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Isotachophoretic separation of alkali and alkaline earth metal cations in water–polyethylene glycol mixtures

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SUMMARY

The influence of polyethylene glycol (PEG) on the effective mobilities of alkali and alkaline earth metal cations was investigated. It is shown that appropriate proportions of water and PEG in the leading electrolyte enable a complete separation of this group of cations to be achieved by capillary isotachophoresis (ITP). The high dynamic concentration ranges of the ITP determinations achieved in the operational systems based on water–PEG mixtures are illustrated by practical examples (water samples, urine, aqueous extract of apple flesh).

INTRODUCTION

Considerable attention has been paid to the separation and determination of alkali and alkaline earth metal cations by capillary isotachophoresis (ITP) because ITP is a promising method for their determination in waters[1–3], in pharmaceuticals[4,5], in industrial control[6], in biological materials[7,8] and in plant nutrition[9].

The ionic mobilities of some of the alkali and alkaline metal earth cations in aqueous solutions are close[10], so that complex equilibria play a key role in their ITP separations. In this respect, mainly negatively charged ligands[1,4,9,11] and electroneutral crown ethers[3,6,12–14] are effective in the cationic mode of the separation. The separation of alkaline earth metal cations in the anionic mode has some inherent disadvantages (a restricted pH range of the separation[2,15,16] and close effective mobilities of the metal chelates[16]).

Methanol is a suitable solvent in ITP separations of alkali and some of the alkaline earth metal cations[9,17,18], as their ionic mobilities in this solvent differ sufficiently. It was reported recently[7] that addition of a polyoxyethylene ether-type detergent (Triton X-100) to the leading electrolyte improves their separations in this solvent. This effect of Triton X-100 is understandable when the stability constants of linear polyethers with alkali metal cations in methanol are considered[19,20].

Crown ethers are useful in extractions of alkali and alkaline earth metal cations from aqueous solutions into organic solvents (for a review see, *e.g.*, refs. 20 and 21). Considerably cheaper polyethylene glycols (PEG) are also effective in this respect[22]. In both instances, the equilibria involved assume the complexation of the cations with polyethers in the aqueous phase. While the stability constants for crown ethers in the aqueous solutions are known[19–21] the corresponding data for their linear analogues (*e.g.*, PEG) are not available. Hence a reasonable assessment of the potential applicability of PEG in ITP separations of alkali and alkaline earth metal cations in aqueous operational systems was impossible. However, limited data available for linear polyethers in methanol[19,20] allow the speculation that the stability constants of PEG with the cations in aqueous solutions should be 10–10^3 lower in comparison with the cyclic polyethers. This indicates that a much higher concentration of PEG may be required to achieve effects equivalent to those of crown ethers.

The above facts led us to investigate in detail the influence of the concentration of PEG on the effective mobilities of alkali and alkaline earth metal cations and thus to assess its applicability in the ITP separations of this group of cations. The results of this investigation and relevant ITP separations of model and practical samples are presented.

EXPERIMENTAL

Instrumentation

A CS Isotachophoretic Analyzer (VVZ PJT, Spišská Nová Ves, Czechoslovakia) was used in the column-coupling configuration of the separation unit[23]. The separation unit was assembled from modules supplied by the manufacturer and others made in our laboratory. The lengths of the zones from the conductivity detectors were measured electronically[24].

Chemicals and purification

The chemicals used for the preparation of the leading and terminating electrolytes were obtained from Serva (Heidelberg, F.R.G.), Loba-Chemie (Vienna, Austria), Janssen Chimica (Beerse, Belgium) and Lachema (Brno, Czechoslovakia).

Hydroxyethylcellulose 4000 (Serva), after purification on an Amberlite MB-1 mixed-bed ion exchanger (BDH, Poole, U.K.), was used as an additive to the leading electrolytes.

Water obtained from a Rodem-1 two-stage demineralization unit (OPP, Tišnov, Czechoslovakia) was further purified by circulation through a pair of tandem-coupled 200-ml polytetrafluoroethylene (PTFE) laboratory-made columns packed with an Amberlite MB-1 mixed-bed ion exchanger (BDH).

PEG samples were batch demineralized on an Amberlite MB-1 mixed-bed ion exchanger in a polyethylene vessel. Ammonia and acetic acid were purified by isothermal distillation into demineralized water kept in polyethylene vessels cleaned as described in the literature[25].

