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(54) STERILISATION OF MEMBRANES

(71) We, KURARAY CO. LTD., a body corporate organised and existing under the laws of Japan, of 1621 Sakazu, Kurashiki-City, Japan and KAWASUMI LABORATORIES INC., a body corporate organised and existing under the laws of Japan, of 2-7-15, Minamirokugo, Otaku, Tokyo, Japan, do hereby declare the invention for which we pray that a patent may be granted to us and the method by which it is to be performed to be particularly described in and by the following statement: —

This invention relates to the sterilization of semi-permeable membranes. Membranes with a variety of configurations, such as hollow fibres, tubing and flat sheets have been widely used in the treatment of fluids. Typical uses for such membranes are in apparatus for purifying body fluids such as blood, from which they remove endogenous metabolites and exogenous toxins. The artificial kidney is an apparatus designed to purify the blood by use of such a membrane and ascitic-fluid-treating apparatus uses such a membrane for filtration and/or concentration of accumulated ascitic fluid.

The membranes in such medical apparatus must be strictly sterile. The methods previously proposed for the sterilization of membranes includes sterilization by immersion in formalin, sterilization with ethylene oxide gas and sterilization by gamma radiation. The formalin-immersion and ethylene-oxide sterilization techniques have been widely practised, but the use of membranes sterilized by these methods results in the absorption of residual formalin or ethylene oxide into the body fluids and, on repeated use of such membranes, such substances gradually accumlate in the body until, finally, a harmful level might be reached. Under the circumstances, radiation sterilization technique has been proposed and studied but radiation involves health hazards and hence requires complicated and costly equipment. Thus its own problems have to be solved before the technique is well established. It is for this reason that a sterilizing technique safe to patients is in demand.

The present invention provides a method for sterilizing a semi-permeable membrane of regenerated cuprammonium cellulose by subjecting it to wet-heat treatment at a temperature in the range of 105 to 140°C, preferably 110 to 140°C, in the presence of physiological saline.

The present invention also provides a regenerated cuprammonium cellulose hollow-fibre membrane that is suitable for use in an artificial kidney apparatus, has a sonic modulus of at least 6.5×10^{10} dynes/cm^2 and has been sterilized by wet-heat treatment at a temperature in the range of 105 to 140°C, preferably 110 to 140°C,. in the presence of physiological saline.

Wet-heat sterilization is commonly used in the sterilization of other medical materials such as injection syringes and needles and surgical instruments. However, it has never been suggested, nor could it be expected, that it could be applied to the sterilization of semi-permeable membranes in the presence of physiological saline. Thus, because of the special solute-permeation characteristics of membranes made of regenerated cellulose or other polymers, it was previously believed that their micro-structures were greatly different from the internal structures of shaped products intended for other uses, e.g. wrapping film, as possessing comparatively loose internal structures.

For the above reason, it was already known that the micro-structure of such membrane is unstable, delicate and vulnerable to severe conditions, being especially liable to heat. It is for this reason that, in the production of such a membrane and,

for that matter, in any treatment preceding its use, extraordinary care has been taken to ensure that the product will not be subjected to unduly high temperatures. In fact, the aforementioned conventional sterilization techniques have been developed and used because they do not involve the use of a high temperature.

Against the above background, the research leading to the present invention was directed to the question of whether the wet-heat treatment at 105 to 140°C could be applied to the sterilization of polymeric membranes. The research led to the surprising discovery that such a treatment does not impair the solute-permeation and mechanical properties of membrances, provided that the membranes are made of regenerated cuprammonium cellulose.

Detailed study of the changes in such properties under wet-heat conditions revealed that the permeability of such a regenerated cellulose membrane to substances such as urea, uric acid, creatinine, vitamin B_{12}, which are typical middle-molecular-weight substances, was not substantially affected while, depending on the type of regenerated cellulose, its permeability to water was markedly impaired. It was really surprising to find that the alteration in characteristics of a membrane was largely accounted for by a change in water permeability, with its permeability to other substances of low-to-middle molecular weight not being adversely affected.

When a membrane is used in an artificial kidney unit, its water permeability is a factor as important as its dialysing characteristics, and membranes with inadequate water permeability cannot be put to practical use even if they excel in other properties.

