ON THE SURFACE CHEMISTRY OF SODIUM PENICILLIN G

by

A. V. FEW AND J. H. SCHULMAN
Department of Colloid Science, The University, Cambridge (England)

INTRODUCTION

Since the advent of crystalline penicillin salts, produced on a commercial scale, there have been considerable data published concerning the physico-chemical state of aqueous solutions of the salts. From conductivity measurements using sodium penicillin G, WOODBURY AND ROSENBLUM[1] concluded that the salt behaved in aqueous solution as a completely dissociated electrolyte of the 1:1 valency type, with possible deviations due to the penicillin ion size and interactions. These authors studied solutions up to a concentration of 0.047 *M* (16.7 mg/ml). Further conductometric investigations by KUMLER AND ALPEN[2], MCBAIN, HUFF AND BRADY[3], and GOYAN[4], using aqueous systems of several penicillin G salts, confirmed the earlier observations of WOODBURY AND ROSENBLUM[1]. In particular, MCBAIN, HUFF AND BRADY[3], studying solutions up to a concentration of 0.7 *M*, found that the penicillin ions only aggregated and exhibited colloidal electrolyte behaviour above a concentration of 0.25 *M*. The osmotic coefficients of salts of penicillin F, G, and X, determined in concentration range 0–0.1 *M* were found by LUND AND PEDERSON-BJERGAARD[5] to be of the same order as that for other dissociated electrolytes. In addition, light-scattering studies[6] have indicated that 0.037 *M* solutions of potassium penicillin G contain, at the most, associations of only a few molecules even in the presence 0.5 *M* potassium bromide, a condition which would tend to enhance micelle formation.

Whilst the above observations indicate that penicillin G salts exist in aqueous solution as normal dissociated ions at concentrations below 0.25 *M*, surface tension measurements carried out by HAUSER, PHILLIPS AND PHILLIPS[7], HAUSER, PHILLIPS, PHILLIPS AND VAVRUCH[8], and HAUSER AND MARLOWE[9], suggested that aqueous solutions of sodium and potassium penicillin G were highly surface active. These results were in contrast to those of KUMLER AND ALPEN[2], who found that the sodium and potassium salts were only slightly surface active, producing surface tension depressions of only 1–2 dynes at a concentration of about 0.017 *M*.

In view of these discrepancies, the surface tension/concentration relationships have been determined using aqueous solutions of several different batches of commercial sodium penicillin G and also samples obtained by recrystallisation of the commercial product with solutions in the concentration range 0–0.14 *M*. An investigation has been made of the dependence upon pH of the surface tension of dilute aqueous solutions of the purified salt. In addition, the molecular associations produced by the injection of

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sodium penicillin G, at different pH levels, beneath certain orientated phospholipid films have been studied by the monolayer technique at the air/water interface.

The possible biological significance of these associations has been discussed.

METHODS

Surface tension measurements

SUGDEN's[10] modification of the maximum bubble pressure method was used for the surface tension measurements. Determinations were made under thermostat conditions at 25° and 2°.

Monolayer technique

A compensated[11] Langmuir-Adam surface balance was used for the monolayer studies. Surface potential measurements were made using a double tetrode electrometer, to be described elsewhere.

The phospholipids were prepared in 1:9 v/v ethyl alcohol/benzene solutions and were spread on to 0.01 *M* buffer solutions contained in the trough. The force/area (F/A) and surface potential/area (Δ V/A) curves of the phospholipids were determined to the collapse pressure of the monolayers. For the monolayer penetration experiments the phospholipids were spread on 0.01 *M* buffer solutions and the films compressed to 3 dynes/cm surface pressure. Sodium penicillin G solutions were prepared using 20 ml of the same buffer solutions as contained in the trough, and injected beneath the monolayer using the technique described by DOTY AND SCHULMAN[12]. The surface pressure rise and surface potential change were then noted at constant area. The films were finally expanded to large areas and subsequently compressed, and the F/A and ΔV/A curves obtained to the collapse pressure of the film.