To minimize contamination of the leading and terminating electrolyte solutions with alkali and alkaline earth metal cations, they were prepared and stored in quartz or polyethylene vessels.

Samples

In the analysis of demineralized water, the sample was introduced into the injection valve (PTFE-lined) of the ITP instrument with the aid of a thoroughly cleaned disposable polyethylene syringe. The syringe was repeatedly washed with the sample to be analysed immediately before the ITP separation. To eliminate potential contamination of the samples during storage, only samples taken directly from the outlet of the PTFE purification columns were analysed.

Vincentka mineral water was bought in a local supermarket. A precipitate present in the sample was removed by filtration through a dense paper filter (the first 5-ml portion of the filtrate was discarded) and fine particulates in the filtrate were removed by an Anotop 10 0.02-μm disposable syringe filter (Anotec Separations, Banbury, U.K.). The membrane-filtered sample was diluted (1:25) with freshly demineralized water before the ITP analysis.

TABLE I

OPERATIONAL SYSTEMS

Parameter	*System No.*[a]				
	1	*2*	*3*	*4*	*5*
Leading electrolyte					
Solvent	H_2O–PEG	H_2O–PEG	H_2O–PEG	H_2O–PEG	H_2O–PEG
Proportions (v/v)	100–50:0–50	55:45	60:40	55:45	55:45
Leading cation	NH_4^+	NH_4^+	NH_4^+	NH_4^+	NH_4^+
Concentration (m*M*)	10	10	20	20	5
Counter ion	OAc^-	OAc^-	OAc^-	OAc^-	OAc^-
pH_L[b]	5.0	5.0	5.0	5.0	5.0
Additive	HEC	HEC	HEC	HEC	HEC
Concentration (%, w/v)	0.2	0.2	0.2	0.2	0.2
Co-counter ion	–	–	Mal^-	–	–
Concentration	–	–	3	–	–
Terminating electrolyte					
Solvent	H_2O				
Terminating cation	TBA^+ (TEA^+)				
Concentration (m*M*)	3 (5)				
Counter ion	ClO_4^-				

[a] OAc^- = acetate; Mal^- = malate; TBA^+ = tetrabutylammonium; TEA^+ = tetraethylammonium.

[b] The desired amount of PEG was added to a stock aqueous solution of the required electrolyte composition and pH.

An aqueous extract of apple flesh[8] was obtained from the Research Institute of Fruit Production (Bojnice, Czechoslovakia). The extract was diluted (1:5) with freshly demineralized water before the ITP analysis.

RESULTS AND DISCUSSION

Influence of PEG on the effective mobilities of alkali and alkaline earth metal cations

The operational systems used are listed in Table I. Experiments aimed at investigating the influence of the concentration of PEG in the leading electrolyte on the effective mobilities of the cations were carried out in operational system No. 1. In preliminary experiments we found that the molecular weight of PEG (evaluated for molecular weights in the range 200–3000) has no observable influence on the effective mobilities of the cations, and the polymer preparations with average molecular weights in the range 200–300 were arbitrarily used throughout.

To show the effect of PEG on the effective mobilities of alkali and alkaline earth metal cations, their relative step heights as obtained from the response of the conductivity detector in the analytical column plotted against the concentration of the polymer in the leading electrolyte (Fig. 1). Here, the leading ion (NH_4^+) and Li^+ were taken as reference constituents (see ref. 10, p. 307) as they exhibited minimum relative changes in their step heights (when compared with the remainder of the cations) with increasing concentration of PEG.

From the plots in Fig. 1, it is apparent that PEG retarded the alkaline and alkaline earth metal cations in the order Ba > Sr > Ca > Mg, *i.e.*, in order of their extractibilities from the aqueous phase in the presence of the polymer[22]. This retardation order also agrees with that achieved in ITP when 18-crown-6 was used as a complexing agent[12,13]. The plotted data for the alkali metal cations show that PEG is

