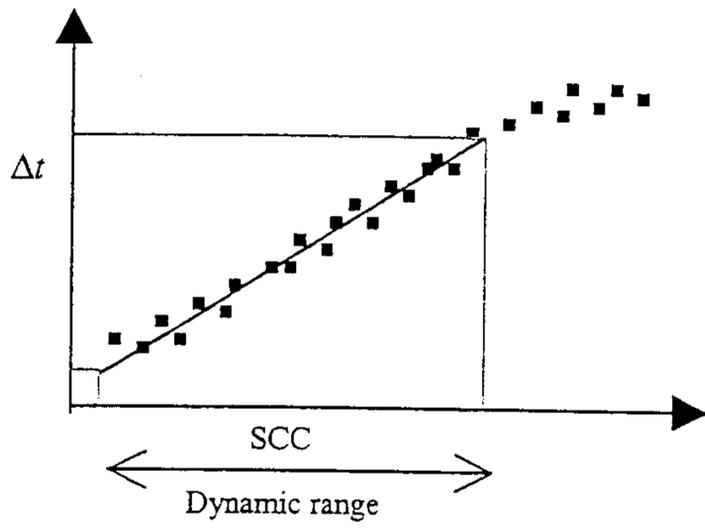


**FIG. 7** Effect of SCC on viscosity. SCC sample were from DRC no 6 dairy analysed within 2 hours of milking, using 1:1 milk: 14% Teepol, time obtained from Gilmont falling ball viscometer.



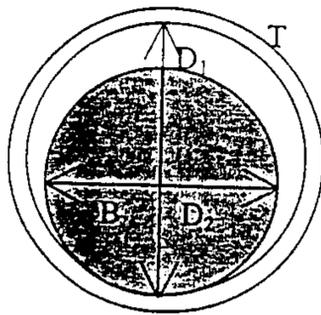
**FIG. 8** Effect of SCC on viscosity. SCC sample were from DRC no 6 dairy analysed within 2 hours of milking, using 2:1 milk: 14% Teepol, time obtained from Gilmont falling ball viscometer.





*FIG. 9*

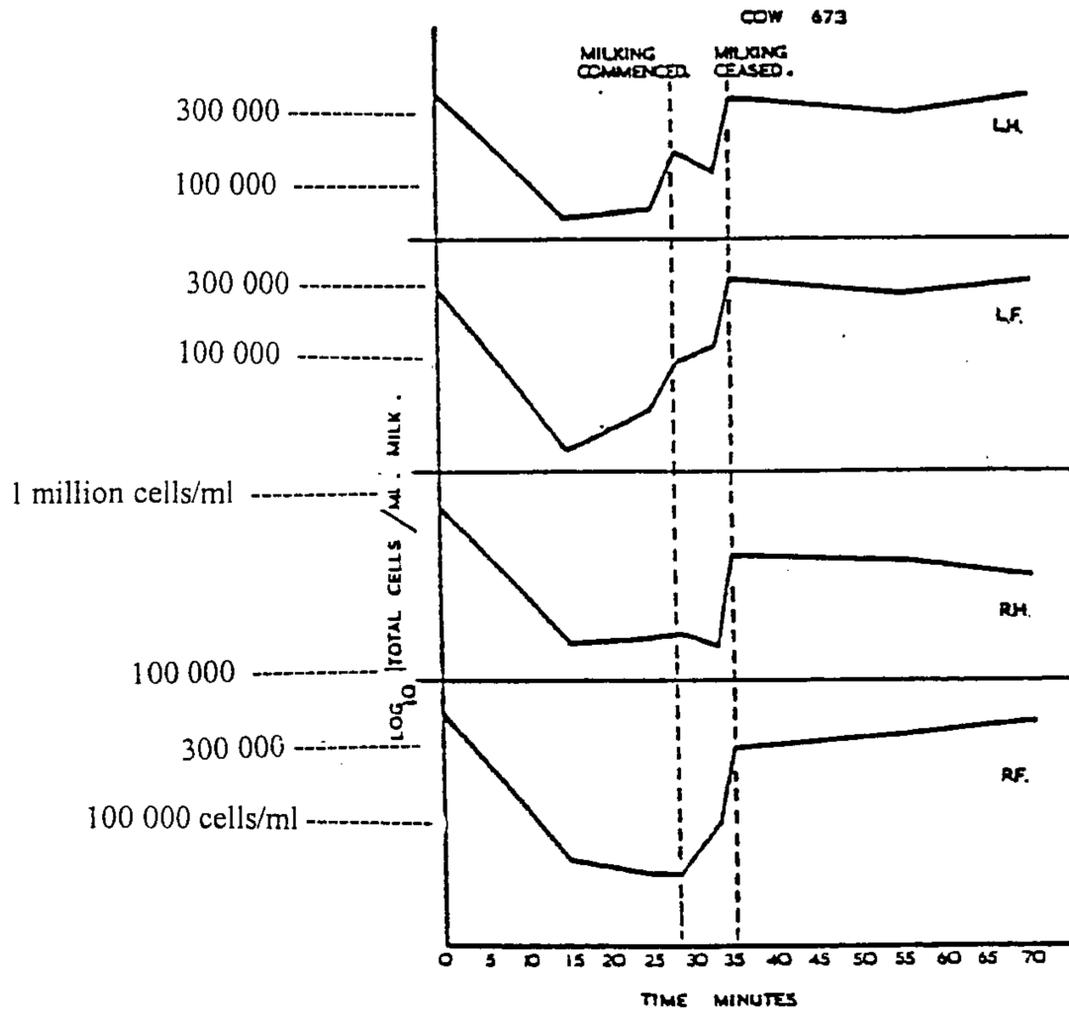
Figure showing an example of a calibration curve