MATERIALS

Sodium penicillin G

Several different batches of commercial sodium penicillin G were found to give different surface tension/concentration (γ/c) curves, presumably due to traces of surface active impurities. In view of this, a large batch of the salt kindly supplied by Messrs. Glaxo Laboratories, Greenford, Middx., was recrystallised. To a filtered saturated solution of sodium penicillin G in 90% v/v acetone were added nine volumes of anhydrous acetone, and after cooling for three hours in a refrigerator (4°) the penicillin salt was filtered and washed with cold anhydrous acetone. Finally the recrystallised sample was dried in a vacuum desiccator for two days. Surface tension curves determined using solutions of this material and a sample obtained by a further recrystallisation were found to give within experimental error identical results, and showed higher surface tensions than solutions prepared from any commercial batch.

Phospholipids

Highly purified samples of lecithin and cardiolipin were kindly supplied by Dr M. PANGBORN, New York State Department of Health.

Cephalin was isolated from a chloroform extract of Ox red cell stroma, obtained from Mr T. SHAW, Department of Physiology, Cambridge, using a modification of the method described by WELCH[13]. Anhydrous acetone was added to the chloroform extract until no more lipid was precipitated. The material was centrifuged, the deposit washed twice with acetone in the centrifuge, and dried in a vacuum desiccator for 2 days. The powder was then dissolved in a minimum quantity of chloroform, centrifuged, and the supernatant recovered, and to this was added an excess of anhydrous ethyl alcohol. The turbid solution was placed in the refrigerator for 2 days to complete the precipitation of the cephalin, which was filtered, dried and weighed. The cephalin was finally dissolved in a known volume of 1:9 ethyl alcohol/benzene.

RESULTS

Surface tensions of solutions of sodium penicillin G

Three commercial samples of sodium penicillin G studied in aqueous solution over a concentration range of 0–0.14 *M* (0–50 mg/ml) were found to give different surface tension/concentration curves (γ/c) (Fig. 1). Whilst these products were of high potency it would be difficult to detect traces of non-penicillin type impurities by bio-assay

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methods and it is quite possible that traces of surface-active impurities were present in these commercial products. Recrystallisation of the commercial salt from acetone

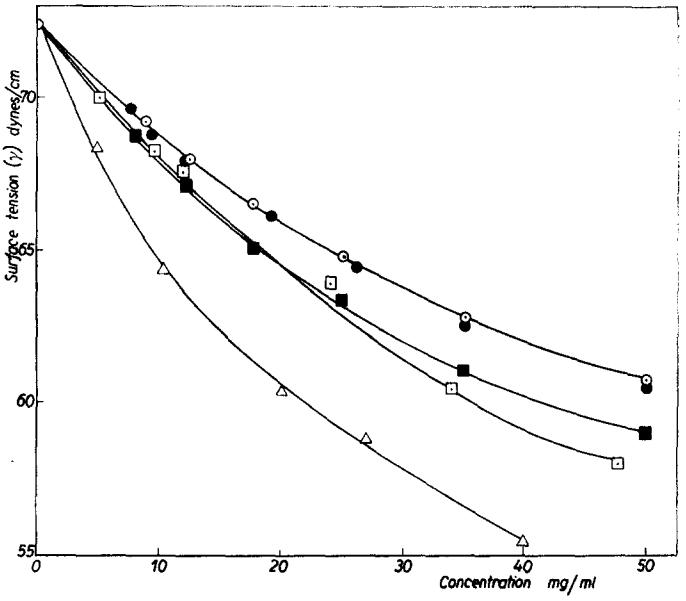

Fig. 1. Surface tension/concentration relationships for aqueous solutions of sodium penicillin G, at 25°. (a) — △ —, (b) — □ —, (c) — ■ — = three different commercial samples of the salt. — ● — = curve obtained using acetone recrystallised material. — ○ — = curve using material obtained after two recrystallisations from acetone. The recrystallisations were carried out using the commercial salt (c).

gave a product solutions of which had a higher surface tension than those of the commercial salt. A further recrystallisation from this solvent gave a product having essentially the same surface tension curve as that obtained with one recrystallisation. These results are also shown in Fig. 1. The lack of any discontinuity in the γ/c curves would indicate that there is little evidence of micelle formation over the concentration range studied.

