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# Production Technology and Physicochemical Properties of Composition Containing Surfactant Proteins

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**Abstract**

The article describes a production method of substance containing great amount of phospolipids (up to 36 %) and surfactant proteins (up to 2 %) in terms of lyophilisate composition. Basic physical and chemical characteristics of the substance (density, viscosity, surface tension and the coefficient of sliding friction) indicate a high lubricant capacity of the derived product. These properties are kept when mixed with native human synovial fluid in the ratio of 1 to 9 inclusive. The obtained data allows to consider the derived composition, containing surfactant proteins and phospholipids, a variety of bionic lubricant suitable for testing as a potential equivalent of synovial fluid which can be used in traumatology and orthopedics, a cosmetic component or agent which increases the stability of the cell suspension during culturing in bioreactors.

**Keywords:** pulmonary surfactant, surfactant proteins, phospholipids, boundary lubrication, viscosity, tribology, articular cartilage; cartilage tissue engineering, surface friction

## 1. Introduction

We are now actively developing technologies of the creation and production of synthetic lubricants and their modifications to apply in different lines of industry, agriculture, medicine and veterinary medicine. Talking about the necessity to provide the contact of lubricant with biological objects, we find the biomimetic approach most effective, as it involves the production and application of the lubricants, which properties are maximally close to natural (Wang, 2014; Hwang et al., 2015; Park et al., 2016). One of the most promising sources of natural lubricants are surfactant mixtures produced from mammals' lung containing specific surfactant proteins (SP) and phospholipids (Sarker et al., 2011; Casals, Cañadas, 2012; Schenck, Fiegel, 2016).

Directly in medicine, veterinary and pharmaceutical biotechnologies lubricants are used to reduce friction between the surfaces of the natural parts of the body and to improve the adaptation of the moving parts (most often - in pulmonology, traumatology and orthopedics) (McNary et al., 2012; Lopez-Rodriguez, Pérez-Gil, 2014; Sui et al., 2016). In particular, the bulk of hyaluronic acid (hyaluronan according to the IUPAK nomenclature) for biomedical use is used as a lubricant for cosmetic preparations and equivalents of synovial fluid (Ayhan et al., 2014; Zhu et al., 2015). Also it is necessary to use lubricants to reduce the friction losses of the cell mass in bioreactors which come as a result of mixing and aeration, etc. (Valentín-Vargas et al., 2012; Knöspel et al., 2016).

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Obviously, there is a constant need for new technologies of creation of new lubricants based on bionic principles. One option of such lubricant may be a composition based on natural surfactants of pulmonary origin.

Physical and chemical properties of lubricant composition, such as density, viscosity, surface tension force and the force of friction (Rantamäki et al., 2011; Hui et al., 2012; Antonov, 2013), form objective criteria to define its effectiveness. Other, but no less important characteristics of the lubricant composition - chemical storage stability, biocompatibility and biosecurity, including the ability of utilization by the natural biological system, in which this lubricant functions, determine the basic advantages of bionic materials (Dicker et al., 2014; Smith et al., 2013).

The goal of this work, based on the above, is an attempt to develop technology to produce compositions with high lubricating qualities from recyclable materials of meat-processing industry and to explore their physical and chemical properties.

## 2. Material and Methods

### Obtaining substance containing surfactant proteins

The experiment involved 16 white male rats of Wistar line 6 months old weighing 180-240 g Protocol of experiments comply with the ethical standards set out in the "Code of Good Practice in Research involving animal experimentation" and Directive 2010/63 / EU of the European Parliament and of the Council of the European Union on the protection of animals used for scientific purposes.