Cross section of rolling ball tube

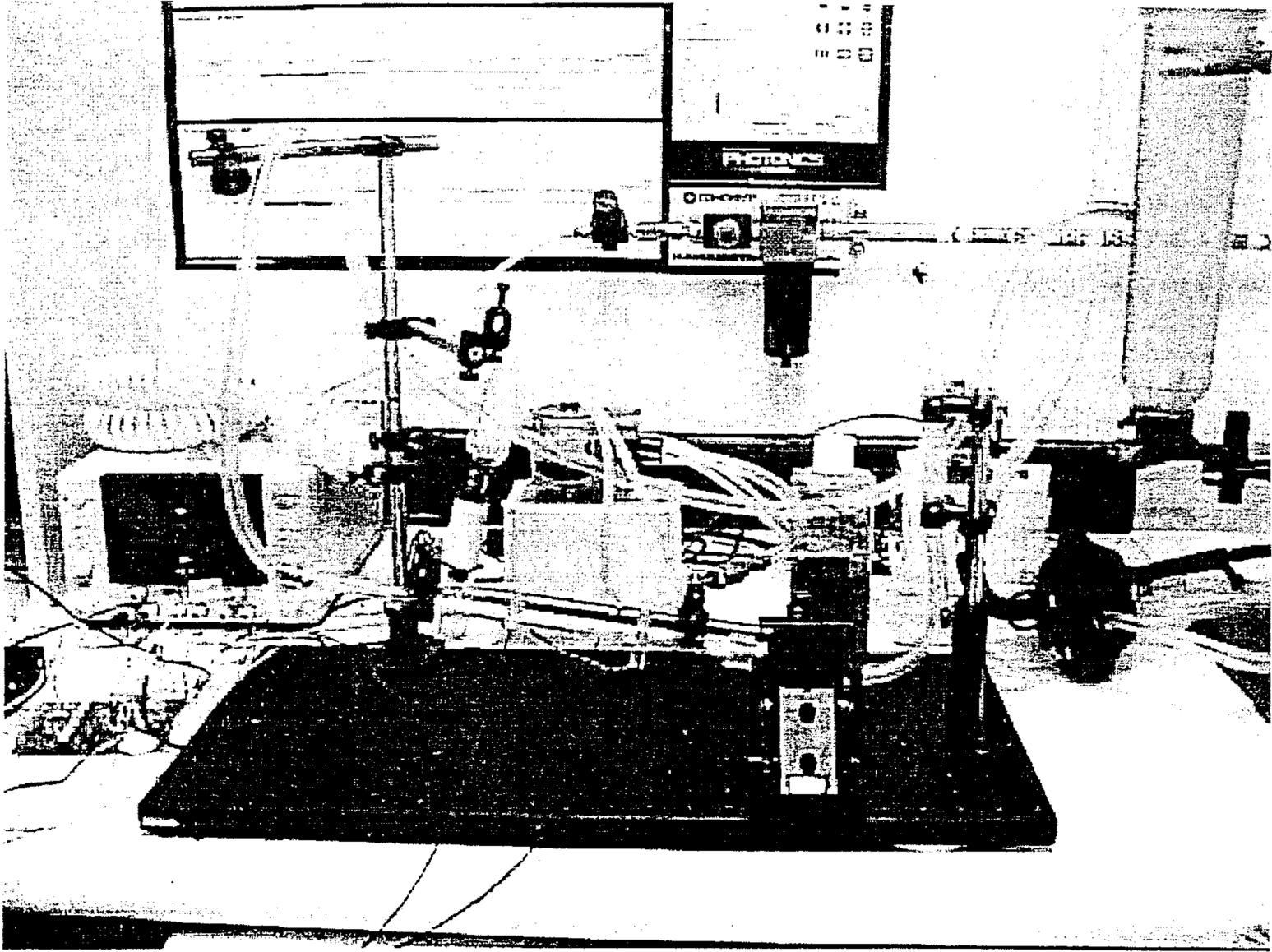
*FIG. 10*

11/18



Cell Counts before, during and after milking:  
(White and Rattray, 1965)