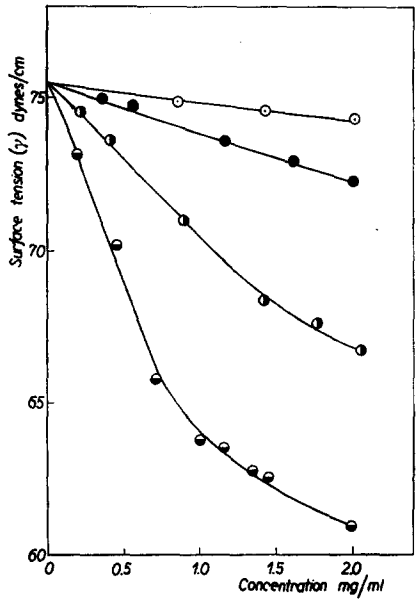

Fig. 2. Surface tension/concentration relationships for solutions of sodium penicillin G in 0.01 *M* buffers at 2°. — ○ — = pH 6.8, — ● — = pH 4.1, — ◑ — = pH 3.2, — ◕ — = pH 2.3.

The surface tension/concentration curves of dilute solutions (0–0.0056 *M*) of the purified sodium penicillin G in 0.01 *M* buffers are recorded in Fig. 2. These results were obtained on solutions cooled to 2° in order to minimise uncertainties due to the decomposition of the penicillin, which according to the data of BENEDICT, SCHMIDT AND COGHILL[14] becomes rapid at room temperature at pH levels below 4. A change in the pH from 6.8 to 4.1 gave only a slight decrease in the surface tension of the penicillin solutions, whilst a further fall in the pH produced a considerable decrease. This rapid decrease occurs in the pH range where considerable proportions of the sparingly soluble penicillin-free acid is formed (pka penicillin G = 2.76)[15]. The lowered surface tensions at low pH levels were not

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due to traces of penicillin decomposition products produced in the acid environment, since studies on partially decomposed penicillin solutions (50% decomposition) showed that the products formed by acid decomposition were less surface active than the original penicillin solutions.

Monolayer studies

Lecithin monolayers were found to give identical force/area (F/A) and surface potential area ($\Delta V/A$) curves on sub-solutions at pH levels between 2.3 and 6.8, and in good agreement with previously recorded values[16,17]. On the other hand, monolayers of cephalin were progressively expanded as the pH of the sub-solution was increased from 4.1 to 9.6, due presumably to an increased ionisation of the phosphoric acid residue present in the molecule which results in an increased electrostatic repulsion of these groups when oriented in adjacent positions in the monolayer. The $\Delta V/A$ curves of this phospholipid also indicate that as the pH of the sub-solution is increased, the potential associated with the monolayer decreases, this corresponding to the removal of a positively charged layer from the surface. The F/A characteristics are similar to those reported by ALEXANDER, TEORELL AND ABORG[18], and the area per molecule at high surface compression, ca 40 A^2/mol, suggests the presence of two saturated hydrocarbon chains. Cardiolipin exhibited a similar behaviour to that of cephalin; an increase of the pH level of the sub-solution giving a slightly expanded monolayer, this occurring in a regular fashion between pH 2.3 and 9.6. Similarly the surface potentials showed a progressive decrease on increase of the pH of the sub-solution, with the exception of those determined on an acetate buffer at pH 4.1. These effects can best be ascribed to an increased ionisation of the phosphoric acid residues in the molecule as in the case of cephalin. The F/A curves of this phospholipid are in good agreement with those determined by DOTY AND SCHULMAN[12] and GLAZER[19]. The latter author found that incomplete spreading of cardiolipin occurred when it was spread from ethyl alcohol solution on to distilled water and solutions containing low concentrations of electrolytes. On sub-solutions of higher electrolyte concentrations the phospholipid gave completely spread monolayers. To explain this phenomenon it was suggested that the incomplete spreading at the interface was due to the presence of strong intramolecular attraction which could, however, be disrupted in the presence of higher concentrations of electrolytes. In the present investigation, using a mixed ethyl alcohol/benzene solution for spreading the phospholipid, completely spread monolayers were obtained on distilled water sub-solutions, suggesting that the incomplete spreading from ethyl alcohol solution observed by earlier authors was due to a partial solution of the cardiolipin in the trough sub-solution, which could however be prevented by the presence of electrolytes. In this respect cardiolipin exhibits similar surface characteristics to those of certain proteins, notably haemoglobin, which only forms monolayers on salt solutions and does not spread on distilled water.

