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DRY ANALYTICAL ELEMENT AND METHOD FOR THE COLORIMETRIC
DETERMINATION OF LITHIUM ION

The present invention relates to clinical
5 chemistry. In particular, it relates to a multilayer
analytical element and method for its use in the
colorimetric determination of lithium ion in aqueous
specimens, such as biological fluids.

Lithium, in the form of lithium carbonate, is
10 administered to manic-depressive patients. However,
the therapeutic range of lithium ion in plasma is quite
narrow, and thus it is very important to accurately
monitor the level of lithium ion in such patients
because of the toxic side effects that appear when it
15 exceeds the therapeutic level. Such determinations are
generally made in the presence of a number of other
ions, such as sodium and potassium ions, in the test
specimen.

A number of analytical reagents and methods
20 have been devised to detect lithium ion
colorimetrically in the presence of other ions. For
example, US-A-5,051,367 describes the use of tetra-
substituted aryl cyclic formazan dyes for this purpose
in either solution or dry assay formulations.

25 More recently, a dry multilayer analytical
element for a colorimetric lithium ion assay is
described in EP-A-0 516 227. The important advance
provided by the invention in that application is the
use of certain 14-crown-4-ether derivative dyes in
30 certain reagent layers of the elements to overcome
problems with interferents. Such dyes are described in
considerable detail in EP-A-0 516 227.

One embodiment of the multilayer element
described above has a first reagent layer containing
35 the 14-crown-4-ether derivative dye and an aromatic
coupler solvent, such as 2,4-di-n-pentylphenol. The

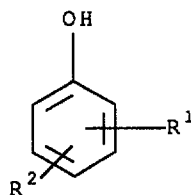
element also contains a second reagent layer, a subbing layer and an outermost porous spreading layer. Coupler solvent is in the first reagent layer only.

Even with the significant improvement
5 represented by the multilayer element described above, there is additional need for improvement in the precision of lithium ion determination. Moreover, a reduction of the background rate of dye development is desired. Background rate refers to dye developed with
10 time which is not related to the presence of lithium ion. Thus, an improved element for lithium ion determination is desired.

We have overcome the problems noted above and further improved a method for colorimetric
15 determination of lithium ion using a dry multilayer analytical element for the determination of lithium ion, the element comprising a support having thereon, in order and in fluid contact:

a) a first reagent layer containing a 14-
20 crown-4-ether derivative dye for lithium ion,

b) a second reagent layer containing a perfluoroalkyl surfactant, and an aromatic phenol organic solvent having the structure (I):



25 wherein R¹ and R² are independently alkyl of 1 to 20 carbon atoms or acyl of 2 to 20 carbon atoms and

c) a porous spreading layer,
the element further comprising, in a layer other than the first or second reagent layers, a buffer

present in an amount sufficient to maintain a pH in the element of at least 9 during an assay,

the element characterized wherein it further comprises a second aromatic phenol organic solvent in the first reagent layer, which second aromatic phenol organic solvent has the structure (I), and the first and second organic solvents can be the same or different.

This invention also provides a method for the determination of lithium ion which comprises:

A. contacting a liquid sample suspected of containing lithium ion with the dry multilayer analytical element described above, and

B) determining the change in spectrophotometric signal in the element from complexation of the 14-crown-4-ether derivative dye with lithium ion in the liquid sample.

The present invention provides a dry analytical element for the effective and specific detection of lithium ion in a relatively short period of time using a colorimetric signal producing chemistry. Background rate of dye development is reduced with the present invention. Thus, the time for evaluating the colorimetric signal can be lengthened without losing precision, thereby providing the advantage of more accurate determination of lithium ion concentration in a narrow therapeutic range. This improvement is achieved by incorporating an aromatic phenol organic solvent in both the first and second reagent layers which are both below the porous spreading layer. The previous element (of EP-A-0 516 227) contained an organic solvent in the bottom reagent layer only. Putting it into both reagent layers unexpectedly improved the precision, by reducing the background rate of dye development. Further advantage

is achieved by having a perfluoroalkyl surfactant in at least the second reagent layer as defined herein.

FIGURE 1 is a graphical plot of reflectance density (D_R) versus time (minutes) resulting from assay of lithium ion using a Control element and several calibrator fluids as described in Example 5 below.

FIGURE 2 is a graphical plot of reflectance density (D_R) versus time (minutes) resulting from assay of lithium ion using an element of this invention and several calibrator fluids as described in Example 5 below.

FIGURE 3 is a graphical plot of reflectance density (D_R) versus time (minutes) resulting from assay of lithium ion using a Control element as described in Example 6 below.

FIGURE 4 is a graphical plot of reflectance density (D_R) versus time (minutes) resulting from assay of lithium ion using an element of this invention as described in Example 6 below.

The element of this invention can be used to determine (that is, detect either the presence, amount or both) lithium ion in biological fluids of animals or humans, but preferably in humans. Such fluids include, but are not limited to, whole blood, plasma, sera, lymph, bile, urine, semen, cerebral spinal fluid, spinal fluid, sputum, perspiration, synovial fluid, lacrimal fluid and stool specimens as well as other biological fluids readily apparent to one skilled in the art. Fluid preparations of tissues can also be assayed. Preferably, human serum is assayed with this invention.

In its broadest embodiment, the dry element of this invention has an inert support with two reagent layers and a porous spreading layer disposed thereon. The support is generally dimensionally

stable, inert to chemical reaction and preferably transparent (that is, radiation transmissive for wavelengths between 200 and 900 nm). However, non-transparent supports can be used if the mode of
5 detection is reflectance spectroscopy instead of transmission spectroscopy. Useful supports are well known in the art, and include but are not limited to polyesters, papers, metal foils and polystyrene, polycarbonates and cellulose esters.

