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DEVICE PERMITTING THE SPREADING OF ONE OR SEVERAL
REAGENTS ON A GEL

The invention relates to a device permitting the spreading of one or several reagents on a gel, in particular an electrophoresis or immunofixation gel.

The invention also relates to a method making use of the aforementioned device.

The techniques of electrophoresis or immunofixation are usually followed, after the migration, by a stage of incubating the gel with a reagent in order to detect and possibly to quantify the protein fractions separated during the electrophoresis.

The most conventional case is the staining of the set of protein fractions separated by electrophoresis by means of a dye which becomes bound specifically on to the latter. In this case, the reagent used (the dye) is a product which is generally cheap and of which it is possible to prepare a solution in a sufficient quantity to immerse the gel totally therein.

However, this method cannot be envisaged in the case where it is necessary to use small quantities of reagent, when it is an expensive reagent, or when it is desired to produce on the same gel incubations on discrete zones with different reagents.

These possibilities relate for example to assaying isoenzymes contained in a serum (isoLDH, isoCK, isophosphatase) where the gel is incubated with a substrate (expensive product) for these enzymes with the aim

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of forming a colored or fluorescent product which permits quantification by densitometry.

The case where it is necessary to carry out, on the same gel, incubations with different reagents is constituted for example by immunofixation where various discrete zones of the gel must be incubated with various antisera of different specificity (expensive reagent) and a protein fixative.

In all these cases, use of a minimum quantity of reagent is sought.

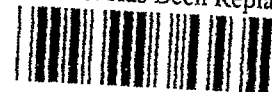
In implementing the abovementioned methods, a sheet of filter paper cut to the size of the gel is impregnated with a quantity of reagent just sufficient for it to be entirely moistened and it is then applied to the surface of the gel.

In the case where various reagents are possibly used on distinct zones of the same gel, as many strips of filter paper are cut as there are zones to be covered by the various reagents, and these various strips are impregnated with the various reagents before being applied to the various zones where the incubation is to take place.

This method has a certain number of drawbacks. It is in fact necessary to avoid trapping air bubbles between the gel and the sheet of filter paper impregnated with reagent, because, in the region of the air bubbles, the reaction would not take place for lack of reagent on the gel. The paper being opaque, it is sometimes difficult to detect the presence of these air bubbles.

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Moreover, during the incubation reaction, a proportion of the proteins which it is desired to develop can be adsorbed onto the paper, or more simply be absorbed by pumping onto the latter.

If an extended incubation time is necessary (30' to 1 hour), this phenomenon is accentuated by the fact that, during the incubation, evaporation from the free surface of the paper occurs. The latter having a tendency, by capillarity, to maintain a constant degree of humidity, a flow of liquid is created, from the gel to the paper, with entrainment of the protein fractions to be assayed initially contained in the gel. This leads to a loss of sensitivity of the development on the gel and, this absorption not being very homogeneous, quantification by densitometry of the developed fractions risks being subject to errors.

Furthermore, and above all, this is a manual process which is very laborious and therefore difficult to implement in routine clinical analysis, and, in current practice, has been abandoned.

Another method also used consists in using a mask constituted by a sheet of flexible plastic, preferably hydrophobic in nature, having approximately the same external outline as the gel and having one or more independent openings of rectangular shape. This mask, once applied to the gel, delimits the zone or zones intended to receive the reactant or reactants.

Use of such a mask also has a certain number of drawbacks.

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After electrophoretic migration, it frequently happens that a proportion of the liquid exudes from the surface of the gel. This exudation is mainly created by electroendosmotic flow.

Before applying the mask to the gel, it is necessary to remove this excess liquid by pumping by means of a thin filter paper, because, if liquid is still present on the gel, it prevents good adhesion of the mask to the gel, as well as a good seal at the periphery of the zone(s) delimited, being assured.

As well as the additional manual operation of pumping, the filter paper risks entraining a portion of the proteins from the gel and of distorting the result.

Furthermore, the gel or the reagents used can, in certain cases, contain surfactants which will facilitate insertion of liquid between the gel and the mask and, under certain conditions, even after removal of the excess liquid which has exuded from the gel at the end of migration, the mask risks not assuring the required seal.

With this type of mask, it is important that the whole of the surface of the mask is perfectly applied to the gel. It is necessary to avoid trapping air bubbles between the gel and the plastic mask during its positioning on the gel. Otherwise, there is a risk of having either a leak of reagent under the mask, or a mixing of adjacent reagents.

An object of the present invention is to propose a device which largely overcomes the preceding drawbacks.

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The subject of the present invention is a device permitting deposition, spreading and incubation of a reagent on a gel, in which quantities of reagent of the same order of magnitude as those of conventional devices are used, the device of the invention being however without the drawbacks of the conventional devices.

Another object of the present invention is to propose a device permitting deposition, spreading and incubation of a reagent on a gel, in which it is possible to incubate different zones with respectively different reagents, without there being mixing of the reagents.

The aim of the present invention is to propose a device permitting deposition, spreading and incubation of a reagent on a gel, in which manual operations are limited to the minimum.

An object of the present invention is to propose a device for deposition, spreading and incubation of a reagent on a gel, permitting accurate results to be obtained as to quantification of the substances fractionated in the gel.

An object of the present invention is to propose a device for deposition, spreading and incubation of a reagent on a gel, capable of being automated.

The subject of the invention is therefore a rigid mask intended for deposition, spreading and incubation of one or several liquids on a gel (of total surface area S_g) according to one or several well-defined zones of the gel, hereafter designated by "incubation surface S_i ", this mask comprising:

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- an upper surface and a lower surface, the distance separating the lower surface and the upper surface constituting the thickness of the mask,

- at least one orifice intended to permit deposition and spreading of the liquid on the incubation surface S_i of the gel, and constituted by a recess crossing the mask over the whole of its thickness, and/or

- at least one slit crossing the mask over the whole of its thickness and intended to permit withdrawal of the extra liquid present on the incubation surface S_i of the gel, this slit being situated in the part of the mask whose projection is either in the incubation surface S_i , or outside the incubation surface S_i , the lower surface of the mask being intended to be laid down in proximity to the surface S_g of the gel, under conditions such that the lower surface of the mask is not in contact with the incubation surface S_i , this lower surface being furthermore such that it does not include, over at least a part of itself designated by S_m , any irregularity on its surface capable of being an obstacle to spreading of the liquid on the incubation surface S_i , the surface S_m being delimited with respect to the rest of the lower surface, so that the liquid can only spread on the surface S_i of the gel which is a projection of S_m , this projection of S_m on the gel being greater than or equal to the area of the incubation surface S_i of the gel.

The invention advantageously permits placing of the mask above the gel comprising the incubation surface

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or surfaces. It also makes it possible to place the mask below the incubation gel. In either case, the surface of deposition, spreading and incubation of the reagents on the gel is situated in proximity to the lower surface of the mask, the latter not being however in contact with the incubation surface S_i .

The liquid is generally the reagent intended to develop the proteins which have previously been deposited on the gel. The reagent is introduced via the recess defined hereinabove, which will also be termed "deposition orifice".

The incubation surface S_i is the zone of the gel which it is essential to cover with the reagent (or reagents) in order that development of the proteins should take place. The length of the incubation surface, that is to say the dimension of the incubation surface parallel to the direction of electrophoretic migration, is designated by L_i .

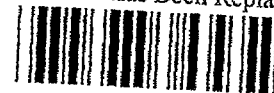
In the case of a single reagent, S_i can be equal to the area S_g or else S_i can be a part of S_g including nevertheless the set of migration tracks, that is to say the zones of the gel where the electrophoretic separations have taken place.

It is necessary however to note that the zone effectively covered by the reagent can be greater than the area S_i (cf. Figure 6f).

In the case of incubation with different reagents, or possibly with the same reagent but on distinct zones of the gel, including for example each of

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the various migration tracks, the incubation surface is defined by Si_1, Si_2, Si_n , n being an integer equal to or greater than 2.