Injection of sodium penicillin G to a final concentration of 0.313 mg/ml beneath lecithin, cephalin and cardiolipin monolayers compressed initially to a surface pressure of 3 dynes/cm produced both a surface pressure rise and surface potential change. Subsequent expansion and recompression of the films resulted in the ejection of the penicillin from the cephalin and cardiolipin; at high compression both the F/A and $\Delta V/A$ curves collapsing on to those obtained in the absence of penicillin G. This occurred when these lipids were spread on both 0.01 M phosphate pH 6.8 and 0.01 M acetate

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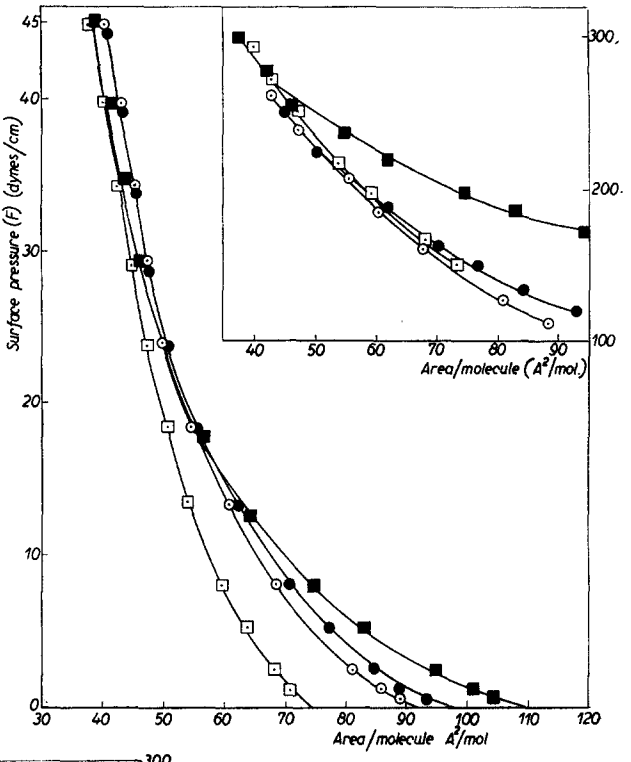

Fig. 3. Effect of injection of sodium penicillin G beneath monolayers of cephalin. Force/area curves of cephalin obtained on 0.01 *M* buffers; — □ — = pH 4.1, — ○ — = pH 6.8. Force/area curves after injection of sodium penicillin G to a final concentration of 0.313 mg/ml; — ■ — = pH 4.1, — ● — = pH 6.8. Insert: Surface potential/area curves, the symbols having the same significance as in the force/area curves.

