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ENZYMATIC TIME-TEMPERATURE INDICATING METHODS AND DEVICES

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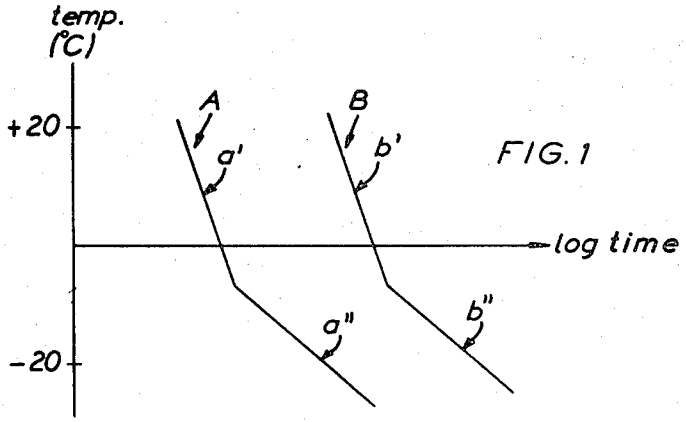


FIG. 1

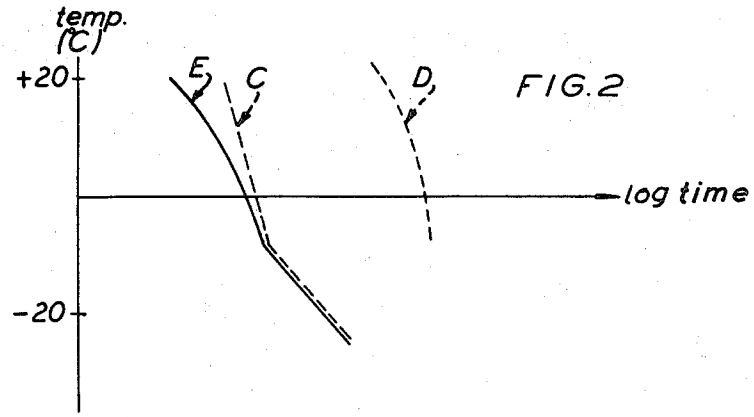


FIG. 2

FIG. 3

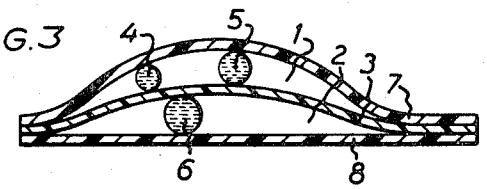


FIG. 4

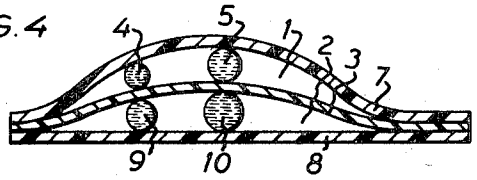
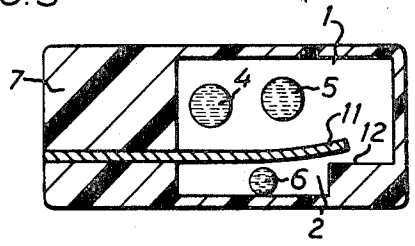


FIG. 5



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ENZYMATIC TIME-TEMPERATURE INDICATING METHODS AND DEVICES

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U.S. Cl. 195—103.5 R 9 Claims

ABSTRACT OF THE DISCLOSURE

Enzymatic time-temperature indicating method and device utilizing an enzyme and its substrate which react with each other in a reaction zone to give a reaction product that is indicated by the visible change of an indicating substance and, to adjust the time needed for visible change at high temperatures, a source of said reaction product supplying an additional amount of reaction product to the reaction zone in response to time and temperature.

The present invention relates to a method and device for time-temperature responsive indication.