The useful lower surface of the mask is designated by " Sm ", which has no irregularity on its surface capable of constituting an obstacle to the spreading of the reagent on the incubation surface Si , and in particular neither concave roughness, nor convex roughness, that is to say a surface without a ridge or a hollow which would be capable of preventing the reagent from spreading on the surface Si , this surface Sm being flat or curved or including flat or curved elements.

The surface Sm can include the slit and deposition orifice defined hereinabove.

Advantageously, the surface Sm is flat or curved, in particular cylindrical, with a radius which is large with respect to the dimensions of Si .

The surface Sm can also be composed of elementary facets, which are flat or curved, in particular cylindrical, connected together, these various facets, as well as their connections, being such that there is no obstacle to the spreading of the liquid on the incubation surface Si .

The length Lm is the dimension of the surface Sm parallel to the direction of electrophoretic migration. The width of the surface Sm , which is dealt with hereinbelow, corresponds to the dimension perpendicular to the length defined hereinabove.

When this surface Sm having a recess permitting

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deposition of a reagent is positioned in proximity with the surface of a gel, it must make it possible to keep by capillarity, between itself and the surface S_i to be incubated, a reagent introduced via the recess.

This deposited liquid can then spread between S_m and the surface of the gel until it encounters a discontinuity, which can be either the edge of the gel, or the edge of the mask, or a groove, or a concave difference in height, and which delimits the surface S_m .

Spreading of the liquid is taken to mean advance or displacement of the liquid on the surface S_i , due only to capillary forces (resulting from the proximity of the lower surface of the mask and the incubation surface S_i) and possibly to gravity forces.

In the case where several reagents must be spread, the mask surfaces are defined by $S_{m_1}, S_{m_2}, S_{m_n}$, n being an integer equal to or greater than 2. [sic] as many surfaces which are flat, curved, in particular cylindrical, coplanar, cocylindrical or composed of elementary facets which are flat or curved, in particular cylindrical, these various elementary facets being connected together.

The surface [sic] S_{m_1} to S_{m_n} are delimited by concave differences in height or grooves.

As concerns the slit, this can be situated in the mask, so that its projection is in the incubation surface S_i , one [sic] outside the incubation surface S_i , but adjacent to one of the edges of the said incubation surface S_i , or adjacent to one of the edges of the

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surface effectively covered by the reagent. Advantageously, the slit is adjacent to the edge of the surface Si which edge is opposite to the vicinity of the deposition orifice.

The slit is advantageously situated outside the incubation surface Si. In fact, if it is situated in the incubation surface Si, it must have dimensions such that it is not capable of being an obstacle to the spreading of the liquid. Under these conditions, the slit is narrow (less than or equal to approximately 1 mm) and makes it possible to keep by capillarity an excess of reagent.

The topography of the upper surface is a variable parameter. However, the upper surface is advantageously flat. In the text that follows, in order to simplify the description, it will be considered that the upper surface is flat, and that the useful lower surface Sm, of length Lm

- either is substantially parallel to the upper surface,

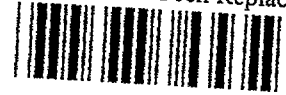
- or forms with the upper surface, when the latter is used horizontally, an angle less than approximately 3° , or

- or else is substantially parallel to the upper surface over a part of its length Lm and over the rest of its length Lm, in the extension of the parallel surface, forms an angle with the upper surface, this angle being such that it is not capable of preventing spreading of the liquid on the incubation surface Si.

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hereinabove relating to the respective position of the lower surface with respect to the upper surface, the lower surface is advantageously substantially parallel to the upper surface over a part of its length L_m which corresponds to the length of the incubation surface L_i . The extension of this parallel surface by a part forming an angle with the upper surface is advantageously such that the angle between the lower surface and the gel is an acute angle of approximately 1° to approximately 90° , which will favor removal of the excess of reagent, when this angle is in the vicinity of the slit (cf. Figure 6d).

The surface S_m of the mask can also be curved, advantageously cylindrical.

When the surface of the mask is constituted by a cylindrical surface, it is necessary for its diameter to be greater than or equal to the square of L_i .

When the cylindrical surface only relates to a fraction f of the incubation length L_i , the diameter must be greater than or equal to the square of the length $f \times L_i$.

The invention also relates to a mask intended for deposition, spreading and incubation of one or several liquids, in which the lower surface of the mask comprises at least two differences in height or two grooves, the differences in height or grooves being spaced apart from each other in order to delimit at least a part of the mask S_m , which part does not include any irregularity on its surface capable of being an obstacle to the spreading

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of the liquid on an incubation surface S_i , and whose projection on a gel is equal to or greater than the area of the incubation surface S_i of the gel on which surface the liquid can be spread, the differences in height and the grooves having a height sufficient for the liquid to be able to be kept by capillarity on the aforesaid incubation surface S_i of the gel.

In this embodiment of the invention, the part S_m of the mask is limited neither by the edge of the gel, nor by the edge of the mask, but by at least two differences in height or two grooves, which have a height sufficient, and possibly a width sufficient, for the liquid to be able to be kept by capillarity on the aforesaid incubation surface S_i of the gel and not to go beyond the limits of the incubation surface S_i of the gel or the limits of the projection of the surface S_m on the gel.

The grooves can cross the whole of the mask, and they will be designated by "hollow grooves". In order for the hollow grooves to keep the liquid by capillarity, it is necessary for their dimensions to be such that they cannot act as a reservoir with possible collection of the liquid within the said reservoir, and therefore that they have advantageously a width greater than or equal to 2 mm, and a height greater than or equal to 0.2 mm, advantageously 0.5 mm and preferably 1 mm.

The differences in height or grooves surround a migration track and are advantageously rectilinear.

In general, the migration tracks have the shape

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of a rectangular strip, having for example the following dimensions: length of approximately 10 mm to approximately 100 mm, width of approximately 2 mm to approximately 80 mm. When a migration track is surrounded by two grooves, the two other edges of the migration track can be constituted by a part of two of the edges of the gel or of two of the edges of the mask, or by two other grooves perpendicular to the two other grooves previously defined.

When there are several migration tracks, the latter are advantageously such that their edges of smallest dimension are in the same alignment.

The subject of the invention is also a mask intended for deposition, spreading and incubation of a single liquid, comprising a single recess crossing the mask, intended to permit deposition, spreading and incubation of a single liquid on a gel according to an incubation surface Si of the gel.

This embodiment relates to the case where it is desired to deposit, spread and incubate a single liquid.

In this case, a single recess is contemplated in order to carry out the deposition of the liquid.

This recess can have the shape of an elongated slit, having for example the following dimensions: length 50 mm, width 1.5 mm.

The invention also relates to a mask intended for deposition, spreading and incubation

- of several liquids on a gel according to respective incubation surfaces Si_1 to Si_n or

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- of one liquid on a gel according to ¹⁴ several incubation surfaces Si_1 to Si_n , n ranging from 2 to 50, this mask being such that its lower surface comprises differences in height or grooves, the differences in height or grooves being spaced apart from each other in order to delimit several parts of the mask Sm_1 to Sm_n , n ranging from 2 to 50, including no irregularity on their surface capable of being an obstacle to the spreading of the liquid on the surfaces Si_1 to Si_n , and whose the projection on the gel is greater than or equal to the areas of the incubation surfaces Si_1 to Si_n of the gel, on which surfaces the liquid is to be spread, the differences in height and the grooves having a height sufficient for the liquid to be able to be kept by capillarity on the aforesaid incubation surfaces Si_1 to Si_n of the gel.

This case corresponds to the deposition of several liquids on respective incubation surfaces or of one liquid on several incubation surfaces Si_1 to Si_n .

