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ANALYSIS AND PURIFICATION OF 2-HYDROXYETHYL METHACRYLATE BY MEANS OF THIN-LAYER CHROMATOGRAPHY*

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SUMMARY

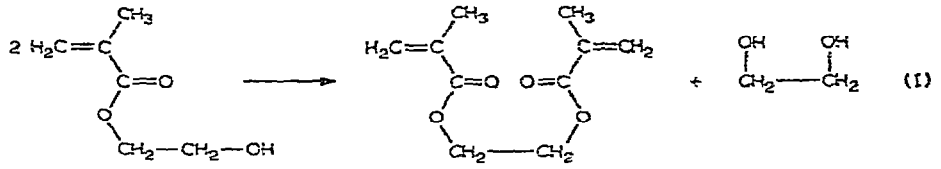
Poly(2-hydroxyethyl methacrylate) is nowadays accepted as a biocompatible, safe and stable hydrogel for medical use. In this paper, the use of thin-layer chromatography for the analysis and small-scale preparation of the initial monomer, 2-hydroxyethyl methacrylate, is described. Development on silica gel, with *n*-hexane–diethyl ether (1:1, v/v) and/or *n*-hexane–isobutyl methyl ketone–*n*-octanol (9:2:1, v/v; saturated with 25% nitric acid) is recommended for qualitative analysis. Preparative-scale work is preferably carried out on sulphuric acid-impregnated silica gel, with *n*-hexane–diethyl ether (1:1, v/v) as mobile phase. Inhibitors are detected by thin-layer chromatography and a drop-test procedure with diazotised sulphanilic acid. The nature of the contaminants present in several commercial samples of 2-hydroxyethyl methacrylate is discussed. n_D^{20} values are reported for the system 2-hydroxyethyl methacrylate–water.

INTRODUCTION

Three-dimensional networks of hydrophilic polymers, the so-called hydrogels, have recently been recognized[1,2] as useful, perhaps unique, materials for biomedical and surgical applications. Implants and other protheses are prepared from such gels, often reinforced with a fabric structure. The research programme of our departments aims at the development of a hydrogel that, combined with a filler, can serve as a substitute for hard tissue (bone), and promotes calcification and/or bone-growth in its matrix, so that the initially soft material is filled up by the body itself with hard tissue. As the hydrogel, poly(2-hydroxyethyl methacrylate) (poly-HEMA) has been selected; this can be prepared from the commercially available monomer, 2-hydroxyethyl methacrylate (HEMA; see Table I) by various methods (see *e.g.*, refs. 3–5).

* Part of the contents of this paper was presented at the Symposium on Hydrogels for Medical and Related Applications, August, 1975, Chicago, Ill., U.S.A.

Poly-HEMA, which has been widely studied and discussed for medical applications, nowadays has largely been accepted[1,6] as a biocompatible, safe and stable hydrogel for medical use. In order to prepare a gel that meets such requirements, the initial monomer should obviously be a high-purity product. Unfortunately, however, commercially available HEMA contains relatively large proportions of methacrylic acid and the diester ethylene dimethacrylate (EDMA; see Table I); EDMA can be formed from HEMA by transesterification, as shown below:



(I)

Further contaminants may well be present, as is discussed later.

The absence of EDMA from HEMA is particularly desirable, because the proportion of diester in the polymerizing mixture affects the nature of the network formed. Since the rates of both polymerization and transesterification reactions increase greatly with temperature, techniques such as distillation and gas chromatography are unsuitable for the ultimate purification and analysis of HEMA samples. Therefore, in the present study, thin-layer chromatography (TLC) was chosen for both qualitative analysis and small-scale preparative work.

MATERIALS AND METHODS

HEMA was purchased from BDH (Poole, Great Britain) and E. Merck (Darmstadt, G.F.R.); these products have a yellow colour. Colourless highly pure (> 99%) HEMA was obtained as a gift from Hydro Med Sciences (New Brunswick, N.J., U.S.A.).

