Scoring of surface parameters of physiological relevance to surfactant therapy in respiratory distress syndrome

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Banerjee, R., and Jayesh R Bellare. Scoring of surface parameters of physiological relevance to surfactant therapy in respiratory distress syndrome. J Appl Physiol 90: 1447–1454, 2001.—The Wilhelmy balance was used for in vitro testing of surface parameters of surfactants used for respiratory distress syndrome therapy. Two commercial protein-free surfactants, ALEC and Exosurf, were compared with pure forms of the three main phospholipids in natural surfactants, dipalmitoyl phosphatidylcholine (PC), phosphatidylglycerol (PG), and phosphatidylethanolamine (PE), and their binary mixtures, PC with PE and PG each in the ratio 2:3. Surface excess films (15 Å/molecule) were compressed at 1.2 cycles/min past collapse to a compression ratio of 4:1. The maximum surface pressure, spreading time, compressibility, respreading ratio, recruitment index, and hysteresis area were compared. A consolidated list of criteria for selection of suitable surfactants was compiled from the literature. A relative scoring system was devised for comparison based on these criteria. PC/PG (2:3) performed the best as it fulfilled all the criteria and obtained the highest relative score. Exosurf also performed well, except on the respreading criterion. ALEC and PE were equivalent in their performance and performed well, except on two criteria: hysteresis area and recruitment index. Thus the scoring system proposed here proved valuable to rate the overall efficacy as well as relative merits of surfactant formulations.

This could be achieved by the introduction of a scoring system for the performance of surfactants. Such a system of comparison would also help in establishing overall relative standards of efficacy for new experimental surfactant formulations. Therefore, this study is aimed at 1) compiling criteria for judging the suitability of surfactant formulations as exogenous therapy in RDS, 2) performing in vitro testing of surface parameters for artificial surfactants and a set of phospholipid formulations that are possible candidates for surfactant therapy, 3) devising a relative scoring scheme based on the criteria to rank the formulations, and 4) comparing the performance of the surfactant formulations based on the criteria and scoring scheme.

Alveolar surfactant is well known for its ability to lower surface tension at the alveolar air-liquid interface to values below 5 mN/m. PC is the prime surfactant, surfactant-lowering component of pulmonary surfactant. PC with PE and PG each in the ratio 7:3, supplied as a lyophilized powder to be reconstituted with 1.2 ml of sterile sodium chloride. The PG component improves the adsorption of PC, which is

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otherwise poor (2). Exosurf consists of PC, 6% tyloxapol, and 9% hexadecanol, is supplied as a lyophilized powder, and is recommended to be used after reconstitution with 8 ml of sterile water. Hexadecanol facilitates adsorption of PC to the air-liquid interface (32). The function of improvement of spreading of PC, in ALEC and Exosurf, is performed by PG and hexadecanol, respectively, and thus they compensate for the lack of surfactant-specific proteins in their composition.

Although ALEC contains a high concentration of PC (PC/PG = 7:3), a recent study by Holm et al. (9) suggested that surfactants with low-PC content would serve as good candidates for exogenous surfactants. In fact, they showed that 40% PC gave the most favorable response in surface pressure-area isotherms compared with higher concentration mixtures. The present study evaluates the performance of certain surfactant formulations with 40% PC and compares them with ALEC.

Apart from surface tension reduction and spreading, other parameters of extreme physiological relevance are the respreading or the ability of surfactant molecules, which are expelled from the interface during compression, to reenter the surface film during expansion, hysteresis area, compressibility, and recruitment. In the absence of high respreading, surfactant would continuously be depleted at the interface during the compression and expansion cycles of respiration (18). However, an absolute maximal increase in respreading results in a small hysteresis area between the compression and expansion isotherms of the monolayer (22). Thus respreading needs to be large enough to allow reentry of surfactant into the interface and yet allow reproducible high-hysteresis areas. Regardless of the actual value of minimum surface tension, a more rapid increase of surface tension of an expanding alveolus is beneficial physiologically as it allows recruitment of many more alveoli with low surface tensions. A low compressibility, as defined by Gaines (4) and applied to the pulmonary surfactant system by King and Clements (11), is desirable to ensure attainment of low surface tension values with minimal changes in area (25). Table 1 summarizes the various parameters and criteria of physiological relevance to exogenous surfactants.