To ensure the deposition, spreading and incubation of the liquid on the surface Si , it is possible to envisage the orifice which is used for deposition having a volume such that it is capable of constituting a reservoir having a capacity for retaining the extra liquid in order to assure spreading of the liquid on the incubation surface Si , even if a slight quantity of reagent has evaporated during incubation (cf. Figure 6a).

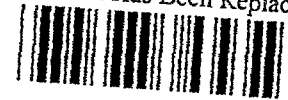
It is possible to proceed otherwise in order to assure the deposition, spreading and incubation of the

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liquid on the incubation surface Si. In fact, if the gel and the mask are kept substantially parallel to each other, it can be useful to incline the gel and mask together by rotation around an axis substantially parallel to one of the edges of the gel or to one of the edges of the mask, so that the liquid descends under gravity in the direction of the incubation surface Si which is to be covered (cf. Figure 6b).

It is also possible, in order to assure the deposition, spreading and incubation of the liquid, to keep the gel in a substantially horizontal position and slightly to incline the mask by rotation around an axis parallel to one of the edges of the mask or to one of the edges of the gel and situated in the vicinity of the edge of the incubation surface so that the distance between the gel and the mask in the region of this axis is smaller than the distance between the gel and the mask in the zone opposite this axis (cf. Figure 6c).

Given that the capillary forces are greater in the vicinity of this axis, the liquid is distributed by capillarity over the incubation surface Si.

The subject of the invention is also a mask such as previously described in which the deposition orifice has a volume such that it is capable of constituting a reservoir having a capacity for retaining the extra liquid in order to assure spreading of the liquid on the incubation surface Si.

According to an embodiment of the mask of the invention, the deposition orifice has an elongated shape

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or a circular shape, which is preferably elongated and has advantageously a width of approximately 1 mm to approximately 2 mm, in particular 1.5 mm, and a length of approximately 4 mm to approximately 80 mm, in particular 5 mm.

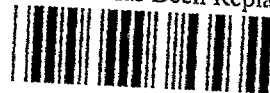
According to another embodiment, the recess of the mask is capable of containing by capillarity a volume of liquid at least equal to $V/10$, V being the volume existing between the incubation surface S_i and the mask, when the mask and the gel are positioned respectively one in relation to the other under conditions such that the liquid deposited in the recess spreads over the gel.

According to another embodiment of the invention, the deposition orifice is situated in the vicinity of the middle of the part S_m of the mask and has the shape of an elongated slit, whose dimensions are such that it cannot prevent the liquid being deposited on the incubation surface S_i , and whose length is advantageously the length of the incubation surface S_i and whose width is advantageously from approximately 1 mm to approximately 2 mm, this deposition orifice also being capable of assuring the function of the slit intended to permit withdrawal of the extra liquid which is spread on the surface S_i .

According to another embodiment of the invention, the slit intended to permit withdrawal of the extra liquid is substantially parallel to the width of the incubation surface and is positioned adjacently to the edge of the incubation surface which is opposite to the

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vicinity of the deposition orifice, and has advantageously a width of approximately 1 mm to approximately 4 mm, in particular 2 mm, and a length greater than or equal to the width of the surface Si, and the internal peripheral surface of the slit is perpendicular to the surface of the mask and preferably inclined with respect to the perpendicular direction of the surface of the mask in order to permit the placing in direct contact of means permitting withdrawal of extra liquid with the said extra liquid.

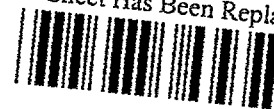
In order to give an idea, the inclination of the internal peripheral surface of the slit with respect to the direction perpendicular to the surface of the mask can be approximately 30°.

According to another embodiment of the invention, the thickness of the mask is approximately 1 mm to approximately 20 mm, in particular approximately 2 mm to approximately 10 mm, and the differences in height have a height at least equal to 0.2 mm, advantageously approximately 0.5 mm to approximately 20 mm, or the grooves have a width sufficient so that, during incubation, each liquid kept by capillarity between the mask and an incubation surface Si does not mix with the liquids kept in the incubation surfaces which are directly adjacent to Si, the width of the grooves being approximately 1 mm to approximately 10 mm.

According to another embodiment of the invention, the mask comprises several mask parts Sm, advantageously approximately 6 to approximately 50, each of the parts Sm

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comprising a deposition orifice, preferably situated in the part of Sm whose projection on the gel corresponds to the anodic part of the gel, this mask comprising a single slit intended to permit withdrawal of the extra liquid which is spread on the surfaces Si of the gel, this slit extending preferably perpendicularly with respect to the largest of the dimensions of the parts Sm of the mask and being preferably situated in the mask at a position such that its projection is in the cathodic region of the gel.

The parts Sm are advantageously constituted by rectangles, having for example a length greater than or equal to approximately 10 mm to approximately 80 mm and a width of approximately 2 mm to approximately 80 mm.

The subject of the invention is also a mask as previously described comprising a single part Sm, whose dimensions are greater than or equal to those of Si, and comprising in the vicinity of one of its ends a recess having an elongated shape, whose length corresponds preferably to the width of the incubation surface Si, and also comprising, in the vicinity of the other end, a slit intended for withdrawing the extra liquid, this slit being advantageously, in length, at least the width of Si.

The invention also relates to a mask comprising means for positioning the mask with respect to the gel such that the minimum distance between the gel and the part Sm of the mask is greater than or equal to 0.1 mm, and the value of the maximum distance is approximately

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0.5 mm to approximately 2 mm, in particular 1 mm.

These means can be constituted by any fastening means, for example screws etc. It is also possible to envisage the mask including, on its periphery, stops capable of resting on the gel, these stops being positioned and having dimensions such that there is no contact between the part S_m of the mask and the incubation surface S_i .

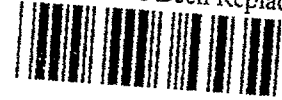
According to another advantageous embodiment of the invention, the upper surface of the mask and its lower surface are not parallel to each other, but form an angle such that, when the upper surface of the mask is positioned parallel to the surface of the gel, the minimum distance between the gel and the mask is greater than or equal to approximately 0.1 mm and the value of the maximum distance is approximately 0.5 mm to approximately 2 mm, in particular 1 mm.

The subject of the invention is also a mask produced so that the orifice for deposition and spreading of the liquid coincides with (is identical to) the slit intended to permit withdrawal of the extra liquid. This embodiment makes it possible in particular to contemplate easier automation of the device for deposition, spreading and incubation of one or several reagents on a gel.

The deposition orifice (orifice (1)) coincides in this embodiment with the slit for removing the reagents and is connected, by means of pipes and a multi-channel valve, to the various reagent solutions to be introduced as well as to a "discharge" flask intended to gather the

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reagents removed. These solutions are conveyed in one direction or the other by means of a pump.

Under these conditions the distinct slit of the orifice (1), previously described and permitting, after the incubation phase, removal of the reagent by pumping by means of a filter paper introduced in this region, is no longer necessary and is dispensed with.

Under these conditions the orifice (1) can have various shapes, and can preferably have a circular shape of small diameter advantageously less than 2 mm and preferably less than 1 mm.

According to this embodiment in which the deposition orifice is also used to remove the reagents after incubation, it is advantageous that:

- 1) the periphery of the orifice (1) is the part of the surface S_m of the mask, capable of being in contact with the various reagent solutions, which is nearest to the surface of the gel without however coming into contact with it (minimum separation greater than or equal to 0.1 mm);
- 2) as the distance from this orifice on the surface S_m becomes greater, that the gel/mask distance increases where [sic] at the limit stays constant.

In the case where the reagent introduced has cohesive forces similar to those of water the maximum distance between the surface of the gel and that of the mask is not to exceed 2 mm (it being possible for this maximum separation to be slightly increased or reduced if the cohesive forces of the liquid introduced are

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respectively increased or reduced).