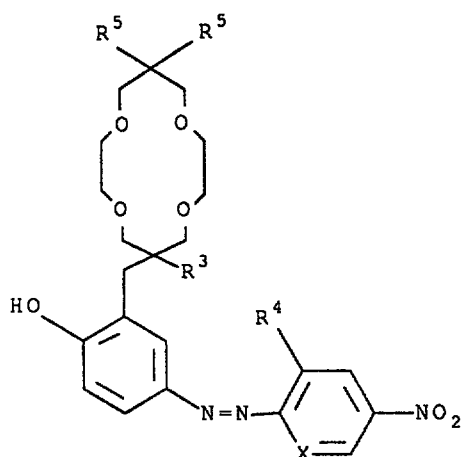
10 The porous spreading zone is prepared from any of the known materials used for such zones as described, for example in US-A-4,292,272, US-A-3,992,158, US-A-4,258,001, US-A-4,430,436 and JP 57(1982)-101760.

15 Preferred spreading zones are those described in US-A-3,992,158 as "blush polymer" zones. Such zones can be formed on a supporting material by dissolving a polymer in a mixture of two organic liquids, one of which is a lower boiling, good
20 solvent for the polymer and other being a high boiling, non-solvent or poor solvent for the polymer. Within the porous zone can be incorporated particulate materials of various sizes to enhance the void volume.

25 The elements have at least two other layers which can contain one or more reagents needed for the assay. Such a layer can also be a second porous spreading layer, if desired. All of the layers are generally in fluid contact with each other, meaning
30 that fluids, reagents and reagent products can pass or be transported between superposed regions of adjacent layers, unless of course, a reagent is immobilized in some manner so it will not migrate within or without a layer.

The reagent layers can be composed of one or more binder materials [such as gelatin and other colloidal materials, hydrophilic polymers such as poly(vinyl alcohol), acrylamide polymers, vinylpyrrolidone polymers and others known in the art] in which reagents are incorporated.

In a first reagent layer nearest the support, is a 14-crown-4-ether derivative which is reactive with lithium ion to provide a colorimetric signal. Such a signal can be a shift in absorbance from invisible to visible (to the human eye), visible to invisible, or from one visible wavelength to another. A number of such derivatives are known in the art, for example JP 62/72683, JP 62/56485 and EP-A-0 516 227. The preferred derivatives are those represented by the structure (II):



wherein R^3 is substituted or unsubstituted alkyl of 1 to 15 carbon atoms (for example, methyl, ethyl, isopropyl, n-butyl, t-butyl, pentyl, hexyl, octyl, decyl, dodecyl, 3-ethyl-5-methyloctyl, benzyl and benzyl substituted with one or more lower alkyl groups such as methyl, ethyl and isopropyl). Also, R^4 is hydrogen, nitro, methylsulfonyl, N-alkylsulfamoyl or N,N-dialkylsulfamoyl wherein the alkyl is substituted or unsubstituted and has 1 to 20

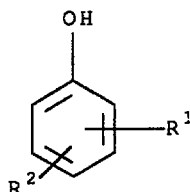
carbon atoms (such as methyl, ethyl, isopropyl, hexyl, benzyl, decyl, dodecyl, octadecyl and eicosyl). R^5 is substituted or unsubstituted alkyl of 1 to 10 carbon atoms (for example, methyl, ethyl, isopropyl, isobutyl, n-butyl, heptyl and decyl), and X is N or CH.

More preferred compounds of structure (II) are those wherein R^3 is methyl, benzyl or dodecyl, R^4 is hydrogen, nitro, methylsulfonyl or sulfamoyl, R^5 is methyl or heptyl and X is CH. Representative compounds are those in the following Table I with Compound 2 being most preferred:

TABLE I

	R^3	R^4	R^5	X
1	n-C ₁₂ H ₂₅	SO ₂ CH ₃	CH ₃	CH
2	n-C ₁₂ H ₂₅	NO ₂	CH ₃	CH
3	CH ₃	SO ₂ N(n-C ₆ H ₁₃) ₂	CH ₃	CH
4	CH ₂ C ₆ H ₅	SO ₂ N(n-C ₆ H ₁₃) ₂	CH ₃	CH
5	CH ₂ C ₆ H ₅	SO ₂ N(CH ₃)-n-C ₁₈ H ₃₇	CH ₃	CH
6	CH ₃	NO ₂	n-C ₇ H ₁₅	CH
7	n-C ₁₂ H ₂₅	H	CH ₃	N

Also present in the reagent layer containing the 14-crown-4-ether derivative is an aromatic phenol organic solvent having the structure (I):



wherein R^1 and R^2 are independently alkyl of 1 to 20 carbon atoms (such as methyl, ethyl, isopropyl, n-butyl, t-butyl, n-pentyl, t-pentyl, hexyl, octyl,

decyl, dodecyl and eicosyl) or acyl of 2 to 20 carbon atoms (such as acetyl, propionyl, valeryl and butyryl). Preferably, at least one of R^1 and R^2 is alkyl, and the groups are in the 2- and 4-positions
5 in relation to the hydroxy group.

More preferably, R^1 and R^2 are independently alkyl of 1 to 10 carbon atoms, or acyl of 3 to 6 carbon atoms. Representative aromatic phenol organic solvents include those described in
10 Research Disclosure, March, 1978, pages 23-24, and US-A-2,835,579. Particularly useful solvents include 2,4-di-n-pentylphenol, 2,4-di-t-pentylphenol, 2-n-octyl-4-pentylphenol, 2-n-nonyl-4-n-pentylphenol, 2-methyl-4-n-decylphenol, 2-n-decyl-4-methylphenol, 2-
15 methyl-4-n-octadecylphenol and 2-n-valeryl-4-n-pentylphenol. Other useful solvents would be readily apparent to one skilled in the art. Most preferred is 2,4-di-n-pentylphenol.

A second reagent layer is disposed on the
20 first reagent layer. The two layers can be immediately adjacent, or separated by a thin subbing layer (described below) as long as the reagents and fluid can readily move between the layers. In this second reagent layer is an aromatic phenol organic
25 solvent which also is defined by structure (I). The solvents in the two reagent layers can be the same or different, but preferably, they are the same compound. A mixture of aromatic phenol organic solvents can be used in either layer if desired.

30 Optionally, but preferably, the porous spreading layer is separated from the two reagent layers with a hydrophilic subbing layer composed of one or more suitable hydrophilic binder materials. Such materials include, but are not limited to
35 gelatin and other colloidal materials, polymers of

vinyl pyrrolidone, vinyl alcohol, acrylamide, N-alkyl-substituted acrylamide (such as N-isopropylacrylamide), including copolymers thereof, and other materials readily apparent to one skilled
5 in the art.