EDMA was obtained from BDH and from Koch-Light (Colnbrook, Great Britain). Methacrylic acid, ethylene glycol, 2-hydroxypropyl methacrylate, methyl methacrylate, hydroquinone and *p*-methoxyphenol, were purchased from Merck. Diethyleneglycol methacrylate has been synthesised[7] by mixing appropriate amounts of diethylene glycol (855 g) and methyl methacrylate (500 g) at a temperature of 60°, adding a 4 *N* solution of sodium methanolate in methanol (10 g) and heating the mixture for 30 min. Subsequently, the mixture is poured into water (400 g), washed with *n*-hexane and extracted twice with diethyl ether. After repeated washings with water, the ether extract is dried, and diethyleneglycol methacrylate is distilled at reduced pressure.

All other chemicals were reagent-grade products, which were used without further purification.

TLC is carried out in Hellendahl staining jars, using pre-coated silica gel plates (Kieselgel 60 F_{254}, Merck) cut into appropriate sizes (4 × 8 cm). Spots are applied with a pointed paper-wick, and chromatography is carried out in a non-saturated atmosphere. After development for approx. 7 cm, detection is carried out by using the methods reported below.

Preparative-scale TLC is carried out on plates (20 × 20 cm) of the same gel

(layer 2 mm thick); 100–150 mg of HEMA, as a 20% (v/v) solution in ethanol, are applied with a Camag chromatocharger equipped with a disposable plastic syringe, and development (for 15–17 cm), without prior equilibration, is done in a normal rectangular tank.

Refractive indices are measured on a thermostatted (20±0.1°) Abbe refractometer, and IR spectra are recorded on a Shimadzu IR 400 spectrometer, using cells with sodium chloride windows.

RESULTS AND DISCUSSION

Reference substances and identification

As stated in the introduction, HEMA generally contains several impurities, notably EDMA, methacrylic acid and ethylene glycol. Moreover, since methacrylic acid and its derivatives tend to polymerize even at room temperature, as a rule an inhibitor such as hydroquinone, *p*-methoxyphenol, phenothiazine or octylpyrocatechol is added to these products[8]. According to the specifications, the HEMA samples obtained from BDH and Merck contain 200 ppm of *p*-methoxyphenol, and 100 ppm of hydroquinone plus 100 ppm of *p*-methoxyphenol, respectively; HEMA from Hydro contains 36 ppm of *p*-methoxyphenol. Hydroquinone is also present in samples of EDMA and methacrylic acid. Methyl methacrylate may also be present as an impurity in commercial HEMA, since it is one of the starting materials in its synthesis. However, during TLC, it evaporates from the chromatoplate (b.p., 100°) and is not usually detected in qualitative analysis; in preparative-scale work, such evaporation during development ensures its removal. Moreover, by developing a sample of pure methyl methacrylate and spraying with water[9] immediately after the run, we have shown its R_F value to be considerably higher than that of HEMA in both solvent systems recommended below. As regards the presence of water, see Fig. 3 and the accompanying text.

As a consequence of the above, as well as HEMA, five compounds were included in our study, *viz.*, EDMA, methacrylic acid, ethylene glycol, hydroquinone and *p*-methoxyphenol (see Table I). The purity of the samples obtained as reference substances was tested, using two or more of the solvent systems discussed below;