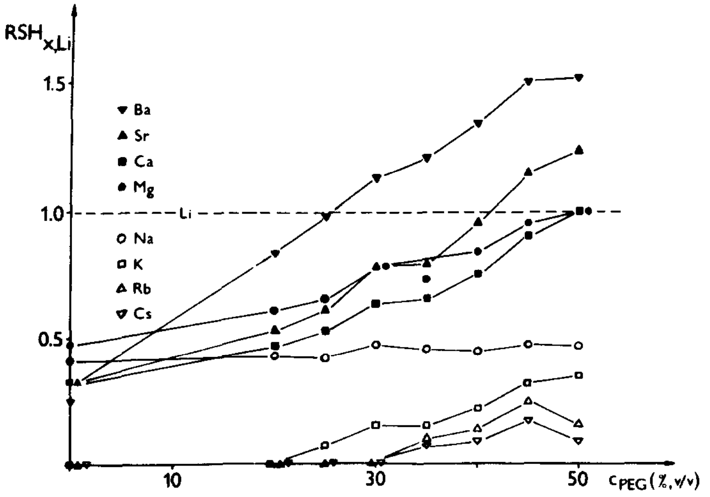

Fig. 1. Dependences of relative step heights ($RSH_{x,Li}$) of the alkali and alkaline earth metal cations on the concentration of PEG in the leading electrolyte. Operational system No. 1 (Table I) was used. The driving current in the analytical column was 45 μA. For further details, see text.

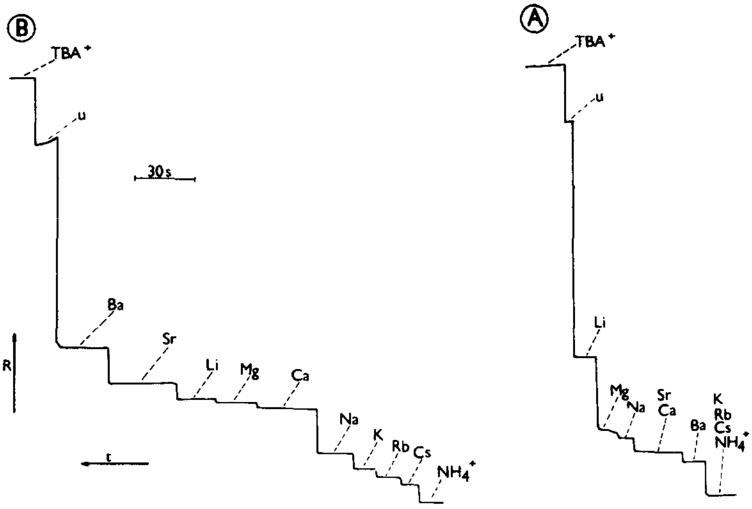

Fig. 2. Isotachopherograms for the separations of alkali and alkaline earth metal cations. Only the records from the analytical column are given. (A) Leading electrolyte without PEG; (B) leading electrolyte containing water–PEG (55:45). The driving currents were 250 and 45 μA in the preparation and analytical columns, respectively. R, t = increasing resistance and time, respectively. u = Unidentified impurity originating from the terminating electrolyte.

responsible also for their differentiation with a relative retardation in the order $K^+ > Rb^+ > Cs^+ > Na^+ \approx Li^+$. Although this is analogous to 18-crown-6[12–14], the extent to which, *e.g.*, K^+ is influenced is lower relative to the effect of the cyclic polyether. This difference can be explained via a macrocyclic effect of the crown ether[20].

From the differences in the relative step heights of the cations (Fig. 1), it is clear that water–PEG mixtures can be effective in separating various groups of alkali and alkaline earth metal cations and that their complete ITP resolution can be expected at 40–45% (v/v) concentrations of PEG in the leading electrolyte. The isotachopherograms in Fig. 2 clearly illustrate such a possibility. To our knowledge, a complete ITP separation of this group of cations by using other separation systems (see Introduction) has not been reported previously.

Anionically migrating constituents forming complexes with alkaline earth metal cations usually retard them in the ITP separations in the order $Ca > Sr > Mg \geqslant Ba$ (see, *e.g.*, ref. 11). The use of such complexing agents in water–PEG separation systems could be effective in enhancing differences in the effective mobilities of alkaline earth metal cations relative to alkali metal cations in general or in changing the ITP migration configurations of the former group of cations. The isotachopherogram in Fig. 3 illustrates such an effect due to the use of malate in the leading electrolyte. Although no detailed study of the applicability of complex equilibria in water–PEG systems was carried out, general rules for their use in ITP (see, *e.g.*, refs. 26 and 27) suggest that they may be useful to complement the differentiating power of PEG in some practical analytical problems.

