

CHAPTER 4

The Nature of Dentine and the use of Dentine in *in vitro* Models

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4.1 Introduction

Dentine fulfills many roles as the largest component of tooth structure. As well as supporting overlying enamel via a complex interface, the dentine-enamel junction, dentine provides open communication to sensory pulpal tissue. Critical events occurring during dentinogenesis elaborate the structure of dentine and so create the morphology required to enable the functions of dentine. Dentine is, therefore, a complex tissue which is still incompletely described. For example, the transmission of sensory stimulus through dentine remains theoretical in Brännström's hydrodynamic theory based on intuitive studies.

The release of TEGDMA from composite resin has been demonstrated in the current work and findings were presented in Chapter 3. Amongst the experimental aims of the current work, is to examine the influence of dentine on TEGDMA release. To do this, an *in vitro* tooth model incorporating dentine was used. To enable the findings to be meaningfully discussed, dentine structure and relevant characteristics, such as dentine permeability and the nature of smear layer, will be discussed in the current Chapter. Then, as several workers have previously used tooth models to examine the interface between dental materials and dentine, these studies will be reviewed.

4.2 The nature of dentine

4.2.1 Dentinogenesis

The embryological development of tooth structure has been often described in stages. Development commences at about the sixth week of life with oral epithelium disposed as basal and surface layers. Thickening and elaboration of these elements continues through stages known as *bud*, and the appearance of the enamel organ, and *cap* with the emergence of the dental sac. The primordial origins of dentine are seen in the *bell* stage. Interactions between epithelial and mesenchymal cells stimulate the differentiation of inner epithelial cells into tall, columnar ameloblasts (Seltzer and Bender, 1965). Underlying this layer, further differentiation of cells of the dental papilla yields the first *odontoblasts*. Specific enzymes characterise these events and include the hydrolytic enzymes: alkaline phosphatase, acid phosphatase, esterases and adenosine triphosphatases. Oxidative enzymes such as lactate dehydrogenase, succinic dehydrogenase and diphosphopyridine nucleotide diaphorase also contribute.

During dentinogenesis the basic cellular framework for dentine is established. This has followed complex cellular and enzymatic interactions within primordial dental structures. As odontoblasts are about to produce dentine they extrude collagen precursor granules whilst a peritubular matrix simultaneously differentiates around their process cell membranes. Progressive calcification of this matrix then occurs but to different degrees in different areas of the matrix so that peritubular dentine becomes more highly calcified than intertubular. This arrangement characterizes mature dentine. However, early dentine is laid down as a fibrous connective tissue, or *predentine*, which develops to a thickness of 15-20 μm before it mineralizes to form dentine. Predentine immediately surrounds the pulp.

4.2.2 Structure of dentine

An elegant description of the structure of dentine has been provided by Pashley (1991). Dentinal tubules extend from the *dentine-enamel junction* toward the pulp in a curved course and vary in diameter from about 2-3 μm at the dentine-pulp interface to about 1 μm at the dentine-enamel junction. Density of tubules varies from 40,000-65,000/ mm^2 at the pulp to 15,000-20,000/ mm^2 at the dentine-enamel junction (Garberoglio and Brännström, 1976) although regional differences in tubule distribution have also been described (Garberoglio, 1994). Variation in tubule density and diameter in deeper dentine is of tremendous significance in restorative dentistry. Pashley has noted that the combined effect of increased tubule diameter and the convergence of the tubules on the pulp confers increased permeability on deep dentine. This difference in permeability of deeper and superficial dentine has tremendous implications for restorative dentistry and is discussed further in a later section. In addition, whilst tubules at the dentine-enamel junction may exhibit branching processes, those tubules closest to the pulp may only show simple structure (Seltzer and Bender, 1965). With age the tubule narrows as further peritubular dentine is deposited although intratubular mineralization also occurs (Thomas, 1985). Peritubular dentine is comparatively more highly mineralized, largely containing hydroxyapatite, and is present in thicker layers in mid- and outer-dentine.

In housing the processes of odontoblasts the dentine is generally considered to be a vital component of the *dentine-pulp complex* such that injury to dentine generally causes an effect on pulp tissue. The extent of the odontoblastic process within vital dentine, however, remains ill-defined

(Maniatopoulos and Smith, 1983; Thomas and Carella, 1984; La Flesche *et al.*, 1985; Thomas, 1985; Weber and Zaki, 1986). The process completely fills the tubule at the predentine-dentine junction, but in more superficial dentine, a space develops around the process, the *periodontoblastic space*, which contains granular material and collagen fibrils (Thomas, 1985). Clinically, as a cavity is prepared in dentine, the resulting pulpal wall is likely to be in a region where most investigators have identified odontoblastic processes by scanning electron microscopy (Pashley, 1989). It has been suggested that this feature of deep dentine may contribute to its protective function by preventing the diffusion of noxious substances (Theilade and Theilade, 1976).

Neural elements within tubules usually extend to only 100-150 μm into the tubules (from the pulpal surface) whereas collagen fibrils may extend considerably further. Dentinal fluid contains proteins similar to plasma and probably originates from terminal capillaries in the pulp and diffuses along periodontoblastic spaces to the dentine-enamel junction (Thomas, 1985).

A variety of analytical tools, including microbiologic assay and chromatography, have been used to clarify the inhomogeneous chemical nature of dentine. The *organic* component of dentine is composed largely of extensively cross-linked type 1 trimer collagen, primarily located in intertubular dentine, although some type V may also be present (Munksgaard and Möe, 1980; Mjör, 1985). Other matrix proteins include phosphoproteins (Krippner and Nawrot, 1977; Termine *et al.*, 1980; Ibaraki *et al.*, 1991) proteoglycans, gamma-carboxyglutamate-containing proteins, acidic glycoproteins, phosphoryns (Fujisawa *et al.*, 1984; Nakamura *et al.*, 1985) and serum proteins; a consideration of these proteins provides good insight into dentinogenesis (Butler, 1992). Non-collagenous proteins have also been identified in the rat (Linde *et al.*, 1980). Dentine has been described as "a matrix composed of collagen protein and sulphated acid mucopolysaccharides into which calcium and phosphorus salts are deposited" (Seltzer and Bender, 1965). Thus dentine has an *inorganic* component, as well as the calcifiable structure of the intertubular and peritubular matrices. The inorganic component of dentine is largely imperfect hydroxyapatite, containing 4% carbonate distributed over the crystal surface and in the apatite. Some trace elements are also present

such as Cl, Mg, Sr, Fe, Zn and Pb. Fluoride has also been found distributed in dentine (Mukai *et al.*, 1994).

Different organic/inorganic proportions characterize different types of dentine. *Physiological secondary dentine* is formed after tooth eruption, deposited by pulp tissue and differs from primary dentine in tubule orientation, but is formed by the same odontoblasts as primary dentine (Seltzer and Bender, 1965). *Sclerotic dentine* equates to hypermineralized dentine following from the development of irregular secondary dentine and does not appear to need the presence of intact odontoblast cells and processes. Sclerotic dentine which is characterized by obliterated tubules and occurs between the exposed dentine and the pulp, may develop as a favourable response of the dentine-pulp complex to a slowly progressing carious lesion. *Irregular secondary dentine* is generally deposited in the pulp in response to pathological processes (Mjör, 1985). Tubules vary greatly in irregular secondary dentine in orientation, cellular inclusions and in degree of mineralization. The chemistry of dentine is also related to its metabolic functions in providing exchange of fluid from the pulp outward into the dentine and from the enamel inward to the dentine. It is this interchange which contributes to the development of sclerotic dentine.

4.2.3 Dentine hydrodynamics

The dynamics of fluid movement in dentinal tubules has been considered in great detail by several workers particularly with respect to dentinal pain. The hydrodynamic theory proposes a cause for dentine sensitivity (Brännström, 1966; Brännström *et al.*, 1967; Brännström and Åström, 1972) and is based on the observations made by Brännström and his co-workers (Brännström, 1960; Brännström, 1961; Brännström, 1962; Brännström, 1963; Brännström and Åström, 1964; Berggren and Brännström, 1965; Brännström, 1966) although other groups have also contributed (Hensel and Mann, 1956; Avery and Rapp, 1959; Winter *et al.*, 1963; Beveridge and Brown, 1965). The theory basically states that "all stimuli producing dentinal pain result in the removal of fluid from the dentinal tubules" (Brännström, 1981a) so that fluid shifts in either direction through dentinal tubules causes deformation of mechanoreceptors in predentine that is responsible for pain. Dehydration of the cut dentinal surface, for example, induces capillary flow outward from the pulp to replace evaporated fluid and it is this outward flow which concomitantly distends neural elements. The

magnitude of dentinal fluid shifts are an important implication of the theory. Brännström and co-workers (1968) calculated that tubule contents move 5-50 μm during the first second of an air blast on the surface and maximum flow rates of 2-4 mm/s have been recorded (Berggren and Brännström, 1965).

Tubules contain tissue fluid which exerts an outward pressure of about 30 mm Hg which is sufficient to empty the contents of a tubule 10 times a day if exposed on the surface. This flow, however, "does not prevent toxins or other substances from reaching the pulp through diffusion in a pulpward direction" and in fact "dentinal tubules are thus accessible not only to pain producing stimuli but also to toxins which may quickly reach the pulp through diffusion" (Brännström, 1981a). The theory also accounts for extensive inward movement of tubule contents (toward the pulp) resulting from stimuli such as heat (Hensel and Mann, 1956; Brännström and Ånström, 1972).

The experiments of Pashley have established several important concepts about dentine hydrodynamics which have challenged the completeness of the hydrodynamic theory. Dentinal tubules act as channels allowing "bidirectional diffusion" of substances across dentine (Pashley, 1991). Pulpal interstitial fluid flows outward, from the pulp, at a rate determined by pulpal tissue pressure and hydraulic conductance (Pashley, 1991). Resistance to this positive hydrostatic pressure is proportional to the remaining dentinal thickness or to tubule diameter. This means that thin dentine is more sensitive to hydrodynamic stimulus than thicker dentine. Clinically, these dynamics are, in part, responsible for the sensitivity of dentine following restorative procedures where deep dentine is approached. Hydrodynamic stimuli induce rapid fluid shifts of tubule contents by convective transport, which activate specific nerves responsible for pain (Pashley, 1991). The condition of the dentine surface and the presence of smear layer are also important factors in dentine hydrodynamics (Pashley, 1985). Smear layer and plugs together form a barrier to hydrodynamic fluid movement. Dentine pre-treatment regimes which remove smear layer and smear plugs effectively removes 86% of the resistance to fluid movement across dentine (Pashley *et al.*, 1978) and the solutions used in these regimes often induce fluid movement outward from the pulp (Pashley, 1991). Regional differences in dentine permeability, despite similar dentine thicknesses, have also been observed (Pashley *et al.*, 1987) and Pashley

attributes this to variation in the tubular contents in different areas. Further comment about dentine permeability is provided in a later section.

4.2.4 Dentine-pulp complex

Pulp is a composite connective tissue including water, cells, neural and vascular elements and extracellular matrix. Its basic chemistry is different from dentine with respect to quantity and type of organic components. Pulp tissue contains type I collagen with some type III collagen, reticular and oxytalan fibres and other components including proteoglycans, glycosaminoglycans and fibronectin and is immediately overlaid with a layer of odontoblasts, a layer of predentine and then dentine (Mjör, 1983). Immediately pulpward of the odontoblast layer is a *cell-free zone* (Mjör, 1983). As pulp and dentine share an embryological origin in the dental papilla and are structurally and functionally intimate, the tissues are often referred to together as the *dentine-pulp complex* and this convention is particularly useful in understanding responses of the tissues to pathological and physiological stimuli (Ten Cate and Torneck, 1982). The laying down of physiological secondary dentine illustrates one type of change in the dentine-pulp complex and many of the functions of dentine depend on the integrity of the interface.