We used bronchoalveolar lavage technique to obtain a substance containing surfactant proteins (SCSP) in the first stage (Blanco, Pérez-Gil, 2007). We used a special eluent to increase the proportion of lipophilic surfactant proteins in lavage fluid. The composition included 2% solution of a phospholipids mixture in a commercial preparation Verolec F-62 (E322) manufactured by Emulgrain SA (Argentina) and Trilon B (EDTA) in phosphate buffer (pH = 7,4). The derived lavage fluid was centrifuged at 500 g for 10 minutes in order to separate the large particles and then directly, with the addition of distilled water, at 12,000 g for 1 hour at 4-6 ° C. The precipitate received was lyophilized and stored at 4 ° C before use.

At the second phase we obtained the mixture enriched with surfactant proteins. Lyophilisate was resuspended in a borate buffer (pH = 8.6) with addition of 1% commercial phospholipids mixture Verolec F-62, it was centrifuged at 3500 g for 15 min. The supernatant was subjected to final purification by preparative column chromatography (gel filtration) on Sephadex G 75 (Nakahara et al., 2009). Such modification of the technology made it possible to lower the concentration of anti-inflammatory peptide impurities, and to ensure the presence in the mixture of two fractions - Water soluble SP-A and SP-D, and cationic amphipathic hydrophobic SP-B and SP-C, associate with surfactant's phospholipids in the composition of the final substance (Fig. 1).

The yield of resulting product was 1.8% -3.2% from the absolute weight of rat's lung mass, the part of SCSP was lyophilized, and then we analyzed the percentage content of lipids, proteins and minerals in it. A similar study was carried out for the commercial preparation "Surfactant BL" manufactured by OOO "Biosurf" (St. Petersburg, Russia). For final use, we resuspended SCSP in sterile saline to 2% concentration of protein and then dispensed it. The ampoules were stored in a refrigerator at 4 ° C.

### Determination of physical and chemical properties of the liquids being tested

We defined density as the ratio of the gained weight "Ohaus DV214CD", Germany), of a plastic syringe filled up to 1 cm 3 SCSP in relation to this volume. The results were expressed in g / cm 3 , the calculated error, taking into account the passport characteristics of the products, was less than 2.0%. To determine the viscosity of the SCSP we used the rotational viscometer DV-II + (Brookfield, USA) with a special adapter for small samples. Index values are expressed in mPas.

