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Optimisation and use of water-in-oil MEEKC in pharmaceutical analysis

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Abstract

Water-in-oil microemulsion electrokinetic chromatography has been applied to the separation of a range of acids, bases and neutrals and is especially suitable for very water-insoluble drug compounds. A number of operating parameters were evaluated. An optimised set of operating conditions allowed separation of a range of pharmaceutical formulations containing water-insoluble compounds. A number of novel applications for W/O microemulsions were developed and ability to quantify drug contents in tablets and a cream was shown with good precision, detector linearity and accuracy. Comparison of obtained data with those determined from a HPLC method showed acceptable agreement.

Keyword: W/O MEEKC

1. Introduction

Microemulsions are made from water, an organic solvent, a surfactant and co-surfactant and contain nanometer-sized spherical droplets. Microemulsions can either consist of water droplets in an oil continuous phase (W/O) or oil droplets in water continuous phase (O/W), which are known as L1 and L2 phases, respectively. Phase diagrams are often used to map the composition of W/O and O/W microemulsions. Fig. 1 shows the L1 and the L2 phases where the composition of the microemulsion is determined. Microemulsions have unique properties as separation media and have been successful in the separation of a range of analytes in both high performance liquid chromatography (HPLC) [1–3] and microemulsion electrokinetic chromatography (MEEKC) [4–18].

W/O microemulsions are directly related to inverse micellar solutions. The basis of the microemulsion structure is obtained from the association of two or three components within the oil. The oil phase consists of long chain alkanes or very water-insoluble alcohols e.g. (pentanol and butanol) which would form two phases without the addition of surfactant. These microemulsions are composed of water, surfactant and possibly a co-surfactant or additional oil phase. The addition of these oils phases decreases the surface tension [19] and forms a more stable microemulsion. A central core of water is surrounded by surfactant (and co-surfactant) molecules, which forms a droplet in the oil [9]. The water solubilising capacity is strongly dependent on the surfactant/co-surfactant ratio. If the co-surfactant concentration is too high this will cause separation into two liquids. Excessive surfactant concentration causes formation of a liquid crystalline phase.

The expected advantage of employing W/O microemulsions is that highly water-insoluble analytes can be readily solubilised due to the high concentration of oil in the W/O microemulsion. For example [6] analysis of highly water-insoluble solutes such as steroids and complex aromatics require modifications to O/W MEEKC compositions as they are insoluble in the typical O/W microemulsions employed.

The use of W/O MEEKC has only recently been reported [19] by us with a preliminary study of the factors affecting selectivity. Separation selectivity in W/O MEEKC was

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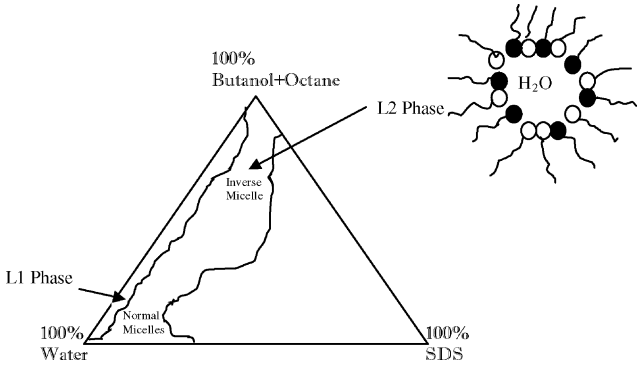

Fig. 1. Schematic representation of a partial ternary phase diagram of the SDS, butanol/octane, water outlining the L1 and L2 phases.

shown to be manipulated by temperature, surfactant and co-surfactant composition. The separation order obtained in W/O MEEKC was not directly related to log *P*. Therefore unique selectivities were obtained when compared to conventional O/W MEEKC. This difference is due to different solute partitioning and dissociation in the W/O and O/W microemulsions.

In the W/O microemulsion there is no solute dissociation as there is no available protons in the predominately non-aqueous microemulsion. Solute dissociation will occur in O/W MEEKC if the solute has a suitable functionality. Separations in O/W MEEKC have electrophoretic and ion-pairing influences for charged solutes. The hydrophobicity (log *P*) of the analyte is an important physicochemical parameter in both W/O and O/W MEEKC. Hydrophilic compounds would be expected to be the most retained in W/O MEEKC as the more hydrophilic analytes will be solubilised by the water droplet and reside predominantly in the microemulsion water droplet.

