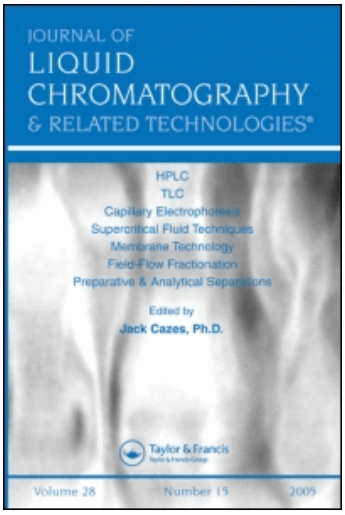

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ELECTROKINETIC DETECTION IN REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY PART I. VOLATILE FATTY ACIDS

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ABSTRACT

Conditions of electrokinetic detection are elaborated for volatile fatty acids (acetic, propionic, isobutyric and valeric) in reversed phase high performance liquid chromatography, HPLC. A simple, open tube capillary electrokinetic detector was constructed. The working unit of the detector was a capillary made of polytetrafluoroethylene, PTFE, or stainless-steel. The output signal of the detector was the streaming potential of the capillary which was measured against earth. When chromatograms were developed in non-buffered polar solution of mobile phase, the retention volume, V_R, of acids increased with the increase of concentration of acids in the sample. The detectability of the detector with PTFE capillary used as a working unit was of the order of 10^{-12} mole for a 5 μl injected sample and the reproducibility was 5% (relative standard deviation, R.S.D., for ten consecutive injections). The linear dynamic range was close to two orders of magnitude of the concentrations of acids.

INTRODUCTION

In the search for a new reliable universal detector for liquid chromatography, electrochemical detectors based on the measurement of streaming current and streaming potential have been recently tested and applied [1-3]. In 1964 a patent was drawn for the first electrokinetic detector for liquid chromatography by A n d o *et al.* [4]. The working unit of that detector is an open tube dielectric capillary or

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packed with a dielectric support. At both ends of that capillary there are Pt or Au electrodes between which the streaming potential is measured. The detector has been used for reversed phase liquid chromatography. In further models of electrokinetic detectors the streaming current was measured. The latter devices were applied both for normal and reversed phase liquid chromatography, LC. The streaming current and streaming potential encoutered under conditions of reversed phase LC [4, 5] are described by the S m o l u c h o w s k i equation [3, 6–9], and for flow conditions relevant to normal phase LC [10–15] the streaming current is described by the G a v i s and K o s z m a n theory [16–20]. It was also shown that the electrokinetic detection signal could be measured directly at a chromatographic column [12–14]. This is advatageous especially for capillary liquid chromatography where extra-column effects of chromatographic band broadening are crucial [12–14]. Only an electrokinetic detector makes it possible to measure the exclusion volume directly during chromatographic elution [11]. Moreover, the electrokinetic detector is useful for studing the so-called "vacant" peaks [11]. The spray impact detector operates on a similar principle as the electrokinetic one [21]. The spray impact detector is based on the L e n a r d effect discovered in 1892 where the electric charge is non-uniformly distributed when liquid droplets are sprayed onto the conducting target.

In the present paper the results are given obtained with the use of our simple electrokinetic detector as applied to the detection of volatile fatty acids in the effluent of the reversed phase chromatographic column. In the detector the streaming potential was measured at the working unit which consisted of an open dielectric or metal tube capillary.

Volatile fatty acids play an important role in food industry when manufacturing wine, cheese or processings fruit as flavour ingredients. Also their determination in blood plasma and lymph may make it possible to follow the mammals metabolism paths [22]. So there is a need for the determination of these acids both in trace amounts and in a wide range of concentrations. Volatile fatty acids were determined chromatographically many times before especially by gas [23–28] and thin layer chromatographies [29, 30]. Classical and high performance LC were also used for the purpose [31–36]. However, serious detection problems were encountered **for acids with short aliphatic chains, which led to the need of applying complex detection methods or on or off line derivatization of substances in the sample.** B u s h *et al.* [22] succeeded to separate volatile fatty acids, i.e. acetic, propionic, butyric, valeric and isovaleric ones, by reversed phase HPLC with UV (210 nm) detection on the C_{18} μBONDPAK column in less than 20 minutes. The quoted authors used MeOH + 0.1 M NaH_2PO_4, pH 3.5 (10 + 90) v/v as the mobile phase. It seemed interesting to repeat their measurements with another type of detection for comparison, since the UV (210 nm) detector is sensitive to even trace amounts of contaminations. Volatile fatty acids $C_4 - C_{22}$ were also recently separated by the reversed phase HPLC on a Nukleosil 7 C_{18} column using electrokinetic detection in which the