As expected, it was found that conventional membranes of other known synthetic polymers, such as polyvinyl alcohol, ethylene-vinyl alcohol copolymer, polyarcrylonitrile, polymethyl methacrylate and polyamides lost their solute-permeation properties when exposed to wet heat treatment and, therefore, could not be subjected to it.

For instance, a technical brochure (published by Enka-Glanzstoff A.G.) on cuprammonium cellulose tubes and hollow fibres, which are examples of regenerated cellulose membrane products, refers to the importance of handling such regenerated cellulose membranes under the conditions of 23°C and 50% R.H., with the admonition that severe temperature conditions deviating from the above should be avoided.

The further research on which the present invention is based shows that there is a certain correlation between the microstructure of a membrane and the alteration in its properties as caused by autoclaving. As a parameter determinant of the premeation performance of a semi-permeable membrane, the micro-structure as established by electron-microscopy has provided the basis for many theorizations and discussions as will be seen from USP 3888771, for instance. In view of the fact that the water permeability of a membrane depends on its micro-structure, it was initially thought that the change in the properties of the membrane could be grasped and explained on the basis of the aforementioned micro-structural parameter. Contrary to this preconception, it was found that this conventional parameter failed to provide any useful information for studying the true changes in the structure of membranes.

A study of a variety of parameters was undertaken and it was recognized that the change in micro-structure of a regenerated cellulose hollow-fibre membrane could be understood on the basis of its sonic modulus.

The sonic modulus of an object such as a fibre or a strip of film is determined from the pulse velocity (the velocity at which a pulse is propagated) and, being independent of the degree of crystallinity at temperatures below Tg, it is a measure of the overall degree of molecular orientation consisting of the degree of orientation of the crystalline region and that of the amorphous region [cf. Journal of Applied Polymer Science *3*, 266-276 (1960)].

A dry hollow-fibre membrane that has been allowed to stand at least overnight at 25°C and 40% R.H. is subjected to the measurement of the velocity of propagation of pulses of 10 kilocycles by means of a KLH pulse-propagation meter.

The sonic modulus *Es* of a test material is calculated from the sonic velocity *C*, which can be determined by the above procedure, and the density ρ which can be determined by the conventional procedure, e.g. the density gradient method, using the following equation:

$$Es = \rho C^2$$

A hollow fibre of regenerated cellulose having an *Es* value of at least 6.5×10^{10} dynes/cm^2 will suffer only a small reduction in water permeability when subjected to wet-heat treatment and is useful for the purpose of this invention. In contrast, a hollow fibre of regenerated cellulose having an *Es* value below 6.5×10^{10} dykes/cm^2 will suffer a reduction of 25% or more in hydraulic permeability and has an absolute permeability value (UFR, ultrafiltration rate) not exceeding 8×10^{-2} ml/cm^2, atm. hr., thus being unusable in artificial kidney applications.

Although there is no critical upper limit

to the sonic modulus of the membrane, it is preferably not higher than 18 x 10^{10} dynes/cm^2 and, for still better results, not higher than 15 x 10^{10} dynes/cm^2. If the upper limit is exceeded, the highly-oriented structure of the hollow fibre membrane would provide a low dialysing performance as well as low water permeability, thus making the membrane unusable as an artificial kidney membrane.

The regenerated cellulose membrane used according to this invention may be a membrane obtained by the regeneration of any cellulose derivative. Thus, for example, regenerated cuprammonium cellulose and saponified cellulose or cellulose ester e.g. cellulose diacetate or triacetate, can be used successfully. The regenerated cellulose membranes include the membranes formed in a tubular or flat shape by conventional techniques. These membranes are assembled, in the case of artificial kidneys for instance, in hollow-fibre, coil and kiil modules. While, normally, the regenerated cellulose membrane is subjected to the wet-heat treatment when already assembled into a module, it is of course possible to so treat the membrane prior to assembling.

The preferred hollow fibre of regenerated cellulose is a fibre of regenerated cuprammonium cellulose having a sonic modulus of at least 6.5 x 10^{10} dynes/cm^2, more desirably at least 7.7 x 10^{10} dynes/cm^2. Other types of regenerated cellulose hollow fibre membranes, such as saponified cellulose acetate hollow fibre membranes, which are currently available on the market, and regenerated cellulose hollow fibre obtained from cuprammonium cellulose and having a sonic modulus below 6.5 x 10^{10} dynes/cm^2 cannot be made into efficient artificial kidney membranes by wet-heat sterilization. The saponified cellulose acetate commercially available today has a sonic modulus below 6.5 x 10^{10} dynes/cm^2 and is unsuitable as aforesaid but if it has been modified so as to give a higher sonic modulus, for example by orienting it to a high degree in the cellulose acetate stage and thereafter saponifying it, such regenerated cellulose can be successfully used for this invention.