The pH of at least 9 in the element is maintained during an assay for lithium ion by one or more suitable buffers. The buffer is incorporated into one or more layers other than the two reagent
10 layers, and preferably, it is in the hydrophilic subbing layer. Useful buffers include, but are not limited to, 3-(cyclohexylamino)-1-propanesulfonic acid, 4-aminobutyric acid, phosphate and arginine. Preferably, the pH is maintained at 10 or above with
15 the upper limit being 14.

It is particularly useful to have one or more perfluoroalkyl surfactants in at least the second reagent layer. Preferably, such a surfactant is placed in both reagent layers. Such surfactants
20 can be anionic, cationic, nonionic or amphoteric in nature. They have one or more fluorocarbon moieties in the molecule, each of which has one or more hydrogen atoms replaced by a fluorine atom. Each fluorocarbon moiety has at least 4 carbon atoms, and
25 can be saturated or unsaturated. When the surfactant has two or more fluorocarbon moieties, each moiety are predominantly straight chain groups having no more than one or two small branches (for example, methyl or ethyl, fluorinated or unfluorinated). The
30 moieties can be connected together by an organic backbone which is linear or branched. When the surfactant has only one fluorocarbon moiety, that moiety can be branched or linear.

More particularly, each fluorocarbon moiety
35 has from 4 to 16 carbon atoms, from 8 to 33 fluorine

atoms, and a ratio of fluorine atoms to carbon atoms of at least 2:1.

Representative surfactants are as follows:

Nonionic Surfactants:

5 Perfluoroalkylpoly(ethylene oxide) alcohols, for example, commercially available as ZONYL™ FSN from DuPont, or as FLUOWET™ OT from American Hoechst, or ethoxylated perfluoroalkyl materials such as SURFLON™ 145 from Asahi Glass
10 (also known as "surfactant F-35").

Cationic Surfactants:

 Perfluoroalkyl quaternary ammonium salts, for example, commercially available as FLURAD™ FC 135 from 3M Corporation, as LODYNE™ 106 from Ciba-
15 Geigy, or as ZONYL™ FSC from DuPont.

Amphoteric Surfactants:

 Perfluoroalkyl betaines, for example, commercially available as SURFLON™ S-132 from Asahi Glass Co., or as ZONYL™ FSK from DuPont.

20 Anionic Surfactants:

 Fluoroalkylsulfates, for example commercially available as FLUORTENSID™ FT-248 from Bayer, or as FLUOWET™ SB from American Hoechst, or fluoroalkylalkylenethioalkylenecarboxylates, for
25 example commercially available as ZONYL™ FSA from DuPont.

 Preferred perfluoroalkyl surfactants include the perfluoroalkyl betaines (such as SURFLON™ S-132 amphoteric surfactant) and the
30 ethoxylated perfluoroalkyl surfactants (such as SURFLON™ S-145).

 The amount of useful perfluoroalkyl surfactant in each reagent layer is generally at least 0.005 g/m² with an amount of from 0.005 to 0.2
35 g/m² being preferred.

The amounts of critical reagents (that is, the 14-crown-4-ether derivative and organic solvents described above) can be readily determined by a skilled worker also. However, the amounts may be
5 within the range of from 0.05 to 2, and preferably from 0.2 to 0.8 g/m², of the 14-crown-4-ether derivative. The organic solvent in the first reagent layer may be present in an amount of from 0.5 to 10, and preferably from 2 to 6, g/m². The organic
10 solvent in the second reagent layer may be present in an amount of from 0.5 to 12, and preferably from 2 to 6, g/m². All amounts are dry weight.

The assay of this invention can be manual or automated. In general, the element is used by
15 physically contacting it with the test specimen (for example from 1 to 200 µl) suspected of containing lithium ion under ambient conditions (although other temperatures can be used). The specimen and reagents become mixed within the layers of the element and any
20 lithium ion complexes with the 14-crown-4-ether derivative to produce a spectrophotometric change as described above. Contact can be achieved in any suitable manner, for example by dipping or immersing the element into the specimen or preferably, by
25 spotting the specimen onto the element by hand, machine or suitable dispensing means.

After specimen application, the element is exposed to any conditioning, such as incubation, heating or otherwise, that may be desirable to
30 quicken or otherwise facilitate obtaining a test result.

Generally within 5 minutes, and preferably within 2.5 minutes, a spectrophotometric measurement is made. This measurement can be made using suitable
35 reflection or transmission spectrophotometric

equipment and procedures as a measure of lithium ion concentration in the test sample.

When the preferred 14-crown-4-ether derivatives listed in Table I above are used in the practice of the invention, the spectrophotometric change is generally measured at a wavelength which is within the range of from 400 to 700 nm, with measurement at a wavelength of from 450 to 650 nm being preferred. The use of other complexing compounds may require measurement at other wavelengths as one skilled in the art could readily ascertain.

Example 1 Preferred Analytical Element for Lithium Ion Determination

15 The preferred element of this invention and amounts of components are illustrated in the structure:

		Dry Coverage (g/m ²)
Spreading Layer	Barium sulfate	100
	Cellulose acetate	8
	ESTANE TM polyurethane	1.4
	TRITON TM X-405 nonionic surfactant	0.7
Subbing Layer	Poly(vinylpyrrolidone)	6
	3-(N-cyclohexylamino)-1- propanesulfonic acid buffer (pH 10.6)	6
Second Reagent Layer	Hardened gelatin	10
	SURFLON TM 132 surfactant	0.02
	2,4-Di-n-pentylphenol	3.2
First Reagent Layer	Hardened gelatin	12
	SURFLON TM 132 surfactant	0.02
	Compound 2 of Table I	0.4
	2,4-Di-n-pentylphenol	3.2
Poly(ethylene terephthalate) Support		

Example 2 Comparative Example Using Alternative
5 Element

5 An element similar to that of Example 1 was
used to detect lithium ion in a serum specimen and
compared to the performance of a Control element which
was like it except 2,4-di-n-pentylphenol was omitted
10 from the second reagent layer. The element of this
invention was like that of Example 1 except: the
polyurethane coverage was 3 g/m², TRITONTM X-100
nonionic surfactant was used in place of TRITONTM X-405

nonionic surfactant, each component of the subbing layer was at 4 g/m², SURFLON™ 145 surfactant was used at 0.01 g/m² instead of SURFLON™ 132, the amount of 2,4-di-n-pentylphenol in the second reagent layer was 4 g/m², and 0.6 g/m² of Compound 2 was used.