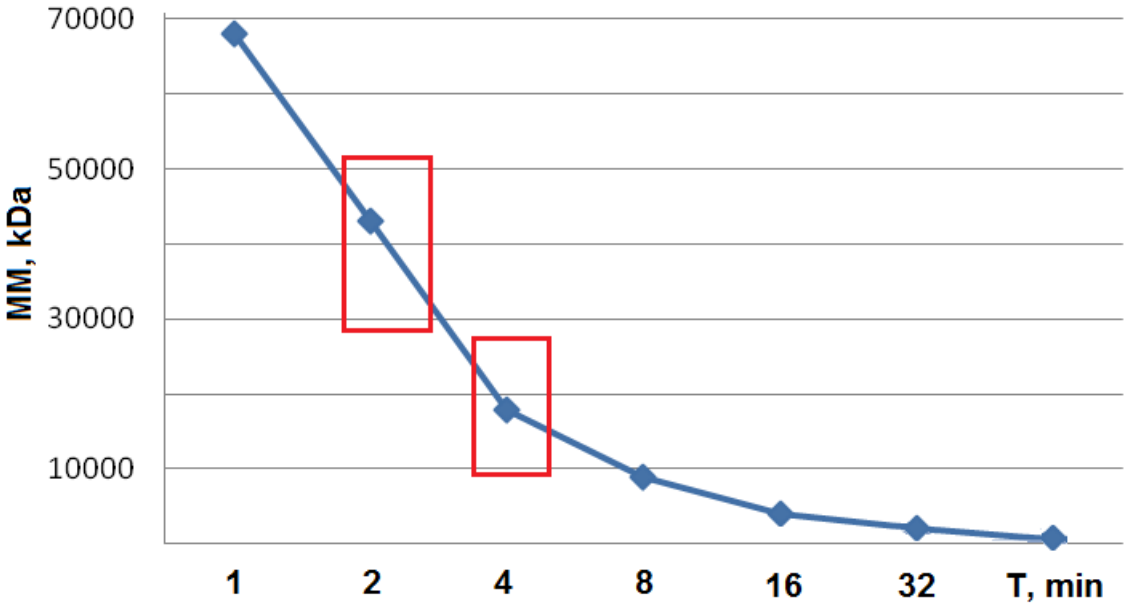


**Fig. 1.** Elution time needed for fractions of surfactant proteins within the evolution reaction performed by the preparative gel filtration with Sephadex G75. The time interval to collect the combined fractions, containing the surfactant proteins and not having the anti-inflammatory peptides, was defined.

To measure the surface tension $k_\sigma$ we used the principle of the drop (stalagmometric) method. At first we sequentially recorded the process of formation and free-fall of three drops of the biological fluid from the plastic capillary with set point inner diameter $D_j$ = 1 mm recording everything on digital video camera Nikon D3200 (Japan) We picked the drops into a plastic mini-cell of the specified weight, then we measured the weight gain with torsion balance, then calculated the average weight of 1 drop ( $m_d$ mg). During the separation of the drop of liquid from the lower end of the vertical tube its weight $m_d g$ was balanced by the surface tension force F, which is equal to the multiplication σ on the drop waist perimeter $\pi D_j$ and prevents its separation.

$$m_d g = \pi D_j \sigma \quad (1).$$

To improve the accuracy of the correction we set up the adjustment factor $k_\sigma$, which can be defined as the ratio of the reference (table) value σ for glycerine to the value obtained in the pilot plant (it was 1.025). Thus, according to the known values of $k_\sigma$ and the inner diameter of the capillary, the final formula for calculating σ (mN / m) becomes:

$$\sigma = k_\sigma m_\kappa g / \pi D_j = 3199\, m_\kappa \quad (2).$$

We used the evaluation formula adapted to the comparative method of Poiseuille's law to measure biological fluids. After substituting the values for the calibration liquid, the calculation formula determining the viscosity of the studied biological fluid (Pa · s) had acquired the form:

$$\eta = 0{,}689\, \rho t \quad (3).$$

The coefficient of sliding friction force M (dimensionless) was determined on a flat surface by fixing the maximum readings of traction micro dynamometer DEP1-1D-0,1R-1 (Pet-Ves, St. Petersburg, Russia) at the beginning of the motion of the reference load weighting 10 g and 1cm base area ² in conditions of contact "glass to glass" with complete wetting by the liquid under study. We referred this value (sliding friction force $F_f$) to the weight of the control weight $mg$ in H:

$$\mu = F_f / mg \quad (4)$$

The described investigative techniques have been used to study the physical and chemical characteristics of the properties of the obtained SCSP, synovial fluid samples of five patients with the second stage of osteoarthritis, which were taken right before Viscosupplementation sessions, as well as their model mixtures in the ratio 1: 1, 1: 2,1: 4 and 1: 9.

**Statistical analysis of the results**

Quantitative data were processed using Statistica 10.0 (StatSoft Inc., USA) with the calculation of the indices adopted to characterize the non-parametric samples in biomedical research. Results were shown as Median [1st quartile ÷ 3rd quartile]. To prove the validity of differences the non-parametric Friedman criterion for multiple groups was applied. P values less than 0.05 were considered significant.

## 3. Results

The composition of SCSP according to the results of the definition included lipids (mostly - phospholipids), fractions of proteins with mass 16-24 kD (including the subunit SP - B, SP - C) and mass32-48 kD (including SP - D and some of SP - A), a small amount of impurity protein fractions and mineral salts. Quantitative results of the composition definition of freeze-dried SCSP are shown in Figure 2.