4.2.5 Functions of dentine

Dentine, as the superstructure around the dental pulp, serves a protective function for this delicate tissue. As the substructure to enamel, the porous nature of dentine causes it to behave as a viscoelastic material, offering resilient and elastic support for occlusion and enabling the absorption of stress (Ten Cate and Torneck, 1982). Intact and unaffected dentine provides excellent structural support for the tooth against occlusal loading. Reparative dentine also provides this function although some of its mechanical properties differ from that of unaffected dentine. The thermal coefficient of expansion, for example, is higher in reparative dentine. The functions of dentine also include the ability to mount a response to insult, consistent with continued pulpal vitality, through the dentine-pulp complex. The implications of the presence of fluid within tubules also adds further function to this tissue which is particularly important to clinical restorative procedures (Pashley, 1991).

Dentinal tubules contain fluid and variable lengths of odontoblastic processes which together endow the tooth with several defence

mechanisms. Amongst these are pulpal inflammation, mineralization of material at exposed dentinal surfaces, formation of sclerotic dentine and irregular secondary dentine. After dentine is exposed to attritive or abrasive trauma it becomes coated with a mineralized layer composed of precipitated components from saliva and dentinal fluid which may block tubule orifices and initiate further mineralization of the dentine within two weeks. Irregular secondary dentine develops relatively slowly in the pulp space and can effectively block pulpal apertures of dentinal tubules (Brännström, 1981).

Some biological responses of the dentine are more favourable than others for continued vitality, and both systemic and local factors influence healing or repair in the tooth. The local factors which influence the type of response include the degree of toxic irritation, the number of tubules effected, proximity to the pulp and the degree of inflammation (Brännström, 1981). A slowly progressing carious lesion, for example, stimulates the development of an effective dentinal barrier. The slow progress allows the cell-rich zone to function under stimulus and produce irregular dentine. Undifferentiated mesenchymal cells of the cell-rich zone are able to differentiate into odontoblasts and replace lost cells (Stanley, 1976). If the lesion progresses rapidly or the host's immune response is more aggressive, the cell-rich zone may itself be destroyed, preventing any formation of dentine.

In addition to the functions outlined above, there are important clinical implications of the structure of dentine. Pashley (1991) has suggested that a "fluid-filled continuum from the cavosurface margins, around gaps between the restorative material and the tooth, to the dentine surface then through dentine via tubules to the pulp" exists whenever an imperfect dentine seal is created during cavity restoration. This "continuum" creates serious problems such as microleakage, poorly efficient dentine bonding and pulpal irritation. The gap, often as large as 20 μm (Saltzberg *et al.*, 1976), and formed between the restoration and cavity walls, enables the development of microleakage and the inevitable colonization of bacteria below restorations placed on prepared dentine. This can be further exacerbated by dentine pre-treatment procedures which remove smear layer. This effect is worse when the dentine is also very deep and when acidic solutions are used (Pashley, 1991). Fortunately, the slow outward fluid flow from the dentine-pulp complex can offer some resistance to bacterial

ingress into tubules (Vongsavan and Matthews, 1991). In addition, the healing capacity of the pulp, which reduces dentinal permeability by the formation of fibrin seals between damaged odontoblasts and dentinal tubules, also reduces the affects of bacterial microleakage (Pashley, 1991).

4.2.6 Smear layer

When dentine is mechanically disturbed, a layer of debris, called *smear layer* (Eick *et al.*, 1970), forms on the surface and extends into the openings of dentinal tubules. The composition of this layer may include amorphous collagen, minerals, macerated odontoblast processes, ground dentine debris, saliva, blood and bacteria and varies from 1 to 5 μm in thickness (Brännström, 1984). Smear layer cannot be removed by water irrigation and is more difficult to remove from dentine prepared with high speed than with low speed or hand instruments (Brännström and Johnson, 1974; Brännström *et al.*, 1980) but can be readily removed by acids so that even organic acids produced by plaque microorganisms or foods can dissolve smear (Absi *et al.*, 1987). Dry preparation of dentine may produce thicker smear layers (Eick *et al.*, 1970) although, whether prepared dry or wet, smear removal procedures are equally effective (Brännström *et al.*, 1980). Changes that occur in smear layer with time are unclear but some studies suggest that smear layer is readily lost if dentine is unprotected (Johnson and Brännström, 1974).

The clinical importance of smear layer is due to its ability to reduce dentine permeability. Smear contributes more than 85% of the total resistance to fluid movement across dentine (Anderson and Ronning, 1962; Pashley *et al.*, 1978). It has other features that may be of importance in restorative dentistry. The layer is hydrophilic, heterogeneous and amorphous. These features complicate dentine bonding techniques which attempt to chemically exploit smear layer in order to enhance bonding and exclude microleakage. Resin bonding systems often employ hydrophilic priming solutions which readily permeate smear layer and so overcome its barrier function, but *in vitro* bond strength tests which show cohesive failure within the smear layer (Pashley, 1991) have suggested that this layer is the "weak link". In this regard, some relatively early experimental work suggested that some chemical bonding may occur with acid-etched dentine (McLean, 1978; Fusayama *et al.*, 1979) although the presence of dentine fluid and collagen probably challenges the occurrence of this *in vivo*. Other research has indicated that smear layer can contain viable microorganisms

which can potentially fill the contraction gap formed between composite resin materials and cavity walls (Brännström and Nyborg, 1971; Brännström and Nyborg, 1973; Qvist, 1975; Beagrie and Brännström, 1977); this seems to support the necessity for the development of cavity cleansing and priming solutions.

The clinical dilemma of whether to remove smear layer or not prior to the placement of a lining or restorative material remains and studies can be found to support either option. Smear layer removal and dentine disinfection, before application of a luting agent or lining material, has been recommended by Brännström and co-workers (1982). Solutions used to remove smear layer include acids such as 5% phosphoric acid applied for 5 to 15 sec (Brännström and Johnson, 1974; Michelich *et al.*, 1978; Nordenvall and Brännström, 1980), and 15-20% EDTA (Brännström and Johnson, 1974; Brännström *et al.* 1980). Alcohols, which have also been investigated, create rapid dentinal fluid outflow when used *in vivo* (Linden and Brännström, 1967). Other workers, however, have suggested that the presence of smear layer can minimize the harmful effects of microbial microleakage and of the chemical toxicity associated with restorative materials (Browne and Tobias, 1986). In addition, the ability of smear layer removal to greatly enhance dentine permeability to monomer released from resin luting agents during crown cementation has been demonstrated (Al-Fawaz *et al.*, 1993).

4.2.7 Changes in dentine and pulp

Changes to the organic and inorganic content of dentine, resulting from pathological and physiological influences, have been discussed by Mjör (1983). Cavity preparation, for example, may cause displacement of odontoblast nuclei into tubules (Langeland, 1957). This can disrupt cell organelles and initiate early degenerative change in the odontoblasts resulting in the formation of dead tracts, irregular secondary dentine and also increase the fibre content of the pulp. The displacement of intratubular mucopolysaccharides into the intertubular matrix also occurs.

Other changes in dentine include those which may affect mineralization, are age-related or are the results of external influence. Both hyper- or hypo-mineralization of dentine are associated with caries (Martens *et al.*, 1959) whereas dentine sclerosis and continued elaboration of peritubular dentine are examples of age changes. Amongst external effects which cause

change is the placement of some dental materials. Accelerated peritubular dentine formation in the periodontoblastic space of the pulpal areas of tubules, for example, is an observed effect following the placement of corticosteroid in prepared cavities (Mjör and Furseth, 1968) and intratubular obturation may be associated with some other cavity liners such as calcium hydroxide (Furseth and Mjör, 1972). Resorption of dentine can be a consequence of pathology or be induced by excessive pressure.

Pulpal tissue is also capable of change due to physiological and pathological influences. The persistence of untreated caries can provide a continuous stimulus for inflammation within the pulp. Trauma during clinical procedures and chemical toxins from restorative materials are just two other potential aetiological factors. The consequences of pulpal inflammation are pertinent to this consideration of dentine, because inflammation, in general, is associated with cellular recruitment from altered vascular permeability and this has the potential effect, in the rigid pulpal space, to raise fluid pressure. However, some animal studies have demonstrated that pulpal blood flow is not significantly influenced by potent vasoconstrictors such as histamine and bradykinin (Edwall *et al.*, 1973; Tønder, 1976) although varied capillary permeability and the absorption of fluid into the blood circulation probably relieve pressure.

4.2.8 Dentine permeability

Dentine can be considered a biological membrane. In this regard, membrane permeability may be considered in a thermodynamic way as the "geometric transition region between two homogeneous compartments" (Kedem and Katchalsky, 1963a). In general terms, this means that a potential exists across the region which drives flow with rate determined by permeability coefficients and that there exist within the region homogeneous areas which can be characterized by simple physical parameters. Although biological membranes, such as dentine, are complex heterogenous structures and overall permeability cannot be determined from partial permeability, it is useful to the current discussion to consider some broad principles.

Dentine contains an array of tubules and forces causing flow across such a membrane largely act perpendicular to the surface (Kedem and Katchalsky, 1963b). Amongst factors to be considered in dentinal flow is pulpal hydrostatic pressure which has been measured in the human in various

normal and pathological states and in response to drugs, although practical difficulties in recording have influenced values reported (Beveridge and Brown, 1965; Brown and Yankowitz, 1964; Luostarinen *et al.*, 1966). In intact teeth, interstitial pressure is considered to be between 15-10 mmHg and is partially dependent on systemic arterial pressure (Brown and Beveridge, 1966; Weatherred *et al.*, 1963), although large pulpal blood pressure rises have been found when large blood vessels are severed (Sticht *et al.*, 1966). Increased pressure, up to about 50-60 mmHg is seen in inflamed pulp (Stenvik *et al.*, 1972; Lervik and Mjör, 1977) and this pressure rise appears to be a local phenomenon only (Van Hassel, 1971). It can be concluded then, that pulpal pressure which influences direction and rate of fluid flow within dentinal tubules is a dynamic property and so may contribute to structure permeability in a definite although limited way.

The most extensive and comprehensive work considering dentine permeability has been completed by Pashley and his co-workers over several years. To evaluate dentine permeability, Pashley and his co-workers established the concept of *hydraulic conductance* or "the measure of the ease with which fluid under hydrostatic or osmotic pressure, can pass through a permeable barrier" (Reeder *et al.*, 1978). These workers calculated the value of this parameter using both an *in vitro* tooth model and animal studies. Variation in the value of this parameter was systematically determined for variables likely to effect dentine permeability such as: presence of pulpal tissue, dentine thickness, molecular size of different diffusing substances, tubular occlusion, smear layer removal, effects of plasma and salivary constituents, distance from the pulp, the effect of a variety of dentine modifying treatments and substances, such as desensitizing dentrifices, cavity varnishes, oxalate, calcium hydroxide and burnishing and air-drying (Pashley *et al.*, 1977; Pashley and Livingston, 1978; Pashley *et al.*, 1978a; Pashley *et al.*, 1981a; Pashley *et al.*, 1982; Pashley *et al.*, 1984a; Pashley *et al.*, 1984c; Pashley *et al.*, 1985a; Pashley *et al.*, 1985b; Pashley *et al.*, 1986; Pashley *et al.*, 1987a; Pashley *et al.*, 1987b; Fogel *et al.*, 1988).