The purpose of this study was to extend the previous preliminary assessment of W/O MEEKC. Further selectivity optimisation of this system is obtained by either varying the microemulsion composition or by adding organic modifiers to the system.

A range of pharmaceutical analysis applications were developed and quantitative determinations demonstrated. Use of W/O MEEKC offers the possibility of drastically reducing preparation time and the large amounts of organic solvents used to solubilise water-insoluble compounds.

Very few examples of the analysis of creams by CE have been reported. Optimised MEKC methods were needed. Levels of the particularly water-insoluble compound hydroquinone, present in skin-toning cream, were determined [20] using SDS-based electrolyte containing 10% methanol. Methanolic solutions of Topsym cream (which contains 0.5% of the steroid, fluocinonide) were directly analysed [21] using a pH 9 phosphate-borate buffer containing 100 mM sodium cholate. The applicability of W/O MEEKC for this type of analysis will be assessed.

2. Experimental

An Agilent 3D-CE capillary electrophoresis system (Waldbron, Germany) was used. Organic chemicals were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). Water was obtained from a Waters Milli-Q system. Capillaries were purchased from Carl Stuart Ltd. (Dublin, Ireland). New capillaries were pre-conditioned with 0.1 M sodium hydroxide for 30 min before initial use. Separations were performed on a 33 cm long, 50 μm internal diameter fused silica capillary (detection window at 24.5 cm). The capillaries were rinsed between injections with 0.1 M NaOH for 2 min followed by 2 min with methanol and finally 2 min of microemulsion buffer. A standard set of operating conditions was developed as a reference point. The effect of varying each operating condition was monitored to obtain an optimum set of conditions.

2.1. Preparation of the microemulsion

W/O microemulsions were prepared by weighing the appropriate ratio of components to form different microemulsion composition. The SDS, butanol/octane, water system was determined from the phase diagram in Fig. 1. The microemulsion was prepared by adding 10% (w/w) SDS, 2% (w/w) octane, 78% (w/w) butanol and 0.07 M sodium acetate pH 8. The order of addition was found to be important in the formation of the microemulsion. Initially the surfactant was mixed with the oil, and then the water was added. This sequence was to ensure that the system does not pass through an emulsion phase, which may be difficult to break. This mixture was sonicated for 30 min to aid dissolution and an optically transparent W/O microemulsion was formed.

2.2. Sample preparation

All components were dissolved in the microemulsion buffer at 1 mg/ml. These were all sonicated for 15 min.

2.3. Preparation of microemulsion with organic modifier

The microemulsion was prepared by adding 10% (w/w) SDS, 2% (w/w) octane, 78% (w/w) butanol, and 10% (w/w) 0.07 M sodium acetate to form a stable microemulsion. Organic modifier was added from 5 to 25%. Addition of 5% organic modifier was added at 5% (w/w). All concentrations from 5 to 25% of methanol, propan-2-ol and acetonitrile were prepared in the same way.

2.4. Separation conditions for MEEKC

Hydrodynamic injection at 50 mbar for 1 s and some quantitation work was carried at 20 mbar for 3 s. The polarity was reversed and a voltage of −30 kV was applied with diode array detection at 200 nm. The reference channel was set

at 450 nm with an 80 nm bandwidth. Reversing the polarity meant that the anode was beyond the detector. The operating temperature was 25 °C unless otherwise stated.

3. Results and discussion

3.1. Method optimisation

3.1.1. Microemulsion composition and physical characteristics

From previous brief studies [19] it was known that temperature, surfactant and co-surfactant concentrations affected selectivity in W/O MEEKC. The effects of other additional operating parameters were evaluated. Microemulsion composition was varied and organic modifiers were added to the W/O microemulsion. A series of microemulsion compositions, were formed in the development of partial phase diagram of butanol, octane, SDS and water. The physical data for the optimisation of W/O microemulsion were determined. From the data an increase in current was shown to depend upon the surfactant water ratio. Increasing surfactant concentration and decreasing water concentration increased the current generated by the system. Maximum current was generated by composition of 6% SDS and 14% water; this was also confirmed from conductivity result. The largest particle size is recorded for the optimal system, which is the most stable, where the least repulsion on the droplets is experienced. A low surface tension is also recorded for this system of 23.65 mN m^{-1}.

3.1.2. Type of oil and additional oil phase

In W/O microemulsion octane is the most frequently employed oil. In this experiment use of the butanol and pentanol as oils also assessed. Octane and heptane were assessed as additional oil phases to both the butanol and pentanol microemulsions to improve microemulsion stability and identify any impact on selectivity.