streaming current was measured [5]. The disadvantage encountered was, however, the strong dependence of the baseline current on the flow rate, J, of the mobile phase. With the increase of J by 0.1 ml min^{-1} the baseline current increased by 1.8×10^{-8} A, while the heights of the chromatographic peaks were only of the order of $10^{-9} - 10^{-8}$ A. Thus a minor instability in J already deteriorated markedly the reproducibility. **Besides, the size of the sample used was as large as 2 cm^3 and was comparable to the dead volume of the column. Such a large sample size is considered disadvantageous in HPLC because of the disturbance to the column it causes during sample injection.**

EXPERIMENTAL

The design and electronic diagram of the electrokinetic detector is shown in Fig. 1. The eluate from the chromatographic column (1) is pumped through a 50–60% porous filter (2), and next through a stainless-steel junction (16 x 1 mm I.D.) (4) to the working capillary (3). A PTFE or stainless-steel 1H18N9T open tube capillary of 0.4 or 0.2 mm I.D., respectively, and 20 mm long was used as the working unit of the detector. The stainless-steel capillary was insulated from the rest of the system by a PTFE tightening ferrule (5). The eluate was drained off to waste by the stainless-steel capilary (6) of 1 mm I.D. which was insulated from the earthed screen (7) by a PTFE connector (8). The chromatographic column and its pumping system, joint and the screen were earthed. The streaming potential, $\Delta\varphi$, as measured as the potential of the capillary (6) against earth using an electronic circuit with an operational RCA-715 amplifier of $10^{11}\,\Omega$ input resistance (9). The potential $\Delta\varphi$ was recorded with a Mera-Tronik V-543 (Warszawa, Poland) multimeter and Radelkis OH-814/1 (Budapest, Hungary) potentiometric recorder. The operational amplifier was fed from a stabilized Unitra-Unima ZT-980-2 (Warszawa, Poland) electric feeder. The Institute of Physical Chemistry, Polish Academy of Sciences (Warszawa, Poland) chromatograph HPLC type 302 was used. The pump of the chromatograph was a syringe type pump with mobile phase flow rate adjusted in the range from 0.06 to 6.0 ml min^{-1}. It was equipped with a 5 μl high pressure injection valve. The column used was a stainless-steel 1H18N9T column 150 x 4 mm I.D. slurry packed by a modified viscous method [37] with LiChrosorb RP-18, 10 μm (E. Merck, Darmstadt, FRG) in a mixture of dioxane + tetrachloromethane (50 + 50) v/v at 42 M Pa, or packed with glass beads 80 mesh (B.D.H., Poole, England) by a dry method. A mobile phase solution was prepared using redistilled water and analytical grade methanol (E. Merck, Darmstadt, FRG). Other chemicals were of analytical grade (P.O.Ch., Gliwice, Poland). The mobile phase was degassed for two hours prior to chromatographic measurements in an ultrasonic bath under a water aspirator.