Although some of these parameters have been considered in the in vitro evaluation of various possible surfactants (13, 30), a comprehensive study of the overall performance of surfactants with respect to all these parameters is required. In fact, an overall index of the performance of the surfactant with respect to all these parameters is required. A relative scoring system is proposed for such a comparison of surfactant formulations, based on their in vitro performance in various parameters of physiological relevance.

Objectives of this study. In the present work, we studied the dynamic surface tension lowering, respreading, compressibility, hysteresis, and recruitment properties of pure films of PC, PG, phosphatidylethanolamine (abbreviated here as PE), and binary mixed films of PC and PE (abbreviated as PCPE) and PC and PG (abbreviated as PCPG). After PC, PG is the second most important phospholipid of the pulmonary surfactant mixture and is said to improve spreading of PC in binary mixtures (12), hence its inclusion in this study. PE is included because of the finding of Yu et al. (33) that PE-based surfactants perform as well as PG-based surfactants. The binary mixtures are made in the ratio of PC to PE of 2:3 or PC to PG of 2:3, based on the suggestion by Holm et al. (9) that surfactants with low PC content would serve as good candidates for exogenous surfactants. In fact, they showed that 40% PC gave the most favorable response in surface pressure-area isotherms compared with higher concentration mixtures. The performance of these films is compared with that of the two synthetic surfactants, ALEC and Exosurf. An attempt is made to score the relative overall performance of each compound after consideration of all the above-mentioned parameters. Although the present study applies the scoring system to parameters determined using the Wilhelmy balance and uses ALEC and Exosurf as controls for comparison, other studies have been conducted for application of a similar system to the pulsating-bubble technique with protein-based surfactants as controls (unpublished observations).

### MATERIALS AND METHODS

PC, PE, and PG were purchased (all >99% purity) from Sigma Chemical. ALEC (artificial lung expanding compound) was a kind gift from Britannia Pharmaceuticals. Exosurf Neonatal was obtained as a kind gift from Burroughs Wellcome. Sterile Ringer-lactate solution was obtained from Albert David Limited, Calcutta, India. Table 2 summarizes the chemicals studied. Other chemicals used in the experiments were HPLC-grade chloroform and acetone (Qualigens Fine Chemicals, Mumbai, India).

<table>
<thead>
<tr>
<th>No.</th>
<th>Parameter</th>
<th>Relevance</th>
<th>Desirable Criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Surface Pressure</td>
<td>Prevent alveolar collapse by its ability to lower surface tension at air-liquid interface</td>
<td>High surface pressure</td>
</tr>
<tr>
<td>2</td>
<td>Spreading time</td>
<td>Spread rapidly to form a monolayer at an air-liquid interface</td>
<td>Low spreading time</td>
</tr>
<tr>
<td>3</td>
<td>Respreading</td>
<td>Ensure reentry of surfactant from subphase to interface and prevent surfactant depletion at interface</td>
<td>High respreading ratio</td>
</tr>
<tr>
<td>4</td>
<td>Compressibility</td>
<td>Ensure attainment of low surface tension without collapse</td>
<td>Low compressibility</td>
</tr>
<tr>
<td>5</td>
<td>Hysteresis</td>
<td>Vary surface tension with area during cycling</td>
<td>High hysteresis area between compression and expansion isotherms</td>
</tr>
<tr>
<td>6</td>
<td>Recruitment</td>
<td>Increase the number of alveoli recruited during inspiration</td>
<td>High recruitment index</td>
</tr>
</tbody>
</table>
SCORING OF SURFACTANTS

Table 2. Compounds studied

<table>
<thead>
<tr>
<th>Compound</th>
<th>Abbreviation</th>
<th>Source</th>
<th>Purity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipalmitoyl phosphatidylcholine</td>
<td>PC</td>
<td>Sigma</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>PE</td>
<td>Sigma</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>Phosphatidylglycerol</td>
<td>PG</td>
<td>Individual phospholipids from Sigma</td>
<td>&gt;99%</td>
</tr>
<tr>
<td>Dipalmitoyl phosphatidylcholine-phosphatidylethanolamine (2:3)</td>
<td>PCPE</td>
<td>&gt;99%</td>
<td></td>
</tr>
<tr>
<td>Dipalmitoyl phosphatidylcholine-phosphatidylglycerol (2:3)</td>
<td>PCPG</td>
<td>Individual phospholipids from Sigma</td>
<td>&gt;99%</td>
</tr>
</tbody>
</table>

Chemicals-Glaxo). Chloroform was used as the spreading solvent.