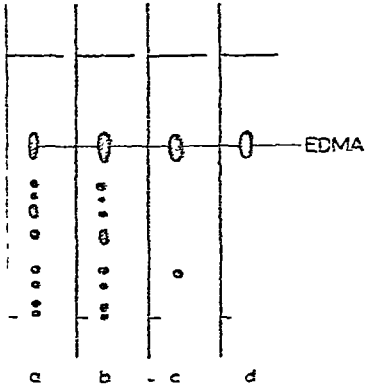


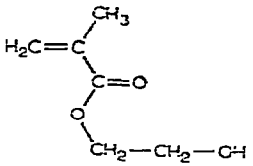
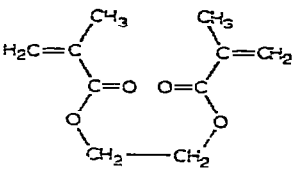
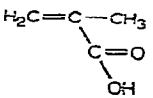
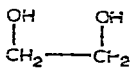
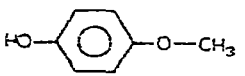
Fig. 1. Chromatograms of EDMA samples: (a) from BDH; (b) from Koch-Light; (c) from BDH, but redistilled; (d) from BDH, but purified by preparative-scale TLC. Layer: silica gel; developing solvent: *n*-hexane–diethyl ether (1:1).

these samples were found to be chromatographically pure (apart from the presence of an inhibitor), with the exception of EDMA. Here, TLC with, *e.g.*, *n*-hexane–diethyl ether (1:1) as mobile phase reveals the presence of some eight additional spots (see a and b in Fig. 1). These contaminants are removed by distillation under reduced pressure (b.p. of EDMA at 4 mm Hg, 90°) or, preferably, by preparative-scale TLC (see c and d in Fig. 1). With the former technique, addition of hydroquinone or of a mixture of potassium chloride, bismuth trichloride and bismuth tri-iodide (ref. 10) is necessary to prevent polymerization reactions.

As regards detection, from a large number of non-specific methods of identification, three were selected for further research. Under UV radiation (254 nm), all compounds except ethylene glycol are visible as dark spots on a fluorescent background; treatment with iodine vapour (red-brown spots on a yellow-white background) is a suitable means of detecting this glycol. Unfortunately, the sensitivity of

TABLE I

STRUCTURE AND DETECTION LIMITS OF HEMA AND ITS CONTAMINANTS

Compound	*Structural formula*	*Detection limit (μg/μl)**				
		UV	I_2	$KMnO_4$	*Echtblausalz B*	*Diazotised sulphanilic acid*
2-Hydroxyethyl methacrylate (HEMA)		30	100	0.6		
Ethylene dimethacrylate (EDMA)		30	15	0.6		
Methacrylic acid		30	60	0.6		
Ethylene glycol		—	15	0.2		
Hydroquinone		15	0.6	0.6	0.2	0.2
p-Methoxyphenol		15	0.6	0.6	0.2	0.2

* For HEMA, d_4^{20} has a value of 1.070–1.074 (ref. 13). Therefore, 1 μg of contaminant per μl of HEMA roughly corresponds to 1000 ppm or 0.1% (w/w).

the combined UV–iodine procedure is rather low (*cf.* Table I). As an alternative, therefore, detection has been achieved by spraying with a 0.2% solution of potassium permanganate in acetone; white spots are formed on a brownish background for all compounds except *p*-methoxyphenol, which shows up as a pale-orange spot. Since permanganate reacts with impurities in the solvent to form manganese dioxide, which mars the detection, the reagent solutions should be freshly prepared. Keeping in mind that controlling the purity of HEMA samples is done by TLC of undiluted HEMA, one may conclude from the data in Table I that spraying with permanganate solution allows the detection of impurities down to at least 0.1%.

Since the inhibitors are present in the various samples of HEMA, EDMA and methacrylic acid in concentrations less than 0.02%, they will not be detected by the above procedures. Therefore, two alternative methods of identification[11,12] have been tested, *viz.*, (1) spraying with a freshly prepared 0.5% aqueous solution of the diazo reagent Echtblausalz B, and then either spraying with 0.1 *N* sodium hydroxide or, preferably, exposure to ammonia vapour; (2) spraying with a mixture of 0.09 g of sulphanilic acid dissolved in 10 ml of 1.1 *N* hydrochloric acid and 10 ml of 4.5% aqueous sodium nitrite kept at 0° and diluted with an equal volume of 10% sodium carbonate solution immediately before use. With both these reagents, brownish spots show up on an almost colourless background. As can be seen from the data in Table I, even the use of these reactions barely suffices for identification of the inhibitors in methacrylic acid and its derivatives. Fortunately, improved results are obtained if sulphanilic acid is used to detect the phenolic compounds by a drop-test procedure, in which one drop of the diazotised reagent is mixed with one drop of sample (dissolved in the minimum amount of ethanol, if necessary) and one drop of 10% sodium carbonate solution; this gives a detection limit of *ca.* 0.01% for hydroquinone (green to yellow spots), and 0.001% for *p*-methoxyphenol (red to green spots).