The assays were performed on the elements (which had been kept at 25°C and 15% relative humidity until use) as follows:

Serum samples (10 µl) containing varying amounts (0.27 to 10.59 mmolar) of lithium ion were spotted onto the spreading layer of individual elements. After incubation for about 48 seconds at 37°C, the reflectance density (D_R) was measured at 600 nm using a standard recording reflectance densitometer. The lithium ion concentrations obtained from these reflectance densities were compared to the predicted concentrations for the various amounts of analyte in the serum samples. Additional densities were measured after about 130 seconds (from specimen spotting) and after about 290 seconds.

Tables II, III and IV below show the reflectance densities measured at 48, 130 and 290 seconds, respectively, for both Control and Example 1 elements. The data include the standard of deviation (S.D.) at each concentration. The standard of deviation is a parameter which indicates the variation from a mean generated from several repeated determinations. It is indicative of precision since a high precision would have a low standard of deviation. The data provided in the 0.27 to 3.71 mmolar concentration is most important since this is the most useful range for determination of lithium ion concentration in patients.

T A B L E II

Reflectance Density (D_p)	Actual Lithium Ion Concentration	Predicted Lithium Ion Concentration	S.D.
-Control-			
0.59038	0.27	0.30	0.0500
0.60684	0.53	0.51	0.1114
0.62593	0.79	0.76	0.0568
0.64594	1.02	1.01	0.0543
0.68924	1.52	1.58	0.1563
0.75296	2.50	2.43	0.1221
0.85375	3.72	3.81	0.2058
0.93319	5.00	4.93	0.2207
-Example 2-			
0.38294	0.27	0.31	0.0356
0.40113	0.53	0.58	0.0579
0.41258	0.79	0.77	0.0631
0.42807	1.02	1.02	0.0560
0.45465	1.52	1.58	0.0435
0.50820	2.55	2.49	0.0452
0.56291	3.71	3.64	0.0644
0.62740	5.00	5.15	0.2099
0.81685	10.59	10.55	0.1966

T A B L E III

Reflectance Density (D_m)	Actual Lithium Ion Concentration	Predicted Lithium Ion Concentration	S.D.
-Control-			
0.78156	0.27	0.31	0.0658
0.80167	0.53	0.57	0.2403
0.81553	0.79	0.76	0.0886
0.83239	1.02	0.98	0.1027
0.87722	1.52	1.58	0.2299
0.93576	2.50	2.38	0.1232
1.04067	3.72	3.82	0.2163
1.11907	5.00	4.92	0.2429
-Example 2-			
0.40299	0.27	0.32	0.0542
0.42751	0.53	0.55	0.0516
0.45080	0.79	0.79	0.0548
0.47036	1.02	1.00	0.0417
0.51114	1.52	1.47	0.0593
0.59127	2.55	2.51	0.0580
0.66887	3.71	3.66	0.1363
0.75481	5.00	5.12	0.2172
1.01130	10.59	10.55	0.1998

T A B L E IV

Reflectance Density (D _r)	Actual Lithium Ion Concentration	Predicted Lithium Ion Concentration	S.D.
-Control-			
1.01963	0.27	0.34	0.0964
1.04389	0.53	0.71	0.4242
1.04574	0.79	0.74	0.1413
1.05871	1.02	0.94	0.1240
1.10091	1.52	1.61	0.3912
1.14445	2.50	2.30	0.1471
1.23757	3.72	3.81	0.3548
1.30090	5.00	4.87	0.3579
-Example 2-			
0.39812	0.27	0.32	0.0480
0.42333	0.53	0.55	0.0518
0.44727	0.79	0.79	0.0541
0.46905	1.02	1.01	0.0335
0.51070	1.52	1.47	0.0457
0.59200	2.55	2.50	0.0542
0.67216	3.71	3.66	0.1163
0.76110	5.00	5.13	0.1737
1.02234	10.59	10.55	0.1798

The data in these tables indicate that placing the aromatic organic solvent in the second reagent layer improves the precision of the assay particularly at the longer evaluation times (130 and 290 seconds) and in the desired concentration range of 0.27 to 3.71 mmolar. This enables a user of the element to accurately determine lithium ion concentration at longer times.

Examples 3 & 4 Elements Having Various Amounts of Organic Solvent in Second Reagent Layer

Elements similar to that shown in Example 2 were prepared having 1 and 5 g/m² of 2,4-di-n-pentylphenol in the second reagent layer (Examples 3

and 4, respectively), and used to detect various levels of lithium ion (0.27-10.59 mmolar) according to the procedure described in Example 2. Precision was acceptable at each concentration of organic solvent, as
5 shown with the data presented in Tables V (Example 3) and VI (Example 4) below.

T A B L E V

Reflectance Density (D ₀)	Actual Lithium Ion Concentration	Predicted Lithium Ion Concentration	S.D.
-48 seconds-			
0.43183	0.27	0.35	0.0164
0.45324	0.53	0.54	0.0282
0.47795	0.79	0.78	0.0430
0.49758	1.02	0.99	0.0400
0.53201	1.52	1.41	0.0597
0.61064	2.55	2.56	0.0989
0.68539	3.71	3.92	0.0574
0.73044	5.00	4.87	0.6131
0.94355	10.59	10.55	0.0630
-130 seconds-			
0.44875	0.27	0.32	0.0189
0.47573	0.53	0.54	0.0298
0.50347	0.79	0.78	0.0431
0.52751	1.02	1.01	0.0451
0.56852	1.52	1.43	0.0555
0.66064	2.55	2.55	0.0787
0.75085	3.71	3.86	0.0370
0.81433	5.00	4.92	0.4491
1.08263	10.59	10.57	0.1380
-290 seconds-			
0.44499	0.27	0.31	0.0152
0.47193	0.53	0.54	0.0309
0.49855	0.79	0.79	0.0463
0.52136	1.02	1.01	0.0458
0.56143	1.52	1.44	0.0567
0.65089	2.55	2.56	0.0786
0.73822	3.71	3.85	0.0392
0.80076	5.00	4.91	0.4259
1.06657	10.59	10.57	0.1352