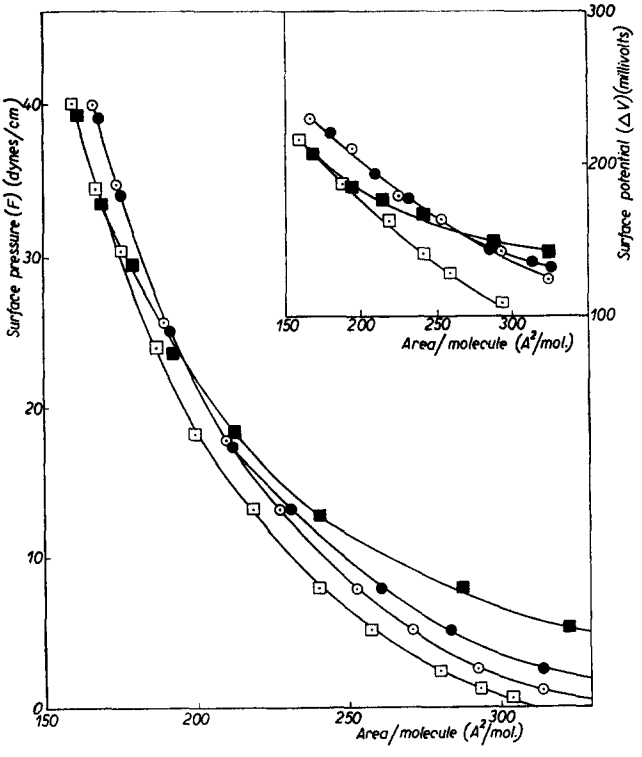

Fig. 4. Effect of injection of sodium penicillin G beneath monolayers of cardiolipin. Force/area curves obtained on 0.01 *M* buffers; — □ — = pH 4.1, — ○ — = pH 6.8. Force area curves after injection of sodium penicillin G to a final concentration of 0.313 mg/ml; — ■ — = pH 4.1, — ● — = pH 6.8. Insert: Surface potential/area curves, the symbols having the same significance as in the force/area curves.

pH 4.1 buffers and is recorded in Figs. 3 and 4. With monolayers of lecithin, on re-compression of the monolayer the penicillin was held firmly in the surface layer up to the collapse-pressure of the film. These results are shown in Fig. 5. Injection of penicillin G beneath monolayers of lecithin on $M/100$ HCl (pH 2.3) gave very large surface pressure increments, and subsequent expansion and compression produced the curve also shown in Fig. 5. In another experiment, after injection of the penicillin G and determination of the F/A curve up to pressures below the collapse-pressure, the film was expanded

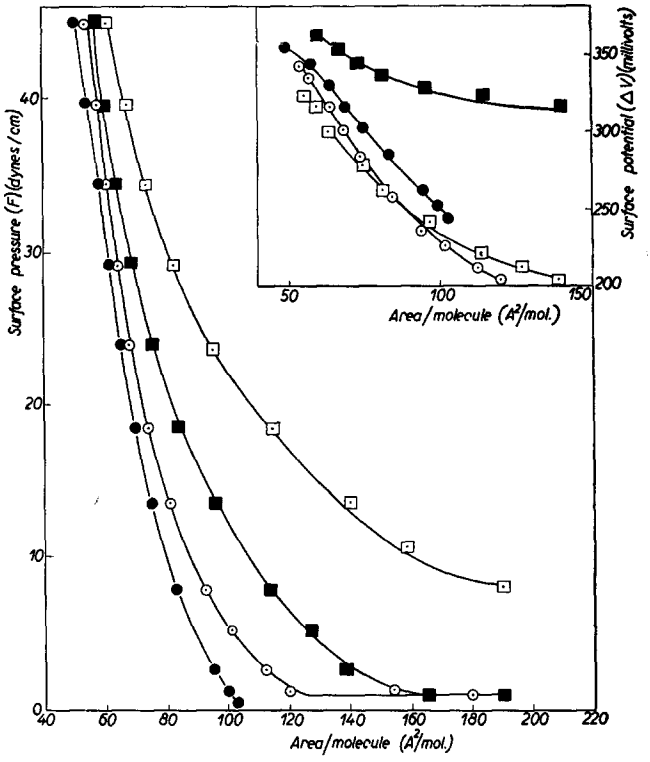

Fig. 5. Effect of injection of sodium penicillin G beneath monolayers of lecithin. Force/area curves obtained on 0.01 M buffers; — ● — = pH 2.3 to 6.8. Force/area curves after injection of sodium penicillin G to a final concentration of 0.313 mg/ml; — □ — = pH 2.3, — ■ — = pH 4.1, — ○ — = pH 6.8. Insert: Surface potential/area curves, the symbols having the same significance as in the force/area curves.