Solvent systems

After several trial runs of the four main components with single-solvent systems ranging from non-polar (*n*-hexane, cyclohexane) to highly polar (diethyl ether, isobutyl methyl ketone (MIBK), acetone), mixtures of *n*-hexane with diethyl ether or other component(s) were selected for further study. Satisfactory results were obtained with *n*-hexane–diethyl ether (1:1, v/v) and *n*-hexane–MIBK–*n*-octanol (9:2:1, v/v) (see a and d in Fig. 2). However, it was observed that the spot due to methacrylic acid tended to "tail", especially in the latter solvent system. Such tailing, which is particularly undesirable in preparative-scale TLC or column chromatography, can effectively be reduced by adding an acidic component to the system. Two alternatives have been investigated. In one, the solvent system was saturated with an equal volume of 25% (v/v) nitric acid; when a higher percentage of acid was used, decomposition of one or more of the compounds under investigation occurred during development, as was shown by the occurrence of a brown streaking zone on the chromatogram. In the second method, development is carried out on a silica gel plate that has been soaked for 15 min in 1% sulphuric acid and subsequently dried overnight at 60°. It can be seen from Fig. 2 that, with each of these techniques, results were improved.

For qualitative analysis, development with a mobile phase saturated with nitric acid should be preferred to TLC on an acid-impregnated support, which is a

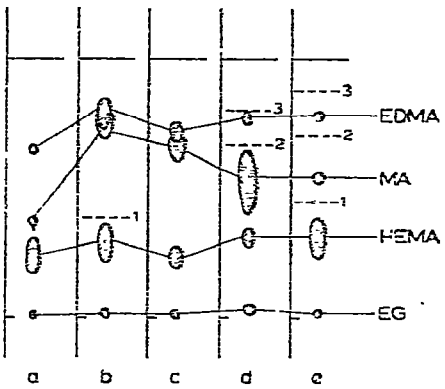


Fig. 2. TLC of HEMA, EDMA, methacrylic acid (MA) and ethylene glycol (EG) in the systems (a) silica gel/*n*-hexane–diethyl ether (1:1); (b) silica gel/*n*-hexane–diethyl ether (1:1, saturated with 25% nitric acid); (c) sulphuric acid-impregnated silica gel/*n*-hexane–diethyl ether (1:1); (d) silica gel/*n*-hexane–MIBK–*n*-octanol (9:2:1); (e) silica gel/*n*-hexane–MIBK–*n*-octanol (9:2:1, saturated with 25% nitric acid). The dashed lines show the fronts for acid (1), *n*-octanol (2) and MIBK (3).

more time-consuming procedure. From Fig. 2, it is evident that *n*-hexane–MIBK–*n*-octanol (9:2:1, saturated with 25% nitric acid) is a powerful solvent system as regards resolution of the four main components. Addition of nitric acid to *n*-hexane–diethyl ether (1:1) also improves the separation of HEMA from methacrylic acid, but the spots due to methacrylic acid and EDMA show an appreciable overlap. Therefore, as a tentative conclusion, we recommend the combined use of both *n*-hexane–diethyl ether (1:1) and *n*-hexane–MIBK–*n*-octanol (9:2:1, saturated with 25% nitric acid) for qualitative analysis.