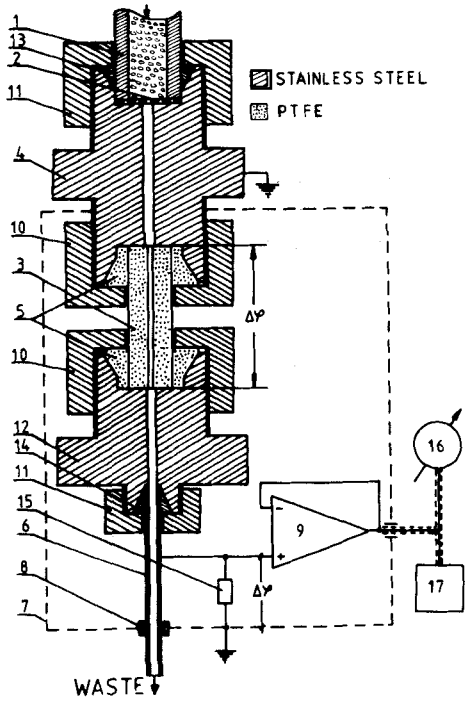

FIGURE 1. Diagram of the electrokinetic detector and its electronic set-up for the measurement of streaming potential.
1 – HPLC column packed with LiChrosorb RP-18, 10 μm,
2 – porous filter, 3 – working PTFE capillary, 4 – stainless-steel M12/M12 joint,
5 – PTFE tightening ferrule, 6 – stainless–steel draining capillary, 7 – earthed shield, 8 – PTFE connector, 9 – operational amplifier RCA–715, 10 – M12 nut,
11 – M6 nut, 12 – stainless-steel M12/M6 joint, 13 – stainless-steel 5/10 ferrule,
14 – stainless-steel 4/3.5 ferrule, 15 – 10^{11} Ohm resistor, 16 – digital voltmeter,
17 – X–t recorder.

RESULTS AND DISCUSSION

Elektrokinetic measurements were carried out using a PTFE (20 x 0.4 mm I.D.) or stainless-steel (20 x 0.2 mm I.D.) capillary. The results, if not stated otherwise, refer to the PTFE capillary.

Preliminary experiments performed without the chromatographic column showed (Fig. 2) that peak heights, $\Delta(\Delta\varphi)$, decrease with the increase of the flow rate of the mobile phase of the composition MeOH + H_2O (10 + 90) v/v which is further

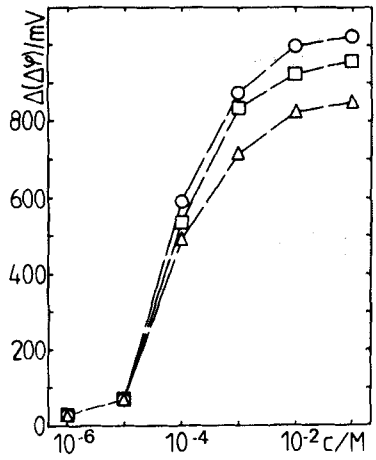

FIGURE 2. Dependence of the streaming potential changes (heights of chromatographic peaks), $\Delta(\Delta\varphi)$, on the concentration of propionic acid obtained using the electrokinetic detector with PTFE capillary (20 x 0.4 mm I.D.) without chromatographic column. Mobile phase: MeOH + H_2O (10 + 90) v/v, sample size – 5 μl. Flow rate: 0.6 (○), 1.8 (□), 3.0 (△) ml min^{-1}.

used, from 0.3 to 3.0 ml min^{-1}. The value of $\Delta(\Delta\varphi)$ was measured as the difference between the streaming potential value at a peak maximum and the background potential, $\Delta\varphi_b$. The detection limit of acids per 5 μl injection size was close to 10^{-12} mole (10^{-6} *M*) for the PTFE capillary and 10^{-10} mole (10^{-4} *M*) for the stainless-steel capillary. A similar dependence or peak heights on the flow rate and concentration has been observed for other studied substances [38]. It was found that the detectability of the detector is nearly by two orders of magnitude higher for ionic compounds (e.g. aliphatic or aromatic carboxylic acids or inorganic salts) than for nonionic ones (e.g. ketones).

Fig. 3 shows the HPLC reversed phase chromatograms of mixtures containing 10^{-4} *M* and 10^{-3} *M* of each of the acids: acetic, propionic, isobutyric and valeric in Figs. 3 a and 3 b, c, respectively. The mobile phase used was of the composition indicated earlier and the flow rate was 1.2 (Fig. 3 a, b) and 4.2 ml min^{-1} (Fig. 3 c). As it is shown in Figs. 3 b and 3 c, $\Delta(\Delta\varphi)$ changes its sign from positive to negative when the flow rate increases above a certain value, J_0. At the same time $\Delta\varphi_b$ increases. Sample injection is accompanied by two spikes, the first being negative and the second one positive (in Fig. 3 they are not separated and are indicated by a vertical segment because of the recorder delay). These spikes are caused by the flow rate disturbance which accompanies sample injection. The first negative spike which