Dentine permeability is "non-uniform" around the pulpal chamber and substances pass across dentine by diffusion which, by definition, creates a concentration gradient (with rate of diffusion determined by Fick's equation). Permeability is also related to the size of molecules present (less permeability is seen with larger molecules), is inversely related to

thickness (and therefore the volume through which diffusion occurs) and varies according to the history and condition of the dentine. Acid-etched dentine, for example, is 5-20 times more permeable than smear-coated dentine; in contrast, carious and sclerotic dentine are far less permeable than normal dentine (Miller and Massler, 1962; Sarnat and Massler, 1965). Smear layer reduces permeability and positive pulpal hydrostatic pressure opposes diffusion towards the pulp, although concentration gradients and capillary attraction forces acting clinically may facilitate diffusion of some molecules. Despite the good data provided by permeability studies, the nature of the chemical interactions of diffusing substances with dentine, however, remains to be clarified. Later work showed albumin, for example, reversibly binds with dentine structure, and as a "carrier" protein in blood, may serve a similar function in dentine diffusion (Pashley, 1988).

4.3 Use of tooth models in dental materials research

Tooth models have generally provided valuable data regarding the interaction of restorative materials with dentine but earliest models were limited in the information derived. As better analytical techniques became available, better model design followed. In general terms tooth models have provided good data regarding dentine permeability and have been used to examine biocompatibility of dental materials when interfaced with dentine.

Thomas (1941) examined phenol "penetration" into dentine *in vitro* using a variety of extracted teeth and compared the results with those that occurred *in vivo*. In the tooth model, permeation of a dye, containing phenol, from the pulp chamber through radicular dentine was examined by periodically testing the solution, in which the roots were submerged, with bromine water. Teeth were then sectioned and examined under "high powered" magnification, permeation was evident by dye patterns in the dentine. In the *in vivo* study, occlusal cavities were prepared in each of five teeth, the cavity floor was swabbed with phenol and "sealed" with gutta percha, which was then varnished. Teeth were then extracted after periods of between 20 and 172 h, sectioned and examined histologically for pulp response. There was little standardization of teeth in the Thomas study and it would be difficult to ascribe the observed inflammatory changes in the pulp to phenol penetration rather than to other factors, such as bacterial microleakage around the gutta percha restoration. Despite these reservations, several interesting observations were made. Amongst the

patients, "metamorphosed" dentine was impermeable to phenol, whilst "young" dentine was readily permeable. Permeation followed the curved tubular pattern through dentine (which provided an early appreciation of tubule disposition) and some dilution of the phenol occurred in the permeation. Thomas concluded that penetration of phenol was dependent on several factors; cavity depth, amount of phenol used, degree of dentine "metamorphosis", degree of secondary dentine and condition of the pulp.

Examination of the passage of substances through dentine has been considered both *in vivo* and in *in vitro* models. Some notable *in vivo* observations have included the potential for systemically administered substances to be detectable in dental hard tissue. Mandel and Sarkady (1946), for example, used systemically administered radioactive iodine in rats and detected deposits in enamel and dentine. Similar work has been done by Bartelstone and co-workers (1947) in a description of the physiology of teeth. A notable example in human dental pharmacological history in this regard is systemically administered tetracycline and its incorporation into enamel and dentine (Bevelander *et al.*, 1961; Owen, 1961; Harcourt *et al.*, 1962). Interestingly, the detection of systemically administered substances in dental tissues can be quite rapid. Young and Greulich (1963), for example, found tritiated glycine in odontoblastic cytoplasm 15 minutes after injection in the rat. It was suggested that the exchange of fluid may be either from the pulp to the dentine or conversely from the dentine surface downward to the pulp. Bartelstone (1951) also observed, *in vivo*, downward flow to the pulp in a cat, where the incisal tip of the cat's tooth was immersed in radioactive iodine and "within a short period" iodine was detected in the pulp. A similar observation was made in a later *in vitro* study by Hardwick (1961) who found radioactive glucose in the dentine and pulp of teeth after 1 day of immersion.

Often the use of a tooth model has been suggested to examine clinical observations. For example, during the 1940s, numerous reports of symptoms following restoration of teeth with methyl methacrylate (MMA) (Slack Jr, 1943) suggested that the pulp needed to be sufficiently protected by an insulating base or it would "inevitably be injured by penetration of the monomer" (Crone and Bloch, 1958). This study used extracted teeth in a tooth model and restored occlusal cavities with MMA containing a dye. Stüben (1953) used a similar tooth model to demonstrate that MMA was able to penetrate 1 mm of dentine in 1.5 min when monomer mixed with Sudan

red was placed in a cavity prepared in an extracted tooth. Bloch (1958) applied acrylic resin containing C^{14} -labelled MMA to the floor of cavities on the facial sides of teeth and examined the dentine at 7, 14 and 21 days qualitatively by autoradiography. Dentine thickness was observed to influence the penetration of monomer.

These early studies using tooth models, although poorly standardized and providing little quantitative analysis, stimulated interest in the permeability of dentine. Increasing sophistication has characterized *in vitro* models by utilizing carefully prepared dentine generally positioned in localization devices. Outhwaite and co-workers (1974) used dentine disks ($1.0-1.6 \pm 0.01$ mm thick) from the mid-occlusal portion of freshly extracted unerupted human third molar teeth of patients 16-29 years of age. Disks were cut using a handpiece and the enamel-side surface was also sanded flat with silicon-oxide paper. The disk was then placed between two rubber 'O' rings which located and sealed the disk between two polycarbonate chambers. Each chamber had outlet ports (to allow the application of fluid pressure through polyethylene tubing) or the enamel side was open to atmospheric pressure. Test materials and solutions could thus be applied to the enamel side and precise analysis of the solution obtained on the pulp side indicated the permeation of the substance through dentine. This basic model allowed modifications for the examination of different variables. Outhwaite and co-workers (1976), for example, used radiolabelled iodine to examine permeation in a tooth model. A hydrostatic pressure of 4 mm H_2O , exerted by the volume of iodine solution itself, was applied above the disk. Net flux of the diffusing molecules was the ratio between ^{125}I counts per minute on the pulp side divided by ^{125}I counts per mole on the enamel side. The dentine permeability coefficient (in cm/min) was then calculated from the ratio between the net flux and the product of the difference in iodine concentration between the pulp and enamel sides, and the dentine surface area, which was an adaptation of the Fick equation. For dentine 1.5 mm thick, the permeability coefficient was calculated to be about $4 \text{ cm} \times 10^{-4} \text{ min}^{-1}$. Regression lines, relating dentine surface area and thickness to dentine permeability of iodine, revealed that iodine permeation was directly proportional to dentine surface area, and inversely proportional to thickness. The model represented an elegant improvement over past models, providing early data on controlled diffusion through dentine, and was sensitive to low permeation rates. However, it is uncertain whether diffusion through dentine is, in fact, classically Fickian in nature since

tubule diameter and density are not uniform and the implications of both smear layer and hydrostatic pressure exerted from the pulp side were not evaluated.

Following on from the Outhwaite work, Merchant and co-workers (1977) compared ^{125}I *diffusion* with ^{125}I *filtration* using the split-chamber device which had been modified to enable hydrostatic pressure (240 cm of the iodine solution) to be exerted from the enamel side and so caused filtration of the iodine. After 70 min, hydrostatic pressure was reduced to zero and dentine on both sides of the disk was etched with 50% aqueous citric acid for 2 min. Rate of fluid filtration (or bulk fluid movement) was calculated as the ratio between fluid flux and the product of iodine concentration on the enamel side and the dentine surface area. Both the rate of fluid filtration and the permeability coefficient (at zero hydrostatic pressure) for iodine were calculated before and after etching of the dentine. Hydrostatic pressure from the enamel side doubled permeation in the unetched group, but increased permeation 32X in the etched group; whereas filtration was increased 37X after etching. Although it is likely that bulk fluid movement is resisted *in vivo* by tubular contents and smear layer, the study highlighted the influence of dentine etching on the movement of molecules through dentine.

Pashley and co-workers (1977) used extracted teeth to monitor permeation of isotopes through dentine in a tooth crown model. In this model, roots were removed well below the CEJ and a syringe needle was inserted into the pulp chamber to provide constant bathing of the pulp chamber with a phosphate buffer. Occlusal cavities were prepared in the crowns which were then filled with about 50 μl of water containing labelled substances. Radioactive effluent from the pulp chamber was collected every 5 min to a maximum of 8.5 h and activity recorded. The influence of pulpal tissue and the importance of dentinal thickness, on permeation rate was examined in these experiments. Dentine permeation was found to be little effected by pulp tissue, varied inversely with molecular radius of the permeating compounds and was greatest when dentine was thinnest.

Pashley and Livingston (1978) used dentine disks (1.00 ± 0.05 mm thick), obtained from an area of the crown thought to be devoid of odontoblastic processes, to examine the effect of molecular size on permeability coefficients. A modified split-chamber device was used (Outhwaite *et al.*,

1976). The importance of several factors which determine dentine permeation, such as molecular radius, dentine thickness and the presence of pulpal tissue was defined. This same model has been used in several studies monitoring permeation of substances through dentine, for example, lidocaine (Hastings *et al.*, 1986), metronidazole (Csukas *et al.*, 1987), tetracycline (Ciarlone *et al.*, 1988; Ciarlone *et al.*, 1989) and adrenaline (Ciarlone *et al.*, 1991). Analysis of pulpal side effluent was performed by several different analytical methods including fluorospectrometry. Dentine preparation included sectioning with a diamond saw, immersion in ethylene tetraaminoacetic acid (EDTA) immersion and flushing with 0.005 M hydrochloric acid to clear tubules. It is possible that these procedures had damaged the intrinsic nature of the dentine thereby limiting the usefulness of the data. These studies also applied pure components of dental materials onto the prepared dentine and not the dental material itself, and so removed the influences of pharmacokinetics on permeation. The studies, however, did provide basic data on the quality of dentine as a biological membrane.

Pashley and his co-workers used a tooth model to examine several parameters important to dentine hydrodynamics, including hydraulic conductance. The model consisted of a dentine disk, obtained from the mid-crown of a freshly extracted tooth, secured within a split-chamber device (Outhwaite *et al.*, 1974; Reeder *et al.*, 1978). The device permitted ready access to the "enamel-side" of the disk, which allowed application of materials and procedures typically used in restorative dentistry. Hydrostatic pressure was determined by connection of the system, via polyethylene tubing, to a pressurized buffer reservoir. The model offered several advantages over models used earlier by previous workers in standardizing important variables, such as dentine thickness (0.99 ± 0.03 mm), and enabling sensitive manipulation of test variables, such as hydrostatic pressure. This split-chamber model has been used to conduct a systematic examination of variables likely to effect dentine permeability such as: presence of pulpal tissue, dentine thickness, molecular size of different diffusing substances, tubular occlusion, smear layer removal, effects of plasma and salivary constituents, distance from the pulp and air-drying (Pashley *et al.*, 1977; Pashley and Livingston, 1978; Pashley *et al.*, 1978a; Pashley *et al.*, 1981a; Pashley *et al.*, 1982; Pashley *et al.*, 1984a; Fogel *et al.*, 1988). The effect of a variety of dentine modifying treatments and substances, such as desensitizing dentrifices, cavity varnishes, oxalate, calcium hydroxide and burnishing, have also been examined (Pashley *et al.*, 1984c; Pashley and

Galloway, 1985; Pashley *et al.*, 1986; Pashley *et al.*, 1987a; Pashley *et al.*, 1987b).