to 15 dynes/cm surface pressure and left for 2 hours, during which time no collapse of the film occurred, even though the penicillin in the sub-solution was undergoing decomposition in the acid environment and would be expected to possess only about 5% of its original biological activity. Evidently the penicillin in association with the lecithin in the surface is protected from decomposition at this pH. A subsequent spread of the phospholipid upon the decomposed penicillin showed little interaction at the acid pH and none after adjustment of the sub-solution pH to 6.8.

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DISCUSSION

The different surface tension/concentration relationships obtained, using several batches of commercial sodium penicillin G, provides evidence for the presence of more highly surface active impurities in the preparations, which might arise from the use of unsaturated and saturated long hydrocarbon chain acids in the fermentation medium[20]. It is possible that traces of impurities of these types may be present in the commercial crystalline product, and could lead to the discrepancies in the physical properties as observed by earlier authors. Indeed, the surface tension/concentration curves obtained by HAUSER[21], using a sample of sodium penicillin G of potency 1373 i.u./mg, show a pronounced minimum surface tension, generally indicative of the presence of impurity. In a later communication, HAUSER AND MARLOWE[9] obtained curves which did not show a minimum, but which gave surface tension depressions of more than 30 dynes/cm at a concentration of 30 mg/ml, whereas in the present investigation a depression of only 8.8 dynes/cm was recorded at this concentration with an acetone-recrystallised preparation of the salt. MCBAIN, HUFF AND BRADY[3] attributed the low surface tensions of solutions of sodium penicillin G, which they found to be relatively independent of concentration, to the presence of small amounts of impurities. The results recorded here using a dynamic method for the determination of the surface tensions are in good agreement with those of KUMLER AND ALPEN[2], and experiments using the static drop volume method gave essentially the same results as those determined with the maximum bubble pressure apparatus. It would appear probable therefore that sodium penicillin G is only slightly surface active and shows little evidence of colloid electrolyte behaviour at least below a concentration of 0.25 *M*.

The effect of variation of the pH level of dilute aqueous solutions of sodium penicillin G revealed that, whilst little change occurred by a decrease from pH 6.8 to 4.1, a further lowering brought about a rapid increase in the surface activity; this occurring in the pH region where increasing amounts of the penicillin free acid were formed.

The increased interaction of penicillin with lecithin monolayers brought about by a lowering of the pH of the sub-solution is paralleled by the effect of pH on the uptake of radioactive penicillin by washed bacterial suspensions[22]. The uptake of penicillin by the organisms was only slightly increased by a change of the suspension pH from 7 to 4.4, but further lowering resulted in a considerably increased uptake by both sensitive and resistant organisms at pH levels below pH 3.5. Similarly, ABRAHAM AND DUTHIE[23] have detected an increase of the antibacterial activity of penicillin on lowering the pH of the assay medium. In addition it is reasonable to expect that an increase in the surface activity of a drug would give rise to an increased Gibbs adsorption or oil solubility, thus enabling the agent to reach a toxic concentration at its site of action at a lower bulk concentration. This is in fact observed for the penicillins differing only in the nature of their side chains; the activities based on molecular activity when determined *in vitro* using a simple growth medium increase in the order *p*-hydroxybenzyl, 2-pentenyl, benzyl and heptyl penicillin[24], which is also the order of expected increase in surface activity. However, *in vivo* the antibacterial activity is in the reverse order, and this was suggested by TOMPSETT, SHULTZ AND MCDERMOTT[25] to be due to an increasing tendency of the penicillins, in the above order, to associate with the albumin present in the serum.

The association detected between penicillin and certain lipid monolayers and their

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protection against acid decomposition is being investigated in the bulk phase to determine the significance of lipids in the environment of cells on the biological activity of penicillin.