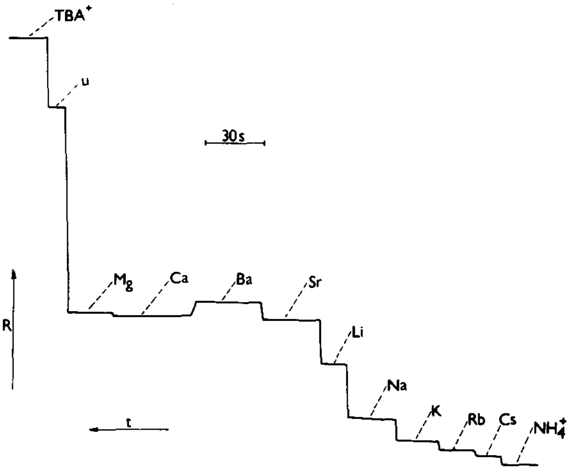

Fig. 3. Isotachopherogram for the separation of alkali and alkaline earth metal cations in a water–PEG electrolyte system containing malate as a complexing co-counter ion (system No. 3, Table I). The driving currents were 350 and 75 μA in the presentation and analytical columns, respectively. Symbols as in Fig. 2.

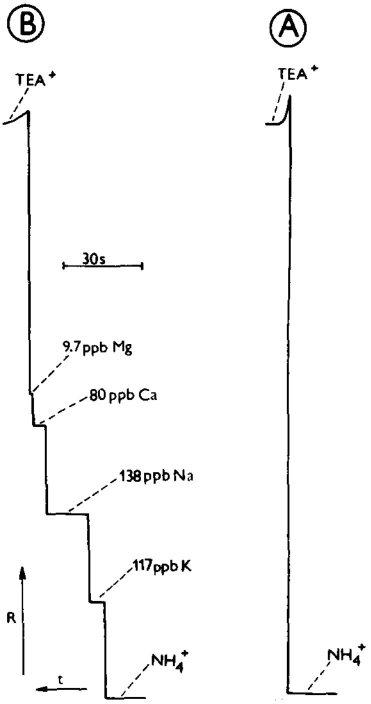

Fig. 4. Isotachopherogram from the analytical control of water demineralization. (A) Blank run (the terminating electrolyte solution was injected); (B) a 300-μl volume of demineralized water further purified by a two-fold recirculation through the columns packed with a mixed-bed ion exchanger (for details, see Experimental). The 300-μl sample volume was injected in 30-μl aliquots (the volume of the internal sample loop of the injection valve) by a 10-fold repetition of the injection. Each sample aliquot was transferred into the preseparation column by applying the driving current (250 μA) for 10 s. Approximately 150 s after the injection of the last aliquot, a steady state was achieved. The driving current in the analytical column was 50 μA. Symbols as in Fig. 2.

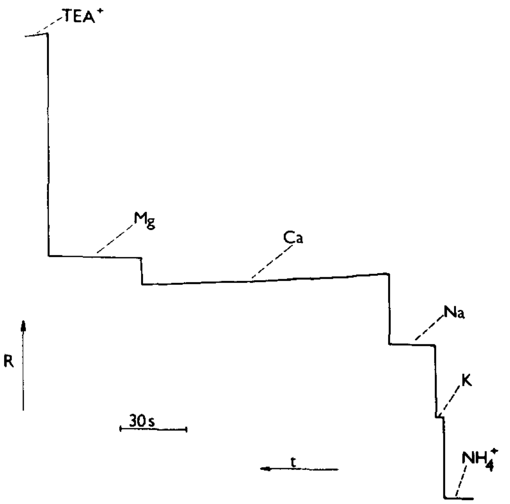

Fig. 5. Determination of K^+, Na^+, Ca^{2+} and Mg^{2+} in tap water. Only the record from the analytical column is given. The sample taken for the analysis was diluted with demineralized water (1:4). The driving currents were 250 and 50 μA in the preseparation and analytical columns, respectively. Symbols as in Fig. 2.