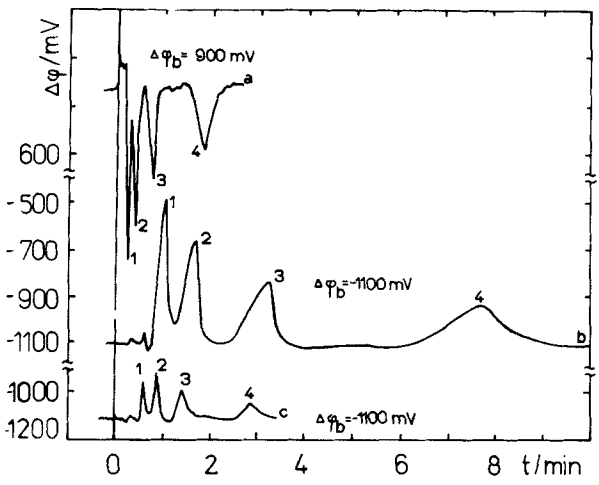

FIGURE 3. HPLC chromatograms of volatile fatty acids recorded with the use of the electrokinetic detector with PTFE capillary (20 x 0.4 mm I.D.), separated on stainless-steel column 150 x 4 mm I. D., packed with LiChrosorb RP-18, 10 μm. Mobile phase: MeOH + H_2O (10 + 90) v/v, sample size – 5 μl. Concentration of acids, in ***M***, and flow rate, in ml min^{-1}: a – 10^{-3}, 4.2; b – 10^{-3}, 1.2; c – 10^{-4}, 1.2. The peaks: acetic (1), propionic (2), isobutyric (3), and valeric (4) acid.

appears is attributed to the decrease of the flow rate, and the following positive one is due to the increase of flow rate caused by the instantaneous increase of pressure. Heights of both spikes increase with the increase of the mobile phase flow rate. Fig. 4 shows the $\Delta(\Delta\varphi)$ ***vs J*** plot for all 10^{-3} ***M*** acids. It is seen that for the smallest available flow rate, i.e. for J = 0.06 ml min^{-1} the sign of $\Delta(\Delta\varphi)$ is positive, and it decreases with the increase of flow rate. With the increase of J, $\Delta\varphi_b$ always increases. For J close to 2.7 ml min^{-1} all peaks disappear in the chromatogram, and for $J > 2.7$ ml min^{-1} the peak changes its sign. With the further increase of J the absolute value of $\Delta(\Delta\varphi)$ also increases. This is presumably due to some constant potentials, e.g. potential from the electrochemical cell or changes of the potential during sample injection (as a result of changes of capacity) interfering with the streaming potential. When the flow rate exceeds 5.4 ml min^{-1} a decrease in the absolute value of $\Delta(\Delta\varphi)$ is observed. Fig. 5 shows the dependence of the height (Fig. 5 a) and surface area (Fig. 5 b) of peaks on the logarithm of concentration of acids. The detectability for the detector with the PTFE capillary (20 x 0.4 mm I.D.) at the signal to noise ratio of two and J in the range from 0.6 to 1.2 ml min^{-1} was close to 10^{-12} mole. The peak to peak reproducibility of signals calculated as a relative standard deviation (R.S.D.) of separate measurements of the peak height in ten consecutive

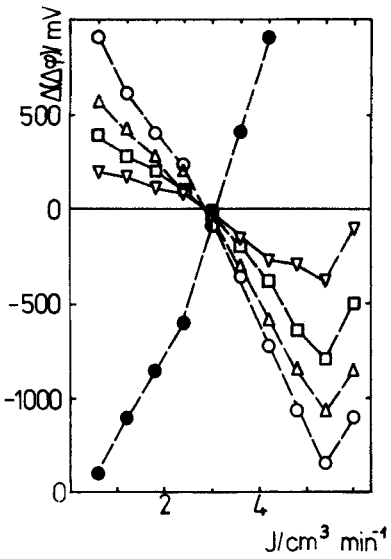

FIGURE 4. Plot of chromatographic peak height, $\Delta(\Delta\varphi)$, *vs.* mobile phase flow rate, *J*, of 5 μl sample of mixture of 1 m*M* acetic (○), propionic (△), isobutyric (□), and valeric (▽) acid. Other conditions as in Fig. 3.