For the handling and storing of sensitive substances and products whose shelf-life depends upon the observance of suitable time-temperature conditions, it is highly important that one can supervise and establish whether or not the substance or product has been subjected to the proper conditions, and various methods and devices have been suggested for this purpose. The supervision has been based upon mechanical, electrolytical or bacterial indication, but the methods and devices previously proposed suffer from certain disadvantages, for which reason they have been no commercial success. Thus, they have either been too expensive or not functioned to satisfaction. Above all, it has been difficult to obtain, for time-temperature responsive indications, a suitable time responsiveness of the indicator. However, this problem has largely been solved by the use of an enzymatic process in which an enzyme is caused to act upon a substrate, resulting in the formation, as a function of time and temperature, of a reaction product that is utilised for indication. One of the substances employed for this purpose is ureas which is allowed to act upon urea while forming ammonia, the presence of which is established by means of a pH indicator, the temperature and the time logarithm being linearly interrelated. By adding a buffer, the change-over time of the indicator can be prolonged, i.e. the linear interrlation is parallely offset.

However, the interrelation obtained by means of such an enzymatic indicator is not always the desired one. For instance, if a suitable interrelation exists between the change-over time of the indicator at low temperatures, the change-over time at high temperatures will frequently be too long. To some extent, this deviation of the time-temperature responsiveness of the indicator from the desired value is corrected in that the enzymatic indicator at low temperatures may exhibit a linear interrelation of temperature and log time which has a different, less negative coefficient of inclination than the linear interrelation at high temperatures. The inclination of these curves at low temperatures is determined by the chemical mechanism and can be varied within rather narrow limits only. In view hereof, it is desired that one should be able better to adapt the time-temperature responsiveness of the indicator to the desired one, i.e. to the time-temperature responsiveness of the substance to be supervised. By this adaptation, it is intended to obtain a more rapid change at high temperatures.

The prior art technique within the field of the invention is set forth in Swedish Pat. 346,162 which relates to an enzymatic indication of the above-mentioned type, and Swedish patent application 9,715/70 which relates to a time-temperature responsive indication, the substances adapted to react with one another being kept apart by a semi-permeable diaphragm, the permeability of which increases with an increase in temperature.

By the present invention, it has now been contrived to obtain a satisfactory adaptation of the time-temperature responsiveness of the indicator and thereby to eliminate the disadvantages of the prior indicators. The present invention is based upon the utilisation of an enzymatic indicator, and to adapt the time-temperature responsiveness of the indicator, i.e. in order to obtain a more rapid change at high temperatures, the indicator is supplied with and additional amount of the reaction product that is being indicated.

The invention relates to a time-temperature responsive indication method in which an enzyme as a function of time and temperature in a reaction zone acts upon a substrate, while forming a reaction product that is established by means of an indicating substance, said method being characterised in that there is introduced into the reaction zone an additional amount of the reaction product, increasing with an increase in temperature.

The invention also comprises a device for carrying the method into effect, said device comprising two chambers interconnected by means of a temperature-controlled valve means, one of said chambers constituting a reaction zone containing a time-temperature responsive enzymatic indicator consisting of an enzyme, a substrate, a solvent, an indicator and, if desired, a buffer, said device being characterised in that said second chamber contains a source of the reaction product that is generated by the enzymatic reaction within said first chamber.

In the indicator of the present invention, the enzyme preferably is urease, the substrate urea, the reaction product ammonia, and the indicating substance is a pH indicator.

The above-mentioned source of reaction product may consist of the reaction product which is to be supplied to the reaction zone or of the same enzyme and substrate as in said reaction zone which, as a function of time and temperature, produce the reaction product which is then supplied to said reaction zone.

The temperature-controlled valve means preferably is a semi-permeable diaphragm, the permeability of which increases with an increase in temperature, or a bimetallic spring.

The invention will now be described in more detail with reference to the accompanying drawings in which

FIG. 1 shows indication characteristics of a known enzymatic indicator;

FIG. 2 shows the characteristic of an indicator according to the invention;

FIGS. 3–5 show different embodiments of the device according to the invention.

