

The invention relates to a tube, referred to as a small straw-like tube portion, for cryogenically preserving biological samples, in particular viral cultures, formed by a tubular casing portion of substantially transparent, biologically neutral polymer material and provided with a seal at each of its two ends and comprising in the vicinity of a first end a sliding stopper comprising an aqueous gel between two plugs of porous elastic material.

The invention also relates to a process for filling a tube or small straw-like tube portion of that kind.

This type of small straw-like tube portion which is formed by a casing portion which is about twelve centimetres long and about three millimetres in diameter, with a wall thickness of some tens of millimetres, has long been known: the sliding stopper which is also referred to as a three-part plug means was described in the document FR-A-995 878 of 20th September 1946. Since then various improvements have been made therein, which related essentially to the end seals, in conjunction with the polymer material from which the casing is extruded. In fact the closure process must afford effective sealing integrity, it must not involve any excessive risk of deterioration in regard to the biological sample contained in the tube portion and, at least for some applications, it must be adapted to the manipulations required for such applications (preservation and use of gametes and in vitro fertilisation).

Moreover the choice of casing material was guided by considerations concerning compatibility with the biological substances, transparency and ease of shaping. In practice all materials which comprise plasticising agents that are likely to migrate will be excluded.

The document FR-A-2 128 374 described a sealing effect involving ultrasonic welding of straw-like tube portions of a material which is not specified. The welding operation is carried out between jaws of complementary V-shapes, one being connected to the ultrasound generator and the other forming an anvil; that therefore results in a seal which is inscribed in the cylinder which corresponds to the shape of the tube on the outside.

The document FR-A-2 592 797 describes a closure process involving the injection of a heat-fusible material in the pasty state in the interior of the end of the tube; the straw-like tube portion is of polyvinyl chloride and the heat-fusible material is a mixture of a polyolefin and an ethylene and vinyl acetate copolymer.

The document FR-A-2 618 452 describes a straw-like tube portion which is closed off at the end opposite to that which adjoins the sliding stopper by a plug member of elastomer which is capable of being pierced by a needle and remaining sealed after the needle has been withdrawn. The elastomer plug member is either introduced by force or formed in situ by vulcanisation of a suitable composition.

The document FR-A-2 623 205 describes a straw-like tube portion for fertilisation which does not comprise a sliding stopper and which is provided at each end with a plug member of self-sealing elastomer.

The progress in microbiology has given rise to an extension in the amount of use of such straw-like tube portions, in correlation with work relating to biological samples which are sometimes rare and often dangerous, in particular in the case of viral cultures. It became absolutely imperative to revise the design of the straw-like tube portion and in particular the end seals thereof in order on the one hand to avoid the use of injection needles which are likely to cause wounds on the part of the personnel called upon to operate them, and facilitate automation of the operations of filling and recovering the samples, and on the other hand in order severely to limit the risk of a loss of sealing integrity on the part of the filled straw-like tube portions under the effect of stresses and thermal shocks linked to freezing by immersion in liquid nitrogen and rapid defrosting.

Although the current straw-like tube portions have a failure rate under cryogenic conditions which is acceptable in terms of current work, that failure rate becomes excessive insofar as the failure of a filled straw-like tube portion can cause a research programme to fail or the risks of infection due to leakage of a sample are of a dramatic character.

The applicants therefore set themselves the aim of providing a tube or straw-like tube portion which would afford a considerably enhanced degree of reliability in regard to cryogenic preservation, including in the sealing zones, and which could be easily filled and sealed on an automatic assembly which would not involve handling on the part of an operator.

The applicants consider that they have attained that aim by proposing a tube, referred to as a small straw-like tube portion, for cryogenically preserving biological samples, in particular viral culture, formed by a calibrated tubular casing portion of substantially transparent, biologically neutral polymer material and provided with a seal at each of its two ends and comprising in the vicinity of a first end a sliding stopper comprising an aqueous gel between two plugs of porous elastic material, characterised in that the polymer material is an ionomer resin, the end seals being formed by autogenous welding of the tube over a limited axial extent.