Wilhelmy balance measurements. Dynamic surface pressure-area isotherms of solvent spread interfacial films were measured with a Wilhelmy surface balance (LB 5000, KSV Instruments). Highly pure triply distilled and deionized water (Milli-Q UV Plus system, Millipore) and acetone were used in all balance cleaning procedures. For subphase formation, sterile Ringer-lactate solution, which contains (in meq/l) 131 Na\(^+\), 5 K\(^+\), 4 Ca\(^{2+}\), 29 bicarbonate as lactate, and 111 Cl\(^-\), was used after adjusting to a pH of 7.4 by adding (dropwise) concentrated NaOH. This choice of subphase solution is in accordance with the chemical composition of the alveolar fluid as studied by Nielson (17).

Surfactants were dissolved in chloroform to a concentration of 1 mg/ml, and the solution was spread dropwise from a syringe at the air-water interface under surface excess conditions. This refers to a high initial surface concentration in which the amount of surfactant spread was greater than that required for a monolayer coverage (15 \(\text{Å}^2/\text{molecule}\)). Experimental temperature was maintained at 37 \(\pm 0.5\) \(^\circ\)C. A time span of 10 min was allowed for solvent evaporation before dynamic cycling was commenced. Surface pressure was monitored continuously from the force on a sand-blasted platinum slide. Seven successive cycles of compression-expansion at the interface were recorded between maximum and minimum areas of 772.5 and 193.1 cm\(^2\) (compression ratio of 4:1) at a constant speed of 1.2 cycles/min. The compression ratio of 4:1 is much higher than that found in normal respiratory cycles (15, 16). However, this ratio was used in the present study to obtain information regarding the respreading capability of the surfactants. This was done by comparison of collapse plateau ratios, which could be obtained only on maximum compression of the films past collapse. The speed of 1.2 cycles/min was the highest possible by the Wilhelmy balance used and was thus chosen for the experiment. However, speeds of 40 cycles/min, as is possible in a pulsating bubble surfactometer, would be more relevant to the respiratory cycle of neonates (23).

The maximum surface pressure on end compression was also noted. Figure 1 shows a schematic diagram of a surface pressure-area isotherm as measured for cycles 1 and 7 and defines the parameters calculated. Spreading of surfactant films at the air-liquid interface was compared by the time taken to reach a surface pressure of 45 mN/m. Compressibility was calculated as \((dA/d\pi)/A\), where \(\pi\) is the surface pressure (in mN/m) and \(A\) is the corresponding trough area (in cm\(^2\)) (10). By modifying the definition by Hallman et al. (7), we could calculate the compressibility between trough areas of 300–500 cm\(^2\). This would permit comparison at 54% area reduction, which is comparable to a change in area from total lung capacity to functional residual capacity and would hence be of physiological relevance (5, 19, 21, 24). Dynamic respreading was characterized by collapse plateau ratio criteria of Turcotte et al. (28). On a given \(\pi-A\) compression curve, a vertical line is drawn at the point of steepest slope and a horizontal line is drawn at the postcollapse region. The distance from the intersection of these lines to the end of the compression stroke is defined as the collapse plateau length. The respreading ratio is given by the ratio of the lengths of these plateaus for the seventh and first cycles. A ratio of 1 indicates complete respreading, and a ratio of 0 indicates no respreading. Area under the curve for the first compression cycle was calculated as a measure of hysteresis. The slope of the initial steep part of the expansion curve of the seventh cycle was calculated as the recruitment index, a measure of recruitment (20). These calculated parameters were the bases of the relative scoring system that is described next.

Devising a relative scoring system. To compare the surfactants based on their overall performance, a relative scoring system was designed based on six parameters. Each parameter varies over a range from zero to a maximum endpoint value, described later for each parameter. The range of each parameter from zero to the maximum value was divided into 10 equal subranges. Each subrange was then given a relative score from 1 to 10 in the order of increasing desirability. This was also in order of increasing magnitude for all parameters,

![Fig. 1. Schematic of a surface-pressure area isotherm defining the parameters studied. \(\pi\), Surface pressure (in mN/m); \(A\), corresponding trough area (in cm\(^2\)).](http://jap.physiology.org/Downloadedfrom/10.23033)
with the exception of spreading time and compressibility. Because a quicker spreading (lesser time in seconds) and a lower compressibility are desirable (6), these parameters were scored from 1 to 10 in the order of decreasing magnitude. Although the setting of endpoints of the range (as described next) is arbitrary, the scores give a common relative method of comparison for overall performance of the various surfactants. Table 3 shows the details of the relative scoring system.