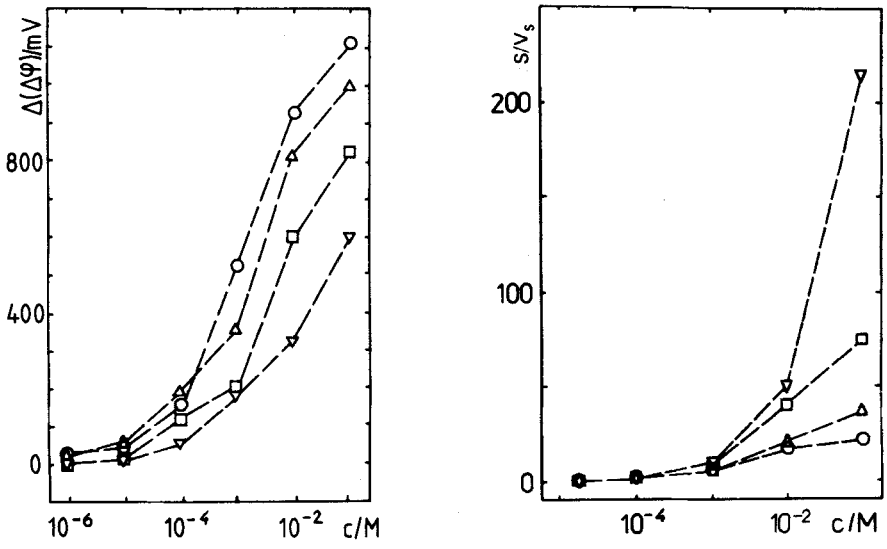

FIGURE 5. Dependence of height (a) and area (b) of the chromatographic peaks on the logarithm of concentration of volatile fatty acids: acetic (○), propionic (△), isobutyric (□), valeric (▽). Flow rate 1.2 ml min^{-1}. Other conditions as in Fig. 3.

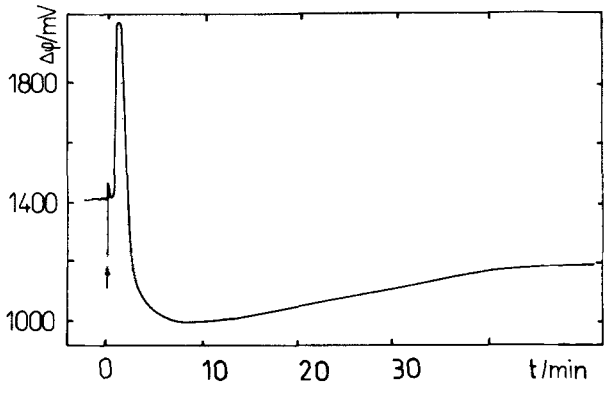

FIGURE 6. HPLC chromatogram of 1 *M* propionic acid, flow rate – 1.2 ml min^{-1}, column 150 x 4 mm I.D., packed with glass beads *ca.* 80 mesh. Other conditions as in Fig. 3.

injections each containing 1 m*M* of every acid was better than 5%, and for lower and higher concentrations was somewhat poorer and varried in the range from 5 to 15%. For smaller concentrations it was limited by the noise level of $\Delta\varphi_b$ and for higher concentrations it resulted most probably from the irreversible adsorption of acids on the surface of the capillary inner wall. Day to day reproducibility was not as good as that mentioned above. However, it could be improved by repeated sample injecting. Adsorption equilibria are then probably attained. The first peak in a series is usually lower than the subsequent ones. The value of $\Delta\varphi_b$ does not assume its orginal value when acids of concentration higher than 10^{-3} *M* are injected but remains at lower level. This creates some difficulties in peak height or surface area determinations. The orginal value of $\Delta\varphi_b$ is reached after several up to several dozen minutes. This is probably the result of the irreversible adsorption of acids. Such a bahaviour was exemplified in Fig. 6 for 1 *M* propionic acid at J = 1.2 ml min^{-1}, (150 x 4 mm I.D.) column packed with glass beads. The largest peak surface area was obtained for valeric acid (Fig. 5 b) to which corresponds the broadest band in the chromatogram (Fig. 3). The reproducibility of the peak surface area was poorer than the corresponding peak height and was approximately equal to 10% (R.S.D. for ten consecutive injections) for 1 m*M* propionic acid at J = 1.2 ml min^{-1}. The linear dynamic range was close to two orders of magnitude of the acid concentration.