**FIG. 11**



*FIG. 12*

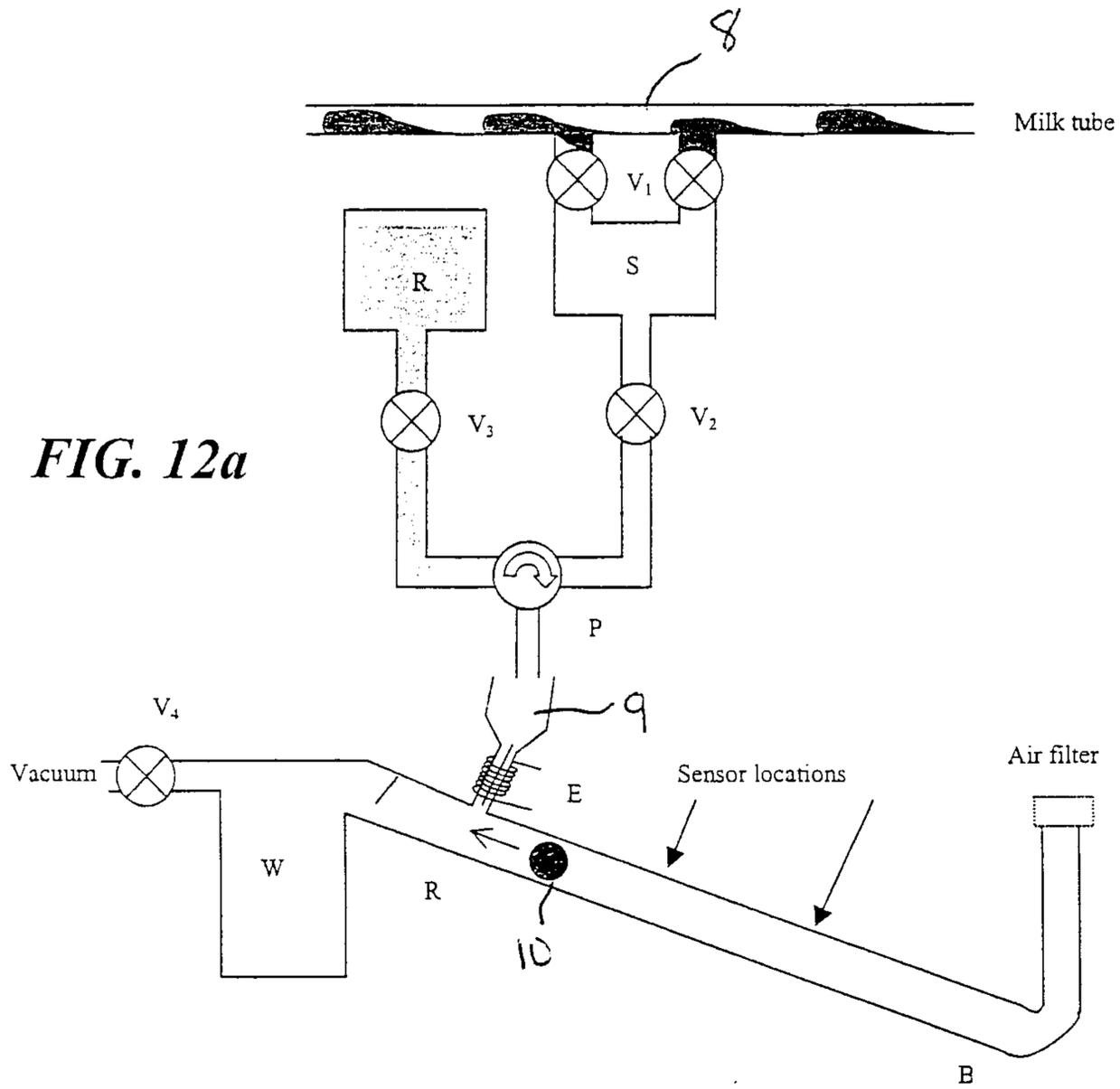