The maximum endpoint of parameters having a definite, natural, absolute maximum value was the maximum value itself. For example, for surface pressure, the value of 70 mN/m at 37°C is equivalent to a minimum surface tension of <1 mN/m. This is the ideal for a surfactant, and, accordingly, 70 mN/m was taken as the maximum endpoint of surface pressure and 0 as the minimum endpoint. The range from 0 to 70 was divided into 10 subranges with the 63–70 subrange being allotted the highest possible relative score of 10. For criteria of respreading by plateau, a value of 1 would indicate maximal respreading. This is an absolute maximum, and by definition the ratio cannot be greater than 1; hence, the value of 1 was taken as the maximum endpoint. The range from 0 to 1 was divided into 10 subranges, and the 0.9–1.0 subrange was allotted a relative score of 10.

For other parameters, however, the maximum value is not defined in absolute terms in the literature, but certain values are known to be high. In such cases, the maximum value obtained among the compounds tested was used for subrange estimation for each relative score. Also, the subrange being allotted the highest possible relative score of 10 was left open ended to accommodate for compounds that have higher values than the maximum obtained experimentally by the test compounds. For hysteresis area, in terms of values than the maximum obtained experimentally by the test ended to accommodate for compounds that have higher values than the maximum obtained experimentally by the test compounds, the subrange allotted the highest possible relative score of 10 was left open ended as ≥0.54 to accommodate values even higher than 0.60. Similarly, for spreading time, a subrange of 18–20 s would accommodate the highest experimental value obtained; however, a relative score of 1 was allotted to an spreading time >18 to accommodate values even higher than 18 s.

Although a relative score of 10 is allotted to a respreading ratio of 0.9–1.0, as mentioned earlier by Notter et al. (20), it is not maximal respreading but a good respreading that is required to allow reentry of molecules into the interface without adversely affecting the hysteresis area. Hence, a respreading ratio of 0.8 might prove better than that of 1.0 when taking into account the performance with respect to both the respreading as well as the hysteresis area. The present relative scoring system would allow determination of the correct trade-off between hysteresis and respreading to obtain high relative scores in each of these two criteria.

A surfactant was considered to fail a particular criterion if it obtained a relative score in that criterion of <5 on a maximum possible scale of 10 (i.e., it performed less than half of the maximum possible). A total relative score was defined as the sum of the relative scores obtained in each of the criteria, and the maximum obtainable total relative score was 60. An average surfactant efficiency index was defined as the total relative score divided by 6, and this index had a maximum possible value of 10.

RESULTS

Figure 1 shows a schematic diagram of the typical surface pressure-area isotherm as measured for cycles 1 and 7 and defined the parameters calculated. Figure 2 shows the resulting actual values of the maximum surface pressure, spreading time, compressibility, respreading ratio, hysteresis area, and recruitment index of the various surfactants and mixtures studied, as measured and calculated from our experiments.

PC and PCPG were found to have very high surface pressure values, above 63 mN/m (Fig. 2A). Exosurf had a slightly lower surface pressure than that of ALEC, but both these surfactants had values lower than those of PC and PCPG. On comparison of spreading time (Fig. 2B), both PC and PE were found to have poor spreading time individually, whereas a binary mixture of the two, PCPE, had a very quick spreading (spread-

Table 3. A relative scoring system for comparison of overall efficacy of the surfactants