Given the flatness of the gel, the constraints of minimum separation of 0.1 mm in the region of the orifice (1) and on the other hand of maximum separation of approximately 2 mm at the periphery of the incubation zone S_i define the shape of the surface S_m of the mask which is for example in a centered or off-centered pyramidal shape with a rectangular or square base, or alternatively in the shape of a centered or off-centered conical surface of revolution with a circular or elliptical base and whose apex corresponds to the orifice (1).

The angles at the apex of this pyramid or of this conical surface of revolution depend on the dimensions of the surface S_i of the gel to be incubated.

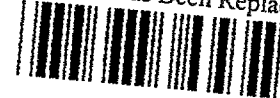
For example in the case of a circular incubation surface of 90 mm diameter the angle at the apex of a mask having a surface S_m of centered conical shape with a circular base must be between 175 and 179°.

Under these conditions, during the introduction of a reagent brought to the orifice (1) by means of pipes and a pump, the liquid will be distributed on the surface of the gel starting from the orifice (1), the successive outlines of the zones of the gel covered by the reagent being contour lines of equal distance of the surface S_m with respect to the surface of the gel which is kept in a horizontal position. In the case where the surface of the mask is a centered conical surface of revolution with a circular base these will be concentric circles centered

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on the orifice (1).

It is expedient to introduce the various reagents free from air bubbles (because in the location of an air bubble, the reagent not being in contact with the gel, the incubation will not take place in that region) and at sufficiently slow flow rates of the order of (50 to 1000 $\mu\text{l/s}$) in order to avoid damaging the gel facing the orifice (1) in consequence of applying hydraulic pressures incompatible with the mechanical strength of the gel.

After the incubation phase the reagent is recovered via the orifice (1) by means of the pump. The orifice (1) being the surface on the zone nearest to the gel, it is in this region that the capillary forces for the liquid introduced between the mask and the gel will be the maximum, and as the liquid is drawn up the latter will collect in this zone of minimum gel/mask spacing. During this operation, the successive outlines of the zones of the gel still covered with liquid will be in inverse order the same as those obtained during introduction, and correspond to the contour lines of equal distance between the mask and the gel. Insofar as the drawing up of the liquid is sufficiently slow (50 μl to 1000 $\mu\text{l/s}$) in order not to break the cohesion of the liquid (especially at the end of the drawing up) the latter will be drawn up continuously and practically completely.

In order that removal of the reagent previously introduced should be as complete as possible it is

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important:

- 1) that the diameter of the orifice (1) flush with the surface of the mask should be of small size (diameter of the order of 1 millimeter);
- 2) that it should be at a small distance from the surface of the gel, of 0.1 to 1 mm and preferably of 0.2 to 0.6 mm;
- 3) in the aim of maintaining cohesion of the last drop which will be drawn up, and it is preferable, that the surface of the mask which surrounds the periphery of the orifice (1) should have a flat surface parallel to the surface of the gel over a zone of 10 to 200 mm², preferably of 25 to 100 mm².

In this embodiment placing of the mask below the surface of the gel can be contemplated.

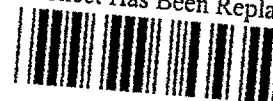
The gel being kept by an appropriate means (for example by drawing up under vacuum by means of a porous plate) in a horizontal position with the free face of the gel directed towards the base of the mask, the gel is then underneath and the operation of the whole is similar to that previously described.

It nevertheless appears under these conditions that the removal of the last drop of liquid previously introduced is facilitated since it has in addition a tendency by gravity to collect in the region of the intake orifice (1).

In the case where a single reagent is introduced according to this method on the same gel it is expedient to calibrate the volume of reagent delivered by the pump

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in order to cover the zone S_i of the gel which is to be incubated.

If the area of the surface S_i is equal to the area of the surface of the gel beyond the volume contained between the surface of the gel and the surface of the mask opposite, an excess of reagent will still be kept. Additional extra liquid can nevertheless escape the capillary forces without however entraining the rest of the solution contained between these two gel and mask surfaces. It is sufficient under these conditions to have a recovery system, for example a gutter, at the periphery of the mask with a system for disposal of this extra reagent, in order for it to have no detrimental effect on the development process.

In the case where several different reagents are introduced according to this method onto the same gel it is imperative not to have any mixing of the reagents.

Each of the zones $Sm_1, Sm_2..Sm_n$ of the mask corresponding to the various incubation zones $Si_1, Si_2..Si_n$ of the gel is then in the shape of a pyramid with a rectangular or square base or alternatively of a centered or off-centered conical surface of revolution with a circular, ellipsoidal or rectangular, etc., base each having an orifice (1) at its apex and separated from each other by furrows and all the orifices being situated preferably at the same distance from the gel.

Independent pumps simultaneously (or successively) inject calibrated volumes of the various reagents via each of the orifices so that the gel surfaces $Si_1,$

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$Si_1..Si_i$ are covered.

An excess of reagent can be tolerated insofar as the various surfaces $Sm_1, Sm_2..Sm_i$ are slightly larger than the surfaces $Si_1, Si_2..Si_i$ to be incubated and as the gel/mask spacings in the region of the periphery of the zones Si_1, Si_2 and Si_i are less than 2 mm (in the case of liquids introduced whose cohesive forces are similar to those of water) in order to permit keeping by capillarity a possible excess of reagent beyond this periphery.

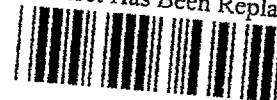
Removal of the reagents after the incubation phase is carried out in the same manner as previously described.

The subject of the invention is also a set comprising a mask and a gel, permitting implementation of an incubation on a gel previously subjected to electrophoresis or permitting crossed (cross-dot) reactions to be carried out.

The subject of the invention is also a set comprising means permitting the parts Sm of the mask to be sufficiently close to the gel for the liquid intended to be spread on the incubation surfaces Si to be kept by capillarity on the incubation surfaces Si of the gel in projection of the parts Sm of the mask and for the parts Sm of the mask to be parallel to the gel or to be inclined with respect to the gel, preferably according to an angle of approximately 0.5° to approximately 3° , advantageously of approximately 1.5° , the minimum distance between the mask and the gel being advantageously in the vicinity of the slit and the

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maximum distance between the mask and the gel being advantageously in the vicinity of the deposition orifice.

The invention also relates to a method of deposition, spreading and incubation of one or several liquids on a gel (of surface S_g) according to one or several well-defined zones of the gel, hereafter designated by "incubation surface S_i ", characterized in that

- a mask is positioned, according to the invention, with respect to the gel, so that the part S_m of the mask should be sufficiently close to the incubation surface S_i for the liquid intended to be spread on the surface S_i to be kept by capillarity on the said surface S_i , and in that:

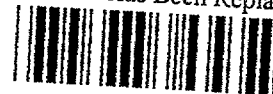
- the liquid is introduced into the deposition orifice defined hereinabove,

- the mask is kept with respect to the gel in the position as indicated hereinabove, or, if the mask and the gel are parallel to each other, the mask is inclined with respect to the gel in order that the liquid should be distributed by capillarity on the surface S_i , or the gel and mask set is inclined with respect to the horizontal so that the liquid should be distributed on the incubation surface S_i by gravity, for a sufficient time for the reaction between the liquid and the components, in particular proteins, deposited on the gel, in particular by electrophoresis, to take place,

- at the end of the incubation, the extra liquid spread on the well-defined incubation surface of the gel

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is withdrawn via means arranged through the previously defined slit, in particular with the aid of filter paper,

- the mask is withdrawn from the gel.

The subject of the invention is also a method in which the mask is kept parallel with respect to the gel, and the set is inclined according to a sufficient angle with respect to the horizontal for the liquid to spread by gravity on the incubation surface Si, and advantageously according to an angle ranging from approximately 5° to approximately 90°, advantageously approximately 30°.

The invention also relates to a method in which the mask is kept according to a sufficient angle with respect to the gel for the liquid to spread by capillarity on the incubation surface Si, and in particular according to an angle of approximately 0.5 to approximately 3°, advantageously approximately 1.5°.