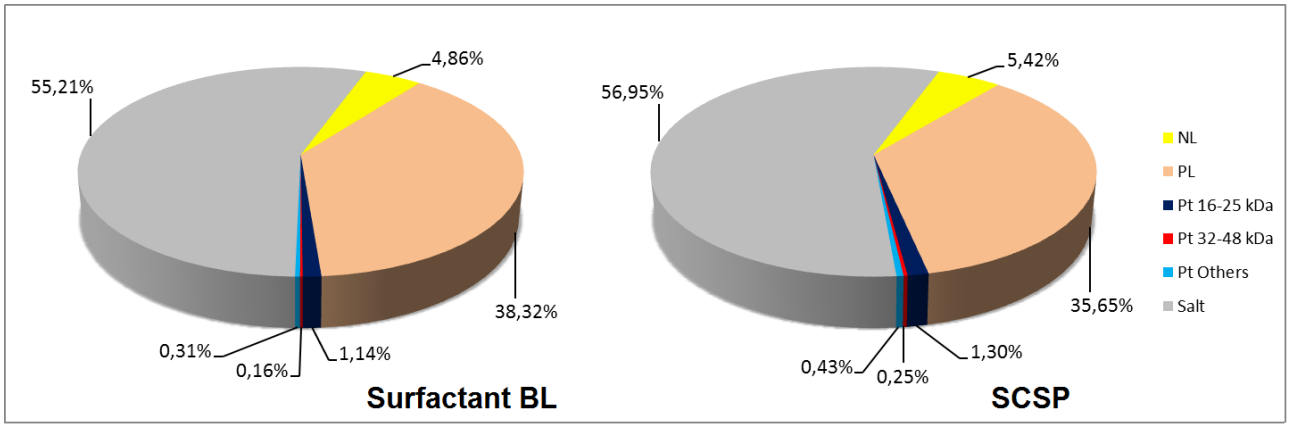


**Fig. 2.** The chemical composition of the market image drug Surfactant BL manufactured by Biosurf (St. Petersburg, Russia) and the original substance containing surfactant proteins (%).

Quantitative analysis of the selected SCSP showed that the resulting mixture contains more neutral lipids by 10%, but the content of the phospholipids fraction is reduced by 7% in comparison with commercial preparation "Surfactant BL». The concentration of proteins weighing 16-24 kD was bigger by 12.3% and of proteins weighing 32-48 kDa, was respectively, bigger by 36% in comparison with commercial preparation "Surfactant BL", the content of other associated proteins was also increased by 27.9%. Such differences may be caused by the difference in phases and the quality of purification of proteins. Salts in SCSP and commercial preparation "Surfactant BL" had no any significant differences and amounted to 3% only.

Figure 3 summarizes our findings in physicochemical properties of the biological fluids under study.

The obtained results of physical properties of synovial fluid and SCSP showed that synovial fluid has higher viscosity, but the friction force and surface tension is less by 15-20%. These figures are provided by key components, due to them synovial fluid performs its main function during the articular surfaces motion.

Mixing SCSP and synovial fluid in various ratios showed that different concentrations of certain components significantly alter the biological fluid properties. The best result was achieved by mixing in ratio of 1 to 4. The viscosity index decreased by 45% in comparison to synovial fluid, this result can be affected by the effective dilution of SCSP of synovial fluid. The surface tension decreased with adding of SCSP to synovial fluid, this reduction is caused by surfactant proteins SP-B and SP-C content in SCSP, the maximum reduction was achieved with ratio of 1 to 1. (Fig. 3).