For preparative-scale TLC, development on silica gel impregnated with sulphuric acid is preferred to development with a mobile phase saturated with nitric acid, because sulphuric acid (but not nitric acid) has a negligibly small solubility in the solvent used to elute HEMA from the support material (see below). *n*-Hexane–diethyl ether (1:1) has been selected as mobile phase (rather than the *n*-hexane–MIBK–*n*-octanol mixture) on account of the relatively long time of development needed with the latter system (*ca.* 4 h, compared with 2.5 h for a 16-cm run). Also, owing to the low volatility of *n*-octanol, its complete removal from the layer is not easily effected, and part of it is eluted together with HEMA.

Next, attention should be paid to the selection of a solvent for eluting the separated component(s) from the silica gel. At first sight, this choice does not appear to be critical, because HEMA dissolves freely in such solvents as water, acetone, diethyl ether and carbon tetrachloride and, though less so, in *n*-hexane. However, when using any of the first three highly polar solvents, the acid used to impregnate the silica gel is extracted together with HEMA. Moreover, when development is carried out on silica gel F_{254}, zinc (originating from the manganese-doped zinc silicate present as fluorescing indicator) is also extracted, as its sulphate. The presence of both the acid and its zinc salt in purified HEMA have been demonstrated by TLC on plain silica gel, with *n*-hexane–diethyl ether (1:1) as developing solvent. Both compounds remain at the origin and are identified by spraying with a suitable acid–base indicator [*e.g.* thymol blue (pH range, 1.2–2.8)] and 4-(2-pyridylazo)resorcinol,

respectively. As a consequence, the relatively non-polar carbon tetrachloride is preferred as eluent.

When considering the TLC data for evaluation of systems suitable for column chromatography, one should consider that development with a mobile phase saturated with nitric acid causes the formation of an acid front at R_F values only slightly higher than those of HEMA (see b and e in Fig. 2). As a consequence, in chromatography on columns equilibrated with nitric acid-saturated solvent systems, the separation of HEMA from contaminants having higher R_F values will probably be marginal; preference should therefore be given to chromatography on silica gel impregnated with sulphuric acid. For obvious reasons, *n*-hexane–diethyl ether (1:1) is again recommended as mobile phase.

Application

Thin-layer chromatograms of all reference substances and HEMA samples are shown in Fig. 3; further information is recorded in Table II. The chromatograms demonstrate that —apart from the inhibitors— ethylene glycol and/or other polar material, and EDMA are present in HEMA samples from both Merck, and BDH; the BDH sample also contains methacrylic acid. Detectable amounts of methacrylic acid and EDMA are absent from HEMA obtained from Hydro, but even this highly pure product contains at least two impurities. The spot with R_F 0.0 is attributed to ethylene glycol and/or other polar material. As for the second spot, a compound displaying R_F approx. 0.10 in both solvent systems used in the present study has also been observed in the HEMA samples obtained from Merck and BDH, and milligram amounts of it have been collected by preparative-scale TLC and column chromatography. On the basis of its mass spectrum (*m/e* value of 144; presence of three oxygen atoms) the unknown compound was initially thought to be hydroxypropyl methacrylate. However, comparison of the TLC behaviour of the unknown compound and a sample of pure 2-hydroxypropyl methacrylate showed that this assumption was incorrect. Recently, from information obtained from Hydro, it has become evident that HEMA samples may contain *ca.* 0.4% of diethyleneglycol methacrylate. This compound was synthesised in our laboratory (*cf.* above) and was shown to be identical with the unknown compound by both TLC and mass spectrometry*. In addition, we noted that a sharp separation of HEMA from diethyleneglycol methacrylate is obtained by development with pure diethyl ether instead of *n*-hexane–diethyl ether (1:1); the R_F values are 0.80 and 0.55, respectively.