Butanol W/O microemulsion showed better separation of analytes compared to pentanol microemulsion. Butanol has a lower viscosity and therefore generates a significant current compared to pentanol. Ions are not able to move fast in higher viscosity solutions and therefore less current is generated. Fig. 2 shows the separation of a test mixture using butanol as the oil with octane as additional oil phase. These five components co-eluted in a pentanol/octane system.

The replacement of octane with heptane as additional oil phase markedly changed the selectivity of the butanol system and slightly reduced the migration time of the butanol heptane system as seen in Fig. 3.

The addition of heptane to the pentanol system improved the separation and gave some resolution. The sum of the carbon chain lengths of both heptane and pentanol equals the chain length of surfactant SDS with a carbon chain of 12. This makes the composition of pentanol and heptane more stable then pentanol and octane and which gave rise to better separation between the components in the mixture. The additional oil phase is beneficial in the microemulsion system and depending on carbon chain length changes the selectivity of the components in the mixture.

3.1.3. Surfactant and water concentration

The surfactant SDS concentration was previously reported [19] to have a profound effect on the retention and the resolution of the test mixtures in W/O MEEKC. There is a relationship between the water concentration and SDS concentration. As SDS concentration is decreased it must be balanced by an increase in water concentration. Altering the surfactant and water concentrations affects (Table 1) the physical properties of the W/O microemulsion.

From the physical property results in Table 1 and experimental evaluations of SDS, butanol, octane, and water compositions it was observed that the lowest amount of water required to form a microemulsion was 8% (w/w) determined from the phase diagram. It was necessary to increase SDS content to accommodate lower water contents. Increasing

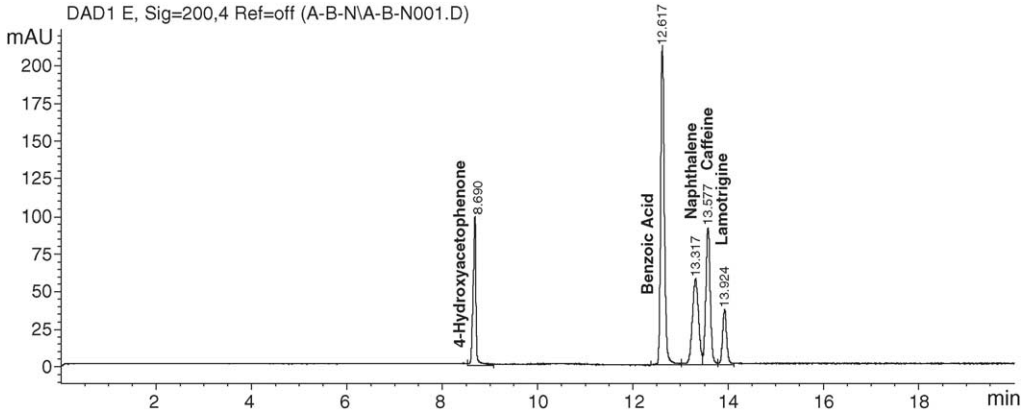

Fig. 2. Separation of the test mixture by W/O MEEKC. 1 mg/ml acid, basic and neutral separation test-mix. Separation conditions: sample injected for 1 s at 50 mbar 10% (w/w) sodium dodecyl sulphate (SDS), 78% (w/w) butanol, 2% (w/w) octane, 10% (w/w, 0.07 M) sodium acetate buffer pH 8, −30 kV, 33 cm × 50 μm ID capillary (detection window at 24.5 cm), 25 °C, 200 nm.