T A B L E VI

Reflectance Density (D _a)	Actual Lithium Ion Concentration	Predicted Lithium Ion Concentration	S.D.
-48 seconds-			
0.42546	0.27	0.31	0.0221
0.45121	0.53	0.54	0.0277
0.47606	0.79	0.78	0.0450
0.49740	1.02	0.99	0.0096
0.54326	1.52	1.49	0.0560
0.62680	2.55	2.53	0.0400
0.70933	3.71	3.72	0.0605
0.79057	5.00	5.05	0.1284
1.05804	10.59	10.57	0.1515
-130 seconds-			
0.41903	0.27	0.32	0.0246
0.44748	0.53	0.55	0.0310
0.47560	0.79	0.79	0.0534
0.49669	1.02	0.98	0.0156
0.54473	1.52	1.45	0.0526
0.64198	2.55	2.55	0.0431
0.72740	3.71	3.67	0.0354
0.82192	5.00	5.09	0.1384
1.11149	10.59	10.56	0.1590
-290 seconds-			
0.41180	0.27	0.33	0.0254
0.43846	0.53	0.55	0.0329
0.46573	0.79	0.79	0.0575
0.48559	1.02	0.98	0.0161
0.53145	1.52	1.45	0.0534
0.62512	2.55	2.54	0.0499
0.70829	3.71	3.67	0.0254
0.80133	5.00	5.10	0.1465
1.08220	10.59	10.56	0.1270

Example 5 Comparison of Calibration Curves

This example shows a comparison of calibration curves generated at various concentrations (0.27-10.59 mmolar) of lithium ion using an element of this invention and a Control element which is outside the scope of this invention. The element of this invention was that shown in Example 1 and the Control element was similarly prepared except that the organic solvent was omitted from the second reagent layer. The procedure for determining reflectance density was like that described in Example 2. FIGS. 1 and 2 show the calibration curves for various analyte concentrations over time for the assays using the Control (FIG. 1) and invention (FIG. 2), respectively.

The amounts of lithium ion concentration used in these assays were (as defined in the FIGURES):

Fluid A: water (no lithium ions),
Fluid B: 0.27 mmolar,
Fluid C: 0.53 mmolar,
Fluid D: 0.79 mmolar,
Fluid E: 1.02 mmolar,
Fluid F: 1.52 mmolar,
Fluid G: 2.50 mmolar,
Fluid H: 3.72 mmolar,
Fluid I: 3.72 mmolar, and
Fluid J: 5.00 mmolar.

Example 6 Effects of Fluorinated Surfactants

This example demonstrates the importance of having perfluoroalkyl surfactants in the second reagent layer with the organic solvent to stabilize the suspension of solvent in the coating formulation.

The element of Example 1 was used to assay samples of various fluids containing various amounts (0- 2.4 mmolar) of lithium ion. A Control element was similarly prepared except that the SURFLONTM 132

surfactant was replaced in both reagent layers with ALKANOLTM XC nonionic surfactant (0.02 g/m²). The results are illustrated in FIGURES 3 (Control) and 4 (Invention). The fluids used were as followed:

- 5 Fluid A: Pool of patient samples, 0.05 mmolar lithium ion,
 Fluid B: Pool of patient samples, 0.9 mmolar lithium ion,
 Fluid C: Pool of patient samples, 2.4 mmolar
10 lithium ion,
 Fluid D: 0.55 mmolar lithium ion,
 Fluid E: 1.6 mmolar lithium ion,
 Fluid F: Pool of patient samples, 0.23 mmolar lithium ion,
15 Fluid G: Pool of patient samples, 1.05 mmolar lithium ion,
 Fluid H: Pool of patient samples, 2.47 mmolar lithium ion,
 Fluid I: Control fluid, 0.71 mmolar lithium
20 ion,
 Fluid J: Control fluid, 0.82 mmolar lithium ion,
 Fluid K: Pool of patient samples, no lithium ion,
25 Fluid L: Pool of patient samples, 0.89 mmolar lithium ion,
 Fluid M: Pool of patient samples, 2.36 mmolar lithium ion.

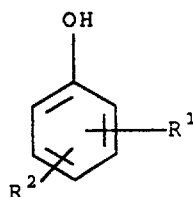
30 It is clear that the element of this invention showed a reduced rate of background dye development (that is, dye development from sources other than lithium ion) compared to the Control element.



CLAIMS:

1. A dry multilayer analytical element for the determination of lithium ion comprising a support having thereon, in order and in fluid contact:

- 5 a) a first reagent layer containing a 14-crown-4-ether derivative dye for lithium ion,
 b) a second reagent layer containing a perfluoroalkyl surfactant, and an aromatic phenol organic solvent having the structure (I):



10

wherein R^1 and R^2 are independently alkyl of 1 to 20 carbon atoms or acyl of 2 to 20 carbon atoms, and

- c) a porous spreading layer,
the element further comprising, in a layer
15 other than the first or second reagent layers, a buffer present in an amount sufficient to maintain a pH in the element of at least 9 during an assay,

the element characterized wherein it further comprises a second aromatic phenol organic solvent in
20 the first reagent layer, which second aromatic phenol organic solvent has the structure (I), and the first and second organic solvents can be the same or different.

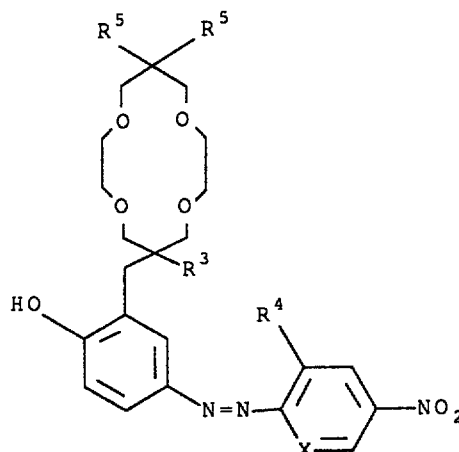
2. The element as claimed in claim 1
25 further comprising a subbing layer between the second reagent and porous spreading layers, and wherein the buffer is in the subbing layer.

