Determination of Dissolved Oxygen in Heterogenous Systems Particularly in Emulsions and Oily Liquids[1]

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Abstract: The content of dissolved oxygen was determined by four independent methods in a series of non-aqueous or heterogenous systems. The Lex-O_2-Content Analyzer represents a fast and simple apparatus that employs a coulometric oxygen assay with Hersch cell detection. A comparison of the results with different methods demonstrates the reliability of the Lex-O_2 in the determination of oxygen dissolved in heterogeneous or non-aqueous systems. Therefore, this apparatus can be recommended for the measurement of oxygen in oxygenator or perfusion fluids, as well as in blood substitutes or other oxygen transporting systems.

The accurate and simple determination of dissolved oxygen in complex systems like pharmaceutical dosage forms, in oxygenator liquids or similar systems with oxygen transferring functions has proven difficult. There are several chemical and physical methods to measure oxygen in aqueous solutions or in gaseous phases. They generally yield satisfactory and reliable results without major problems. Conditions change, however, when oxygen content is assayed in organic solvents, oily liquids, or heterogenous dispersed systems, for instance in emulsions. Membrane functions of coated electrodes are impaired by solvents or oils, which rules out the use of Clark-type electrodes. Polarographic methods are disturbed by surfactants, emulgators or similar agents, while iodometric titrations are only applicable in clear solutions. UV-spectrophotometric determinations become useless in many cases for the same reason. The van Slyke tonometric determination has the disadvantage of being rather time consuming. Gas chromatography in principle is a suitable method to overcome the assay problems, but there are disadvantages in handling and in column pollution by oily or non-aqueous ingredients.These problems may be controlled by attaching a pre-column or with the use of headspace injection. According to our experience a hermetically closed apparatus for coulometric analysis with the Hersch cell for oxygen reduction as the detector (1) proved to be a very sensitive device to determine oxygen in heterogenous systems in an elegant and economic way (Cavitron/Lex-O_2-Content Analyzer[5]). The reliability of this method was checked by comparing the results of blood oxygen determination with conventional methods (2–5), but it has not yet been validated for heterogenous hydrophilic/lipophilic systems.

Materials and Methods

Description of the Device

The operating principle of the used oxygen analyzer (Cavitron/Lex-O_2-Content Analyzer[5]) is shown in Fig. 1.

After rinsing the apparatus absolutely free of oxygen by a carrier gas consisting in 97 % N_2, 2 % H_2 and 1 % CO, the flow rate of the gas is set at 50 ml/min by means of a flow meter. The removal of every trace of oxygen is ensured by an interposed catalyst. For each determination samples of 20 µl each are injected through the septum into the distilled water of the scrubber by means of a Hamilton syringe. The oxygen contained in the sample is expelled and carried by the gas stream, through the tubular Hersch cell (Fig. 2), where it is reduced, producing an electric current proportional to the amount of dissolved oxygen.

Cathode Reaction

$$O_2 + 2\,H_2O + 4\,e^- \rightarrow 4\,OH^-$$

Anode Reaction

$$2\,Cd + 4\,OH^- \rightarrow 2\,Cd(OH)_2 + 4\,e^-$$

The Hersch cell consists of a carbon cathode and a cadmium anode separated by a synthetic diaphragm, impregnated with potassium hydroxide. In contrast to earlier published determinations with Lex-O_2-Content Analyzers (2–5), the more recent model used here has a more convenient and exchangeable scrubber.

Validation Experiments

The quantitative displacement of dissolved oxygen by the carrier gas from heterogeneous dispersions or non-aqueous liquids was thought to be the most probable error source in applying this indirect method. The following experiments were carried out to test the accuracy and the precision of coulometric

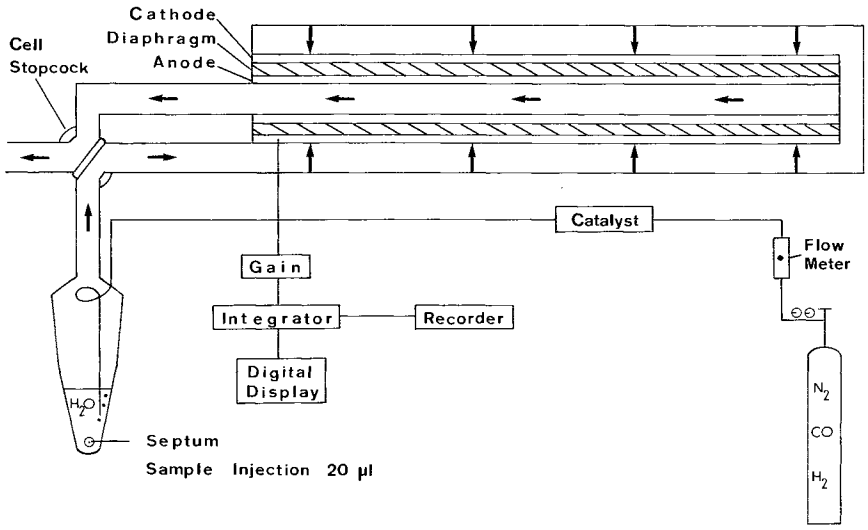

Fig. 1 Block diagram of the oxygen content analyzer (Cavitron/Lex-O_2-Content Analyzer[5]).