The heights of the chromatographic peaks obtained using the detector with the stainless-steel capillary (20 x 0.2 mm I.D.) were about three times smaller than those obtained using a PTFE capillary. The stainless-steel capillary revealed also worse detectability, equal to *ca.* 5 x 10^{-5} *M*, and reproducibility which was better than 30% (R.S.D. for ten consecutive injections).

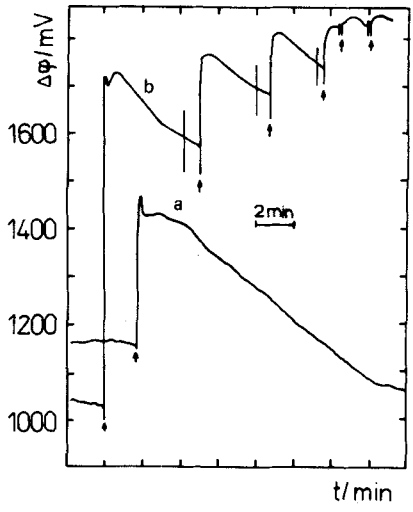

FIGURE 7. Changes of the streaming potential in time for consecutive injections of *ca.* 1 *M* TEA^+ $C_6H_5COO^-$ *ion-pair without a column. Flow rate*-0.6 ml/min, sample size – 5 μl, mobile phase: isopropanol + methylene chloride + hexane (20 + + 60 + 20) v/v/v the detector working unit – stainless-steel capillary 100 x 0.2 mm I.D.

The measurements presented above were performed using a nonbuffered mobile phase. Addition of any electrolyte e.g. buffer to the mobile phase causes a decrease of the heights of chromatographic peaks due to the increase of conductivity which results in the so-called "short circuit effect". This leads to the decrease of reproducibility and detectability. But in the absence of a buffer in the mobile phase the retention volume, V_R, was highly dependent on the concentration of acids in the sample (cf. Figs. 3 b and 3 c) which would be inconvenient for analytical purposes. This effect resulted from retaining of mainly non-dissociated substances by the reversed phase, RP, support. The degree of dissociation of acids and their pH increase with dilution, and the dissociated acids are eluted in shorter time [39]. The most pronounced changes in the V_R were observed for high concentrations of the acid. For concentrations smaller than 10^{-5} *M* these changes were practically independent of the concentration of acid. We tried to eliminate dependence of V_R on acids concentration by:

(i) Applying dilute buffer solutions. However, phosphate or citrate buffers diluted as much as 10^{-4} *M* proved useless, since their buffer capacities are insufficient to prevent V_R of acids from being independent of their concentration. When more concentrated buffers were used, $\Delta(\Delta\varphi)$ decreased drastically due to the "short circle effect".

(ii) Applying the mobile phase of high content of organic solvent MeOH or AcCN in order to decrease the degree of dissociation of acids and to eliminate the necessity of buffering the solution. This resulted in the increase of height of peaks but at the same time the retention volume of all acids decreased so much that they were eluted all together.

(iii) Separating acids using ion-pair normal phase chromatography with a buffer and tetraalkylammonium ions as the stationary phase on the LiChrosorb Si 100, 10 μm support (as it was proposed in [40,41]). However, it appeard that ion-pairs formed of acid anions (injected to the column) and tetrabutylammonium cations, TBA^+, (present in the stationary phase) were irreversibly adsorbed on the inner surface of the capillary wall, what is exemplified in a model experiment in the absence of a column shown in Fig. 7. After the injection of benzoic acid $\Delta\varphi$ returned to the value characteristic for the baseline potential after a long time (curve a). In the succesive injections smaller peaks were obtained (curve b).

(iv) Separating acids using ion-pair reversed phase chromatography. The addition of the tetraethylammonium cation, TEA^+, to the mobile phase resulted in the decrease of peaks and also of the detectability as well as of reproducibility as a result of the irreversible adsorption of TEA^+ on the inner surface of the capillary wall. We made therefore an attempt to separate acids in very dilute solutions of $TEAClO_4$ without buffers what will be described in part 2 of this work [42].

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