The difference between the sonic moduli of such a membrane before and after the wet-heat treatment has been found to be comparatively small, 2 x 10^{10} dynes/cm^2 at most.

The hollow fibre of regenerated cellulose having such a desired sonic modulus can, for instance, be manufactured by adjusting such conditions as the formulation of the spinning solution, conditions of coagulation and regeneration, conditions of stretching subsequent to the spinning stage, and conditions of heat treatment. While these conditions can be varied rather widely, it is generally advantageous to set most of these conditions within an easily practicable range and vary one or two remaining conditions, such as the spinning draft ratio or/and wet-heat stretching ratio, so as to obtain the desired sonic modulus. Thus, a typical procedure may involve attenuating the ordinary spinning dope in the air, passing it into an alkaline coagulation bath, stretching it between the coagulation bath and an acid regeneration bath at the rate of about 0 to 50 percent, and rinsing and drying the regenerated hollow fibre.

The ultrafiltration rate (UFR) of the regenerated cellulose hollow fibre obtained according to this invention features an extremely small reduction by wet-heat sterilization. As mentioned hereinbefore, the water permeability or ultrafiltration rate of a membrane is one of the basic parameters of artificial kidney performance and, if the UFR of a membrane is below a certain level, the membrane has no practical utility as an artificial kidney. When the difference between the UFR values before and after the wet-heat treatment of a regenerated cellulose hollow fibre membrane is expressed as a retention rate, the retention rate of the semi-permeable membrane of this invention is not less than 75 percent and preferably not less than 80 percent.

Thus, a membrane having a sonic modulus of at least 6.5 x 10^{10} dynes/cm^2 has a UFR retention rate not less than 75 percent and a membrane having a sonic modulus of at least 7.7 x 10^{10} dynes/cm^2 has a UFR retention rate not less than 80%. It is necessary that, after the wet-heat treatment, the membrane should have a UFR value of at least 8 x 10^{-2}ml/cm^2.atm.hr. while satisfying the above-mentioned retention rate requirement.

The single figure of the accompanying drawing is a graphic representation of the correlation between the sonic modulus and the UFR retention rate of the regenerated cellulose hollow fibre.

The wet-heat treatment according to this invention is carried out in the presence of physiological saline at an elevated temperature in the range of 105 to 140°C and, preferably 110 to 140°C, and particularly 110 to 130°C. The desired treatment may be accomplished by heating the membrane with physiological saline in the range of 110 to 140°C. This wet-heat treatment is carried out in an autoclave or any other suitable pressure-resistant gas-tight equipment. While there is no particular limitation on the heating time provided that it is sufficiently long to effect the necessary sterilization, 20 to 40 minutes is the usual

time. Because of the presence of heated steam, the wet-heat treatment features a large quantity of heat, a ready release of heat and an efficient penetration of heat into intricate spaces, thus providing for effective sterilization.

The conventional wet-heat treatment is generally carried out by one of the following alternative procedures: (a) heating the material to be sterilized in boiling water at 100°C, (b) heating the material as the latter is immersed in water at 105 to 140°C as described above, and (c) sterilizing the material with heated steam only. A detailed study of the influence of such wet-heat treatments on the performance characteristics of the membrane material has shown that there are significant and unexpected differences among the above three sterilizing techniques in terms of the permeation properties of the sterilized membrane as well as in the adaptablity of the membrane to medical application.

Thus, one of the differences is concerned with the change in hydraulic permeability caused by a wet-heat treatment. In other words, the aforementioned correlation between sonic modulus and UFR retention holds true only with sterilization in water and, in the case of autoclaving with use of steam alone, the UFR of the membrane generally decreases so drastically as to make the membrane useless.