FIG. 12a

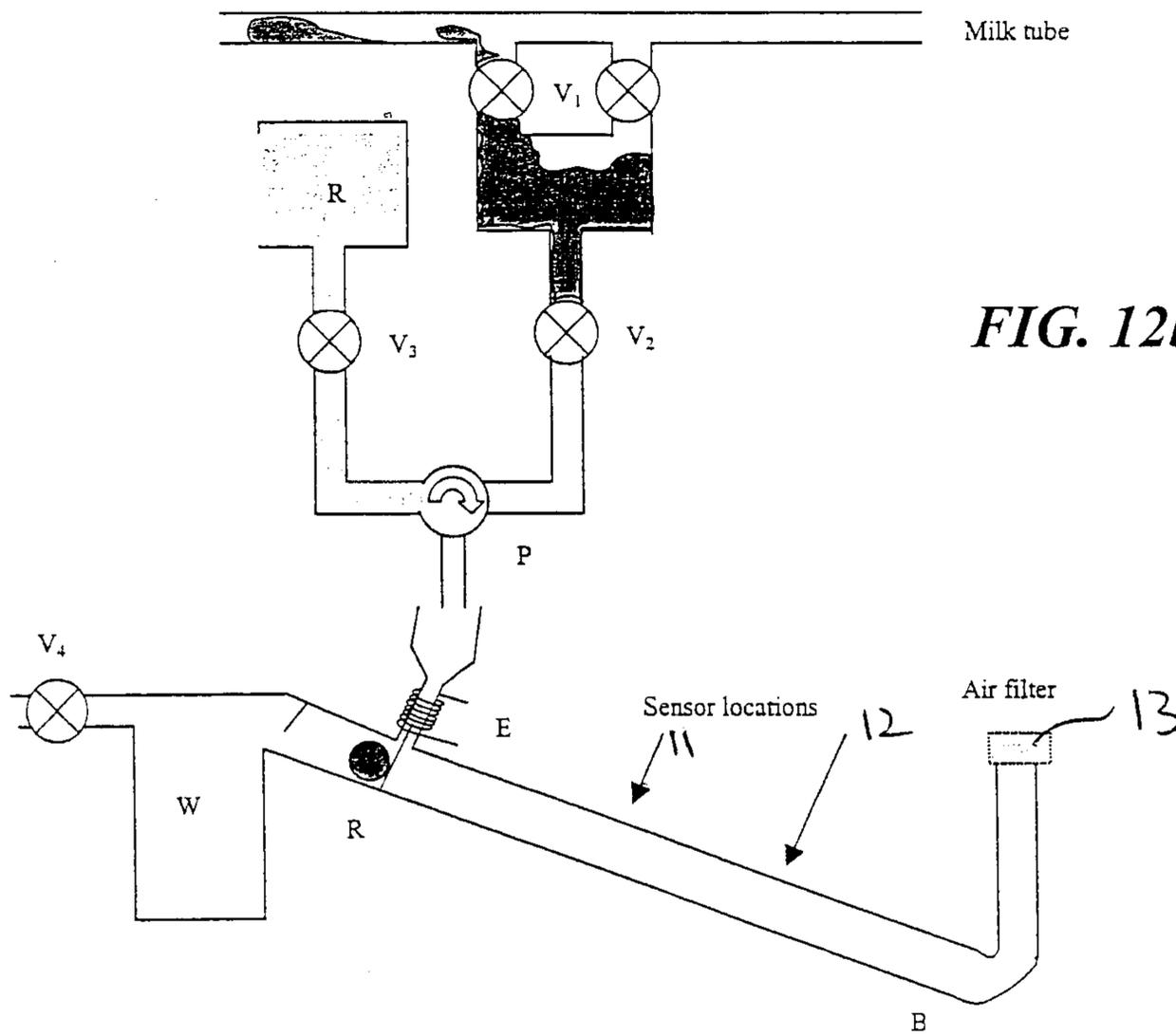


FIG. 12b

FIG. 12c