The ionomer resins which are formed by the association of a copolymer of ethylene and carboxylic acid with a metal cation have the property of behaving like a thermoplastic material above a transition temperature zone which is in the range of from 40 to 90° while below that transition zone they behave like a cross-linked material, the metal cations forming transverse bonds between straight copolymer chains. The transformation is reversible. Autogenous welding of the tube is a simple and effective operation above the transition temperatures; cooling after welding induces only few internal tensions, solidification of the resin by ionic cross-linking not being accompanied by substantial variations in volume.

Ionomer resins of that kind were described in French patents Nos 1 336 164, 1 350 608 and 1 430 478 lodged by the company Dupont de Nemours on 28th August 1962, 23rd January 1963 and 26th February 1965 respectively. Those resins are marketed under the trade mark 'Surlyn'.

Most solids have an embrittlement temperature below which creep flow is practically inhibited. Thermoplastic polymer materials are in general highly sensitive to embrittlement in the cold condition, in particular when they do not comprise any plasticising agent. Now, ionomer resins have very low embrittlement temperatures, always below 213K, and for most of them lower than 173K. For certain 'Surlyn' resins it has not been possible to demonstrate any embrittlement temperature.

It will be appreciated that operations involving the handling of conventional straw-like tube portions when cooled to the temperature of liquid nitrogen, 77K, are delicate and are poorly suited to automatic manipulation. In addition, if internal stresses still remain in the welds, cracks and splits may occur in the seal. Those difficulties are attenuated or disappear with the straw-like tube portions according to the invention.

The cross-linked structure of the 'Surlyn' resins at ambient temperature imparts attractive mechanical behaviour to the straw-like tube portions; the straw-like tube portions do not have a tendency to creep flow under their own weight and they remain straight. The position of the ends of the straw-like tube portions, when placed on a suitable support, will be precise, and the introduction of a needle or a conical nozzle for the filling operation is thus made easy without the need for the intervention on the part of operators, even on an occasional basis.

It will be noted that conventional straw-like tube portions have been satisfactory over a long period, the losses of sealing integrity due to the seals breaking or cracks due to embrittlement appearing to

be compatible with the acceptable level of inevitable errors and handling accidents. It is only recently that the need to lower the thresholds in terms of acceptable levels of waste and rejects led the applicants to analyse the causes of possible accidents in order to remedy same. It can be supposed that originally the manufacturers of straw-like tube portions chose the materials which are normal in the industry of biological laboratory materials, the behaviour of which was known, both in regard to biological samples and cryogenic uses.

It will be appreciated that the 'Surlyn' resins have suitable qualities of transparency and biological neutrality. It will also be noted that those resins accept marking inks, which makes it possible to identify the filled tube portions without the risk of error.

Preferably the ionomer resin will be of the type which is marketed under the name 'Surlyn' 8921. That resin comprises a sodium metal cation and it has not been possible to determine an embrittlement temperature thereof. In relation to the transition zone the temperature of bending under load of 0.46 MPa is 45°C, the VICAT temperature (speed B) is 48°C, the melting temperature is 84°C and the solidification temperature is 52°C.

In another aspect the invention proposes a process for filling a tube referred to as a small straw-like tube portion for cryogenically preserving biological samples, in particular viral culture, formed by a calibrated tubular casing portion of substantially transparent, biological neutral polymer material, in which process a sliding stopper is set in position in the vicinity of a first end of the tube by successively inserting a first porous elastic plug, a quantity of powder capable of gelling upon coming into contact with an aqueous phase, and then a second porous elastic plug, the stopper being pushed beyond a zone of the tube which is reserved for the seal for the first end, the biological sample is injected into the previously sterilised tube through the second end and beyond a zone reserved for the seal for said end while the atmosphere in the tube is evacuated through the stopper and the first end until the aqueous phase passing through the first plug causes gelling of the powder and therefore sealing integrity of the stopper, characterised in that, the tubular casing being formed by an ionomer resin, the ends of the tube are sealed by crushing the tube in the reserved zones in such a way as to apply two parts of the internal peripheral layer of the tube against each other and raising at least said two parts to a sufficient temperature for them to fuse.

The use of an ionomer resin for the tubular casing, besides the specific advantages afforded by the resin for the tube referred to as a straw-like tube portion for cryogenically preserving a biological sample, makes it possible to carry out the sealing operation in a

simple manner which is easy to automate, without the risk of causing deterioration of the content by overheating of the sample, while ensuring sterilisation of the weld joint in the true sense, by virtue of the temperature at which autogenous welding is effected (in the range of 90°C to 110°C).