Another difference is that, compared with sterilization by steam autoclaving, heat sterilizing in an aqueous medium according to the present invention yields an improved dialysing performance. In the case of steam autoclaving, the dialysing performance is found to be considerably affected: an artificial kidney unit incorporating a membrane of this invention displays a high dialysis performance *in vivo* which is 20 to 50 percent higher than the performance of the conventional artificial kidney unit sterilized with ethylene oxide gas. The improved performance thus obtained is presumed to have brought about a swollen structure to the membrane by the application of the aforementioned conditions and it is quite surprising that whereas sterilization with steam alone considerably impairs the permeability characteristics of the membrane to make it unusuable, wet-heat sterilization in water or physiological saline yields an excellent membrane performance even surpassing the performance hitherto available.

The sterilizing treatment in boiling water at 100°C entails the evolution of bubbles, which adhere to the hollow-fibre membrane. The air bubbles formed in the lumen of the fibre not only interfere with blood flow but also tend to cause clotting of blood. Therefore, the formation of air bubbles should be avoided as much as possible and such a treatment is undesirable as a method for sterilizing artificial kidneys.

Hitherto, regenerated cellulose membrane tended to become brittle on drying so that a substantial amount of a plasticizer such as glycerin is incorporated, with the consequent danger of this being eluted from the membrane when it is used in an artificial kidney apparatus. As previously mentioned, there may also be the problem of elution of the formalin sterilant.

The membrane according to this invention contains no such additive or sterilant. When the membrane is treated in water or physiological saline at above 105°C, any contaminants in the membrane that might tend to elute during use at a lower temperature than the said treating temperature, e.g. room temperature, are almost completely removed beforehand.

Furthermore, a preliminary clinical evaluation of membranes of this invention has shown that, compared with membranes sterilized with ethylene oxide gas, they have less adverse effects upon the blood, so that there is, unexpectedly, reduced possibility of clotting and thrombosis. None of these results can be accomplished by heat sterilization by steam autoclaving alone.

If the membrane treated by the sterilzing method of the present invention is cooled as sealed, it can be maintained in sterile condition. If necessary, after the sterilization treatment, aseptic replacement may be carried out, with distilled water for injections or physiological saline, and the product may then be kept sealed till use. Of course, it is possible to apply this membrane to an artificial kidney made of an appropriate material for such a treatment and the thus obtained module may be subjected to the wet-heat treatment. In the sterilizing method according to this invention, physiological saline is safe to use because it is isotonic with body fluids. Where the elution from the membrane or potting agent is considered to be a problem, it is preferable to carry out a preliminary heat treatment in water or physiological saline at 70 to 120°C, drain the membrane, reimmerse it in physiological saline and then carry out the sterilization treatment in the above manner. Since the module incorporating the membrane according to this invention contains only physiological saline, it is not necessary to rinse it to remove the sterilant before use and the module can be immediately put to use. A conventional membrane sterilized with formalin or ethylene oxide gas must be subjected to a preliminary rinse of about 30 minutes.

Thus, since the method described here-

inbefore simplifies the complicated preparatory procedure required in the current dialytic therapy and helps rationalise the work of the personnel in charge of such therapy, the invention has considerable advantages and significances in biomedical and clinical fields.

The following Examples are intended to illustrate the invention. Comparative Examples illustrate methods and membranes not in accordance with the present invention.

EXAMPLE 1

A regenerated cellulose hollow fibre produced by the cuprammonium cellulose process (manufactured by Enka-Glanzstoff A.G.) was washed with ethanol to remove the isopropyl myristate that it contained, and was dried. The hydraulic permeability (UFR) of this membrane was found to be 19.1 x 10^{-2}ml/cm^2.atm.hr. The sonic modulus of this membrane was 11.4 x 10^{10} dynes/cm^2. Using an autoclave, the membrane, immersed in physiological saline, was heat-sterilized at 120°C for 20 minutes. The UFR of the sterilized membrane was 17.1 x 10^{-2}ml/cm^2.atm.hr., and its UFR retention was 89.5%. The *Es* value of the membrane was 10.7 x 10^{10} dynes/cm^2.

EXAMPLE 2

A regenerated cellulose hollow fibre manufactured by the cuprammonium cellulose process was rinsed with distilled water and dried. The sonic modulus and UFR of the dry membrane were 8.99 x 10^{10} dynes/cm^2 and 14.4 x 10^{-2}ml/cm^2.atm.hr., respectively.

This membrane was sterilized under wet-heat conditions as in Example 1. The UFR and sonic modulus of the sterilized fibre were 12.2 x 10^{-2}ml/cm^2.atm.hr. and 9.24 x 10^{10} dynes/cm^2, respectively. The UFR retention was 85%.