A subject of the invention is an electrophoresis apparatus characterized in that it comprises a mask as previously described, intended for spreading and incubation of one or several reagents.

The invention can be illustrated in the light of the description which follows.

This new type of mask of the invention (floating mask or suspended mask) can be constituted by a flat and rigid piece of plastic which is preferably transparent (for example Plexiglas), having for example a thickness of approximately 2 to approximately 10 mm and in which the lower surface comprises at least one part Sm whose

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area corresponds at least to that of the surface of the gel to be covered by the reagent, also called "incubation surface Si". This piece of plastic is drilled right through and perpendicularly to its surface with a deposition orifice, preferably of elongated shape, situated inside or outside the incubation zone. This deposition orifice permits introduction of the reagents by means of a pipette.

The part Sm of the lower surface of the piece of plastic corresponding to the incubation surface is characterized by the fact that it has no surface irregularities (polishing). It is arranged by appropriate support means in proximity to the surface of the gel, either parallel thereto or slightly inclined, but without there being any contact between the incubation surface Si and the part Sm of the mask.

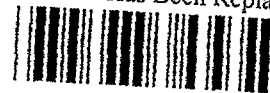
Given the small distance between the surface of the gel and the surface of the plastic (0.1 to 2 mm), the liquid introduced by means of a pipette through the deposition orifice is distributed by capillarity between the two flat surfaces (part Sm and surface of the gel) and covers the zone of the gel which is in projection beneath the part Sm of the piece of plastic.

The volume introduced must be at least equal to the volume Vo of the clear space between the two surfaces in order for the incubation surface Si to be covered.

Extra liquid (reagent) with respect to this volume Vo being able to be up to 1.2 or 1.3 times; the volume Vo being preferred. In fact, it permits covering

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of the gel according to the incubation surface S_i to be assured even if a slight evaporation of the reagent takes place during the incubation phase.

In fact, this excess of reagent will be mainly kept by capillarity in the region of the deposition orifice, insofar as the latter has an elongated shape of small width (1 to 2 mm) (in order for capillary effects to develop there) and of a length which is sufficient (greater than or equal to 4 mm) to assure a sufficient volume of liquid kept by capillarity (see the examples hereinbelow).

If a quantity of reagent slightly greater ($\times 1.1$) then the sum of the volume V_0 and the volume of liquid capable of being kept by capillarity in the region of the deposition orifice is added, through the deposition orifice, this excess will be distributed homogeneously at the periphery of the incubation surface of the piece of plastic, leading to a slight increase in the area of the surface of the gel subjected to incubation, but this occurs regularly, this excess always being kept in the region of the piece of plastic by capillarity.

In the case of using a single reagent, the only drawback is an unnecessary consumption of reagent.

In the case of incubation of adjacent zones with different reagents, it is sufficient for the spacing between the contiguous incubation surfaces to be sufficient to tolerate this increase in area of treated surface, without there being a risk of the surfaces treated by two different reagents becoming joined.

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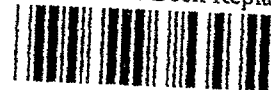
In any case, the separation between the zones must be greater than or equal to 2 or 3 mm, in order for the phenomena of diffusion of the reagents in the gel taking place during the incubation of the various reagents in the case of longer incubation times, for example 1 h, a spacing greater than or equal to 4 or 5 mm will be necessary, but this is equally true in the case of the other types of method previously used.

The elongated shape of the deposition orifice, in addition to its role, mentioned hereinabove, as a reserve of reagent, has the advantage of permitting easy introduction of the reagent onto the gel, for example by making the liquid flow on the vertical wall of the orifice, at one end of the latter.

In the case of a circular orifice of the same diameter as the width of the preceding orifice, it is necessary to assure a seal by friction between the tip of circular cross-section of the pipette and the orifice, and to inject the reagent onto the surface of the gel with a slight pressure, this pressure having a value such that it is equal to or greater than that necessary to overcome the capillary forces which develop in the region of the circular orifice of small cross-section. In this case, it is advantageous that the area of the surface S_m should be greater than S_i and that the volume of the quantity injected should be less than the volume contained between the surface S_m and the gel.

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The width of the deposition orifice is such that the end of the frustoconical tip of a pipette can penetrate therein, without however permitting it to descend beyond the plane of the surface of the plastic situated opposite the gel. The end of the pipette can therefore in no case damage the surface of the gel during introduction of the reagents.

Another advantage of the device of the suspended mask is that the phenomena of evaporation which can take place during the incubation are greatly reduced, since they can only take place at the periphery of the incubation surface and in the region of the deposition orifice.

After the incubation phase, it is expedient to remove the excess of reagent.

In the case where several reagents are used on contiguous zones, it is possible to proceed as follows.

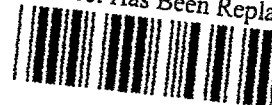
The various incubation surfaces are generally rectangles whose long sides are parallel and whose short sides are colinear.

By making along these short sides a slit going from one side to the other of the piece of plastic, it is possible to introduce through the latter a piece of filter paper which is to be applied on to the gel and to be flush with the liquid kept between the plastic mask and the gel. By capillarity, the liquid rises into the filter paper and is removed via the incubation surface or surfaces.

However, in order to facilitate this removal of the liquid by pumping by means of filter paper, it is

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important for the suspended mask not to be perfectly parallel to the gel, but to make a dihedron with a very small angle, of 1 to 2°, the vertex of which dihedron is on the side where it is desired to pump the liquid through the filter paper. In fact, under these conditions, the capillary forces are stronger on the side where the gel/mask distance is smallest and the liquid interposed between these two surfaces has a tendency to collect therein and therefore to facilitate its removal through the filter paper, when the latter is introduced into the slit intended for this purpose.

DESCRIPTION OF THE FIGURES

- Figure 1 represents a cutaway view and a plan view of a mask of the invention intended for deposition, spreading and incubation of a single liquid on an incubation surface Si.

(1) represents the deposition orifice and (2) represents the slit intended for pumping the excess of reagent.

Sm represents the useful surface of the mask, delimited on one side by the slit (2) and on each of the other three sides by a difference in height.

- Figure 2 represents:

- * a cutaway view C-C according to the direction of a groove,

- * a cutaway view D-D passing through a deposition orifice, and

- * a plan view

of a mask intended to receive several liquids which are

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to cover various incubation surfaces, in this case six.

(1) corresponds to the various recesses, (2) corresponds to the slit and (3) corresponds to the grooves intended to delimit the various incubation surfaces Si_1 to Si_6 . The useful surfaces Sm_1 to Sm_6 corresponding to the incubation surfaces Si_1 to Si_6 are indicated.

- Figures 3a and 3b represent a gel/mask set intended for deposition of a single reagent on the whole of the gel.

In these two figures, (1) represents the deposition orifice. The perimeter of the mask is dotted and the perimeter of the gel is in solid lines.

The slit, not represented, intended for pumping the liquid is either adjacent to the edge of Si opposite to the vicinity of the deposition orifice (in the case of Figure 3a), or is situated inside Si (in the case of Figure 3b).

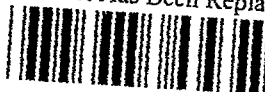
The hatched part corresponds to the incubation surface Si , which, in these two cases, is equal to the surface Sg of the gel.

- Figure 3a represents the case where the useful surface of the mask Sm is greater than the surface Sg of the gel.

- Figure 3b represents the case where the useful surface Sm of the mask is equal to the surface Sg of the gel.

- Figures 4a, 4b and 4c represent a gel/mask set for deposition of a single reagent on a part of the

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surface S_g of the gel. In these cases, the incubation surface S_i is smaller than the surface S_g of the gel.

The perimeter of the mask is in dotted lines and the perimeter of the gel is in solid lines.

The hatched part corresponds to the incubation surface S_i .

In these three figures, (1) represents the deposition orifice.