Of the two indication characteristics illustrated in FIG. 1, A generally is a characteristic of an indicator having a low buffer strength, i.e. it has no or but an insignificant addition of buffer, whereas B is a characteristic of an indicator having a high buffer strength, i.e. it has a higher addition of buffer. As will appear from the figure and as has been mentioned before, the indication characteristic is parallelly offset towards longer change-over times by the addition of a buffer. The figure also shows that the indication characteristic is composed of two curve branches which substantially correspond to straight lines, and for the characteristic A the portion a' is the high temperature indication characteristic, whereas the portion a'' is the low temperature indication charac-

teristic. The same applies to the characteristic B and the curve branches *b'* and *b''*, respectively.

As has been pointed out above, the indication characteristic could previously only be affected by the addition of a buffer, i.e. by parallelly offsetting the characteristic. The inclination of the curve branches *a'* and *b'*, respectively, could not be affected, whereas the inclination of curve branches *a''* and *b''*, respectively, could be affected to a small degree only.

What is desired is an indicator having a characteristic largely corresponding to A and B, respectively, but having at higher temperatures a shorter change-over time than A and B, respectively, i.e. a different inclination of the curve branches *a'* and *b'*. To this end, the enzymatic indicator, as has been mentioned before, is supplied as a function of the temperautre with an additional amount of reaction product from a source which is or generates such a reaction product. The result is a composite indication characteristic which at low temperatures corresponds to the above-mentioned characteristic according to FIG. 1 but which at high temperatures, because of the additional amount of reaction product, obtains different inclination, as will appear from FIG. 2, curve E. FIG. 2 also shows how this new indication characteristic E is composed of the characteristic C of the "main indicator" according to FIG. 1, and of curve D for the additional amount of reaction product. The appearance of curve D will depend upon the special design, described in the following, of the indicator according to the present invention, by which design an increasing addition of reaction product at increasing temperature is made possible. It will be appreciated that by changing the form of curve D the indication characteristic E can be changed in different ways so that the desired appearance will be obtained.

Several preferred embodiments of the device according to the present invention will now be described in the following with reference to FIGS. 3–5.

The indicator according to FIG. 3 is a container comprising two chambers **1** and **2** separated by a partition **3**. The chambers **1** and **2** are defined outwardly by outer walls **7** and **8** of impermeable material, such as a suitable plastics material. Within the chamber **1**, there are provided two capsules **4** and **5** containing enzyme and substrate, respectively, a solvent, an indicating substance and, if desired, a buffer. The chamber **2** contains a capsule **6** with the reaction product, i.e. the substance that is formed by the reaction between the enzyme and the substrate in capsules **4** and **5**. The above-mentioned partition **3** is formed of a semi-permeable diaphragm material, such as low density polyethylene or cellulose acetate, the permeability of such diaphragm material increasing with an increase in temperature.

The function of the indicator according to FIG. 3, in which the enzyme is urease, the substrate is urea, the resulting reaction product is ammonia, and the indicating substance is a pH indicator, is as follows. The indicator is activated by crushing the capsules **4**, **5** and **6**, whereby urease and urea are brought together within the chamber **1**, and the enzymatic process starts by forming ammonia. The enzymatic indication reaction within the chamber **1** has a characteristic corresponding to curve A, B or C in FIGS. 1 and 2, respectively. The chamber **2** simultaneously contains ammonia that has been released by the crushing of the capsule **6**, and this ammonia passes through the diaphragm **3** into the chamber **1** as a function of the temperature. If the time it takes for a given amount of ammonia to penetrate the diaphragm **3** into the chamber **1** is taken as a function of the temperature, a curve corresponding to D in FIG. 2 will be obtained.

As will appear from FIG. 2, the addition of ammonia at low temperatures is negligible, whereas the addition at high temperatures actively influences the indication reaction within the chamber **1**. An indication characteristic corresponding to curve E is obtained.