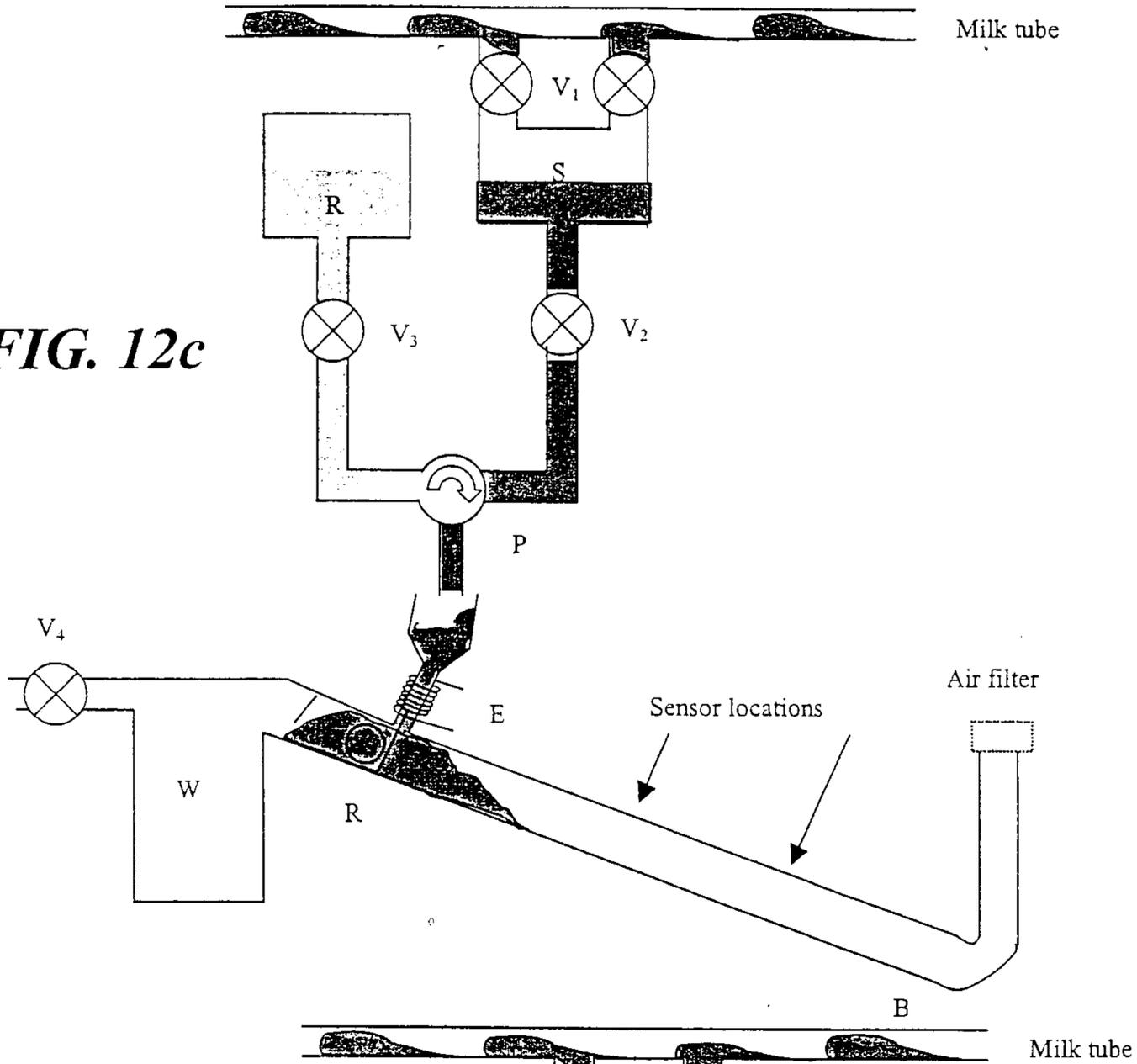
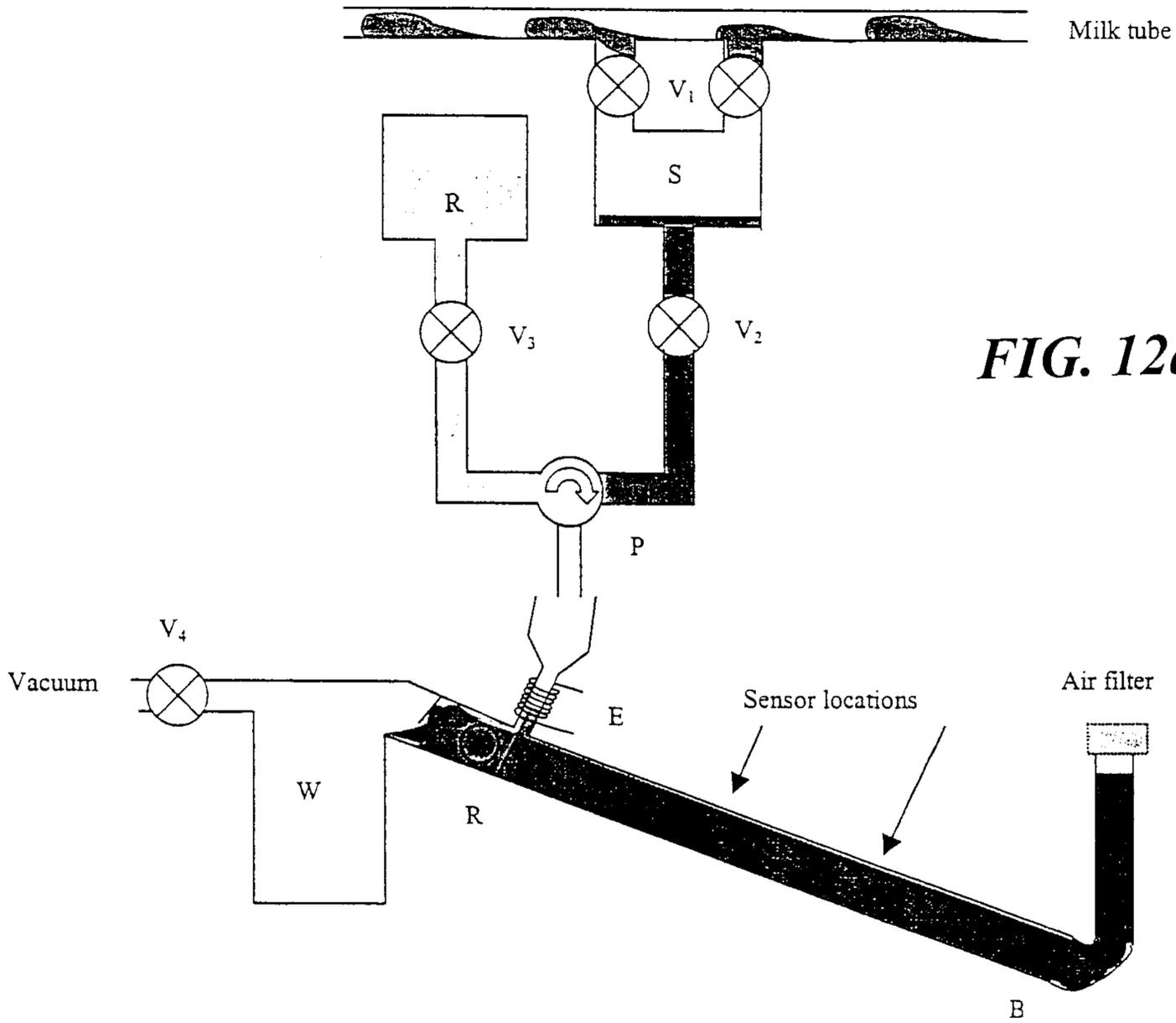