3. The element as claimed in either of
claims 1 or 2 wherein the buffer is present in an
30 amount sufficient to maintain a pH in the element of at least 10 during an assay.



4. The element as claimed in any of claims 1 to 3 wherein the buffer is 3-(cyclohexylamino)-1-propanesulfonic acid, 4-aminobutyric acid, phosphate or arginine.

5. The element as claimed in any of claims 1 to 4 wherein the 14-crown-4-ether derivative dye has the structure (II):



wherein R³ is substituted or unsubstituted alkyl of 1 to 15 carbon atoms, R⁴ is hydrogen, nitro, methylsulfonyl, N-alkylsulfamoyl or N,N-dialkylsulfamoyl wherein said alkyl is substituted or unsubstituted and has 1 to 20 carbon atoms, R⁵ is substituted or unsubstituted alkyl of 1 to 10 carbon atoms, and X is N or CH.

6. The element as claimed in any of claims 1 to 5 wherein the first and second aromatic phenol organic solvents are the same.

7. The element as claimed in any of claims 1 to 6 wherein R¹ and R² are the same and are butyl, pentyl or hexyl.

8. The element as claimed in any of claims 1 to 7 wherein the first and second aromatic phenol organic solvents are both either 2,4-di-n-pentylphenol or 2,4-di-~~t~~-pentylphenol.



-25-

10. A method for the determination of lithium ion comprises:

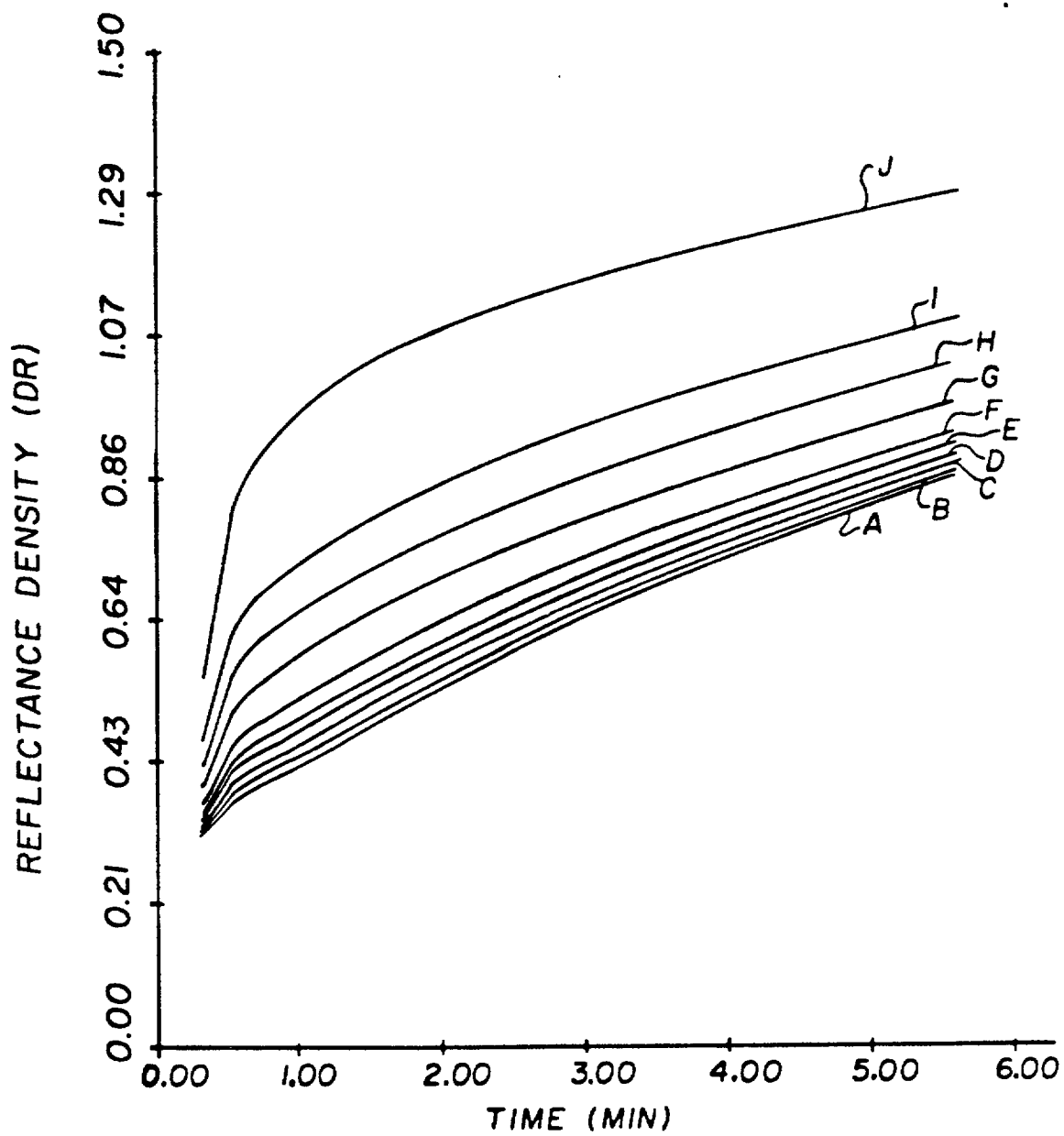
- 5 A. contacting a liquid sample suspected of containing lithium ion with a dry multilayer analytical element as claimed in any of claims 1 to 9, and
- B) determining the change in spectrophotometric signal in the element from complexation of the 14-crown-4-ether dye derivative with lithium ion in the liquid sample.