<table>
<thead>
<tr>
<th>Relative Score</th>
<th>Surface Pressure, mN/m</th>
<th>Spreading Time, s</th>
<th>Compressibility, mN/m</th>
<th>Respersading Ratio</th>
<th>Hysteresis Area, $10^{-4}$ mN/m²</th>
<th>Recruitment Index, $10^{3}$ mN/m³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0–7</td>
<td>≥18</td>
<td>≥0.54</td>
<td>0–0.1</td>
<td>0–1,500</td>
<td>0–0.06</td>
</tr>
<tr>
<td>2</td>
<td>7–14</td>
<td>16–18</td>
<td>0.48–0.54</td>
<td>0.2–0.3</td>
<td>1,500–3,000</td>
<td>0.06–0.12</td>
</tr>
<tr>
<td>3</td>
<td>14–21</td>
<td>14–16</td>
<td>0.42–0.48</td>
<td>0.3–0.4</td>
<td>3,000–4,500</td>
<td>0.12–0.18</td>
</tr>
<tr>
<td>4</td>
<td>21–28</td>
<td>12–14</td>
<td>0.36–0.42</td>
<td>0.4–0.5</td>
<td>4,500–6,000</td>
<td>0.18–0.24</td>
</tr>
<tr>
<td>5</td>
<td>28–35</td>
<td>10–12</td>
<td>0.30–0.36</td>
<td>0.5–0.6</td>
<td>6,000–7,500</td>
<td>0.24–0.30</td>
</tr>
<tr>
<td>6</td>
<td>35–42</td>
<td>8–10</td>
<td>0.24–0.30</td>
<td>0.6–0.7</td>
<td>7,500–9,000</td>
<td>0.30–0.36</td>
</tr>
<tr>
<td>7</td>
<td>42–49</td>
<td>6–8</td>
<td>0.18–0.24</td>
<td>0.7–0.8</td>
<td>9,000–10,500</td>
<td>0.36–0.42</td>
</tr>
<tr>
<td>8</td>
<td>49–56</td>
<td>4–6</td>
<td>0.12–0.18</td>
<td>0.8–0.9</td>
<td>10,500–12,000</td>
<td>0.42–0.48</td>
</tr>
<tr>
<td>9</td>
<td>56–63</td>
<td>2–4</td>
<td>0.06–0.12</td>
<td>0.9–1.0</td>
<td>12,000–13,500</td>
<td>0.48–0.54</td>
</tr>
<tr>
<td>10</td>
<td>63–70</td>
<td>0–2</td>
<td>0–0.06</td>
<td>≥13,500</td>
<td>≥13,500</td>
<td>≥0.54</td>
</tr>
</tbody>
</table>
ing time <3 s). The presence of hexadecanol in Exosurf also improved the spreading time of PC and was found to spread faster than ALEC. PCPG had a quicker spreading than that of ALEC, and thus a binary mixture having 40% PC was found to spread quicker than one with a higher PC concentration (ALEC).

Both of the commercial surfactants tested had very low values of compressibility, <0.06 m/mN (Fig. 2C). In fact, all the compounds tested except for PE had low values of compressibility. PC was found to have a very low respreading ratio (Fig. 2D), which was not improved even by the addition of hexadecanol. This was evidenced by the poor respreading ratio of Exosurf. ALEC, PCPE, and PCPG were found to have high respreading ratios; hence, addition of PE or PG to PC was found to be beneficial with regard to respreading. PC, PCPG, and Exosurf had high hysteresis areas (Fig. 2E). On comparison of the recruitment index (Fig. 2F), PC and Exosurf were found to have high recruitment indices, whereas ALEC had a very poor recruitment index. On the basis of these results, an index of the overall performance of these surfactants and test compounds was obtained by calculating their average surfactant efficiency indices as discussed below.

**DISCUSSION**

Figure 3 shows the relative scores obtained by the various surfactants by using the scoring system described earlier. The six parameters, surface pressure (A), spreading time (B), compressibility (C), respreading ratio (D), hysteresis area (E), and recruitment index (F), are plotted for each surfactant as a bar chart. The horizontal line at a relative score of five is the border of the pass-fail criteria. Thus it is seen from Fig. 3 that ALEC was found to fail in two of the criteria, namely, the hysteresis area and the recruitment index. The other artificial surfactant, Exosurf, was found to fail in only one criterion, namely, the respreading ratio.

On the basis of the individual relative scores, an average surfactant efficiency index was calculated for the systems studied, and the results are presented in Fig. 4. The average surfactant efficiency index of Exosurf was superior to that of ALEC (Exosurf obtained 7, whereas ALEC obtained 5.7).

PC also had a high average surfactant efficiency index (7, same as that of Exosurf). However, it is considered to have performed worse than Exosurf because, apart from failing to meet the respreading cri-
In the spreading time category, it obtained a relative score of five, which can be considered to be a borderline performance. This is in accordance with previous findings of poor spreading time (33) and poor respreading (14) of PC. Because ALEC performed well in both of these parameters, it is seen that a binary film of PC/PG in the ratio of 7:3 causes an improvement over that of PC with respect to spreading time and respreading.