Tyas (1977, 1978) used horizontal dentine slices, $150 \pm 10\mu\text{m}$ thick, prepared from the mid-occlusal dentine of third molar teeth. The slice was placed within a test apparatus and acted as the separation layer between test material (for example, restorative material) and culture medium all housed within a Petri dish. A Pyrex cylinder simulated a cavity. The apparatus thus tested membrane permeability in a cell culture system and toxic effects were determined by quantifying changes in enzyme activity in the culture medium, using scanning and integrating microdensitometry. Dental materials were tested in the state and amount in which they are used clinically, and the system allowed the determination of cytotoxicity after 2h. Unlike previous cytotoxicity studies, the method examined the effect of dentine which was found to produce a marked "amelioration" of the irritant effect of zinc phosphate cement. The study did not describe the preparation of the dentine slice and whether this was achieved in a close simulation of clinical procedure. The time intervals of examination were sufficiently broad to perceive significant changes in microdensity but were not sensitive to non-uniform changes, such as degradation of the test material, which may have occurred during the intervals. Although the system could not identify the exact cytotoxic element of the test material, it did highlight the importance of dentine. This basic model has been used in a modified form by several authors (Meryon and Browne, 1983; Meryon and Johnson, 1989).

Although the Pashley model (1977) examined the rate of permeation (unlike that of Tyas) both models used dentine as a physiological barrier and both were able to demonstrate intrinsic functions of dentine. The Pashley model offered several advantages. Since permeation was examined before and after the experiment for each tooth, each tooth provided its own internal comparison, which was important in view of the great variation in permeation between teeth found by the authors. Dentine was prepared using a carbide bur, thus creating a smear layer which probably more accurately simulated clinical events.

Permeability and fluid movement studies have been reported in the dog (Pashley *et al.*, 1981a; Pashley *et al.*, 1981b; Pashley *et al.*, 1981c; Pashley *et al.*, 1983a; Pashley *et al.*, 1984b; Potts *et al.*, 1985; Maita *et al.*, 1991) and in the

cat (Vongsavan and Matthews, 1992), and comparisons between *in vivo* and *in vitro* animal studies have also been completed (Pashley *et al.*, 1981b; Pashley *et al.*, 1981c; Pashley *et al.*, 1988; Vongsavan and Matthews, 1991). Pashley found good correlation between *in vivo* and *in vitro* dentine permeability rate in the dog and also found rapid systemic absorption of radiolabelled iodine applied to dentine. Pashley and co-workers (1988) found no significant difference between *in vivo* and *in vitro* composite resin bond strengths in the dog. The *in vitro* studies provided clear recommendations intended to improve animal studies, such as the importance of clinical simulation of dentine treatment, the necessity for storage of samples under positive hydrostatic pressure and the importance of a dentinal interface prepared with a bur rather than abrasive paper (Pashley, 1991).

Veaco and co-workers (1982) used a form of the Pashley tooth model (Pashley *et al.*, 1977) to examine the movement of ^3H -eugenol through dentine. Rather than using dentine slices, entire crowns from freshly extracted non-carious teeth were used by removing roots and coronal pulp tissue and then perfusing the pulp chamber with 0.1 ml/min saline. Occlusal cavities were prepared in the teeth using high speed rotary instruments and the labeled eugenol was applied to the dentine cavity floor either as a solution or mixed with zinc oxide. Perfusates from the pulpal side were assayed by liquid scintillation spectrometry. Eugenol appeared in the perfusate from the first minute after placement in the cavity and reached maximum about 1 h after placement. This model, like the Pashley tooth crown model, simulated cavity preparation and the creation of smear layer but, in contrast to the Pashley model which monitored labelled iodine, water, urea and technetium, the Veaco model examined diffusion of a component, eugenol, from a dental material. This was an appreciation of not only diffusion through dentine but of the availability of component release from a dental material.

Meryon and Browne (1983) used the Tyas model but replaced the dentine slice with a 1 mm layer of compacted dentine powder (particle size <250 mesh) obtained from extracted teeth from which crowns, cementum and pulp had been removed. A chemically-initiated composite resin, a zinc phosphate cement, a silicate cement and a zinc oxide-eugenol mixture were individually assessed. As listed in the previous sentence, this order corresponded to the order of increasing severity of adverse reaction in cell

culture medium. The modifying effect of the dentine powder, it was suggested, was either due to absorption of toxic components or due to attenuation of response associated with reduced concentration. Dentine powder allows standardization of this substrate but its relevance is questionable in an *in vitro* model if structure and permeability are to be examined. Meryon (1984) varied the thickness of the compacted dentine powder and found an inverse relationship between thickness and toxicity of zinc oxide-eugenol. The individual components of dentine are readily obtained from dentine powder and the interaction of these components with restorative materials has also been examined when used in this tooth model. Meryon (1988) found hydroxyapatite absorbed the majority of available zinc from zinc oxide eugenol and that fluoride was selectively bound by collagen. This model has also been used to examine the cytotoxicity of bonding agents that permeate through a dentine slice (Meryon and Brook, 1989) and in the assessment of antibacterial properties of restorative materials using a 0.45 μm - pore size filter in place of the dentine disk (Meryon and Johnson, 1989).

Permeation through human radicular dentine has also been investigated (Martin *et al.*, 1968; Fraser and Laws, 1976; s'Gravenmade *et al.*, 1981). Lekka and her co-workers (1984) used human third molars, prepared in clinical simulation, and placed volumes of formaldehyde or glutaraldehyde in the prepared cavity. They spectrophotometrically examined aldehyde diffusion through radicular dentine over 3 days. Each tooth was then immersed below the DEJ in a container of saline which was monitored for aldehyde. The concentration of intra-cavity aldehyde was compared with that in the saline. Less than 5% of the available formaldehyde and less than 0.1% of the glutaraldehyde had diffused by 1 day. Permeation of formaldehyde continued throughout the 3 days. The data confirmed that aldehyde diffused poorly through radicular dentine and that the presence of pulpal tissue was probably responsible for the reduced glutaraldehyde diffusion. The model did not, however, allow standardization of dependent factors between teeth, for example the dentinal diffusional area, and did not allow identification of the time of highest diffusion within the first 24 h. The analytical technique was also not sufficiently sensitive for quantitation of small amounts found in later intervals.

Hume (1984b) used a tooth crown model, based on that of Veaco and co-workers (1982), to simultaneously examine ^3H -eugenol release and diffusion

through dentine. The occlusal cavity was restored with radiolabelled zinc oxide-eugenol material and both occlusal and pulpal sides of the teeth, which were bathed separately in buffered saline, were assayed by scintillation spectrometry. A comparison was made of the amount and time course of eugenol in the enamel side with detection in the pulpal side. The findings illustrated the influence of dentine on diffusion of released eugenol. In addition, extraction of eugenol from samples taken from the dentine floor allowed the observance of a concentration gradient through dentine below a cavity restoration. Movement of eugenol through dentine was markedly less than the release of eugenol from the restoration surface. In addition, a decreasing concentration of eugenol was found in the deeper regions of the dentine. The study provided classic and early data in support of an explanation of the apparent equivocation between cytotoxicity of dental materials and their beneficial effect *in vivo*.

Hume (1985) developed a tooth model which united some features of previous models and provided a method of simulating more closely the use of both clinical procedures and restorative materials. The model incorporated a tooth crown from which the roots and pulp chamber had been removed and replaced with a plastic chamber containing 2.5 ml of culture medium. Cavity preparation, dentine pre-treatment and placement of restorations could all be carried out in a clinically appropriate manner, using labelled restorative materials including glass ionomer cements (Hume and Mount, 1988). The contents (culture medium) of the "pulp chamber" were removed for examination by fibroblast-labelled thymidine uptake after 1, 3 and 10 days and the time course for pulp chamber sampling was considered to coincide with the arrival of permeated substances at the pulpal surface. Acid etching of dentine (60 s phosphoric acid) increased the toxic potential of composite resin; a finding which supported the observations of Pashley and Livingston (1978). Increased toxicity was seen with the 3-10 day samples and Hume suggested that this was associated with cytotoxic products of necrosis of culture cells. It may also have been due to the developing toxic potential of the restorative material as it degraded or to physical changes in the material. The model offered improvement on previous models in enabling the examination of the relative toxicity of restorative procedures in addition to that of the restorative materials themselves. This Hume model was a modification of earlier models (Veaco *et al.*, 1982; Hume, 1984; Hume and Wang, 1986; Wang and Hume, 1988). By attaching a second chamber to the tooth crown into which test solutions were placed, analysis

of the pulpal chamber contents was able to be used to determine the diffusion through dentine. In addition, by removing the intervening dentine and analysing its contents, a pH gradient across dentine was observed. Data suggested that dentinal hydroxyapatite was a highly effective buffer; a finding which presented an explanation for the lack of pH change below strongly acidic phosphate-type cements.

Hume and Wang (1986) used the principle of the split-chamber device but preserving the tooth crown as described by Hume in 1984, to examine the diffusion of hydrogen ions through dentine. Several acids of different strengths were added to the chamber on the cavity side of a prepared tooth crown and the pulpal side fluid was monitored for pH changes up to 2 weeks. Large differences were noted in the pH change between the acids, directly related to their respective dissociation constant, that is, stronger acids showed slower change in pH. The study illustrated the efficient buffering capacity of dentine for strongly dissociating acids.

Tjan and co-workers (1987) used a dentine disk (mean thickness 1.4 ± 0.1 mm) in another split-chamber diffusion device which allowed the exertion of pressure (20 psi for 15 min) to a test solution (TEGDMA with a fluorescent dye, rhodamine) in the "enamel" side of the device. The effect of cavity varnishes on monomer penetration to the pulpal side was then qualitatively determined spectrophotometrically.

A similar model to that of Lekka, but with roots only, was used to study the release and diffusion of corticosteroid and tetracycline from a therapeutic topical dental drug, Ledermix®, through radicular dentine (Abbott *et al.*, 1988; Abbott *et al.*, 1989a; Abbott *et al.*, 1989b; Abbott *et al.*, 1989c). Highest release rate was seen within 1 day. Diffusion was enhanced when either smear layer or cementum was removed and was directly related to diffusional surface area of dentine. A clinically-used preparation of the drug was applied to dentine and the diffusion of the labelled components released from the drug was examined. This gave both valuable information about drug pharmacodynamics, relative to dentine, which are exploited for clinical benefit and provided good data on rate and time of release of components from materials on diffusion through dentine.

Hanks and his co-workers (1988, 1994) used a modification of the model suggested by Outhwaite and co-workers (1974) to examine cytotoxicity of

composite resins. The model incorporated several split chambers containing dentine disks ($0.5 \pm 0.02\text{mm}$ and $1.5 \pm 0.02\text{ mm}$ thick) which separated composite resin from a "pulpal" chamber containing Balb/c 3T3 cells. Enamel-side dentine was cut with a diamond wheel and hand sanded, and a positive hydrostatic pressure of 1 cm H₂O (that is, outward from the pulpal side) was maintained during composite resin placement in the cavities. The pulpal-side contents were removed at 24, 48 and 72 hours for cell function analysis. Release from the light-activated composite resins was responsible for depression of protein synthesis only during the first 24 hours after activation; this did not occur if thicker dentine disks were used. The model provided advantages over earlier models in allowing closer simulation of clinically relevant variables such as appropriate dentinal thickness and positive hydrostatic pressure. However, it is uncertain whether the smear layer produced in preparation of the enamel-side dentine was a reasonable representation of that created clinically and whether the use of cell culture directly in contact with pulp-side dentine may not have protected against pulpward diffusion from the composite resin by itself being drawn up into the tubules by capillary attraction.

Cox (1990) strongly supported consideration of the "diffusion factor of dentine" in biomaterials usage tests and, in this light, several intermediate tests incorporating a tooth model have been used to evaluate dental materials. The common feature in these studies has been the incorporation of a dentine component in the basic model interposed between test restorative material and cells in culture so that the ability of diffusable compounds to pass through dentine and exert toxic influence distant from the restorative material is also tested. The models have attempted to "bridge the gap" between cytotoxicity tests and usage tests but more importantly can be used to provide unique data about the character of dentine. Some good recommendations have been made by Prati (1994) in regard to future *in vitro* permeability testing; that the role of proteins in dentine fluid be established and that consideration be given to the buffering capacity of mechanisms that regulate permeability.