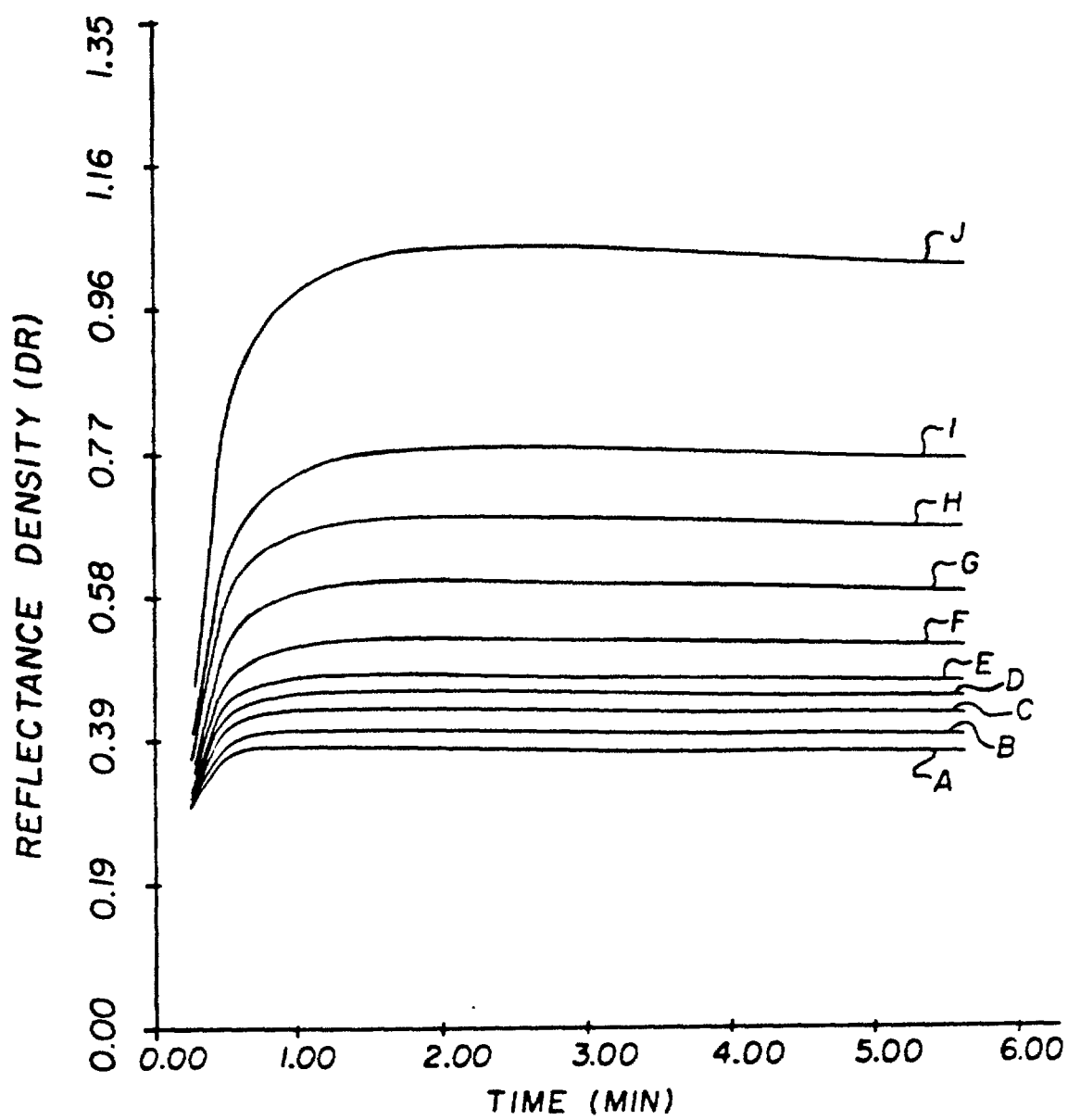
**DRY ANALYTICAL ELEMENT AND METHOD FOR THE
COLORIMETRIC DETERMINATION OF LITHIUM ION**