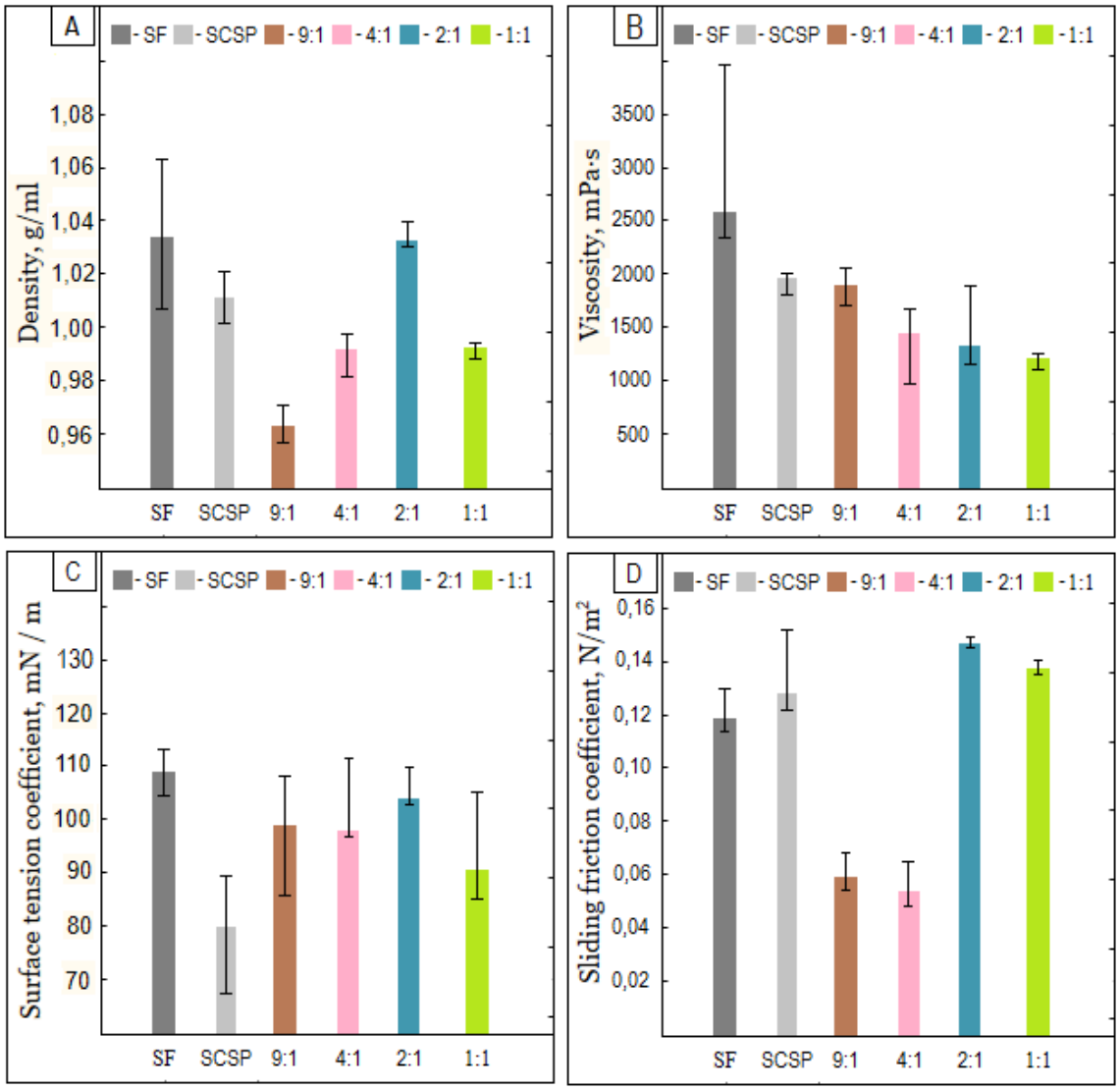


**Fig. 3.** The physicochemical properties of synovial fluid, substance containing surfactant proteins, and its mixtures in model suspensions. A. Density, g/ml. B. Viscosity, mPa s. C. Tension coefficient the Surface, mN/m. D. Sliding friction coefficient, N/m² . Quantitative results are represented in the form of Me [Q1 ÷ Q3].