The following Chapters examine the effect on release of components from resin restorative materials when these materials are applied to dentine.

CHAPTER 5

Diffusion of Components of Composite Resin through Dentine, *in vitro*

5.1 Introduction

5.2 Experimental method

- 5.2.1 Tooth model
- 5.2.2 Materials
- 5.2.3 Procedure
- 5.2.4 Sample analysis
- 5.2.5 Statistical analysis

5.3 Results

5.3.1 Component identification

5.3.2 TEGDMA quantification

5.3.2.1 Cumulative diffusion of TEGDMA from Foundation through dentine *in vitro*

5.3.2.2 Rate of diffusion of TEGDMA from Foundation through dentine *in vitro*

5.3.3 Comparison between TEGDMA release and TEGDMA diffusion, from Foundation, through dentine *in vitro*

5.3.3.1 Comparison between cumulative amount of TEGDMA released and cumulative amount of TEGDMA diffused, from Foundation, through dentine *in vitro*

5.3.3.2 Comparison between rate of release of TEGDMA and rate of diffusion of TEGDMA, from Foundation, through dentine *in vitro*

5.4 Discussion

5.1 Introduction

An important clinical implication of component release from composite resin is the potential of released TEGDMA to exert toxic influence. In fact, there is strong but indirect evidence that composite resins release cytotoxic components into a tissue culture medium (Qvist and Stoltze, 1982; Qvist, *et al.*, 1989). It is also apparent that intervening dentine protects pulpal cells from some or all of the toxic effects of many restorative materials, including composite resins, *in vitro* (Hanks *et al.*, 1988; Wataha *et al.*, 1994).

It is important to determine how dentine exerts this protective effect. Data already presented have examined the pattern of release of components from composite resin restorative material into an aqueous phase. The work described below examined diffusion of released components through dentine *in vitro*, using a tooth model, and compared this diffusion with the release of components from the material directly into an aqueous phase.

There may be, in addition, circumstances and conditions which influence the protection afforded by dentine, so it was of interest to examine diffusion under these circumstances. Some of these factors are considered in subsequent Chapters, and include dentine pre-treatments in Chapter 6, different composite resin formulations in Chapter 7, dentine bonding systems in Chapter 8 and the influence of intrapulpal hydrostatic pressure in Chapter 9.

5.2 Experimental method

5.2.1 Tooth model

The tooth model used is based on that of Hume (1985) which has been further developed for use in the current study. Human teeth were obtained with consent from patients of either sex, aged 18 to 24 years. The teeth were intact and non-carious third molars, recently removed by surgical means, for therapeutic reasons unrelated to the study. Infection control procedures for the handling and management of human tissues were followed in accordance with the policies of the institutions where the studies were carried out and included immediate post-extraction sterilization overnight by gamma-irradiation using a caesium source (475-curie CS-137 Irradiator). Teeth were stored in freshly obtained double distilled water at 4°C for up to 5 days until used. Water was provided by a water purification unit (MilliQ Plus, Millipore Corporation, Milford, MA, USA). This same grade

of water was used as one of the HPLC mobile phases, as the sample medium in all experiments and as the irrigant for cooling the handpiece during tooth preparation. All cavity preparations were completed using a high-speed air turbine contra-angled dental handpiece (Kavo Super-torque 630C, Germany) and water coolant was used at all times. Dentine pre-treatment and composite resin placement were carried out using conventional restorative dentistry procedures, methods and instruments. Dental materials used were prepared, placed or used in accordance with manufacturer recommendations.

Teeth with crowns of comparable size were collected, divided into groups for the experiments described and returned to the storage solution at 4°C. Teeth in each group were prepared on the same day. All teeth in a group were prepared by first removing the root systems below the cemento-enamel junctions using new tapering long shank carbide burs 1 FG 1169L (Midwest, Ontario, Canada) and a dental handpiece. Pulpal tissues were carefully removed with a dental explorer (K6, Ivoclar, Sydney, NSW, Australia), root systems were discarded and all crowns were returned to storage in water at 4°C. Each crown was then prepared individually and, when all teeth in a group were prepared, the experiment for that group was started with teeth tested sequentially.

An occlusal cavity was prepared in each tooth crown with a new pear-shaped H 245 008 carbide bur (Komet, Lemgo, Germany). The cavity diameter, measured using a periodontal probe (Hu-Friedy PCP11, Chicago, IL, USA), was approximately 6 mm. The cavity depth was progressively increased to leave remaining dentinal thickness (RDT) to the pulp chamber within the range of 1.6 to 2.0 mm. This measurement was determined at a minimum of 10 points on the cavity floor using outside prosthodontic calipers (R. Jagmssons, Göthenburg, Sweden). Teeth showing RDT less than 1.6 mm were discarded.

Following preparation of each crown, a 2 ml capacity polypropylene vial (Cryovial, Sigma, Los Gatos, CA, USA) was trimmed so as to accommodate only 1 ml of water and then fitted close to the cemento-enamel junction of each crown. This vial was attached there with dental sticky wax (Associated Dental Products Ltd., Kemdent Works, Purton, Swindon, UK) thus simulating a pulpal space area. 1 ml of distilled water was then introduced, through a prepared hole (2 mm in diameter), into the chamber using a disposable

plastic syringe (Terumo, Luer Lock, USA) and needle (25G, 0.50 x 19 mm, Luer. Becton Dickinson, New York, NY, USA). A second hole acted as a vent by allowing escape of air during filling of the vial with water. Both sample and vent holes were sealed with sticky wax between sample removal times. Prepared teeth, with chambers, were stored hydrated in closed containers at 4°C.

A diagram of the tooth model is presented in Fig. 5.1. Although the vent hole is shown opened in Fig. 5.1, it was only this way when the sample of water was being removed or replaced. Teeth were held vertically during cavity preparation and restorative procedures and were stored horizontally, as depicted in Fig. 5.1, for all other stages of the experiments.

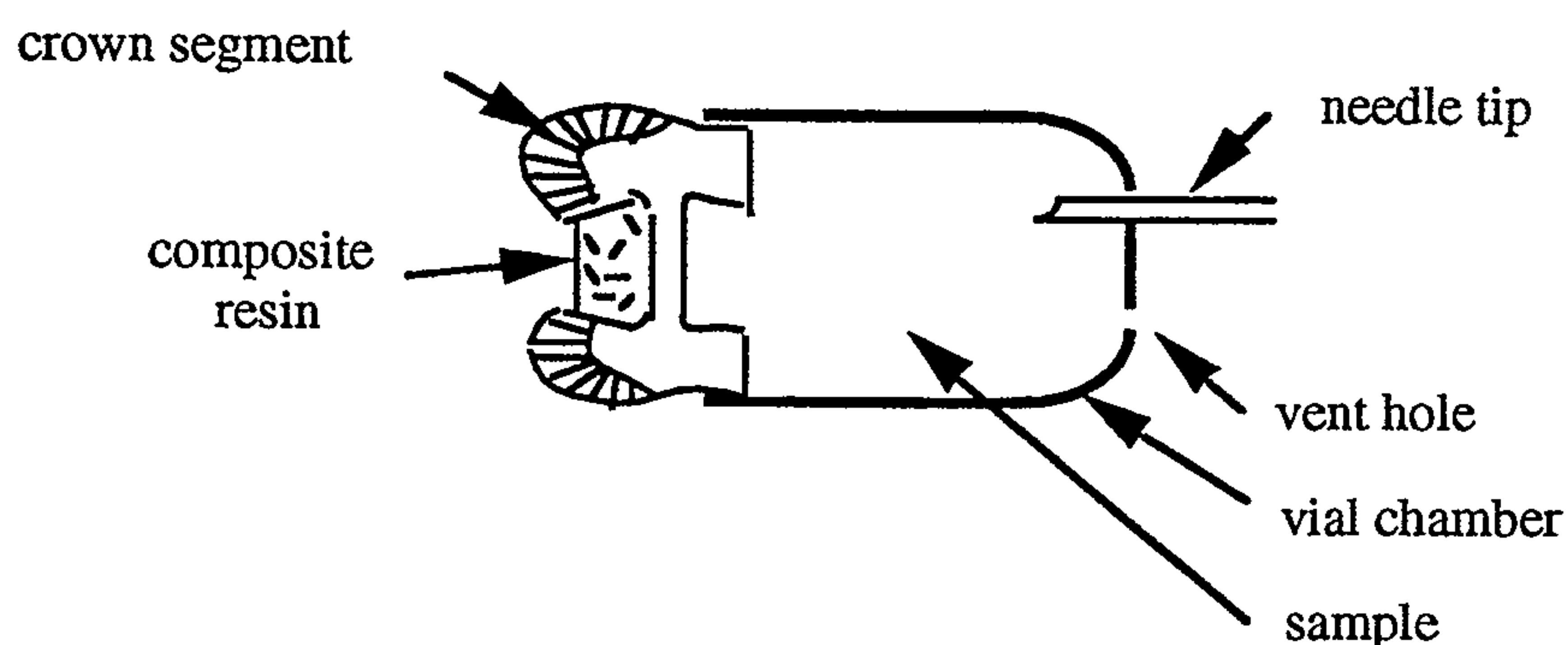


Figure 5.1 Diagram of the tooth model used to examine the diffusion of components of composite resin through dentine, *in vitro*

5.2.2 Materials

The composite resin used to restore the cavities was the same as that used in the release study (Chapter 3), namely Foundation (Nulite Systems International, Hornsby, NSW, Australia). Dentine pre-treatment consisted of the application of a gel etchant, 37% phosphoric acid (3M Dental Products, St. Paul, MN, USA). Experimental preparation took place on a benchtop at $22 \pm 1^\circ\text{C}$ and prepared teeth (with attached chamber containing 1 ml water) were placed in an incubator (Labec, Sydney, NSW, Australia) at $37 \pm 1^\circ\text{C}$ for conduct of the experiment.

5.2.3 Procedure

Following cavity preparation, the cavity of the tooth crown (with attached chamber containing 1 ml water), was irrigated with distilled water from an air-water syringe and dried lightly with air from the same syringe. Dentine pre-treatment was carried out as suggested by Fusayama (1990), that is, 37% phosphoric acid gel was applied to enamel and dentine of the cavity for 60 s using a disposable dental bristle brush. The cavity was then irrigated with distilled water for 30 s and dried with air. Increments of composite resin were then applied to the dentine, to arrive at a material thickness of 2 mm which was then light activated for 30 s using a dental activating visible light (Optilux 400, model 401, Demetron Research Corporation Danbury CN, USA). No attempt was made to restore the cavity beyond this thickness of composite resin. Thus, the thickness and diameter of composite resin placed in each tooth was approximately standardized, measuring approximately 6 mm in diameter and 2 mm in thickness. Each tooth was assigned a number; a labelled syringe and needle carried that number for exclusive use with that tooth throughout the experiments.

Immediately at the end of the period of light activation of the composite resin in the cavity, the tooth was placed in a 37°C biological incubator and a stop watch was activated (time zero). All sample retrieval was timed from that moment. Ten samples were removed, at 4.32, 14.4, 43.2, 144 and 432 min and 1, 3, 10, 30 and 100 days from time zero, from the "pulp chamber" of each tooth. Each of these samples was retrieved as described below.

At the end of each time interval, the tooth was removed from the incubator and the wax seal in both sample and vent holes was punctured using that tooth's needle. The entire sample in the "pulp chamber" vial was then aspirated into the tooth's syringe. The needle was then removed and it was replaced with a 0.22 µm filter tip (Millipore Waters, Milford, MA, USA); the sample was then filtered directly into a 1 ml capacity glass HPLC vial used in the autosampler unit. The tooth's needle was then attached to a new syringe filled with fresh water and 1 ml was introduced into the tooth's "pulp chamber". Both sample and vent holes were then resealed with wax and the tooth was returned to the incubator until the next time interval. Each tooth was checked periodically for evidence of chamber volume loss or leakage around the restoration. Teeth demonstrating either of these problems were discarded. A diagram of the experimental procedure is shown in Fig. 5.2.