In these three figures, the slit intended for pumping the extra liquid is situated in the vicinity of the axis xx' .

• More precisely, Figure 4a corresponds to the case where the useful surface S_m of the mask is greater than S_i and the useful surface S_m of the mask is greater than S_g , the deposition orifice (1) being inside or outside S_i .

xx' corresponds to the lower limit of the surface S_i .

yy' corresponds to the upper limit of S_i .

zz' corresponds to the upper limit of the gel.

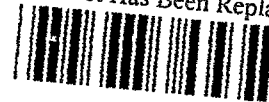
• Figure 4b corresponds to the case where the useful surface S_m of the mask is equal in area to the incubation surface S_i , and the useful surface S_m of the mask is smaller in area than the surface of the gel S_g , the deposition orifice (1) being inside S_i .

xx' corresponds to the lower limited [sic] of S_i .

• Figure 4c corresponds to the case where the

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useful surface S_m of the mask is greater than the incubation surface S_i , and the useful surface S_m of the mask is smaller than the surface S_g of the gel. The deposition orifice is inside or outside the incubation surface S_i .

xx' corresponds to the lower limit of S_i or of S_m .

yy' corresponds to the upper limit of S_i .

zz' corresponds to the upper limit of S_m .

- Figures 5a and 5b represent the case of several reagents or of a single reagent on several distinct incubation surfaces S_{i_1} to S_{i_n} of the same gel.

The deposition orifices are designated by (1). The incubation surfaces S_{i_1} to S_{i_n} are hatched. The surface of the gel is delimited by the solid lines and useful surfaces S_{m_1} to S_{m_n} of the mask are delimited by the dotted lines.

In Figure 5a,

xx' represents the alignment of the lower edges of the incubation surfaces S_{i_1} to S_{i_n} .

In Figure 5b,

xx' represents the alignment of the lower edges of the incubation surfaces S_{i_1} to S_{i_n} ;

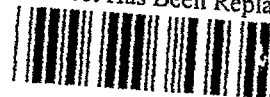
yy' represents the alignment of the upper edges of the incubation surfaces S_{i_1} to S_{i_n} ;

zz' represents the alignment of the upper edges of the surfaces S_{m_1} to S_{m_n} .

In Figures 5a and 5b, the slit, not represented, is in the vicinity of the axis xx' .

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- Figures 6a, 6b, 6c, 6d, 6e, 6f, 6g, 6h and 6i represent cutaway diagrams of various possible configurations of the masks of the invention.

Figure 6a corresponds to the case where the surface S_m is flat and parallel to the gel, the gel and the surface S_m being in a horizontal position.

The orifice for introduction of the reagent has been represented by (1), the distance separating the surface S_m from the gel has been represented by (e) and the surface S_i which is to be subjected to incubation has been represented by hatching. In this case, the volume of reagent to be introduced must be greater than or equal to $S_m \times e$.

It is noted that the surface S_m is limited, on the one hand, by the slit (2) intended for pumping the extra liquid, and, on the other hand, by a concave part with a difference in height.

Figure 6b represents the case where the surface S_m is parallel to the gel, as indicated in Figure 6a, with the sole difference that the gel and mask set is inclined on the side of the slit (2) intended for pumping the extra liquid, the angle formed between the mask and gel set with respect to the horizontal being approximately 15° . In this case, the volume of the reagent introduced must be greater than or equal to $S_i \times c$.

Figure 6c represents the case where the surface S_m makes a small angle of approximately 1 to approximately 2° with the gel, which is itself in a horizontal position. The minimum distance between S_m and the surface

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Si has been represented by (e1), and the maximum distance between Sm and Si has been represented by (e2). In this case, the volume of the reagent introduced is greater than or equal to $Si \times \frac{(e1 + e2)}{2}$.

Figure 6d corresponds to the case where the mask is constituted by a flat upper surface and a flat lower surface, which form an angle between them (dihedral). The minimum distance between Sm and Si has been represented by (e1) and the maximum distance between Sm and Si has been represented by (e2).

In this case, the volume of the reagent introduced is greater than or equal to $Si \times \frac{(e1 + e2)}{2}$.

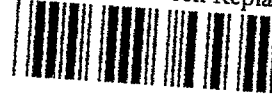
Figure 6e corresponds to the case where the mask is constituted by two flat surfaces forming between them an angle (dihedral). In this case, the surface Sm of the mask is equal to (S'm + S''m). The surface S'm is the surface which is parallel to the gel, which itself is in a horizontal position, and the surface S''m makes an angle α with the surface S'm. The minimum distance between the surface S''m and the surface Si is e1 and the distance between the surface S'm and the surface Si is e2. In this case, the volume of the reagent introduced is greater than or equal to S'm $\times$ e2 with in addition the condition that:

$$S'm \times e2 \geq (Si - S''m \cos \alpha) e2 + S''m \cos \alpha \frac{(e1 + e2)}{2}$$

In Figure 6e, the case where the incubation

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surface goes up to the slit (2) has been represented, but it is equally possible to contemplate that the incubation surface stops at the intersection (3) of the surfaces $S'm$ and $S''m$. This configuration is represented in Figure 6f.

In fact, under these conditions, the quantity of reagent per unit surface area spread on the incubation zone is constant, which is not the case in Example 6e.

Now, this constant value of the quantity of reagent per unit surface area of the gel subjected to incubation can be necessary mainly when the incubation is prolonged (because the incubation reaction kinetics are slow) and when the reagent is not in large excess with respect to the requirements of the reaction.

Under these conditions, in order to obtain the same result whatever the position of the incubation zone, it is important to have in the region of the incubation zone an equal distance between the gel and the mask. The zone corresponding to the narrowing of the gel/mask space is subjected to incubation, although this is not necessary, since it is, in this case, outside the incubation zone and leads to a slight over-consumption of reagent.

Figure 6g corresponds to the case of a mask with a flat surface $S'm$ parallel to the gel in a horizontal position extended by a cylindrical surface $S''m$ of radius R , which is tangent to the surface $S'm$. The radius R is of the same order of magnitude or greater than the length L_i of S_i . (1) represents the deposition orifice and (2) the slit.

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Figures 6h and 6i correspond to the case of a mask in which the upper surface and the lower surface both comprise a cylindrical surface.

When the upper surface is concave, Figure 6h is referred to and when it is convex, Figure 6i is referred to.

In both cases, the diameter of the lower cylindrical surfaces is greater than the square of the length L_i of S_i .

- Figures 7a, 7b and 7c illustrate a gel/mask set in which the mask is produced so that the deposition orifice of the reagent or reagents also constitutes the orifice (called slit when it [lacuna] of the reagents after the incubation. The gel is poured onto a plastic support.

In the case of Figures 7a, 7b and 7c, the lower face of the mask is inclined with respect to the surface of the gel, itself parallel to the upper surface of the mask.

In the case of Figures 7b and 7c, the gel is placed above the mask. In this case, the gel is kept in proximity with the mask without being in contact with the latter, for example by means of a device by [sic] vacuum suction (5), for example a perforated plate connected to a vacuum supply tap. The gel therefore has its face downwards. It is to be noted that whatever the embodiment, when it is a question of the relative position of the mask with respect to the gel, the face of the gel corresponds to the surface of this gel on which the

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incubation takes place and the so-called lower and upper surfaces of the mask correspond in every case to the surface nearest the gel (in the case of the lower surface of the mask) and to the surface furthest from the gel (in the case of the upper surface of the mask).

In this embodiment, the reagents are brought to the surface of deposition and of incubation by means of a pump supplied with reagents via channels or pipes (4) (multichannel in the case of various reagents).

Figure 7c illustrates a gel/mask device permitting deposition, spreading and incubation of reagents in the region of several separate surfaces of the gel.

EXAMPLES

Example I: Mask intended for deposition, spreading and incubation of a single reagent on the whole of the surface of the gel:

Under these conditions, the surface of the gel can be either smaller than the surface S_m of the mask (Figure 3a), or equal to the surface S_m of the mask (Figure 3b).