Further features and the advantages of the invention will become apparent from the following description by way of example with reference to the accompanying drawings in which:

Figure 1 is a partly sectional view of a tube or straw-like tube portion according to the invention,

Figure 2A is a view of a first procedure for filling a straw-like tube portion,

Figure 2B is a view of an alternative form of the filling operation,

Figure 3A shows a procedure for sealing a straw-like tube portion,

Figure 3B is a view in longitudinal section of a filled and sealed straw-like tube portion,

Figure 4 is a diagrammatic elevational view of a bed for filling and sealing straw-like tube portions,

Figure 5 is a plan view of the bed shown in Figure 4,

Figures 6A and 6B are views in two orthogonal planes of a first type of seal,

Figures 7A and 7B are similar views of a second type of seal, and

Figures 8A and 8B are similar views of a third type of seal.

In the embodiment selected and shown in Figure 1 a tube 1, referred to as a straw-like tube portion, is provided for cryogenically preserving biological samples, in particular viral cultures, at the temperature of liquid nitrogen, namely 77K. It is designed to ensure a high degree of safeguard against leakage of samples in spite of the stresses produced by the low temperatures and to permit automatic handling, in particular in the filling operation, in order to minimise the danger of infection to the personnel using it. The tube 1 comprises a tubular casing 10 produced by extrusions

of an ionomer resin marketed by the company Dupont de Nemours under the trade mark 'Surlyn' 8921. The properties of that resin and in particular its lack of embrittlement at 77K and above have been referred to above.

- 5 The tube is of a length, prior to filling, of 90 mm, an outside diameter of about 3 mm and an inside diameter of 2.5 mm.

10 Conventionally the tube 1 contains a sliding stopper 2 in the vicinity of an end 11 which will be referred to as the first end. The stopper comprises on respective sides of a quantity 20 of hydrophilic polyvinyl alcohol powder which is capable of gelling upon coming into contact with an aqueous phase, two porous plugs 20 and 21 formed by portions of interwoven cotton roving. The length of the stopper is about 18 mm, each of the elements 20, 21 and 22 occupying a length of about 6 mm. The useful internal volume is 250 μ l. Calibration of the tubular casing 10 upon extrusion and in particular calibration of the internal section thereof permits reproducibility of the internal diameter.

20 The operation of filling the straw-like tube portion is illustrated in Figure 2A. The tube 1 which has been previously sterilised is engaged between two frustoconical nozzles 41a and 42a which respectively pass into the ends 11 and 12; the frustoconical shape of the nozzles 41a and 42a, combined with a tightening force towards each other, ensures sealing integrity of the connection. The nozzle 41a, at the end of a conduit 41, is connected to a reduced-pressure reservoir while the nozzle 42a is at the end of a conduit 42 which is immersed in a test tube 35 containing the biological sample 30.

30 The atmosphere in the tube 1 is evacuated through the sliding stopper 2 and the biological sample 30 is drawn in through the tube 42 and passes into the tubular casing 10 until it reaches the stopper 2. The biological sample 3 infiltrates into the plug 21 and comes to hydrate the powder 20 which forms an impermeable gel by virtue of hydration thereof. The filling operation is thus stopped. When the nozzles 41a and 42a are withdrawn the biological sample 3 remains in the form of a continuous column and the end 12, over the depth of penetration of the nozzle 42a, does not contain any sample and remains available for the subsequent seal, as the end 11, which has not been reached by the sample.

45 It will be appreciated that the relative rigidity of the tube 1 of 'Surlyn' at ambient temperature permits the casing 10 to be subjected to longitudinal compression as is required to achieve sealing integrity and that the maintenance of a precise shape without flexing below the transition zone of the ionomer resin permits the

precise automatic introduction of the nozzles 41a and 42a into the ends 11 and 12 of the straw-like tube portion 1.

The arrangement shown in Figure 2B is similar to that shown in Figure 2A except however that the tubes 41 and 42 are replaced by capillary tubes 45 and 46 which are fixed in grippers 45a and 46a. Sealing integrity as between the tubes 45 and 46 and the ends 11 and 12 is ensured by elastomer plug members 47 and 48 which are fitted over the capillary tubes 45 and 46 and which bear at the end against the end faces 11 and 12 of the straw-like tube portion 1. The filling procedure is unchanged. It will be noted however that the capillary tube 46 penetrates sufficiently deeply into the end 12 of the straw-like tube portion 1 for that end 12 to remain empty over a length substantially corresponding to the depth of engagement of the plug 22 into the end 11.