Examples of ITP separations in water–PEG operational systems

The determination of residual alkali and alkaline earth metal cations is often required in the analytical control of water demineralization. ITP has inherent advantages for this type of application, since a low load capacity of the separation

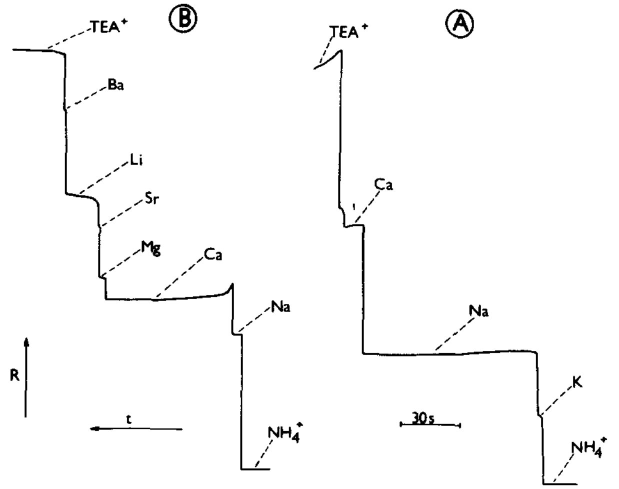

Fig. 6. Isotachopherograms from (A) the preseparation and (B) the analytical columns obtained in the analysis of Vincentka mineral water. A 30-μl volume of the sample pretreated as described under Experimental was taken for analysis. The driving currents were 350 and 25 μA in the preseparation and analytical columns, respectively. For the other details, see the text. Symbols as in Fig. 2.

compartment is sufficient also when a high sample volume is to be injected while a short analysis time is typical[28]. The isotachopherograms in Fig. 4 were obtained in the analytical control of water demineralization carried out as described under Experimental. Here, an analysis time of less than 5 min could easily be achieved for a 300-μl injection volume with operational system No. 2 (Table I). The blank run in Fig. 4A shows that the electrolyte solutions used contained alkali and alkaline earth metal cations at concentrations that were undetectable under our working conditions, so that they did not introduce (to some extent subtractable) systematic analytical errors.

Very reproducible ITP determinations of K^+, Na^+, Ca^{2+} and Mg^{2+} in drinking water (0.5–1.5% relative standard deviations) were possible by using operational system No. 2 (Table I) with an analysis time of less than 10 min (see also Fig. 5).

Alkali and alkaline earth metal cations need to be determined in mineral waters, which represent samples with typically extremely varying concentrations of cations. When their determinations are to be carried out in one run with minimum sample preparation, a high load capacity of the separation compartment needs to be combined with low detection limits attainable by a universal detector, *i.e.*, a high performance index of the separation compartment[29] is necessary. At present, such contradictory requirements in ITP can be best fulfilled by using a column-coupling configuration of the separation unit[23]. The isotachopherograms in Fig. 6 were obtained from the analysis of Vincentka mineral water containing the cations of interest within a $2 \cdot 10^3$–$3 \cdot 10^3$ concentration span (Na^+ *vs.* Sr^{2+} and Ba^{2+}). Here, the analysis was divided into two stages, *i.e.*, the determination of macroconstituents (K, Na, Ca) in the preseparation column (operational system No. 4, Table I), followed by their removal from the separation compartment, while the microconstituents (Mg, Sr, Li, Ba) were evaluated under more favourable detection conditions (operational system No. 5) in the analytical column. Diffusion of ions into the analytical column in the bifurcation block during their removal (K^+, Na^+ and a major part of Ca^{2+}) from the separation compartment was mainly responsible for the presence of Na^+ in this column (the detected zone corresponded to less than 0.1% of the total amount of Na^+ taken for the analysis). Although this undesirable transfer of the ions into the analytical column is difficult to suppress[30], it did not disturb the determination of the microconstituents in the analytical column. For example, in our particular case, the reproducibilities of determinations were characterized by 0.2–7.0% relative standard deviations with the highest values being typical of Sr^{2+} and Ba^{2+} (occurring in the sample at *ca.* 10^3 lower concentrations than Na^+).

It has been reported[7] that determinations of K^+, Na^+, Ca^{2+} and Mg^{2+} in urine may account for about 50% of all routine analyses of urine samples carried out in some clinical laboratories. Possibilities of using ITP in a methanolic operational system in the determination of these cations in urine has been studied[7]. A high dynamic concentration range may be required when all of these cations are to be determined in one ITP run. The isotachopherograms in Fig. 7 obtained in the analysis of a urine sample with a very low concentration of Mg^{2+} illustrate that the ITP analysis in the column-coupling system with the electrolyte system based on a water–PEG mixture can fulfil these extreme analytical requirements. An appropriate sample dilution was sufficient in this instance, so that no precipitation of the anions before the analysis was needed[7].