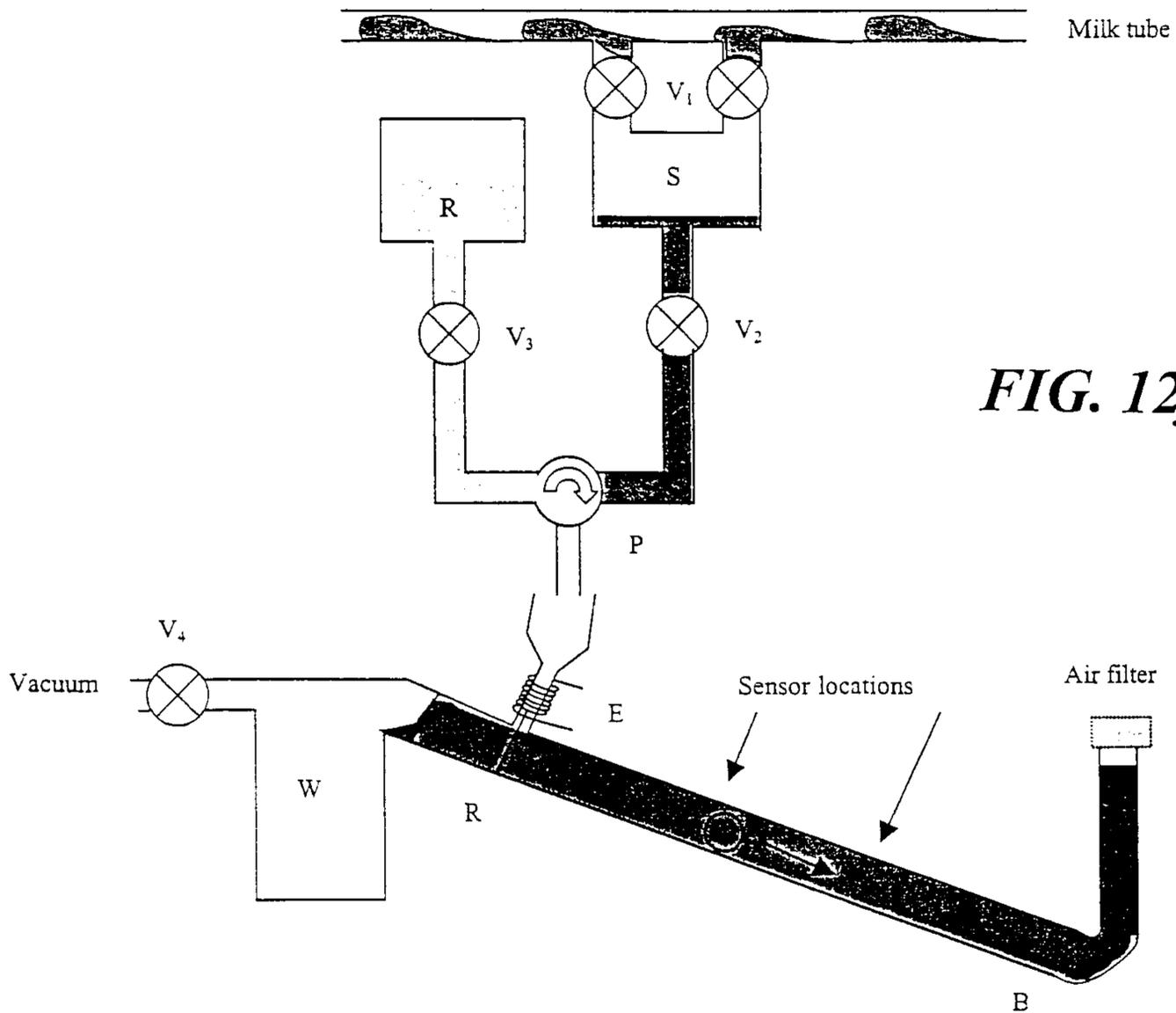