The straw-like tube portion 1 filled with biological sample 3 is then sealed as illustrated in Figure 3A.

The ends 11 and 12 are clamped between pairs of jaws 61a, 61b; 62a, 62b until the tubular casing is sufficiently crushed for the opposed parts of the internal surfaces of the ends 11 and 12 to come into contact. The pairs of jaws 61a, 61b; 62a, 62b are heated to a temperature such that the ionomer resin melts between the jaws and the opposed parts which are in contact fuse.

It will be recalled that the ionomer resin selected has a melting temperature of 84°C and that the transition zone between the heat-fusible state and the cross-linked state is between 45° and 60°C approximately.

After sealing the straw-like tube portion 1 is substantially of the appearance shown in Figure 3B, with flattened sealing zones 11.1 and 12.1 at the two ends 11 and 12. It will be noted that a gas bubble 12a remains between the sealing zone 12.1 and the end of the column 3 of biological sample. Besides the fact that in known manner the gas bubble 12a prevents the straw-like tube portion from rupturing, freezing of the sample being accompanied by an increase in volume, in the sealing operation the bubble 12a prevents contact from occurring between the heated zone 12.1 and the biological sample which could otherwise be adversely affected.

It will be noted that on the other hand, if traces of biological sample had extended between the ends 11 and 12 of the straw-like tube portion, the organic materials of such traces would be essentially destroyed at the melting temperature of the resin, with sterilisation of the accessible ends.

Referring to Figures 4 and 5 which show a filling bed it will be seen that the bed 100 comprises recesses 101, 102, 103 and 104 for fitting the straw-like tube portions in position. A conveyor member 120 is operable periodically to lift the straw-like tube portions, then displace them towards the left by a length between recesses, move them downwardly into the corresponding recess, and move away to begin transportation again after a chosen period.

The sterilised straw-like tube portions are deposited in a vertical loader 101a, the lower straw-like tube portion engaging into the recess 101 or loading recess. After transfer into the recess 102 at the filling position, nozzles 141a and 142a are moved towards each other in order to be introduced into the ends of the straw-like tube portion, the filling operation being effected as described with reference to Figure 2A.

After filling the straw-like tube portion 10 is transferred into the recess 103 where it is welded by heated jaws 161a, 161b; 162a, 162b, by the procedure described with reference to Figure 3A.

After that the straw-like tube portion 10 is transferred into the recess 104 where it is subjected to a marking operation. A marker 111 bearing characters in relief is inked by the passage of an inking roller coupled to a rotary duct plate 113. A transfer pad 110 receives the mark formed by the marker 111 in a withdrawal movement and, in an advance movement, applies the mark to the straw-like tube portion 10 in the recess 104.

In the following action of the conveyor member 120 the straw-like tube portion 10 is removed in the channel 105.

It will be appreciated that all those operations are effected without the intervention of a human operator.

It will be noted that the marking operation could appear a priori a secondary operation, without any risk to a human operator. However it is important that the marking operation takes place just after the straw-like tube portion is personalised in order to avoid any mistake. Moreover that marking operation would have been much more complicated to effect if the material from which the straw-like tube portion is formed were not capable of fixing the marking ink.

Reference will be made to Figures 6 to 8, each constituted by a Figure A taken parallel to the direction of approach movement of the jaws, and a Figure B.

Figures 6A and 6B show the form of seal 11.1 obtained by clamping between flat narrow jaws and which leaves intact the end face

of the straw-like tube portion casing 10. That shape will be the usual one.

5 Figures 7A and 7B show a seal 11.2 in the form of a blade which is produced by clamping between flat jaws which assumed a position of gripping the straw-like tube portion at the end. That shape may be preferred for reasons of greater ease of holding the blade portion, while in addition that shape eliminates the end cavity of the seal 11.1.

10 Figures 8A and 8B show a seal 11.3 of V-shape, similar to what is described in FR-A-2 128 374. This shape which results from clamping between two V-shaped jaws, one of which is of a projecting configuration and the other is of a re-entrant configuration, has the advantage that the seal is entirely contained within the cylinder
15 which corresponds in shape to the external surface of the straw-like tube portion so that fixing of the straw-like tube portion in a chucking member is made easier.