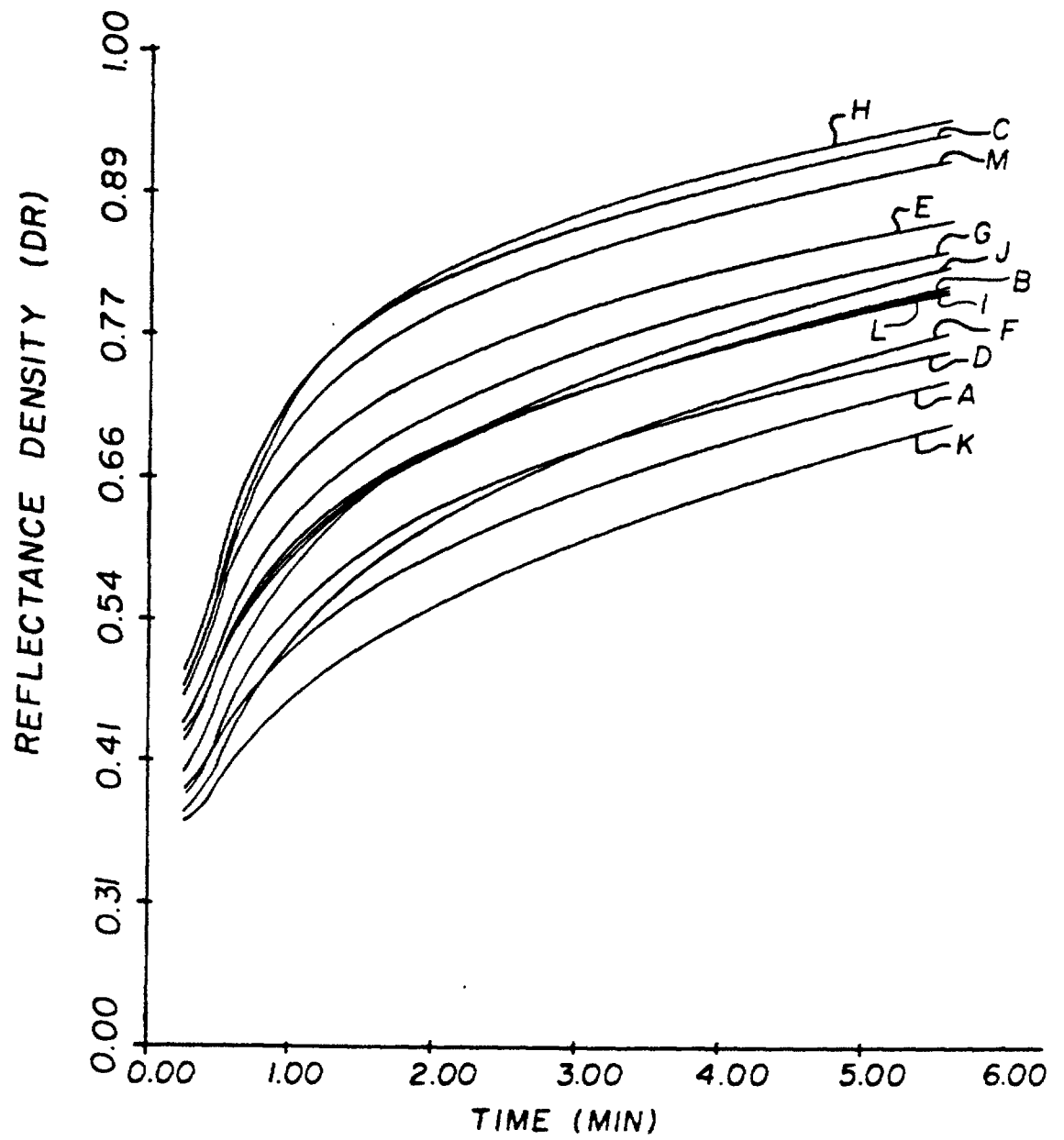
Abstract

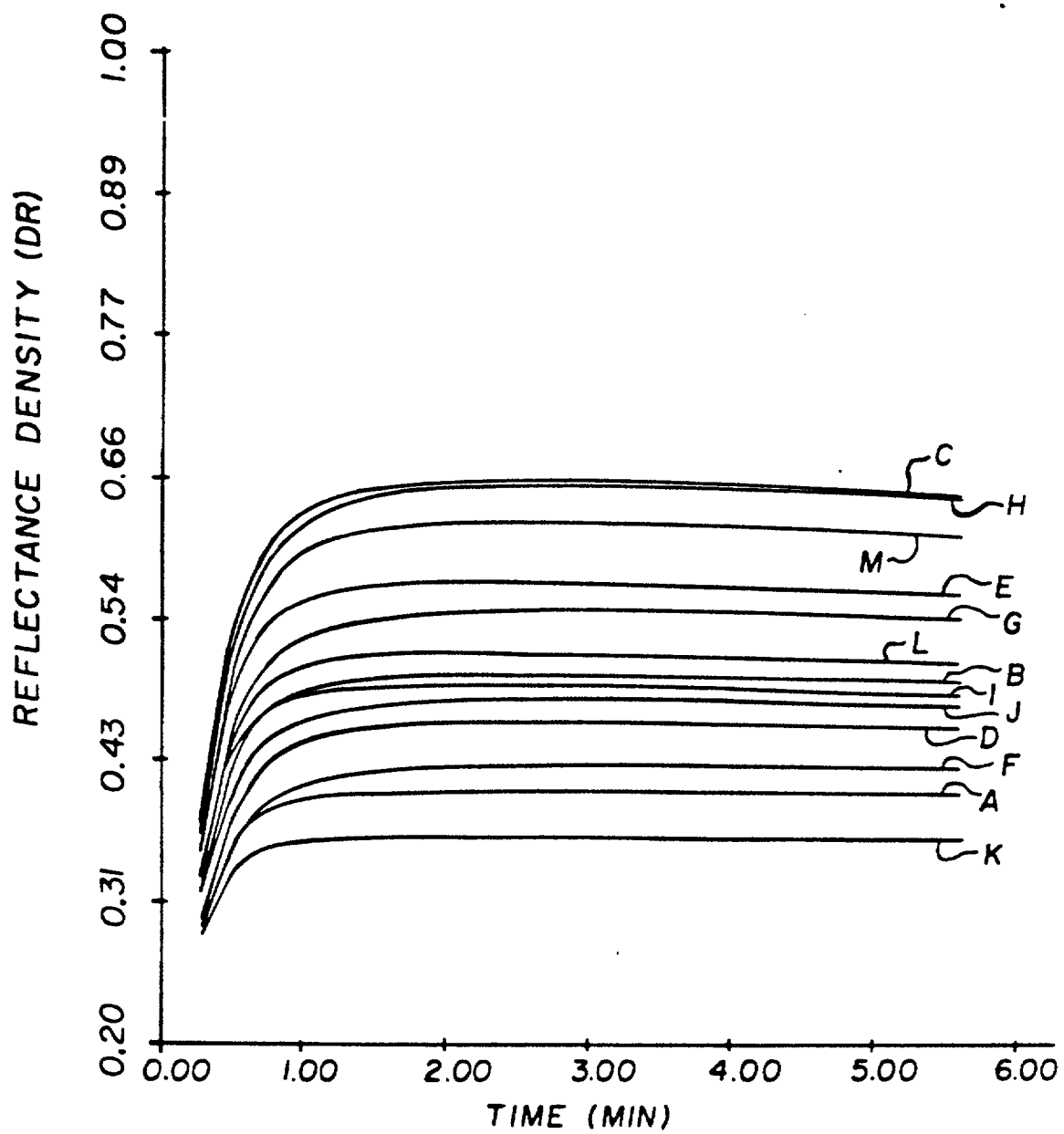
A multilayer analytical element has been
5 prepared for use in the accurate colorimetric
determination of lithium ion found in aqueous
specimens. The element includes first and second
reagent layers underneath a porous spreading layer. A
more precise assay at longer read times of dye
10 formation and reduced rate of background dye
development are achieved because the second reagent
layer contains both an aromatic phenol organic solvent
and a perfluoroalkyl surfactant which were formerly
included only in the first reagent layer. A 14-crown-
15 4-ether derivative dye is used to complex with lithium
ion to provide the colorimetric signal.

(Figure 2)

**FIG. 1**

**FIG. 2**

**FIG. 3**

**FIG. 4**