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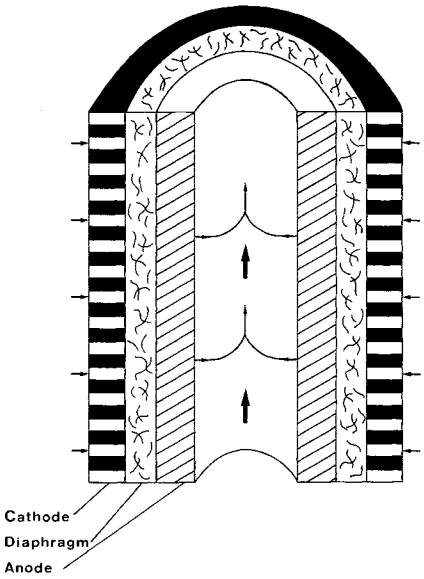

Fig. 2 Construction of the Hersch sensor.

determinations performed with the Lex-O_2-Content Analyzer. The samples were:

a) *Water,* because its oxygen content at certain temperatures and under certain pressures is well known;

b) *Siloxan Tego* (Th. Goldschmidt AG, D-4300 Essen), a silicon surfactant commonly used as a model substance for emulsifying or amphiphilic ingredients;

c) *Silicon oils* (AK 50 and AK 100) (Wacker Chemie GmbH, D-8000 München) as well as two *fatty alcohols,* octanol and isooctanol, as representative models for lipophilic substances;

d) *Silicon emulsion* as a two-phase system;

e) *Sodium sulfite solution* as a blank solution free of oxygen.

Before analysis all these samples were saturated with oxygen, and one water sample with air. In the latter case saturation correlates with the partial pressure of oxygen in air. First these preparations were repeatedly measured with the Lex-O_2 apparatus (at least 6 times and up to 54 times to determine the precision and the reproducibility of this method) (Table I). All preparations were also measured by gas chromatography, except for the silicon surfactant and the silicon oil AK 100. Finally the oxygen contents in the two water samples were determined with a Clark-type electrode (Oxi 521) (WTW GmbH, D-8120 Weilheim), and in four lipophilic samples by the van Slyke tonometric method. These latter determinations should indicate the accuracy of the coulometric method, and further test for complete displacement of oxygen from the samples.

Experimental Conditions

a) Lex-O_2:

20 µl of each sample was injected by a Hamilton syringe into the scrubber of the hermetically closed Lex-O_2 apparatus. One measurement took approximately 5 minutes.

b) Gas Chromatography (6):

A Perkin Elmer F22 gas chromatograph with M3 Integrator was used with headspace injection from 5 ml sealed test tubes, prerinsed with helium. The sample volume was 2 ml (liquid saturated with oxygen), and the sample temperature 80° C. GC conditions were as follows: Column: Perkin Elmer molecular sieve 5 Å; detector: catharometer (thermal conductivity detector) (The detection limit is approximately 10^{-6} mg/ml); detector temperature: 40° C; injector temperature: 40° C. One determination took approximately 20 minutes.

c) Clark-type-electrode (Oxi 521) (7):

The electrode was dipped directly into the oxygen-saturated samples. One measurement required approximately 2 minutes.

d) van Slyke (8):

Samples of 25 ml each were placed under vacuum until no more bubbles could be observed, and subsequently tonometered with oxygen until the pressure remained constant. Depending on viscosity or foaming one measurement took from 2 to 8 hours.

Table I: Summary of the Results of Oxygen Content Obtained with Four Different Methods

Substance	Average values Lex-O_2 % O_2[a]	n[b]	s_n[c]	GC % O_2	n	s_n	Single values Clark-type electrode (Oxi 521) % O_2	n	van Slyke % O_2	n
Water, air saturated 18° C	0.71	54	0.03	0.69	16	0.17	0.65 0.69 0.64	3	–	–
Water	2.35	26	0.08	2.19	17	0.40	2.29 2.25 2.22	3	–	–
Siloxan Tego®	9.26	6	0.22	–	–	–	–	–	8.99/8.17 9.81	3
Silicon oil AK 50	22.18	27	0.77	15.05	22	0.87	–	–	–	–
Silicon oil AK 100	20.60	6	0.51	–	–	–	–	–	22.00 19.30	2
Silicon emulsion	7.06	24	0.28	6.26	22	0.35	–	–	7.50	1
Octanol	14.73	20	0.43	9.31	17	0.65	–	–	13.67/12.72 14.63/14.63	4
Isooctanol	16.05	21	0.20	10.62	17	1.48	–	–	–	–
Sod. sulfite sol.	0.00	10	0.00	–	–	–	–	–	–	–

[a] = vol % O_2/1013 hPa/37° C; [b] = number of determinations; [c] = standard deviations.