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SUMMARY

Different batches of commercial sodium penicillin G give different surface tension/concentration curves. Recrystallisation of the salt from acetone removes certain more highly surface-active impurities. The surface tensions of aqueous solutions of the purified salt differ only slightly from that of water, with no evidence of micelle formation up to a concentration of 0.14 *M*. In buffered solutions of sodium penicillin G the surface tensions are slightly decreased by a change in the pH level from 6.8 to 4.1, whilst a further lowering of the pH produced a considerable decrease in surface tension.

A characteristic difference exists between the effect produced by injection of penicillin G beneath monolayers of lecithin, cephalin and cardiolipin. Compression of the mixed monolayers results in an ejection of the penicillin from the surface in the case of cephalin and cardiolipin, but with lecithin monolayers the penicillin remains in the surface phase. A decrease of the pH of the sub-solution produces an increased association of the penicillin with the three lipid monolayers. In addition, there is evidence of the protection from acid decomposition of penicillin, when present in association with lecithin monolayers.

RÉSUMÉ

Des échantillons divers de pénicilline G du commerce (sel de sodium) donnent des courbes tension superficielle/concentration différentes. Par recristallisation dans l'acétone l'on peut éliminer certaines impuretés à tension superficielle élevée. Les tensions superficielles de solutions aqueuses de sel purifié ne diffèrent que légèrement de celle de l'eau; jusqu'à une concentration de 0.14 *M* il n'y a pas d'indication de la formation de micelles. Dans le cas de solutions tamponnées, la tension superficielle diminue légèrement lorsque le pH passe de 6.8 à 4.1; lorsque le pH diminue davantage, la tension superficielle décroît considérablement.

Il y a une différence caractéristique entre les effets produits par une injection de pénicilline G sous une couche monomoléculaire de lécithine, de céphaline et de cardiolipine. Dans le cas de la céphaline et de la cardiolipine une compression des couches monomoléculaires mixtes a pour effet une éjection de la pénicilline, tandis que dans le cas de la lécithine, la pénicilline reste dans la phase superficielle. Une diminution du pH de la solution sousjacente a pour effet que la pénicilline s'associe davantage aux trois couches monomoléculaires. De plus, l'association de la pénicilline avec des couches monomoléculaires de lécithine semble protéger la pénicilline contre l'action décomposante des acides.

ZUSAMMENFASSUNG

Verschiedene Ansätze von Handelspenizillin G (Natriumsalz) geben verschiedene Oberflächenspannung/Konzentrations-Kurven. Durch Umkristallisieren des Salzes aus Aceton kann man verschiedene Verunreinigungen mit hoher Oberflächenaktivität entfernen. Die Oberflächenspannungen wässriger Lösungen der gereinigten Salze unterscheiden sich nur wenig von der des Wassers; bei einer Konzentration, welche 0.14 *M* nicht übersteigt, ist kein Anzeichen von Micellenbildung vorhanden. In gepufferten Lösungen von Natriumpenizillin G nehmen die Oberflächenspannungen ein wenig ab, wenn das pH von 6.8 auf 4.1 sinkt; sinkt es tiefer, so nimmt die Oberflächenspannung bedeutend ab.

Es besteht ein charakteristischer Unterschied zwischen der Wirkung einer Penizillin G-Injektion unter monomolekulare Schichten vom Lecithin, Cephalin und Cardiolipin. Werden die gemischten monomolekularen Schichten zusammengepresst, so wird im Falle von Cephalin und Cardiolipin das Penizillin herausgestossen, während es bei Lecithinschichten in der Oberflächenphase bleibt. Eine Verminderung des pH der unterstehenden Lösung verstärkt die Assoziation des Penizillins mit den drei monomolekularen Lipoidschichten. Ausserdem scheint es, dass die Assoziation des Penizillins mit den monomolekularen Lecithinschichten das Penizillin gegen Säurezersetzung schützt.

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