When the gel is in a horizontal position and parallel to the lower surface of the mask, the distance between the gel and the mask is e . As a consequence the volume of reagent to be introduced is at least equal to $S_i \times e = S_g \times e$.

In the case of an excess of reagent, which may be necessary in order to compensate for the losses due to evaporation during incubation, the latter is distributed on the one hand in the region of the deposition orifice,

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insofar as the latter has dimensions such that capillary forces can develop, and, on the other hand, regularly at the periphery of Si.

Example II: Mask for deposition, spreading and incubation of a single reagent on a part of the gel:

It is possible to envisage the following configuration.

The surface S_m of the mask is greater than the incubation surface S_i and the surface of the mask S_m is greater than or equal to the surface S_g of the gel, the deposition orifice being inside or outside the incubation surface S_i (Figure 4a).

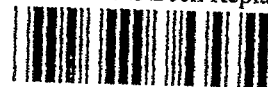
If the gel and the mask are parallel to each other and separated by a distance e , the volume of reagent introduced is $S_i \times e$.

In order to ensure the presence of the reagent on the incubation zone S_i , the gel and mask set can be inclined by rotation around an axis parallel to xx' , so that the reagent descends under gravity on the side of the zone of the gel to be covered, that is to say between xx' and yy' .

In order to ensure covering of the incubation surface S_i by the reagent, another possibility is to keep the gel in the horizontal position and slightly to incline the mask by rotation around an axis parallel to xx' , so that the gel/mask distance e , in the region of xx' is less than the gel/mask distance e , in the region of yy' . The capillary forces being stronger in the vicinity of xx' , the reagent is distributed by

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capillarity between xx' and yy' .

In this case, the mask then makes an angle α with the plane of the gel. If Li is the distance in mm between xx' and yy' , $\tan \alpha$ is less than or equal to $\frac{2}{Li}$.

$\frac{2}{Li}$

Under these conditions, the volume of reagent to be introduced in order to cover Si is:

$$\frac{Si(e_1 + e_2)}{2}$$

e_1 being greater than or equal to 0.1 mm because no contact must be created between the gel and the mask, e_2 being less than or equal to 2 mm in order for the capillary forces to be sufficient.

An excess of reagent, permitting compensation for the effects of evaporation which can be produced during the incubation, is distributed starting from yy' , between yy' and zz' .

Example III: Mask for deposition, spreading and incubation of a single reagent on a part of the gel:

The surface Sm of the mask is equal to the incubation surface Si , and the surface Sm of the mask is smaller than the surface Sg of the gel, the deposition orifice being inside the incubation surface (Figure 4b).

If the gel is in a horizontal position and is parallel to the mask, at the distance e , the volume of liquid to be introduced is $Si \times e = Sm \times e$.

A slight excess of reagent is distributed, on the one hand, in the region of the deposition orifice (if it has dimensions appropriate for capillary forces to

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develop therein) and, on the other hand, uniformly at the periphery of Si. In this case, it is necessary neither to have to incline the gel and mask set nor to create an angle between the plane of the gel and that of the mask. Example IV: Mask for deposition, spreading and incubation of a single reagent on a part of the gel:

The surface Sm of the mask is greater than the incubation surface Si, and the surface Sm of the mask is smaller than the surface Sg of the gel, the deposition orifice being inside or outside the incubation surface (Figure 4c).

a) If the gel and the mask are parallel, separated by a distance e, the volume of reagent introduced is $S_i \times e$.

In order to assure the presence of the reagent between xx' and yy', the gel and mask set is inclined by rotation around an axis parallel to xx', so that xx' is in a lower position than yy' and that the liquid descends by gravity to xx' and collects between xx' and yy'.

The maximum angle allowable in order for the liquid to stay kept by capillarity between xx' and yy' increases as the distance e decreases. It can theoretically be 90°, for a distance e of 0.2 mm, in the case where the distance Li between xx' and yy' does not exceed 7.5 cm and if the reagent is water (Jurin's law).

b) If the gel is in a horizontal position, in order to assure the presence of the reagent between xx' and yy', the mask can be slightly inclined. This can be done so that the gel/mask separation e, in the region of

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xx' is less than the separation e_2 in the region of yy' (cf. Example II). The volume of reagent is then:

$$\frac{Si(e_1 + e_2)}{2}$$

Example V: Case of distribution of several reagents, or possibly of the same reagent, but on several distinct incubation surfaces of the same gel:

The latter case (same reagent on several distinct surfaces) can be envisaged in order to limit consumption of the reagent.

If the gel and the mask are horizontal and parallel to each other, separated by a distance e , the volume of reagent to be introduced on to each incubation surface (Si_1 to Si_n) is $Si_1 \times e$, $Si_2 \times e$, etc. (Figure 5a).

This example is similar to Example III.

Example VI: Case of distribution of several reagents, or possibly of the same reagent, but on several distinct incubation surfaces of the same gel:

a) If the gel and the mask are parallel to each other, separated by a distance e , the conditions are similar to those of Example IVa.

b) If the gel is in a horizontal position and the mask is slightly inclined, the conditions are similar to those of Example IVb.

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CLAIMS

1. Rigid mask intended for deposition, spreading and incubation of one or several liquids on a gel (of total surface area S_g) according to one or several well-defined zones of the gel, hereafter designated by "incubation surface S_i ", this mask comprising:

- an upper surface and a lower surface, the distance separating the lower surface and the upper surface constituting the thickness of the mask,

- at least one orifice intended to permit deposition and spreading of the liquid on the incubation surface S_i of the gel, and constituted by a recess crossing the mask over the whole of its thickness, and/or

- at least one slit crossing the mask over the whole of its thickness and intended to permit withdrawal of the extra liquid present on the incubation surface S_i of the gel, this slit being situated in the part of the mask whose projection is either in the incubation surface S_i , or outside the incubation surface S_i ,

the lower surface of the mask being intended to be laid down in proximity to the surface S_g of the gel, under conditions such that the lower surface of the mask is not in contact with the incubation surface S_i , this lower surface being furthermore such that it does not include, over at least a part of itself designated by S_m , any irregularity on its surface capable of being an obstacle to spreading of the liquid on the incubation surface S_i , the surface S_m being delimited with respect to the rest of the lower surface, so that the liquid can only spread

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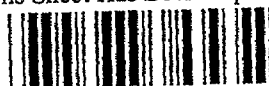
on the surface S_i of the gel which is a projection of S_m , this projection of S_m on the gel being greater than or equal to the area of the incubation surface S_i of the gel.

2. Mask according to Claim 1, in which the surface S_m is flat or curved, in particular cylindrical, of diameter greater than or equal to the square of the length of the incubation surface, or is composed of flat or curved, in particular cylindrical, elementary facets which are connected together, these various facets, as well as their connections, being such that there is no obstacle to the spreading of the liquid on the incubation surface S_i .

3. Mask according to Claim 1, intended for the deposition, spreading and incubation of one or several liquids, in which the lower surface of the mask comprises at least two differences in height or two grooves, the differences in height or grooves being spaced apart from each other in order to delimit at least a part of the mask S_m , which part does not include any irregularity on its surface capable of being an obstacle to the spreading of the liquid on the incubation surface S_i , and whose projection on a gel is equal to or greater than the area of an incubation surface S_i of the gel on which surface the liquid can be spread, the differences in height and the grooves having a height sufficient for the liquid to be able to be kept by capillarity on the aforesaid incubation surface S_i of the gel.

4. Mask according to Claim 1, intended for the

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deposition, spreading and incubation of a single liquid, comprising a single recess crossing the mask, intended to permit the deposition, spreading and incubation of a single liquid on a gel according to an incubation surface Si of the gel.