The coefficient of sliding friction decreased with ratio of 1 to 9 and 1 to 4, when SCSP had been added to synovial fluid, and started to rise with ratio of 1 to 2 and 1 to 1, hypothetically the friction coefficient may have been affected by rising concentration of phospholipids, as adhesion of sliding surfaces takes place, and their movement is possible only when the force exerted on the load increases.

## 4. Discussion

Protein-containing bionic lubricants are able to provide a so-called boundary lubrication that means the lubrication, which is effective at high pressures and minimum layer of lubricating substance (McNary et al., 2012; Lu, 2009; Greene et al., 2011). Thus, hyaluronic acid is responsible for hydrodynamic lubrication at moderate pressures and the sufficient layer of the synovial fluid layer between the joint surfaces, and in case of direct contact of cartilage and high pressure of the contact - specific glycoprotein lubricin weighting 227 kDa (Flannery et al., 2009; Novochadov et al., 2014; Ludwig et al., 2015). Similar mechanisms are required in terms of maintaining the elasticity of the alveoli, which is provided by the surfactant lung system having a high grade of homology with lubricin, primarily due to the presence of mucin domain fragment (Blanco, Pérez-Gil, 2007, Fathi-Azarbayjani, Jouyban, 2015).

The Data, Obtained by us at study of SCSP , indicates that surfactant-associated proteins content in conjunction with phospholipids provides a biological fluid with high rate of force coefficients of surface-tension and sliding frictional force, having reduced viscosity at the same time. That in turn can allow using of SCSP as improved lubricating composition.

Known physical and chemical characteristics of the resulting substance (alone or in various dilutions with human synovial fluid) are comparable by their values to the characteristics of drugs currently used as a synovial fluid prosthetic in joint diseases (Shen, Chen, 2014; Zhang et al., 2016). One of the SCSP's advantages is that it has functionally active association of phospholipids and proteins, which is, in modern concepts, necessary for mediating not only frictional, but also signal anti-inflammatory properties of natural lubricants of a human body (Andrades et al., 2012; Kosinska et al., 2013). The surfactant proteins listed above, as well as their association with phospholipids, of course, fulfills the function of reducing the surface tension and the coefficient of sliding friction. Therefore, the resulting lubricant composition can be used as an additive for cosmetic products industry, as well as for bioreactors to reduce the cell loss in cultivation them in the form of suspensions. This technology can be successfully scaled to manufacture the product from the arm animal's lungs saving the ability to use the remaining by-product as a raw material for the food industry and other biotechnologies.

## 5. Conclusion

The resulting product has composition with basic properties of a lubricating composite. The main components of SCSP are surfactant-associated proteins and phospholipids. Physical and chemical properties correspond to the characteristics of natural lubricant – the synovial fluid of articular cartilage. Therefore, this product can be tested as a potential component of synovial fluid prosthetic and other biomedical products.

## 6. Acknowledgements

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## Технология получения и физико-химические свойства композиции, содержащей белки сурфактанта

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**Аннотация.** В работе описана методика получения из легких млекопитающих композиции с высоким содержанием фосфолипидов (до 36 %) и белков сурфактанта (до 2 %) в пересчете на лиофилизат. Основные физико-химические характеристики композиции (плотность, вязкость, коэффициент поверхностного натяжения и коэффициент трения скольжения) свидетельствуют о высокой лубрикативной способности полученного продукта. Эти свойства сохраняются при смешивании с нативной синовиальной жидкостью человека в разведении до 1: 9 включительно. Полученные данные позволяют считать полученную композицию, содержащую фосфолипиды и белки сурфактанта, вариантом бионического лубриканта, пригодного для проведения испытаний в качестве потенциального протеза синовиальной жидкости в травматологии и ортопедии, компонента косметических средств или средства, увеличивающую стабильность клеточной суспензии при культивировании в биореакторах.

**Ключевые слова:** легочный сурфактант, белки сурфактанта, фосфолипиды, граничная смазка, вязкость, трение скольжения, трибология, суставной хрящ; тканевая инженерия хряща.

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