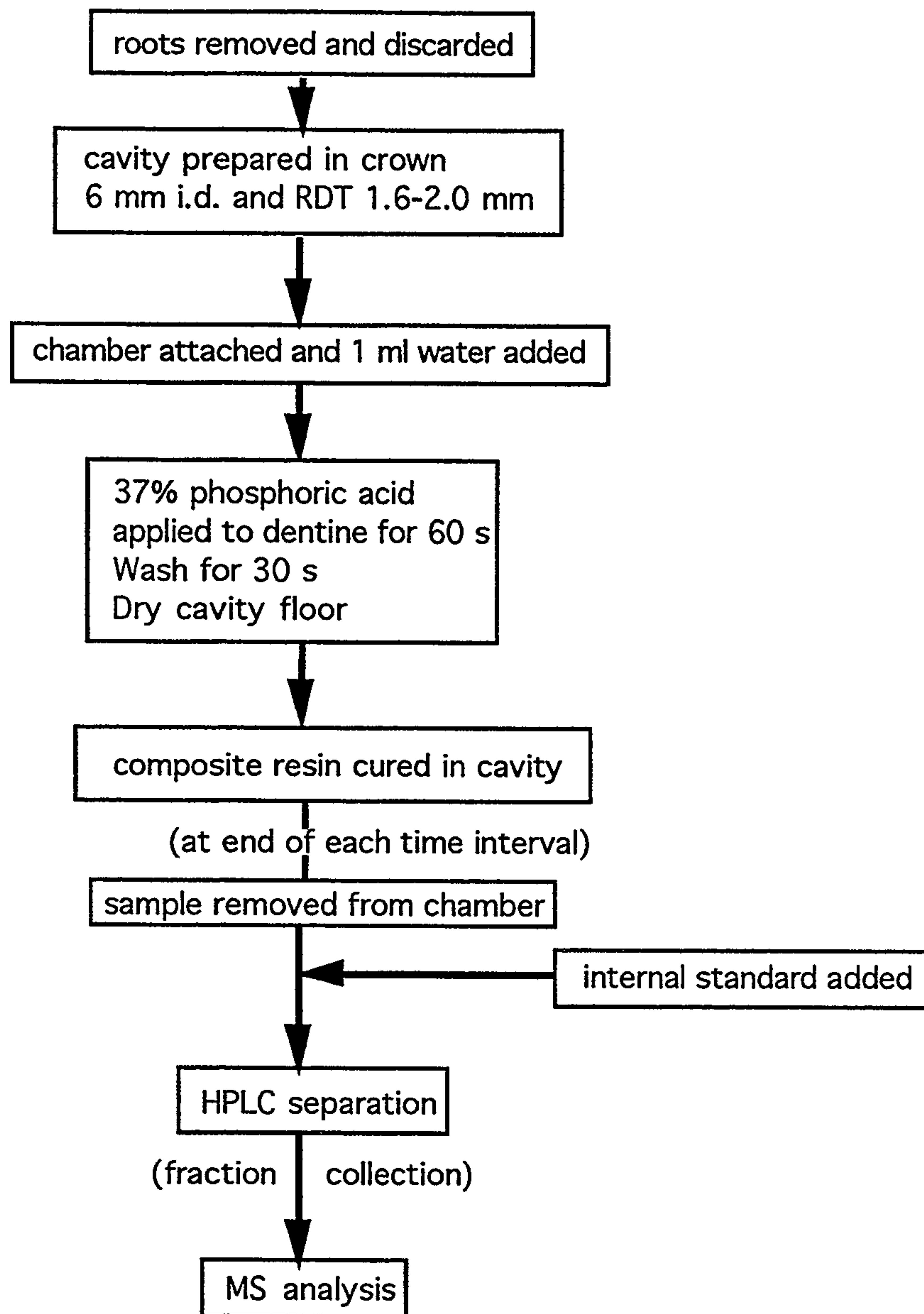


Figure 5.2 Diagram of the experimental procedure used in the tooth model experiments

5.2.4 Sample analysis

To each filtered water samples retrieved from the pulpal chambers a volume of internal standard solution (methyl paraben) was then added. These samples were analysed immediately or stored at 4°C for no more than 3 days before analysis. A 200 µl volume of each 1 ml sample was used for each individual HPLC analysis.

The theory and experimental requirements of the separation of an aqueous sample into its components by HPLC and the confirmation of component identification by MS has been described in Chapter 2. The application of HPLC/MS to analysis of aqueous eluates from composite resin in the current work has also been described in Chapter 2. In brief, this required comparison of sample analyses to those of standard solutions and control solutions. This also necessitated that all samples were run under identical analytical conditions.

A standard solution containing each component of Foundation resin was freshly made for each analysis using concentrations shown in Table 2.6. A series of dilutions of this "stock" solution was prepared. Control solutions included water samples from a resinless tooth model and samples of HPLC mobile phases. Injectionless HPLC runs served as instrument controls. A typical series of samples for HPLC separation thus included controls, standard dilutions and then, following integration of these chromatograms and the generation of calibration curves, the assaying of samples.

MS spectra were generated for the prepared individual resin components and for 500 μ l sample fractions collected from the HPLC at times corresponding to retention times which matched those of peaks of the components. Peaks in each standard chromatogram were then integrated in order to generate calibration curves for the internal standard and for each individual composite resin component in the standard solutions. These curves related signal response (or peak height) to the quantity of component in the injected volume of the standard. Response signal or "peak" in the sample was then fitted to this curve to determine the amount (in μ g) of the component in the injection volume (200 μ l). Each amount represented the accumulated amount over that interval and this was converted to nmol by relating the amount detected to the volume of the sample (4 ml) and to the molecular weight of the component.

The cumulative diffusion for each interval was calculated by adding the amount in that interval to the total amount of the previous intervals. The cumulative diffusion for 43.2 min, for example, was equal to the sum of the amounts accumulated after 4.32, 14.4 and 43.2 min. Diffusion rate was calculated by dividing the amount diffused (in nmol) in that interval by the length of the interval (in minutes). For example, the diffusion rate between 4.32 min and 14.4 min was derived from the amount found in that interval,

that is, the 10.08 min between 4.32 and 14.4 min. Cumulative amounts diffused and diffusion rates over the time course are presented in graphic form.

5.2.5 Statistical analysis

Means plus or minus standard deviations about the means for cumulative amounts and rates of release were derived. Assumptions of normality of the data were checked. Comparisons between data at different time points for each group were made using one factor ANOVA-repeated measures and Scheffé statistical tests at $p < 0.05$.

5.3 Results

The term release was used to describe the movement of components of mold-confined composite resin into water. The term diffusion was used to describe the movement of released components from composite resin through dentine *in vitro*.

5.3.1 Component identification

A typical chromatogram of an unknown sample taken from the tooth model at 1 day is presented in Fig. 5.3. Fig. 5.4 shows a chromatogram of a control sample from the resinless series also taken at 1 day. A comparison of the retention times of peaks in these chromatograms with those in the chromatogram of the standard solution shown in Fig. 2.2 indicated that only one detectable component was present in the unknowns, the monomer TEGDMA. This was confirmed by MS analysis of the fraction collected at the TEGDMA retention time. Other components were below the threshold for detection by HPLC.

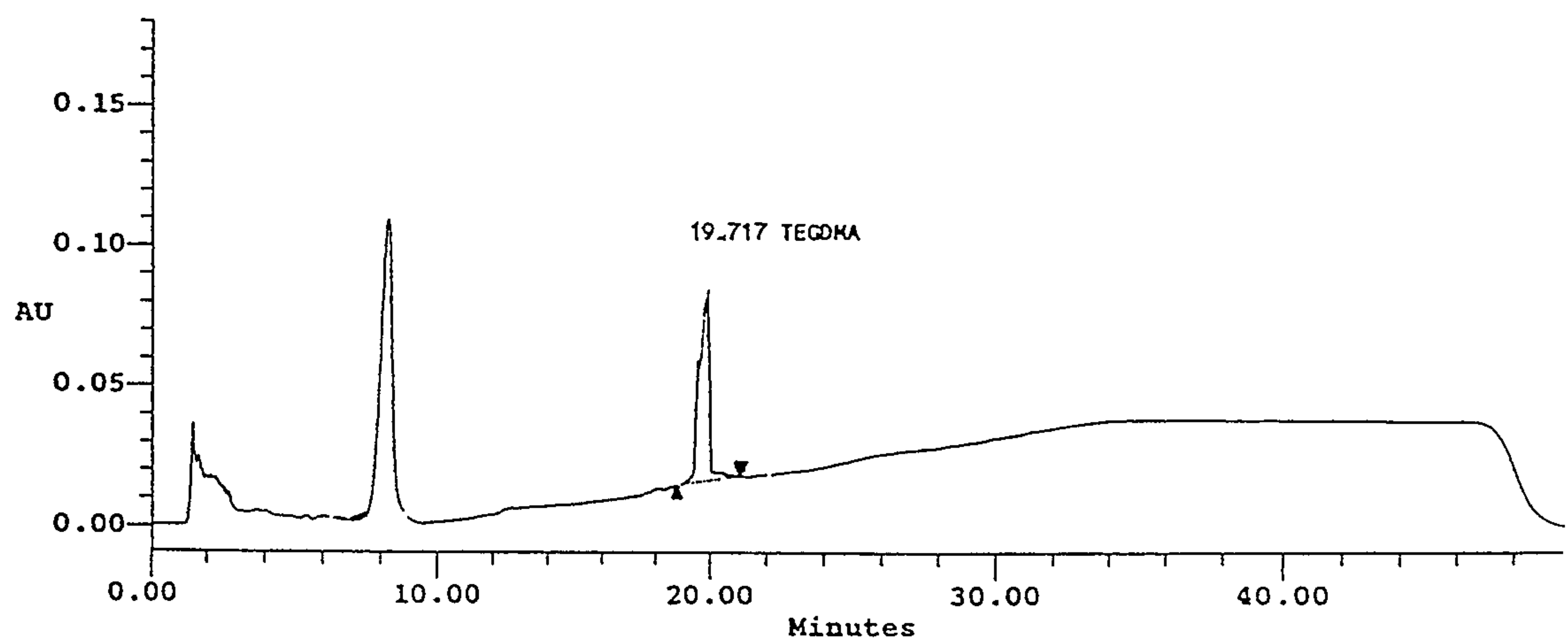


Figure 5.3 Chromatogram of an "unknown" sample from the tooth model (taken at 1 day)