As regards the inhibitors, with *n*-hexane–diethyl ether (1:1), hydroquinone displays an R_F value approximately equal to that of HEMA and cannot be detected with either Echtblausalz B or diazotised sulphanilic acid. When using *n*-hexane–MIBK–*n*-octanol (9:2:1, saturated with 25% nitric acid) as mobile phase, hydroquinone has a slightly faster migration rate than HEMA, its spot overlapping that of methacrylic acid. However, detection of small amounts of hydroquinone is not successful. In separate experiments, we have demonstrated that this is due to severe streaking of the hydroquinone spot, caused by the presence of the large excess of

* As regards the discrepancy between the mass of diethyleneglycol methacrylate (174) and the value quoted above (144), it is well known that, in mass spectrometry, polyglycols easily loose a part of their molecule having a mass of 30, *i.e.*, the *m/e* value of 144 should be attributed to $[M - CH_2O]^+$.

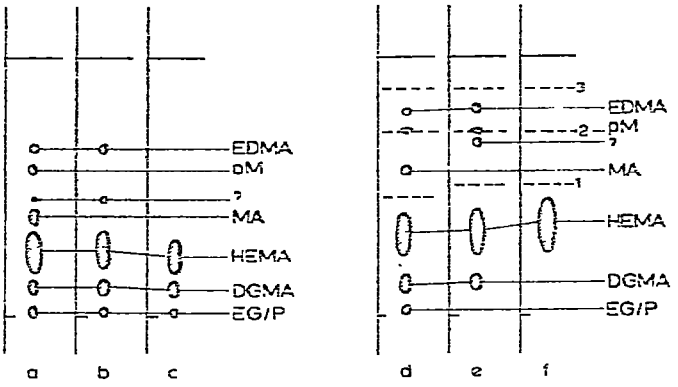


Fig. 3. TLC of three HEMA samples on silica gel, using *n*-hexane–diethyl ether (1:1) (a–c), and *n*-hexane–MIBK–*n*-octanol (9:2:1, saturated with 25% nitric acid) (d–f) as mobile phase. HEMA samples obtained from BDH (a, d), Merck (b, e) and Hydro (c, f). For abbreviations, see Table II. The dashed lines show the fronts for acid (1), *n*-octanol (2) and MIBK (3).

HEMA. In consequence, hydroquinone can only be detected in isolated instances (methacrylic acid). No such difficulties are encountered with *p*-methoxyphenol; with this inhibitor, identification by TLC is usually successful. Still, with both compounds, in view of the rather unfavourable detection limits quoted in Table I, we recommend that 2–4 microlitres of sample solution be applied to the chromatoplate and that development be for 10–15 cm. Alternatively, pre-concentration of the inhibitors by means of treatment of the sample solution with Amberlyst A-27 resin (see below) may be used. In conclusion, it is worthwhile to add (as is evident from Table II) that the drop-test procedure with sulphanilic acid is well suited to detect the presence, although not the type, of an inhibitor.

TABLE II

R_F DATA FOR HEMA AND ITS PRINCIPAL CONTAMINANTS IN TLC ON SILICA GEL WITH *n*-HEXANE–DIETHYL ETHER (1:1) AS MOBILE PHASE

A positive drop test indicates the presence of an inhibitor.

Compound	*Abbreviations (cf. Fig. 3)*	*hR$_F$*				
		Pure compound	*HEMA (BDH)*	*HEMA (Merck)*	*HEMA (Hydro)*	*Methacrylic acid*
EDMA	EDMA	70	70	70	—	—
p-Methoxyphenol	pM	60	60	60	60	—
Methacrylic acid	MA	40	40	—	—	40
Hydroquinone	Hy	25	*	*	*	25
HEMA	HEMA	25	25	25	25	—
Diethyleneglycol methacrylate	DGMA	10	10	10	10	—
Ethylene glycol/ polar material	EG/P	0	0	0	0	—
Drop test			+	+	+	+

* Not detectable; see text.