Results and Discussion

The oxygen contents measured by the four methods are summarized in Table I.

The results with oxygen and air saturated water samples demonstrate that the Lex-O_2 method is precise and compares well with Clark-type electrode measurements. Because of the higher viscosities and more complicated handling the other samples show increased standard deviations.

In comparison with the other methods, the Lex-O_2 assay appears to provide realistic results under various conditions. The results with the Clark-type electrodes cannot be directly compared in each case, because these electrodes work only in aqueous systems. In principle the GC-headspace method is also suitable to determine oxygen contents in lipophilic substances or in heterogeneous systems. The precision of this method is dependent on the detection limits of the catharometer, which is in the same range as that of the Lex-O_2. The results are very similar to those obtained with the Lex-O_2, but the standard deviations are often considerably higher. This is certainly due to the more complicated procedure, e.g. the need for absolute exclusion of ambient air by rinsing with helium, and the transfer of the oxygen-saturated samples by means of a syringe into the oxygen-free and sealed test tubes. Even most careful processing during these manipulations cannot prevent certain losses of oxygen, as the samples cannot be applied directly. Another disadvantage is the fact that no internal standard can be used, because each internal standard will get into solubility competition with oxygen. In this case less oxygen would be dissolved.

The oxygen values measured with the van Slyke method also correspond well with those of the Lex-O_2, but this method is extremely time consuming particularly in the case of foaming or highly viscous systems. Another disadvantage is the possibility of water losses during the evacuation phase if there is not sufficient cooling.

Conclusion

The reported results of oxygen content determinations in selected aqueous, oily, and heterogeneous multi-phase systems by means of four different methods demonstrate that the coulometric oxygen assay with Hersch cell detection is simple, fast and accurate. Moreover, it can be applied to non-aqueous systems and complex multi-phase dispersions like emulsions or suspensions.

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Chemistry of Primaquine I: Acylation of the Quinoline Ring Portion

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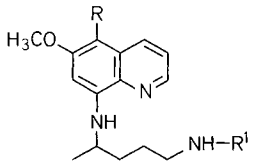
Abstract: Investigation of derivatization of primaquine (**1**) with various perfluoroacylating reagents revealed that the quinoline ring portion of the drug undergoes unexpectedly facile acylation. Chemical and spectral evidence in support of the assigned structures is presented and discussed. Results of the evaluation of these new primaquine derivatives for antimalarial activity is reported.

In the course of an ongoing program to study the chemistry and metabolic fate of primaquine (**1**), a known radical curative agent for relapsing malaria, it was reported that the 5 position of the quinoline ring is unexpectedly reactive (1–3). This high reactivity was indicated by the rapid exchange of H-5 in D_2O (1) and by the formation of an unusual bistrifluoroacetyl derivative (**2**) on treatment of primaquine with excess trifluoroacetic anhydride (2). Finally, the formation of a novel 5-5 methylene linked dimer (3) by certain microorganisms and its ready chemical synthesis provided additional confirmation of this chemical reactivity of the 5 position. Raj Gopalan et al. (4) have reported the formation of an *N,N*-bisheptafluorobutyramide derivative on treatment of primaquine with heptafluorobutric anhydride (HFBA). However, they did not give any spectral evidence in support of their assigned structure. This prompted us to reinvestigate the reaction of primaquine with heptafluorobuytric anhydride and compare the outcome with our results of formation of bistrifluoroacetate **2**. We also wished to prepare sufficient quantities of these materials for biological evaluation as antimalarials.



1 R = R^1 = H
2 R = R^1 = $COCF_3$
3 R = R^1 = $COCF_2CF_2CF_3$
4 R = $COCF_2CF_2CF_3$; R^1 = H
5 R = H ; R^1 = $COCF_2CF_2CF_3$
6 R = $COCF_3$; R^1 = H
7 R = H ; R^1 = $COCF_3$

Materials and Methods

TLC was done on Sil G-25 UV_{254} (Brinkman Instruments, Inc.) plates using A) methanol: chloroform (3:97) and B) (8:92) as solvents. The compounds were

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