COMPARATIVE EXAMPLE 1

A regenerated cellulose hollow fibre produced by the resaponification of cellulose acetate and having a sonic modulus of 5.53 x 10^{10} dynes/cm^2 (manufactured by Cordis-Dow Co) had a measured UFR value of 9.83 x 10^{-2}ml/cm^2.atm.hr. This hollow fibre was sterilized under wet-heat conditions as described in Example 1. The UFR and sonic modulus of the sterilized fibre were 6.84 x 10^{-2}ml/cm^2.atm.hr. and 5.77 x 10^{10} dynes/cm^2, respectively. The UFR retention of this membrane was 70%. The membrane was unsuitable for use as an artificial kidney.

COMPARATIVE EXAMPLE 2

Hollow fibre of the same type as in Example 1 was washed with ethanol, dried and sterilized in an autoclave using heated steam at 120°C for 20 minutes. The UFR of the sterilized membrane was 3.92 x 10^{-2} ml/cm^2.atm.hr.

EXAMPLE 3

The hollow fibre of the same type as in Example 1 was washed with ethanol, dried and assembled into a cylindrical housing made of polycarbonate to fabricate a hollow fibre dialysing module. The module was filled with distilled water and sterilized in an autoclave at 120°C for 20 minutes. After confirmation that it met artificial kidney standards, the following clinical trial was carried out in routine manner.

Case: female, 36 years of age, with chronic renal failure. Before dialysis, BUN 138.0 mg/dl, creatinine 10.0 mg/dl and uric acid 12.0 mg/dl. After 5 hours of dialysis, BUN 28.9 mg/dl, creatinine 3.4 mg/dl and uric acid 2.9 mg/dl were obtained. The rates of removal were 79.1%, 66.0% and 75.2%, respectively. Thus, the membrane was found to have an excellent dialysis performance.

COMPARATIVE EXAMPLE 3

The hollow fibre washed with ethanol and dried, which was of the same type as used in Example 3, was assembled into a module and sterilized with ethylene oxide gas. This module was tested in the same clinical trial as described in Example 3. The rates of removal were 50.5% for BUN, 47.6% for creatinine and 57.4% for uric acid.

EXAMPLE 4

An artificial kidney module fabricated in the same manner as Example 3 was filled with physiological saline and heat-treated in an autoclave at 120°C for 20 minutes. After cooling, the saline was aseptically replaced with fresh physiological saline and the module was sterilized in the autoclave at 120°C for 20 minutes. This sterilized product was used in a dialysis trial. Whereas the amount of elution (in water at 70°C for 60 minutes) from the hollow fibre washed with ethanol and dried and thereafter treated in water at 70°C for 60 minutes was 8.3 ml (the consumption of potassium permanganate), the amount of elution from the hollow fibre after the above mentioned wet-heat sterilization was reduced to 0.20 ml.

WHAT WE CLAIM IS:

1. A method of sterilizing a semi-permeable membrane of regenerated cuprammonium cellulose that comprises subjecting the semi-permeable membrane to a wet-heat treatment conducted at a temperature in the range 105 to 140°C in the presence of physioligical saline.

2. A method as claimed in claim 1 in which the semi-permeable membrane is a hollow fibre membrane.

3. A method as claimed in claim 1 or 2 in which the membrane is preliminarily heat treated in water or physiological saline at a temperature of from 70 to 120°C

before being subjected to the said wet-heat treatment at a temperature from 105 to 140°C in the presence of physiological saline.

4. A method as claimed in claim 3 in which the second wet-heat treatment is conducted at a temperature of 110 to 140°C.

5. A hollow fibre membrane of regenerated cuprammonium cellulose that has a sonic modulus of at least 6.5×10^{10} dynes/cm^2 and has been sterilized at a temperature in the range 105 to 140°C in the presence of physiological saline.

6. A method as claimed in any one of claims 1 to 4 as applied to the production of a hollow fibre membrane as claimed in claim 5.

7. A method as claimed in claim 1 substantially as hereinbefore described in any one of Examples 1 to 3.

8. A membrane obtained by a method as claimed in any one of claims 1 to 4, 6 and 7.

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1 SHEET *This drawing is a reproduction of the Original on a reduced scale*