Lastly, comparison of the overall picture of the series of chromatograms obtained with *n*-hexane–diethyl ether (1:1), and the series obtained with *n*-hexane–MIBK–*n*-octanol (9:2:1, saturated with 25% nitric acid) demonstrates that a smaller number of spots is observed when the latter solvent system is used. This is chiefly due to the fact that *n*-octanol, owing to its low volatility, cannot easily be removed quantitatively from the chromatogram before identification. In consequence, on spraying with potassium permanganate solution, the colour of the background turns to brown rather rapidly, thus obscuring several of the smaller spots. To summarize, *n*-hexane–diethyl ether (1:1) should be preferred for qualitative analysis, both on account of its faster migration rate and the better detection achieved for all components of the HEMA and EDMA samples.

Preparative-scale TLC on silica gel impregnated with sulphuric acid has successfully been carried out with HEMA samples from Hydro and BDH. (For all compounds studied, the R_F values in this system are equal to, or slightly higher than, those in the system silica gel/*n*-hexane–diethyl ether, saturated with 25% nitric acid; *cf.* Fig. 2). After development with *n*-hexane–diethyl ether (1:1) and elution with carbon tetrachloride, the samples are chromatographically pure, except for the presence of hydroquinone. However, an inhibitor will certainly be added to the purified samples in order to protect them against undesired side reactions up to the time of their actual use. Only after polymerization of HEMA to poly-HEMA are the inhibitors quantitatively removed by prolonged washing with water. Thus, the presence of hydroquinone in the purified HEMA is not harmful. However, should quantitative removal of hydroquinone at an early stage be imperative, then two consecutive treatments of the sample to be purified with Amberlyst A-27 resin (Rohm and Haas, Philadelphia, Pa., U.S.A.) suffice to remove up to 0.2% of hydroquinone, as well as other polar compounds; these can subsequently be eluted with methanol. The concentrated extract so obtained is well suited for detecting the presence of one or more inhibitors, as was referred to above.

A final word about two further criteria used to assess the purity of HEMA and EDMA, *viz.*, the refractive index and the IR spectrum. According to our experience, n_D^{20} values generally quoted in the literature are not a very reliable criterion, since the impurities normally present in HEMA exhibit n_D^{20} values that may be higher (for EDMA and diethyleneglycol methacrylate) or lower (for methacrylic acid and ethylene glycol) than that of HEMA itself. As an illustration, a series of data is recorded in Table III. Also, the refractive index of HEMA decreases as the water content increases (see Fig. 4). Infrared spectroscopy has been recommended for

TABLE III

VALUES OF n_D^{20} FOR HEMA AND SOME OF ITS CONTAMINANTS

Compound	n_D^{20}	*Reference*
HEMA	1.4525	1, 2, 4
Diethyleneglycol methacrylate	1.4568	7
EDMA	1.4549	2, 4
	1.4549–1.4553	1
Ethylene glycol	1.4318	5
Methacrylic acid	1.4314	4, 5
Methyl methacrylate	1.4142	5

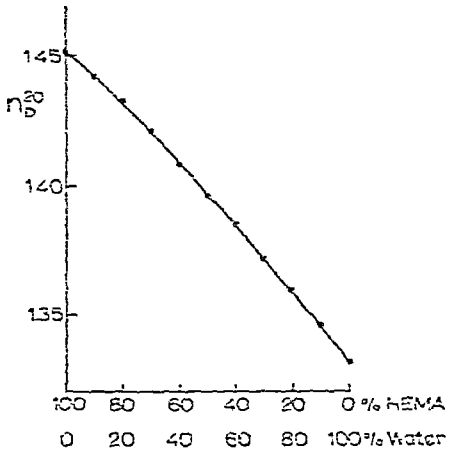


Fig. 4. Dependence of n_D^{20} on water content of HEMA.

checking the purity of EDMA. Our results indicate that compounds such as HEMA and ethylene glycol can be detected down to approx. 1% (w/w) from the strong absorption of their hydroxyl groups (broad band at 2800–3600 cm^{-1}). However, it should be borne in mind that the IR spectrum cannot easily be used to draw conclusions regarding the nature of the contaminant(s), *e.g.*, HEMA, ethylene glycol or water. Also, a compound such as methacrylic acid will be undetected even at the 1% level.

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