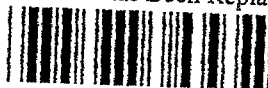
5. Mask according to Claim 1, intended for the deposition, spreading and incubation

- of several liquids on a gel according to respective incubation surfaces Si_1 to Si_n or

- of one liquid on a gel according to several incubation surfaces Si_1 to Si_n , n ranging from 2 to 50, this mask being such that its lower surface comprises differences in height or grooves, the differences in height or grooves being spaced apart from each other in order to delimit several parts of the mask Sm_1 to Sm_n , n ranging from 1 to 50, including no irregularity on their surface capable of being an obstacle to the spreading of the liquid on the surfaces Si_1 to Si_n , and whose projection on the gel is greater than or equal to the areas of the incubation surfaces Si_1 to Si_n of the gel, on which surfaces the liquid is to be spread, the differences in height and the grooves having a height sufficient for the liquid to be able to be kept by capillarity in the aforesaid incubation surfaces Si_1 to Si_n of the gel.

6. Mask according to any one of Claims 1 to 5, in which the deposition orifice has a volume such that it is capable of constituting a reservoir having a capacity for retaining the extra liquid in order to assure spreading

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of the liquid on the incubation surface Si.

7. Mask according to any one of Claims 1 to 6, in which the deposition orifice has an elongated shape or a circular shape, which is preferably elongated and has advantageously a width of approximately 1 mm to approximately 2 mm, in particular 1.5 mm, and a length of approximately 4 mm to approximately 80 mm, in particular 5 mm.

8. Mask according to any one of Claims 1 to 7, in which the recess is capable of containing by capillarity a volume of liquid at least equal to $V/10$, V being the volume existing between the distance from the incubation surface Si and the mask, when the mask and the gel are positioned respectively one in relation to the other under conditions such that the liquid deposited in the recess spreads over the gel.

9. Mask according to any one of Claims 1 to 8, in which the deposition orifice is situated in the vicinity of the middle of the part Sm of the mask and has the shape of an elongated slit, whose dimensions are such that it cannot prevent the liquid from being deposited on the incubation surface Si, and whose length is advantageously the length of the incubation surface Si, and whose width is advantageously from approximately 1 mm to approximately 2 mm, this deposition orifice also being capable of assuring the function of the slit intended to permit withdrawal of the extra liquid which is spread on the surface Si.

10. Mask according to any one of Claims 1 to 9, in

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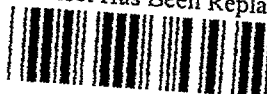
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which the slit intended to permit withdrawal of the extra liquid is substantially parallel to the width of the incubation surface and is positioned adjacently to the edge of the incubation surface which is opposite to the vicinity of the deposition orifice, and has advantageously a width of approximately 1 mm to approximately 4 mm, in particular 2 mm, and a length greater than or equal to the width of the surface Si, and the internal peripheral surface of the slit is perpendicular to the surface of the mask and preferably inclined with respect to the direction perpendicular to the surface of the mask in order to permit the placing in direct contact of means permitting withdrawal of extra liquid with the said extra liquid.

11. Mask according to any one of Claims 1 to 10, in which the thickness of the mask is approximately 1 mm to approximately 20 mm, in particular approximately 2 mm to approximately 10 mm, and the differences in height have a height at least equal to 0.2 mm, advantageously approximately 0.5 mm to approximately 20 mm, or the grooves have a width sufficient so that, during incubation, each liquid kept by capillarity between the mask and an incubation surface Si does not mix with the liquids kept in the incubation surfaces which are directly adjacent to Si, the width of the grooves being approximately 1 mm to approximately 10 mm.

12. Mask according to any one of Claims 1 to 11, comprising several mask parts Sm, advantageously approximately 6 to approximately 50, each of the parts Sm

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comprising a deposition orifice, preferably situated in the part of S_m whose projection on the gel corresponds to the anodic part of the gel, this mask comprising a single slit intended to permit withdrawal of the extra liquid which is spread on the surfaces S_i of the gel, this slit extending preferably perpendicularly with respect to the largest of the dimensions of the parts S_m of the mask and being preferably situated in the mask at a position such that its projection is in the cathodic region of the gel.

13. Mask according to any one of Claims 1 to 11, comprising a single part S_m , whose dimensions are greater than or equal to those of S_i , and comprising in the vicinity of one of its ends a recess having an elongated shape, whose length corresponds preferably to the width of the incubation surface S_i , and also comprising in the vicinity of the other end, a slit intended for withdrawing the extra liquid, this slit being advantageously, in length, at least the width of S_i .

14. Mask according to any one of Claims 1 to 13, comprising means for positioning the mask with respect to the gel such that the minimum distance between the gel and the part S_m of the mask is greater than or equal to 0.1 mm, and the value of the maximum distance is approximately 0.5 mm to approximately 2 mm, in particular 1 mm.

15. Mask according to any one of Claims 1 to 9, 11, 13 or 14, characterized in that the deposition orifice is also intended to permit withdrawal of the extra liquid

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present on the incubation surface Si of the gel.

16. Mask according to any one of Claims 1 to 15, characterized in that it is placed either above the gel or below the gel, the surface for deposition, spreading and incubation of the gel being in each case in proximity to the lower face of the mask.

17. Set comprising a mask according to any one of Claims 1 to 16 and a gel permitting implementation of an incubation on a gel previously subjected to electrophoresis or permitting crossed (cross-dot) reactions to be carried out.

18. Set according to Claim 17, comprising means permitting the parts Sm of the mask to be sufficiently close to the gel for the liquid intended to be spread on the incubation surfaces Si to be kept by capillarity on the incubations surfaces Si of the gel in projection of the parts Sm of the mask and for the parts Sm of the mask to be parallel to the gel or to be inclined with respect to the gel, preferably according to an angle of approximately 0.5 to approximately 3°, advantageously of approximately 1.5°,

the minimum distance between the mask and the gel being advantageously in the vicinity of the slit and the maximum distance between the mask and the gel being advantageously in the vicinity of the deposition orifice.

19. A method of deposition, spreading or incubation of one or several liquids on a gel (of surface Sg) according to one or several well-defined zones of the gel, hereafter designated by "incubation surface Si",

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characterized in that

- a mask is positioned, according to any one of Claims 1 to 16, with respect to the gel, so that the part S_m of the mask should be sufficiently close to the incubation surface S_i for the liquid intended to be spread on the surface S_i to be kept by capillarity on the said surface S_i ,

and in that:

- the liquid is introduced into the deposition orifice defined hereinabove,

- the mask is kept with respect to the gel in the position as indicated hereinabove, or, if the mask and the gel are parallel to each other, the mask is inclined with respect to the gel in order that the liquid should be distributed by capillarity on the surface S_i , or the gel and mask set is inclined with respect to the horizontal so that the liquid should be distributed on the incubation surface S_i by gravity, for a sufficient time for the reaction between the liquid and the components, in particular proteins, deposited on the gel, in particular by electrophoresis, to take place,

- at the end of the incubation, the extra liquid spread on the well-defined incubation surface of the gel is withdrawn via means arranged through the previously defined slit, in particular with the aid of filter paper,

- the mask is withdrawn from the gel.

20. Method according to Claim 19, in which the mask is kept parallel with respect to the gel, and the set is inclined according to a sufficient angle with respect to

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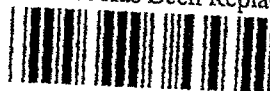


the horizontal for the liquid to spread by gravity on the incubation surface Si, and advantageously according to an angle ranging from approximately 5° to approximately 90°, advantageously approximately 30°.

21. Method according to Claim 19, in which the mask is kept according to an angle sufficient with respect to the gel in order for the liquid to spread by capillarity on the incubation surface Si, and in particular according to an angle of approximately 0.5 to approximately 3°, advantageously approximately 1.5°.

22. Electrophoresis apparatus characterized in that it comprises a mask according to any one of Claims 1 to 16, or a set according to one of Claims 17 or [sic] 18, intended for the spreading and for the incubation of one or several reagents.

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