FIG. 4 shows another embodiment of the indicator according to this invention. The indicator of FIG. 4 largely corresponds to the one shown in FIG. 3, and like parts have been identified by like reference numerals. However, the indicator according to FIG. 4 differs from the indicator according to FIG. 3 in that the source of reaction product within the chamber **2** is not directly a reaction product (ammonia), but the same enzyme and substrate as in the chamber **1**, in this instance urease and urea. The enzyme and the substrate within the chamber **2** are enclosed in capsules **9** and **10** which, upon activation of the indicator, are crushed like the capsules **4** and **5** within the chamber **1**. After activation of the indicator, the enzyme and the substrate within the chamber **2**, like the enzyme and the substrate within chamber **1**, will generate as a function of time and temperature the reaction product ammonia which passes through the diaphragm **3** into the chamber **1**. The indicator according to FIG. 4 thus corresponds to the indicator according to FIG. 3, except that the reaction product within the chamber **2** is not in its finished state, but is generated as the indication proceeds.

FIG. 5 shows a further embodiment of the indicator according to the present invention, and this indicator differs from the two devices previously described substantially in that the diaphragm **3** which acts as a time-controlled valve means, has been replaced by a bimetallic spring **11**. At low temperatures, the bimetallic spring **11** rests against the seat **12** and thus prevents communication between the chambers **1** and **2**. At increasing temperature, the bimetallic spring is bent more and more and rises from the seat **12**, as is shown in FIG. 5. In this manner, a communication passage is established between the chambers **1** and **2**, the size of said passage increasing with an increase in temperature. In this manner, the additional amount of reaction product within the chamber **2** will be supplied to the chamber **1** as a function of the temperature, as in the devices according to FIGS. 3 and 4. Like in the prior art devices, the source **6** of the reaction product within the chamber **2** actually may be a finished reaction product, as is shown in FIG. 5, or enzyme and substrate forming the reaction product.

The invention has been described above with reference to certain preferred embodiments, but it will be obvious that various modifications can be effected without departing from the scope of the invention. Thus, the invention has been described above in connection with the enzymatic system urease-urea, but it will be appreciated that other suitable enzymatic systems may also be employed. Furthermore, the device according to the invention has merely been described with valve means between the chambers **1** and **2** in the form of a semi-permeable diaphragm or in the form of a bimetallic spring, but also in this respect it will be obvious that any valve means can be employed which, as a function of the temperature, controls the communication between chambers **1** and **2**.

The present invention has a wide field of use generally encompassing all kinds of sensitive substances and products, for instance the supervision and indication of food products, medicines, photographic film, storage-sensitive chemical substances, such as explosives, etc.

I claim:

1. In a method of time-temperature responsive indication, wherein an enzyme acts on a substrate in a reaction zone in response to time and temperature to form a reaction product that is indicated with an indicating substance, the improvement comprising:

supplying to the reaction zone an additional amount of said reaction product, which amount increases with increasing temperature.

2. In a device for time-temperature responsive indication, said device having

two chambers

a temperature-controlled valve means connecting said chamber;

a time-temperature responsive enzymatic indicator including an enzyme and its substrate, a solvent, an indicating substance to indicate enzyme activity on the substrate and a buffer in one of said chambers, said first chamber constituting a reaction zone, wherein the improvement comprises:

a source of the reaction product formed during the enzymatic reaction in said first chamber contained in the second chamber.

3. A device according to claim 2, wherein the enzyme is urease, the substrate urea, the reaction product ammonia, and the indicating substance is a pH indicator.

4. A device according to claim 2, wherein said source consists of the reaction product which is to be supplied to the reaction zone.

5. A device according to claim 3, wherein said source consists of the reaction product which is to be supplied to the reaction zone.

6. A device according to claim 2, wherein said source consists of enzyme and substrate of the same kind as those in the reaction zone which, as a function of time and temperature, form the reaction product subsequently supplied to the reaction zone.

7. A device according to claim 3, wherein said source (9, 10) consists of enzyme and substrate of the same kind as those in the reaction zone which, as a function of time and temperature, form the reaction product subsequently supplied to the reaction zone.

8. A device according to claim 2, wherein said valve means is a semi-permeable diaphragm whose permeability increases with increasing temperature.

9. A device according to claim 2, wherein said valve means is a bimetallic spring.

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ALVIN E. TANENHOLTZ, Primary Examiner

U.S. Cl. X.R.

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