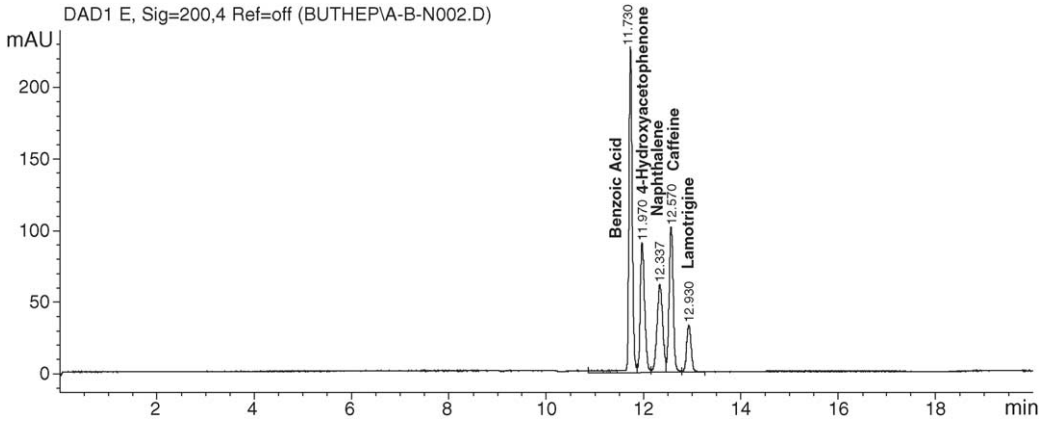

Fig. 3. Separation of test mixture of charged and neutral solutes by W/O MEEKC. Separation conditions same as Fig. 2 except 2% (w/w) heptane instead of octane.

Table 1
Physical data along the 80% line of the butanol, octane, SDS, water phase diagram

Composition	SDS (%)	Butanol/octane (%)	Water (%)	Current (μA at −30 kV)	Conductivity (μS/cm)	Particle size (nm)	Surface tension (mN m^{-1})
1	0.461	80/2	19.539	−2.7	117.6		23.89
2	0.469	80/2	19.531	−3.6	117.2		23.71
3	1	80/2	19	−5.4	220	1.83	24.07
4	2	80/2	18	−8.9	356		24
5	4	80/2	16	−11.2	482		24.05
6	6	80/2	14	−13.6	581	0.55	23.5
7	10	80/2	10	−10	510	2.86	23.65
8	12	80/2	8	−11.3	453		20.65

SDS concentration increases conductivity and lower surface tension. The 12% SDS, 78% butanol, 2% octane, 8% water composition gave the best resolution; however, this formed liquid crystals after a week of storage. The microemulsion containing 10% water was physically stable and gave acceptable separation performance and was considered optimal.

3.1.4. Surfactant type

The choice of surfactant has a marked effect on the separation achieved in MEEKC, as it influences the droplet charge and size, level and direction of the EOF and ion pairing in the system. Sodium dodecyl sulphate (SDS) is the most frequently used surfactant and is anionic with C12 carbon chain length.

Sodium caprylate, anionic surfactant has been used to replace SDS. Sodium caprylate (SC) (chain of C8) gave the same selectivity as SDS, which was previously reported. The separation with sodium caprylate gave much better resolution between the peaks but comprised the short analysis time seen in Fig. 4. The separation of the test mixture was three times longer than with SDS as the surfactant. The current generated with sodium caprylate was lower than SDS

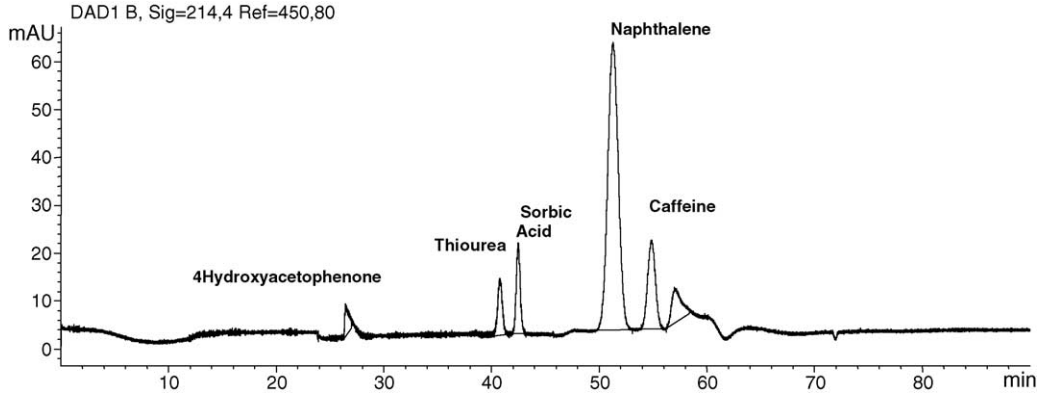

Fig. 4. Separation of test mixture of charged and neutral solutes by W/O MEEKC. Separation conditions same as Fig. 2 except 10% (w/w) SC (sodium caprylate) instead of SDS.

microemulsion. Baseline noise was increased also with use of caprylate.

A cationic surfactant cetyl trimethylammonium bromide (CTAB) was substituted for the anionic surfactant SDS. It successfully formed a microemulsion but did not give good separation. A chiral surfactant deoxycholate was substituted in the composition instead of SDS but failed to give separation.