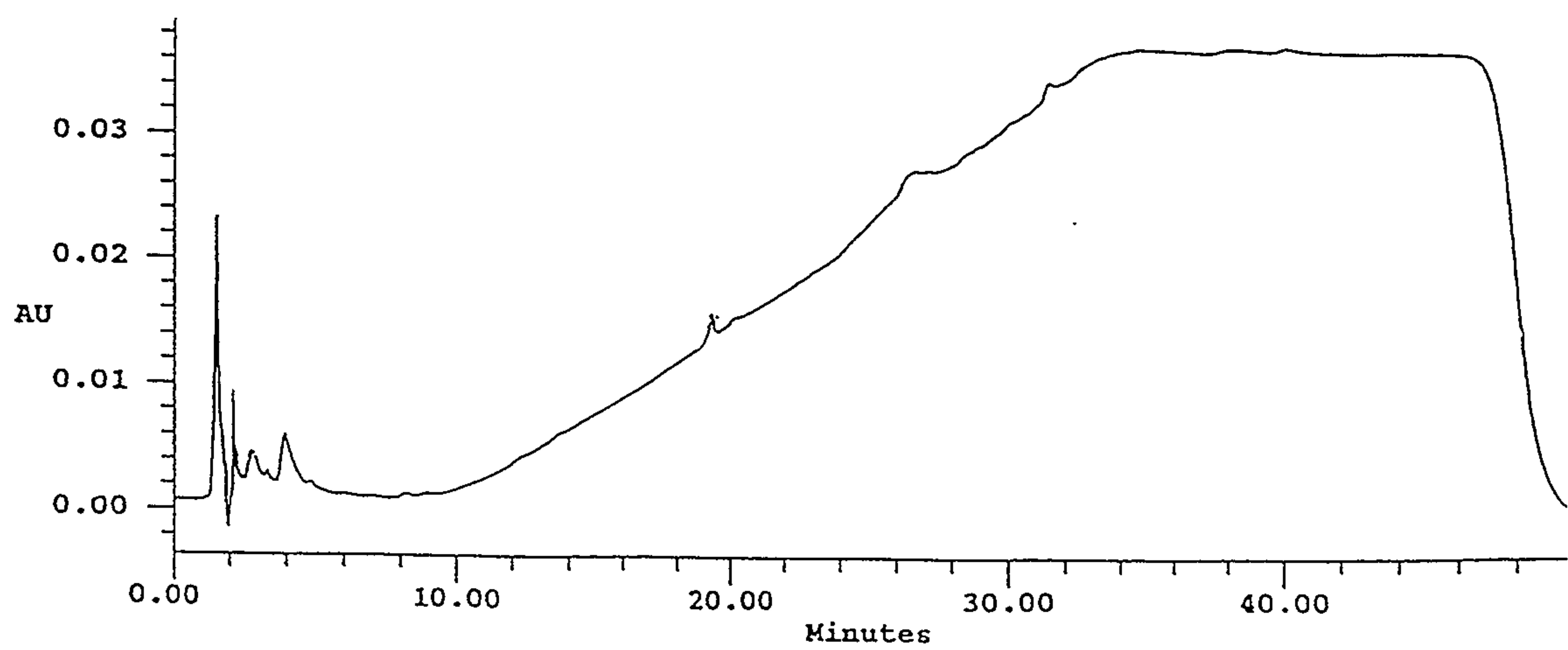


Figure 5.4 Chromatogram of resinless control sample from the tooth model (taken at 1 day)

5.3.2 TEGDMA quantification

5.3.2.1 Cumulative diffusion of TEGDMA through dentine *in vitro*

The cumulative diffusion of TEGDMA is shown in Fig. 5.5. Means and standard deviations about the mean are shown (number of teeth=34). TEGDMA detected in the pulpal space in the first two time periods was well below reliable quantification and earliest traces were detected at 43.2 min. Only small additional amounts were detected after 3 days when cumulative diffusion was 74.21 ± 24.45 nmol.

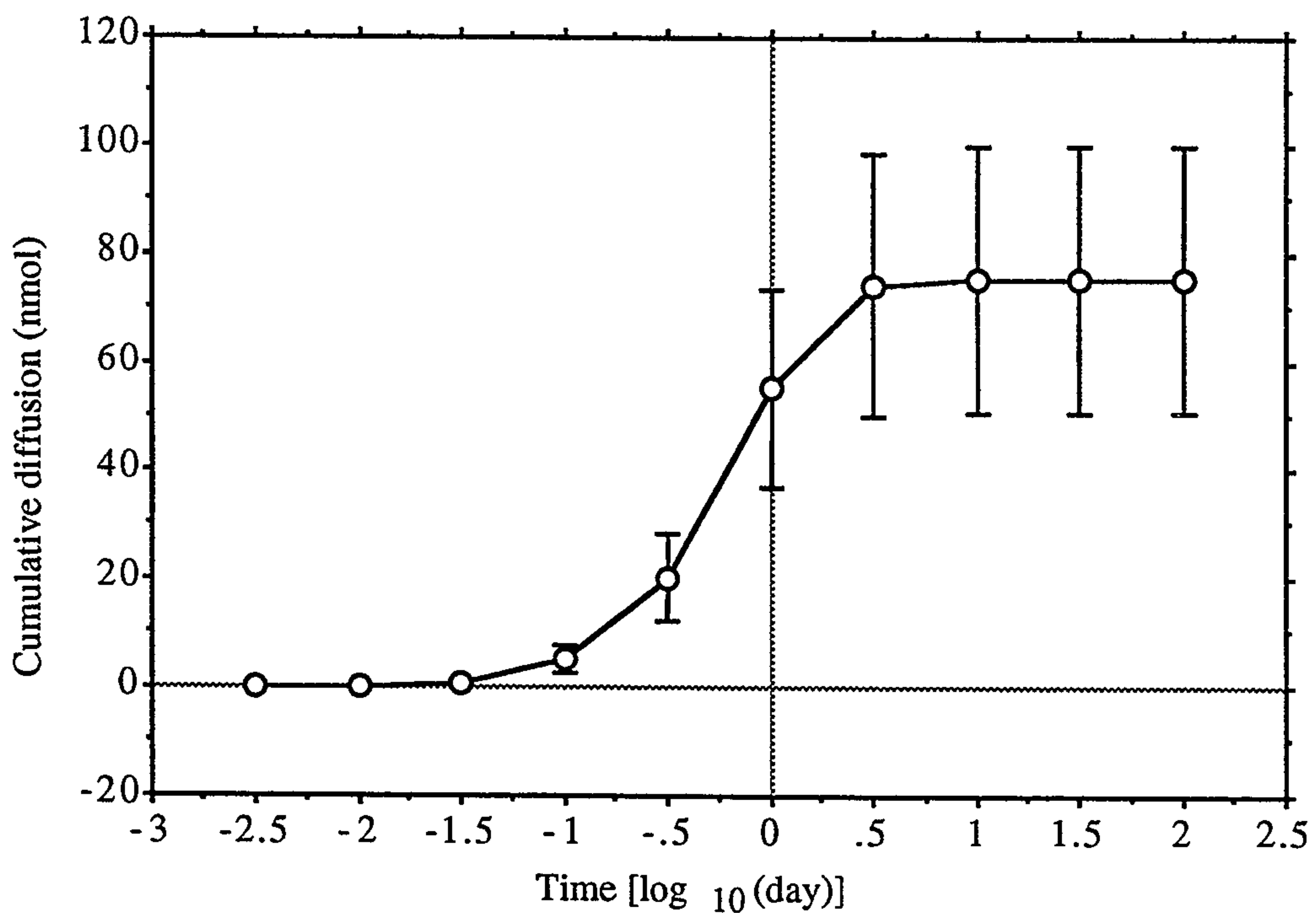


Figure 5.5 Cumulative diffusion of TEGDMA through dentine, *in vitro*

5.3.2.2 Rate of diffusion of TEGDMA through dentine *in vitro*

The rate of diffusion is shown in Fig. 5.6. As the amount of TEGDMA detected in samples from the first two time intervals was well below reliable HPLC quantification, the rates at this time are depicted lower than graph scale. The first high diffusion rate was seen at about 43.2 min, with a rate of 0.05 ± 0.02 nmol/min, although there was no significant difference seen between rates during the intervals of 43 min to 3 days. A significant decrease in rates was seen in the intervals after 3 days (at $p < 0.05$).

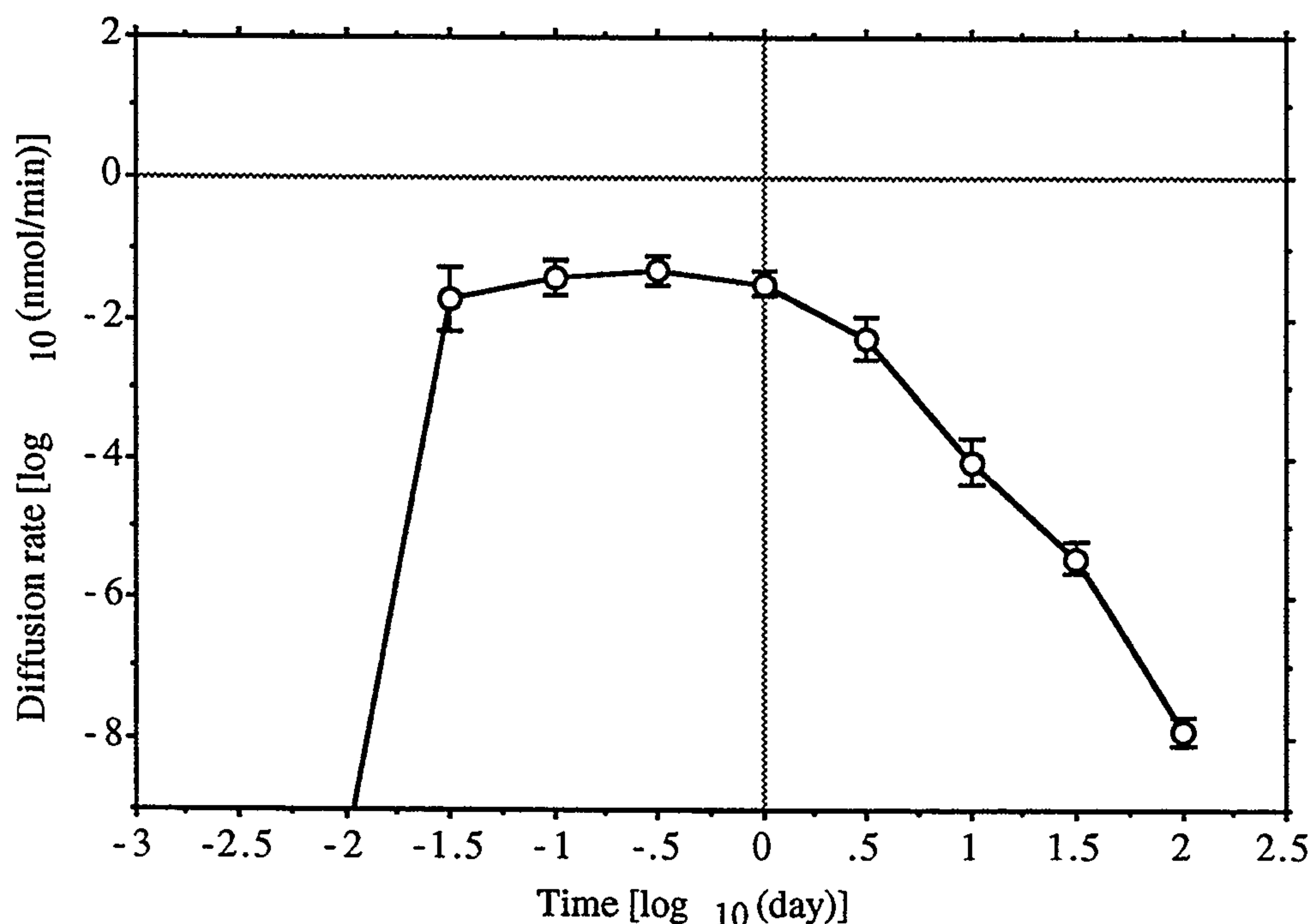


Figure 5.6 Diffusion rate of TEGDMA through dentine, *in vitro*.

5.3.3 Comparison between TEGDMA release and diffusion through dentine *in vitro*.

To evaluate the effect of dentine, it was necessary to compare the *release* of components from composite resin with the *diffusion* of components across the dentine interface. Therefore, the data presented in the above sections were compared with component release data described in Chapter 3. Release and diffusion data are combined in the following graphs. Several aspects of both experiments were standardized to each other to allow this comparison to be made and are considered in the discussion.