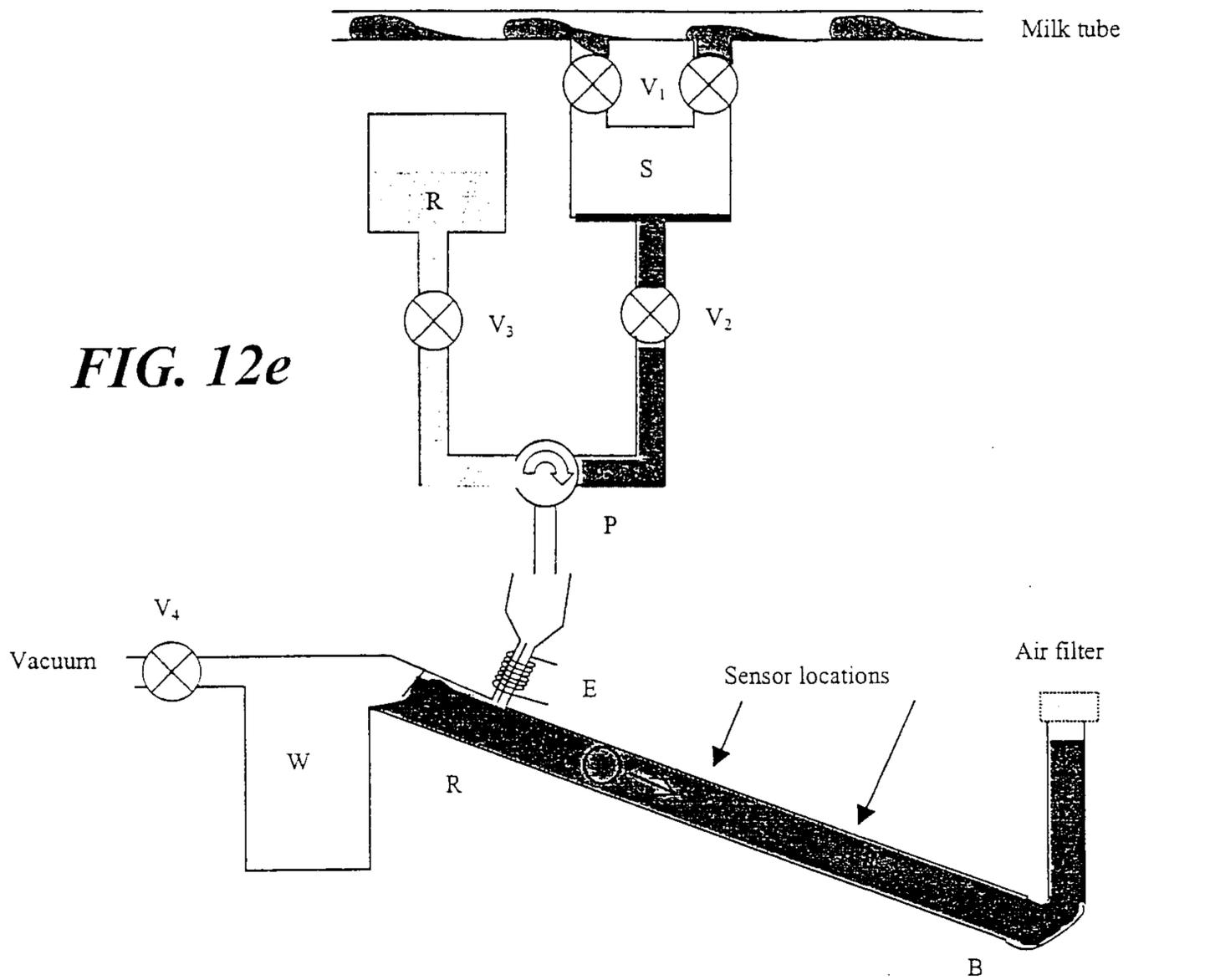
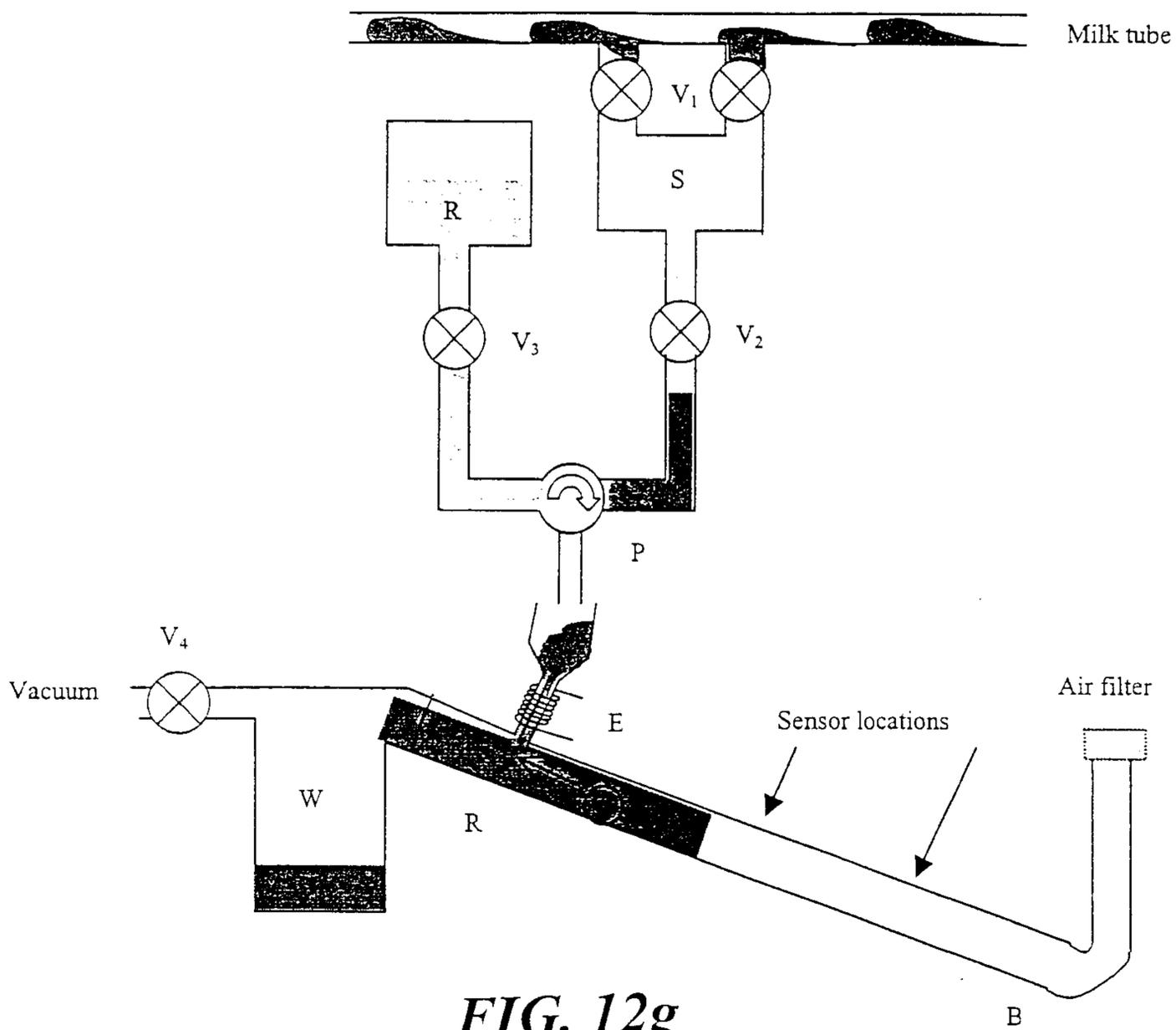