Substitution of anionic SDS with anionic SC changed the selectivity but did not further optimise the system. Substituting cationic surfactant did not give good separation of the analytes. SDS was determined to be the optimal surfactant.

3.1.5. Addition of organic solvents

The addition of organic solvents is usually performed in CE to improve solubility of solutes of high hydrophobicity. In MEKC and O/W MEEKC addition of an organic modifier increases the affinity of the solute for aqueous phase. The addition of organic modifier to the W/O microemulsion partitions with both the aqueous phase and oil phase. The polar nature of these solvents allows them to partition with the aqueous droplets; there is slight partitioning of the short chain alcohol into the butanol continuous oil phase. Organic modifier will change the migration rate and the capacity factor. Methanol and propan-2-ol are more viscous than water and reduce any low residual EOF, which expands the separation window in MEKC and O/W MEEKC. Acetonitrile is less viscous than water and has variable effects on the separation window.

3.1.5.1. Propan-2-ol. As the microemulsion being studied consisted mainly of butan-1-ol the addition of propan-2-ol makes the system more stable as it acts like a co-surfactant and does not disrupt the water droplet. This is different to O/W microemulsion where the addition of propan-2-ol causes the oil droplet to swell and disrupt. A range of concentrations from 5 to 25% of propan-2-ol was added to the W/O microemulsion. Migration times increased with propan-2-ol content. The migration time for bupivacaine was 13.5 min with no propan-2-ol, 15 min with 5% propan-2-ol and 30 min with 25% propan-2-ol. Poor peak shapes and efficiencies were obtained. The use of propan-2-ol was not considered to be advantageous due to the excessive migration time increases and poor peak shape.

3.1.5.2. Methanol. The maximum amount of methanol that can be solubilised in O/W microemulsion is 15% (v/v) [14] before the microemulsion becomes destroyed. This percentage is higher with W/O microemulsion and microemulsions containing 0–25% methanol were assessed. The addition of methanol considerably increased resolution between the peaks at the expense of an increased analysis time has improved greatly with addition of increased concentration of methanol.

The dielectric constant of methanol is also high which generated an increase in current. From a current of −10 μA, for a microemulsion containing no methanol to −28 μA with 25% methanol. The use of methanol addition in W/O MEEKC was considered useful as it gives excellent peak shape and resolution at the expense of increased analysis time. Fig. 5A shows the separation with 5% methanol and Fig. 5B with 25% methanol with excellent resolution.

3.1.5.3. Acetonitrile. The migration times of the analytes decreased slightly with increases of acetonitrile to 15%. Addition of concentration of greater than 15% increased both the migration time of the analytes and resolution between the peaks. The maximum amount of acetonitrile added to the system is 25%. Acetonitrile is considered a useful organic solvent to slightly improve resolution.

3.1.6. Separation of neutral, acidic and basic solutes

In our previous [19] work the solute range was limited to four neutral analytes and one charged (acidic) analyte in W/O MEEKC. In this work the range of analytes has been greatly extended. For example Fig. 6 shows the simultaneous separation of water soluble and insoluble acids (sorbic, benzoic), bases (acyclovir, lamotrigine, bupivacaine) and neutrals (thiourea, naphthalene, 4-hydroxyacetophenone, caffeine). The migration of the neutral solute components depends solely on their interaction with the moving microemulsion droplets. Their migration order was found not to related to their log *P*. The pH of the droplet was between 8 and 9 and therefore the acids should be partially or fully negatively charged whilst the bases should be partially or fully positively charged and migrate with different interaction of the moving droplet and the EOF velocity. Acidic analytes and basic analytes will separate by a combination of mechanisms: free mobility of the solute and partitioning and ion-pair interactions with the moving droplet whilst under the influence of any low residual EOF.

During these investigations a range of other solutes were successfully separated including the highly water-insoluble antibiotic cefuroxmine axetil, Vitamin E and also a range of highly water-insoluble steroids (fluticasone proprionate and beclamethasone di-proprionate)

3.2. Analytical performance and application of W/O MEEKC

The development of a stable water in oil microemulsion and a standard set of MEEKC operating conditions have shown to be applicable to water-insoluble drugs, and creams. These drugs and creams are not readily soluble in aqueous systems and so would normally have to be extracted into organic solvents. Typically a solvent extraction is needed to remove the oil soluble materials to prevent interference and precipitation. Methanol has frequently been used in the sample preparation process. O/W MEEKC do not normally sufficiently solubilise water-insoluble drugs and solvents such as methanol are needed for the preparation step to dissolve the drug prior to dilution with the microemulsion. There can be a