Determinations of Ca^{2+}, Mg^{2+} and K^+ in apple flesh are important as they

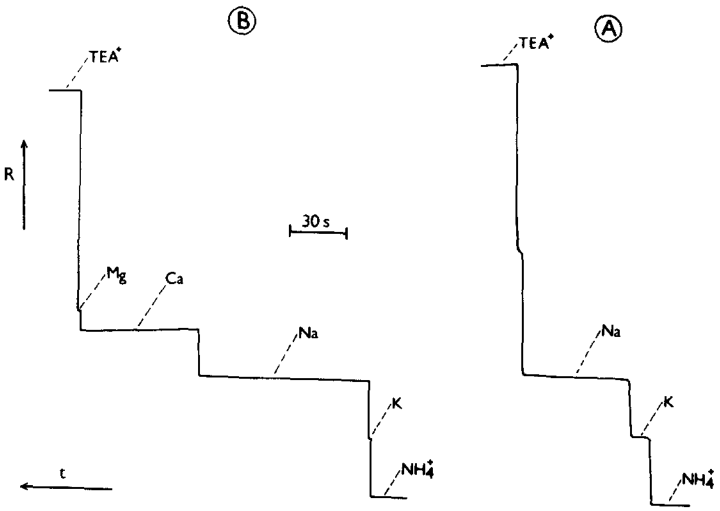

Fig. 7. Isotachopherograms from the analysis of a urine sample. The record from (A) the preseparation and (B) the analytical columns served to evaluate macro- and microconstituents, respectively. The injected sample (30 μl) was diluted (1:100) with demineralized water. The driving currents were 250 and 50 μA in the preseparation and analytical columns, respectively. Symbols as in Fig. 2.

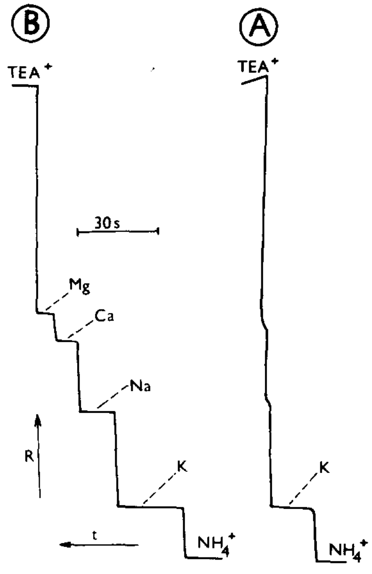

Fig. 8. Isotachopherograms from the analysis of an aqueous extract of apple flesh. K^+ in the extract (see Experimental) was evaluated from the response of the detector in the preseparation column (A) and the remainder of the cations from the response of the detector in the analytical column (B). The driving currents were 250 and 40 μA in the preseparation and analytical columns, respectively. Symbols as in Fig.

provide important data with respect to long-term storage of apples. ITP combined with a simple extraction procedure[8] can be a convenient method for this purpose, especially owing to its high precision in the determination of Ca^{2+} (2% relative standard deviation) since the concentration of this cation is of primary diagnostic value in this respect. The isotachopherograms in Fig. 8 for the analysis of an aqueous extract of apple flesh for alkali and alkaline earth metal cations were obtained with the operational system No. 2 (Table I). Under these conditions, Ca^{2+}, Na^{+} and Mg^{2+} could be determined in one run together with K^{+} also when K^{+} was present in the sample at a 10^3-fold excess.

CONCLUSIONS

This work has clearly shown that water–PEG mixtures are useful for ITP separations and/or determinations of alkali and alkaline earth metal cations. An appropriate ratio of both components in the leading electrolyte enables a complete resolution of this group of cations to be achieved in one ITP run. The favourable price of PEG makes this mixture a convenient alternative to separations based on the use of crown ethers. From the practical point of view, it is also important that the separation need not be performed in the separation compartment made of highly inert materials, as is necessary, *e.g.*, with methanolic operational systems.

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