FIG. 12d



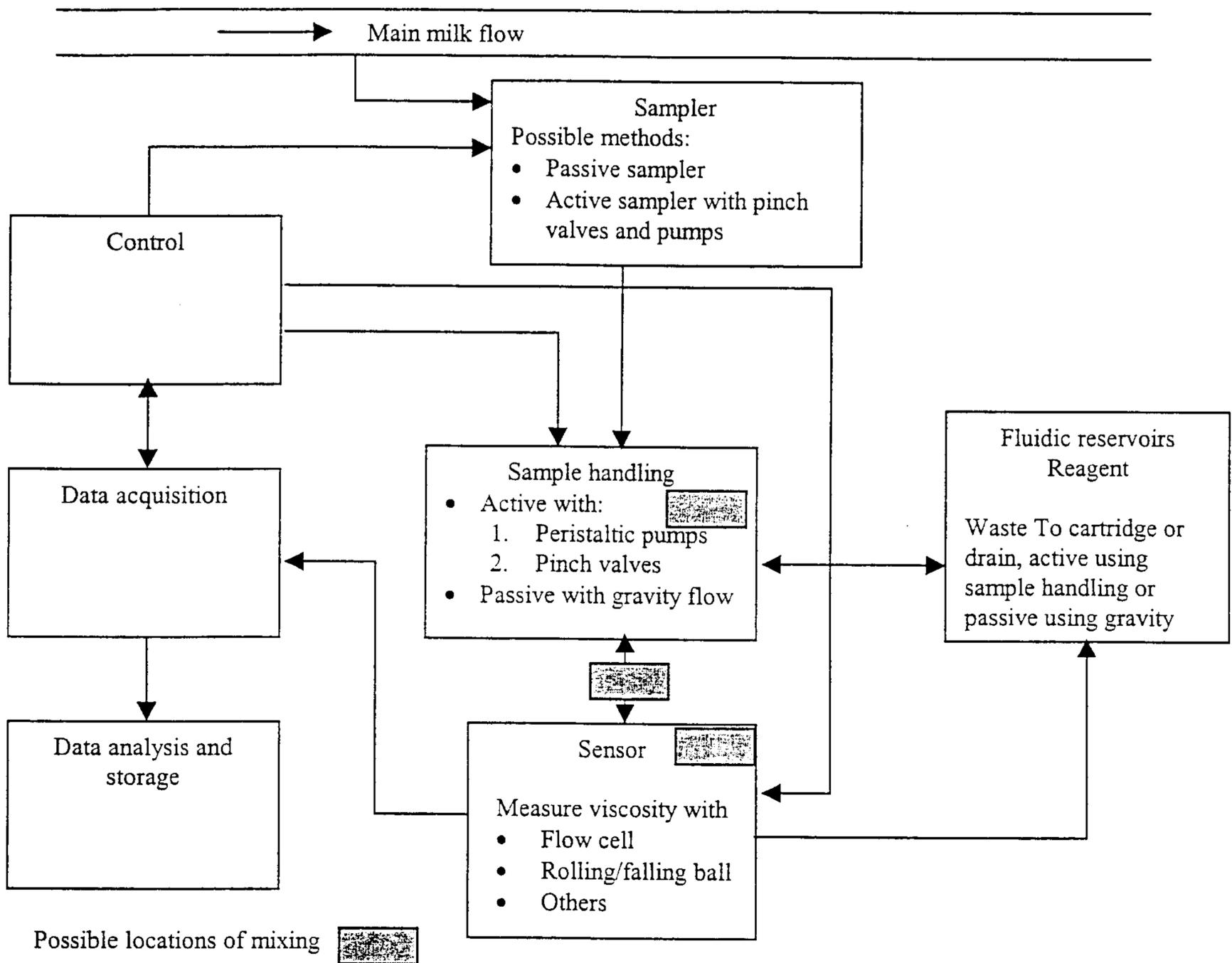




**FIG. 12g**

Diagram of system modules

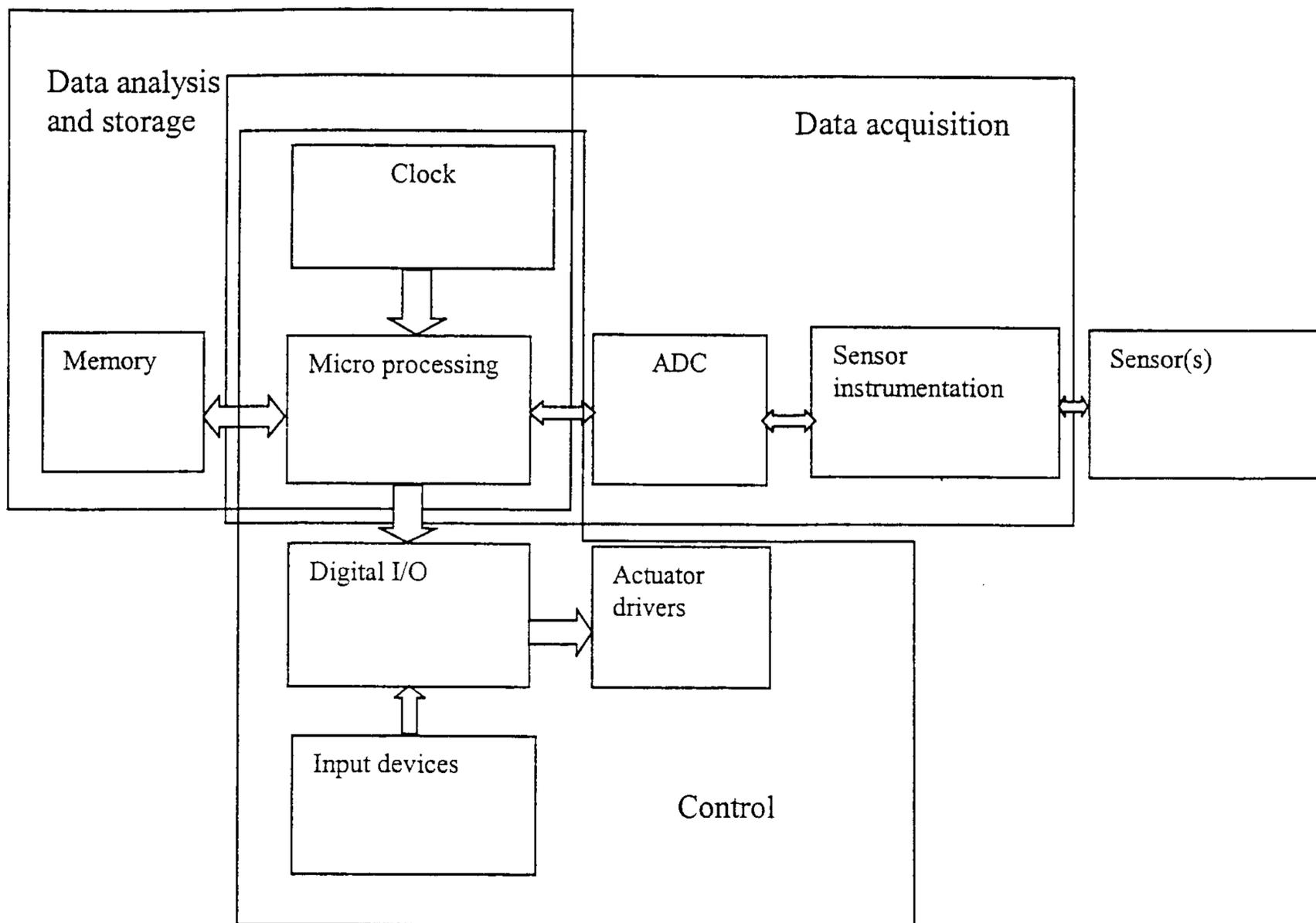
17/18



Could be passive or active mixing, with inline spirals, active shaking or controlled pumping.

**FIG. 13**

Block diagram of electronic systems



**FIG. 14**