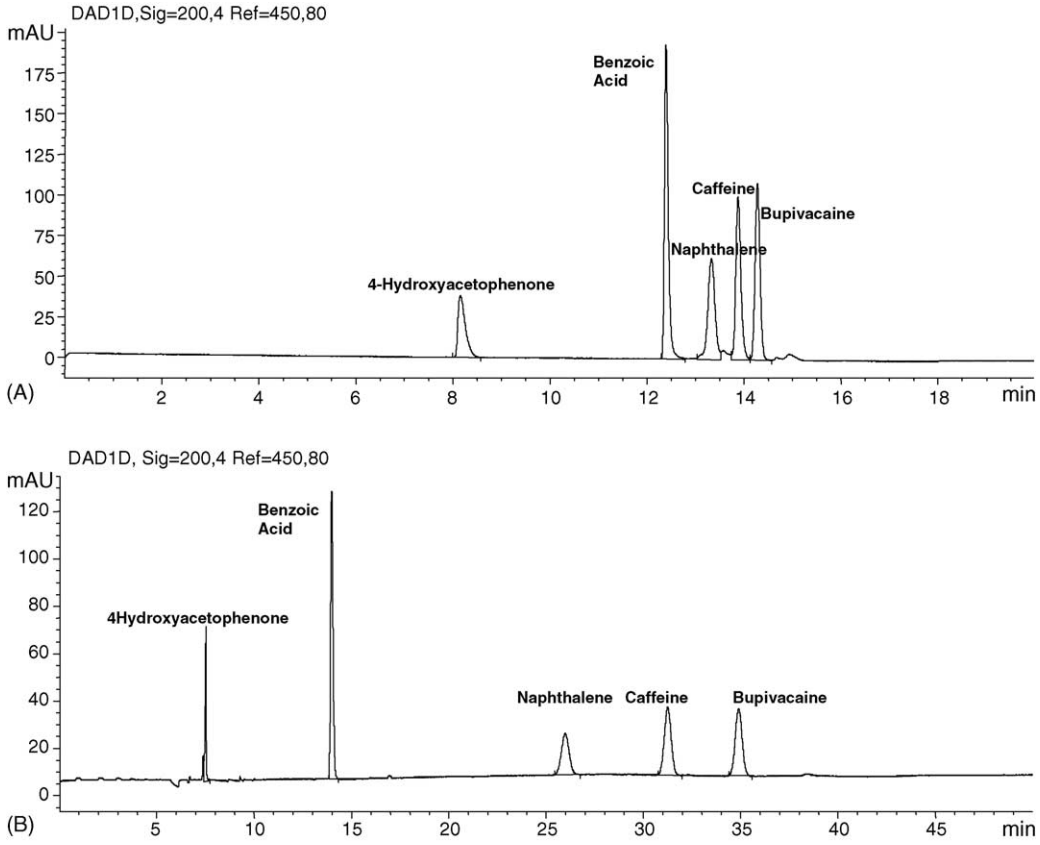

Fig. 5. Separation of acid, base and neutral components in W/O standard microemulsion with the addition of (A) 5% methanol and (B) 25% methanol by W/O MEEKC. Separation conditions same as Fig. 2.

significant problem with this method as O/W microemulsion can only solubilise up to 8% methanol before the microemulsion disrupts.

3.2.1. Applications

3.2.1.1. Fluticasone proprionate in Flonase® aqueous nasal spray. Fluticasone propionate is a highly insoluble neutral steroid and is the active ingredient in Flonase® nasal spray. The nasal spray contains three preservatives including a paraben in the formulation; Fig. 7 shows the separation achieved. The sample was obtained from a commercial source and standards of the preservatives were unable to confirm the identity of the preservative peaks. The highly insoluble nature of the steroid normally requires a solvent extraction process to remove it from the aqueous nasal spray liquid. Use of O/W microemulsion as the sample diluent and separation medium ensured direct compatibility avoiding a sample extraction process.