PG performed poorly in two criteria, hysteresis area and recruitment index. A binary mixture of PCPE also failed these same two criteria (hysteresis area and recruitment index). When PCPE is compared with ALEC, it is seen that they failed the same two criteria and the average surfactant efficiency index of PCPE was slightly higher than that of ALEC (PCPE = 5.8 and ALEC = 5.7). When the performance of PCPE is compared with that of pure PE, it is seen that the poor spreading time and compressibility of PE films were compensated by the binary PCPE mixture (a relative score of 1 each for spreading time and compressibility of PE was improved to 10 and 8 for spreading time and compressibility, respectively, of PCPE). However, no beneficial effects on hysteresis area and recruitment were seen (both PE and PCPE failed these two criteria with the very low relative scores of 1 or 2 each).

The binary film of PCPG did not fail even a single criterion; in fact, the average surfactant efficiency index of this combination was the highest among the compounds tested (average surfactant efficiency index of PCPG was 7.7 with individual relative scores ≥5 for each criterion). It performed better than both of the commercial surfactants (ALEC and Exosurf).

A binary mixture of PC with PG containing a low concentration of PC (40%) was found to be a promising candidate for exogenous surfactant therapy in RDS based on the scoring system described and was applied here. Of course, it is to be remembered that in vitro studies do not always provide an accurate determination of in vivo exogenous surfactant efficacy due to inhibitory effects of plasma proteins and the in vivo metabolism of the exogenously administered surfactant. However, the present scoring system based on in vitro criteria would provide a useful method of screening for possible candidates for successful exogenous surfactant therapy in RDS, which could then undergo further animal and clinical trials.

In conclusion, the present study identified and categorized the various criteria of physiological relevance.
to surfactants as exogenous therapy in RDS. A relative scoring system was devised to obtain an overall picture of the performance of a surfactant formulation as detected by in vitro tests using a Wilhelmy balance. On the basis of the criteria and the relative scoring system, the performances of the compounds were compared and each compound was said to pass or fail criteria on the basis of the relative score obtained by it. The following conclusions were drawn from the relative scores obtained by the surfactants.

1) A binary film of PC and PG in the ratio 2:3 could be a favorable combination as an exogenous surfactant in RDS. It fulfilled all the criteria tested, was the best combination among the surfactants tested, and performed better than both ALEC and Exosurf. The superior performance of PCPG compared with ALEC showed that a mixture with 40% PC performed better than that with 70% PC when combined with PG.

2) A binary film of PC and PE failed two criteria: hysteresis area and recruitment index. ALEC also failed these two criteria. PCPE (2:3) obtained a slightly higher average surfactant efficiency index and can be considered of comparable or slightly higher efficacy than ALEC as detected by in vitro testing.

3) Exosurf was the second most successful combination among the surfactants tested. It performed better than ALEC and failed only one criterion, that of respreading. Although PC obtained the same average surfactant efficiency index and failed the same criterion of respreading ratio as Exosurf, it also had a borderline performance in spreading time and hence was considered poorer than Exosurf as a surfactant formulation.

The relative scoring system devised here gives an idea about the overall performance of surfactants with regard to various in vitro criteria of physiological relevance to exogenous therapy in RDS. Presently, the scoring system has been applied to criteria determined by the Wilhelmy technique. This has also been applied to criteria determined by other in vitro techniques of greater relevance to the neonatal respiratory system, like the pulsating bubble surfactometer (unpublished observations). Also, the present scoring system utilized equal weights of one each for all the parameters considered. However, the exact importance of one parameter relative to the other should determine the strength of each parameter, e.g., for two surfactants failing a single criterion each (respreading and hysteresis, respectively), the better surfactant would be determined by the relative importance of respreading compared with hysteresis area, i.e., a higher weight would be given to the more important parameter. These relative scores now open the possibility of further refinement of the ranking system of surfactants by determining the relative importance of each criterion and assigning suitable weights to them (unpublished observations).

The relative importance of each parameter will obviously differ for the specific clinical condition for which the surfactant is intended. Thus this scoring system can be modified and extended to diseases other than RDS by assigning different weights to the various parameters to obtain disease-specific rankings of surfactants (unpublished observations).

This relative scoring system, as well as further refinements of the same, would be useful screening tests for surfactant formulations that can be used before animal and clinical trials. Furthermore, pitfalls in the properties of existing surfactant formulations can be determined by this system, which would help focus future research for specific improvement of the products.

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