20 It will be appreciated that it may be an attractive proposition for the two end seals of straw-like tube portions to be of different shapes for reasons of expediency. Moreover other forms of seal can be envisaged.

25 It will be of course be appreciated that, in order to recover the biological sample for the purposes of use, the ends 11 and 12 are cut off at the level of the non-deformed zones which are before the seals and the sample is expelled by mechanically or pneumatically pushing the sliding stopper.

30 It will be apparent that the invention is not limited to the examples described but embraces all alternative configurations within the scope of the claims.

CLAIMS

1. A tube referred to as a small straw-like tube portion (1) for cryogenically preserving biological samples (3), in particular viral culture, formed by a calibrated tubular casing portion (10) of substantially transparent, biologically neutral polymer material and provided with a seal (11.1, 12.1) at each of its two ends (11, 12) and comprising in the vicinity of a first end (11) a sliding stopper (2) including an aqueous gel (20) between two plugs (21, 22) of porous elastic material, characterised in that the polymer material is an ionomer resin, the end seals (11.1, 12.1) being formed by autogenous welding of the tube (1) over a limited axial extent.

2. A tube referred to as a small straw-like tube portion according to claim 1 characterised in that said ionomer resin is Surlyn[®] of type 8921.

3. A tube referred to as a small straw-like tube portion according to claim 1 characterised in that the seal (11.1, 11.2) comprises a zone with two flat parallel outer faces which extend perpendicularly to the axis over a width greater than the running outside diameter of the casing.

4. A tube referred to as a small straw-like tube portion according to claim 1 characterised in that the seal (11.3) has V-shaped outer faces, one being of a projecting configuration and the other being of a re-entrant configuration, the seal being completely contained in a cylinder corresponding to the running portion of the tube.

5. A tube referred to as a small straw-like tube portion according to claim 1 characterised in that it contains a given volume of biological sample (3) between the sliding stopper (2) and a bubble of gas (12a) in contact with the seal (12.1) of the second end (12) of the casing (10).



6. A process for filling a tube referred to as a small straw-like tube portion (1) for cryogenically preserving biological samples (3), in particular viral culture, formed by a calibrated tubular casing portion (10) of substantially transparent, biologically neutral polymer material, in which process a sliding stopper (2) is set in position in the vicinity of a first end (11) of the tube (1) by successively inserting a first porous elastic plug (21), a quantity of powder (20) capable of gelling upon coming into contact with an aqueous phase, and then a second porous elastic plug (22), the stopper being pushed beyond a zone of the tube which is reserved for the seal (11.1) for the first end (11), the biological sample (30) is injected into the previously sterilised tube through the second end (12) and beyond a zone reserved for the seal (12.1) for said end (12), while the atmosphere in the tube is evacuated through the stopper (2) and the first end (11) until the aqueous phase passing through the first plug (21) causes gelling of the powder (20) and therefore sealing integrity of the stopper (2), characterised in that, the tubular casing (20) being formed by an ionomer resin, the ends (11, 12) of the tube are sealed by crushing the tube in the reserved zones in such a way as to apply two parts of the integral peripheral layer of the tube against each other and raising at least said two parts to a sufficient temperature for them to fuse.

7. A process according to claim 6 characterised in that the ionomer resin is Surlyn[®] of type 8921.

8. A process according to claim 6 characterised in that for producing the seals (11.1, 12.1; 12.2, 12.3) for the ends (11, 12) the reserved zones are clamped between complementary jaws (61a, 61b; 62a, 62b; 161a, 161b; 162a, 162b) which are raised to a sufficient temperature for the internal peripheral layer parts to fuse.

9. A process according to claim 8 characterised in that the jaws (61a, 61b; 62a, 62b; 161a, 161b; 162a, 162b) have flat parallel clamping faces.



10. A process according to claim 8 characterised in that the jaws are V-shaped, one being of a projecting configuration and the other being of a re-entrant configuration.

11. A process according to claim 6 characterised in that when the tube (1) is filled with the biological sample (3) a bubble of gas (12a) is provided between said sample (3) and the reserved zone of the second end (12).