3.2.1.2. Separation of highly water-insoluble sunblock filters in sunscreen lotion. Previously Klampfi [18] separated the UV filter components such as Eusolex 4360 and Eusolex 6300, which are present in sun tan lotions by both HPLC and O/W MEEKC methods. These filter components are water-insoluble polyaromatic compounds. Both O/W MEEKC and HPLC required extensive sample preparation

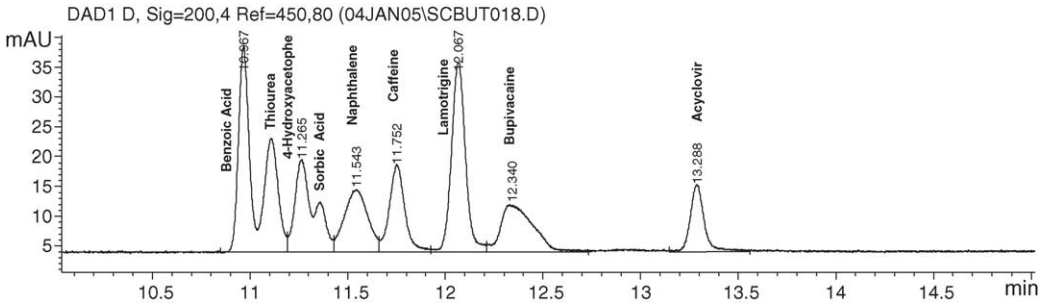

Fig. 6. Separation of acidic, basic and neutral components in W/O standard microemulsion by W/O MEEKC. Separation conditions same as Fig. 2.

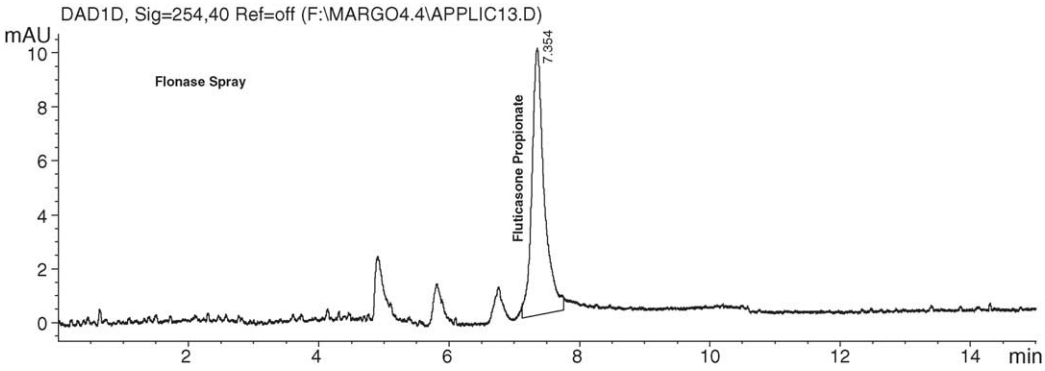

Fig. 7. Separation of fluticasone proprionate in Flonase® aqueous nasal spray in W/O standard microemulsion by W/O MEEKC. Separation conditions same as Fig. 2 except 254 nm.

of the milky lotions which involved extraction and dilution step. For example for O/W MEEKC analysis, the sample was prepared in 20 ml of THF, sonicated for 25 min the sample was filtered and then and the sample was diluted (25:1) with microemulsion before analysis. Analysis of sunscreen samples was found to be directly possible with W/O MEEKC as the samples were completely soluble in the W/O microemulsion. Fig. 8 shows the analysis of a commercial sunscreen lotion sample showing three sunblock filter components. Samples were directly diluted with the W/O microemulsion and injected into the capillary with no sample pretreatment.

3.3. Quantitation of active components in cream and formulations

Etofenamate is a water-insoluble neutral active ingredient present in Flexin® cream. Bumetanide is a water-insoluble neutral compound present in a pharmaceutical drug formulation. O/W MEEKC has previously been used [22] for the separation of bumetanide from its related impurities but the sample has to be first dissolved at 2 mg/ml in 80% methanol. Attempts to analyse the ointment with O/W MEEKC were unsuccessful. Atenolol is a water-insoluble drug, which typically requires the use of glacial acetic acid for sufficient solubilisation.

The quantitative performance of a single W/O MEEKC method for analysis of these active three components was assessed. Internal standards are frequently employed in CE to improve injection precision. Benzoic acid was found to be suitable internal standard for both bumetanide and atenolol. Thiourea eluted before etofenamate and was used successfully as an internal standard for the etofenamate content present in Flexin® cream. Ten injections of various solutions were performed to demonstrate the injection precision of the system. Peak area ratio (PAR) precision was calculated. 0.5 mg/ml solutions of both bumetanide and atenolol were used and a 1 mg/ml etofenamate solution was used. Good precision was obtained. Bumetanide giving a repeatability of 1.07% R.S.D., atenolol 1.1% R.S.D. and etofenamate 0.9% which are all within the acceptance criteria.

The linearity of detector peak area with bumetanide concentration was determined over the range of 25–75% of the nominal sample of 0.5 mg/ml. The use of internal standard improved the injection precision. The relative area of the atenolol peak was also shown to have good linearity over the range 50–150% of the nominal (0.5 mg/ml) concentration with a correlation coefficient value of 0.9992. Etofenamate

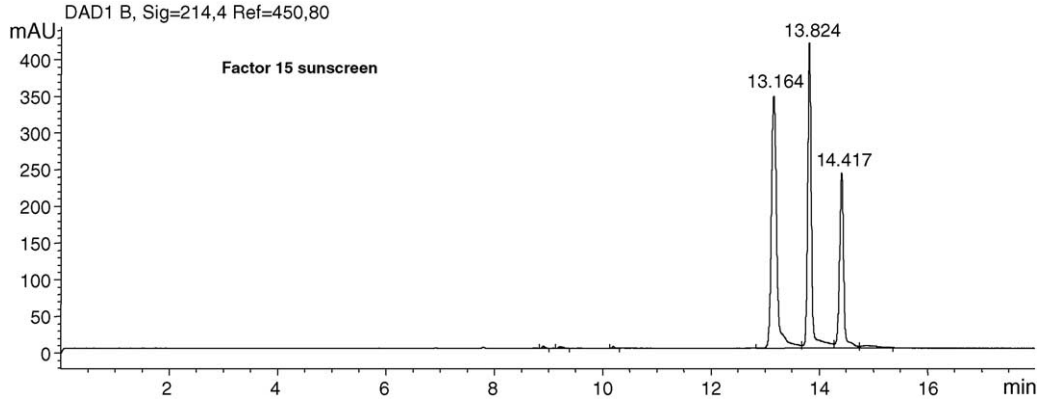

Fig. 8. Separation of sun block filters in sunscreen lotion in W/O standard microemulsion W/O MEEKC. Sample injected for 1 s at 50 mbar. Separation conditions same as Fig. 2 except 214 nm instead of 200 nm.

Table 2
Quantitative results determined by W/O MEEKC compared to label claim and results determined by HPLC

	Label claim (mg/tablet or % (w/w))	Content by W/O MEEKC	Content of active by HPLC	% R.S.D. for response factor ($n = 10$)
Bumetanide tablets	1 mg	0.98	0.97	1.07
Atenolol tablets	50 mg	50	50.5	1.1
Etofenamate cream	10% (w/w)	10.1	9.5–10.5	0.9

also gave good linearity with an r^2 value of 0.999 of 50–150% of the nominal 1.0 mg/ml concentration.

The formulated samples were prepared by dissolving sufficient sample to yield 0.5 mg/ml of both bumetanide and atenolol active in W/O microemulsion. The actives were very soluble in this microemulsion and did not require prior extraction into organic solvent. They were sonicated for 15 min in W/O microemulsion containing the internal standard and directly analysed. A 500 mg portion of Flexin cream® (label claim 10% (w/w) etofenamate) was added directly into the microemulsion and sonicated for 15 min. This was then diluted further to give a working sample concentration of 1 mg/ml. All samples and standards were carried in duplicate with five repeat injections on each standard and sample. Table 2 shows good agreement between label claim and the O/W MEEKC and HPLC results.

4. Conclusions

The use of W/O MEEKC has been found to be useful in the separation of a range of acids, bases and neutrals and especially suitable for very water-insoluble drug compounds. A number of factors were shown to affect the separations achieved in W/O MEEKC. These were surfactant and water content, type of oil and additional oil phase, surfactant type and the addition of organic solvents to the microemulsion.

Acetonitrile, propan-2-ol and methanol were added to the microemulsion system. The addition of large quantities up to 25% (w/w) could be added before disruption of the microemulsion. Increase in current was also noted with the addition of acetonitrile and methanol.

An optimised set of operating conditions allowed separation of a range of pharmaceutical formulations containing water-insoluble compounds. The ability to quantify drug contents in tablets and a cream was shown with good precision, detector linearity and accuracy. Sample extraction and preparation steps, normally needed to remove the oil soluble excipient materials to prevent interference and precipitation, were eliminated compared to HPLC and O/W MEEKC of preparation. W/O MEEKC offers unique selectivity opportunities compared to O/W MEEKC especially for the analysis of highly water-insoluble neutral solutes. The technique offers the possibility of useful quantitative analysis of complex pharmaceutical formulations and merits further investigation and application.

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