5.3.3.1 Comparison between cumulative amount of TEGDMA released and cumulative amount of TEGDMA diffused through dentine, *in vitro*

Fig. 5.7 presents the comparison between the cumulative amounts of TEGDMA released with those amounts diffusing through dentine *in vitro* in the tooth model. This figure unites data from Figs 3.5 and 5.5, illustrating cumulative release ($n=49$) and cumulative diffusion through dentine *in vitro* ($n=34$) respectively. A significant difference in early cumulative amounts was seen up to 1 day (at $p<0.05$). By 3 days, the total cumulative diffusion of TEGDMA through dentine was 60% that of the direct release.

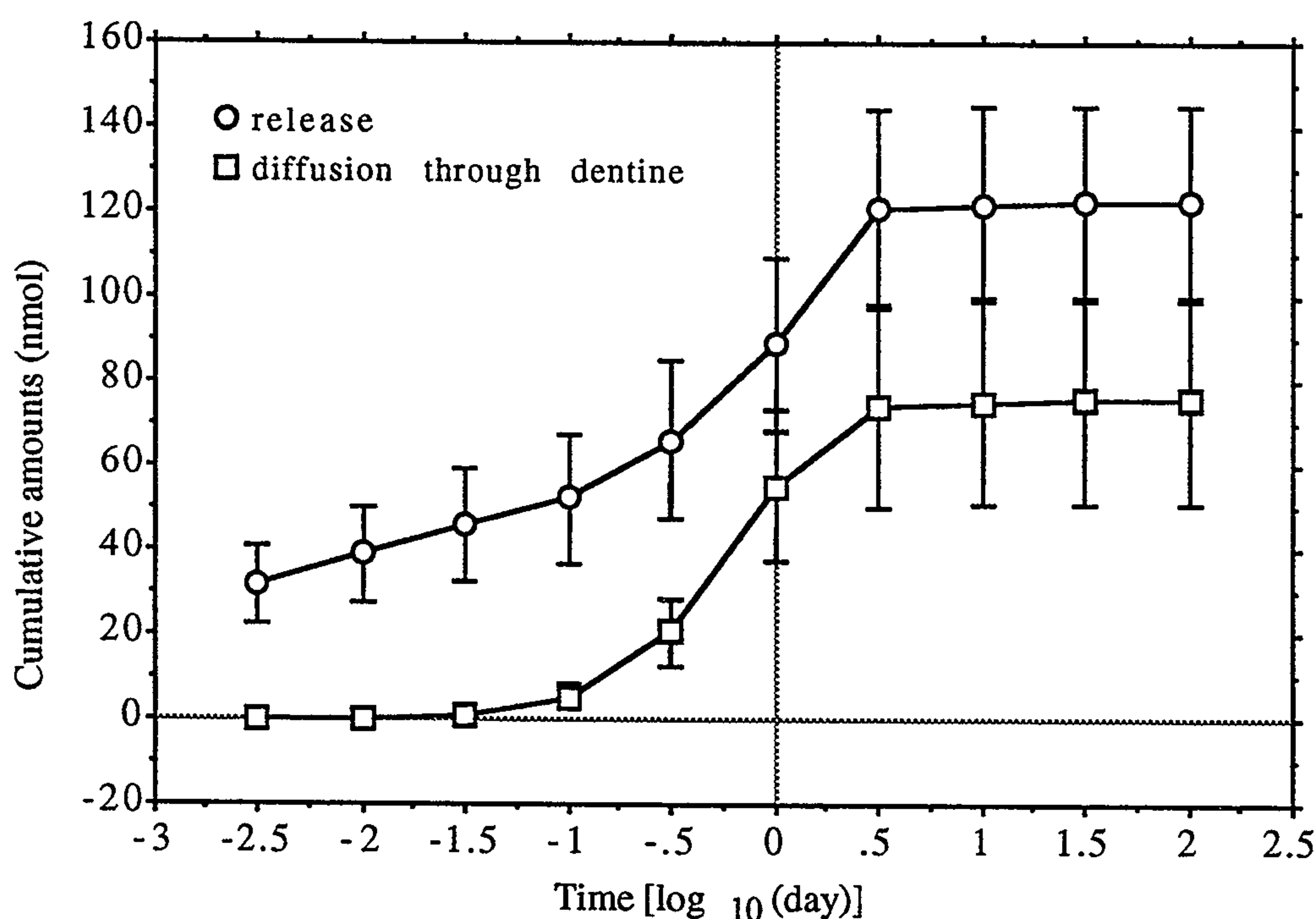


Figure 5.7 Comparison between cumulative amounts of TEGDMA released and diffused through dentine, *in vitro*

5.3.3.2 Comparison between rate of release of TEGDMA and rate of diffusion of TEGDMA through dentine *in vitro*

Fig. 5.8 presents the comparison between the rate of release and the rate of diffusion through dentine, of TEGDMA. This figure unites data from Figs 3.6 and 5.6, illustrating rate of release and rate of diffusion respectively. With the exception of a significant difference seen up to 43.2 min (at $p<0.05$), release rates and diffusion rates were similar for the remainder of the time course. Highest rate of release of about 7.38 ± 2.12 nmol/min was in the period to 4.32 min, and declined thereafter. The interval of highest

diffusion rate was much less well defined than the interval of highest release rate and occurred over a time range of 43.2 min to 1 day.

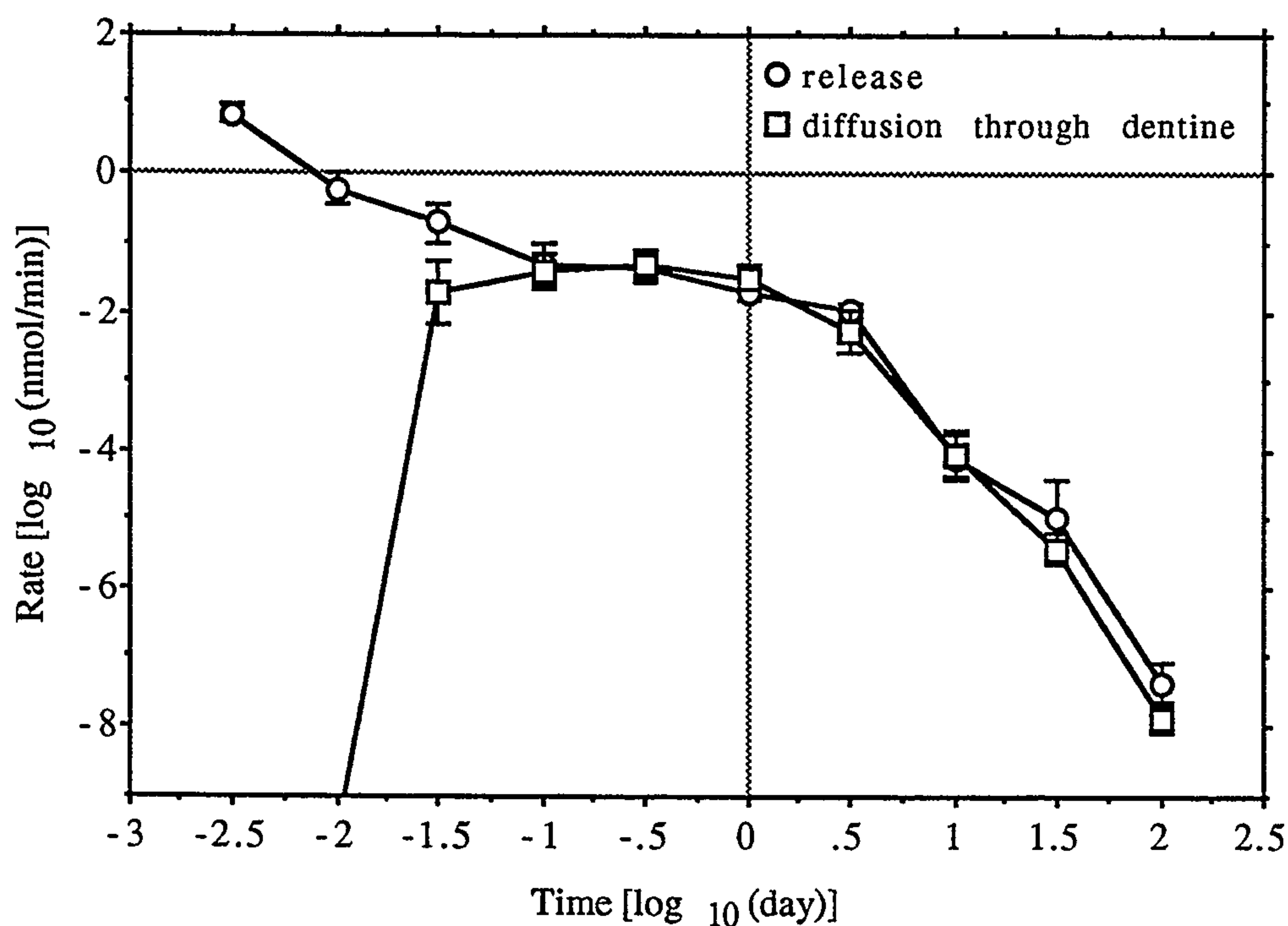


Figure 5.8 Comparison between rates of TEGDMA released and rates of TEGDMA diffused through dentine, *in vitro*

5.4 Discussion

The data showed that dentine did not prevent TEGDMA diffusion but instead delayed the arrival of TEGDMA in the pulp space. This delay represents, very probably, the time taken for TEGDMA to diffuse out of the cured composite resin, traverse dentinal tubules, leave the dentine/pulp space interface and enter the pulp chamber fluid.

These data will be discussed in the light of several observations. First, the intrinsic nature of dentine structure probably influenced TEGDMA diffusion. Secondly, a comparison of component diffusion with component release from the composite resin (Chapter 3) demonstrated the magnitude of the influence of dentine. Thirdly, clinical restorative procedures which modified dentine structure and smear layer have also probably influenced dentinal permeability.

Of the structural elements of dentine, tubules are the most likely avenue for the passage of diffusible substances through dentine. In fact, tubules, which widen in diameter toward the pulp, have been described by Brännström and Åström (1972) as "highways transporting various types of irritants" and so are responsible for the permeability of dentine (Baume, 1980). Tubular structure is also associated with considerable capillary attraction force capable of rapidly moving fluid across dentine (Brännström and Åström, 1972). These structures are not merely hollow tubes, as their fluid content also participates in the overall properties of dentine (Pashley, 1991). The hydrodynamics of intratubular fluid, as described by Brännström, suggests that a tubular pressure gradient caused by fluid flow is directed toward the cavity floor, but "this flow does not prevent toxins or other substances from reaching the pulp through diffusion in a pulpal direction" (Brännström, 1981a). This fluid flow, as a consequence of filtration, describes the bulk movement of fluid propelled by a pressure gradient, without loss of concentration and without the observance of diffusional coefficients (Merchant *et al.*, 1977). It is conceivable that in some circumstances, fluid flow occurs through dentine and, in fact, this has been suggested as the cause for dentinal sensitivity (Brännström, 1966). Fluid flow is also a basic concept in permeability studies where cavity and pulpal sides of dentine disks are fluid-bathed. However, in the current experiment, composite resin was directly applied to dentine, in contact with dentinal fluid without the purposeful creation of a pressure gradient which would have created fluid flow. It is more likely then, that TEGDMA released from the composite resin, by presumably having overcome the solid/liquid interface at the composite resin surface, passively entered intratubular fluid at a rate determined by Fick's laws (Pashley *et al.*, 1978a). In light of these observations, the passage of TEGDMA through to the pulpal space was likely to be that of the *diffusion* of TEGDMA through intratubular fluid and not *filtration* or flux through dentine. Physical laws which assist in the description of diffusion and permeability are considered further in Chapter 10.

Several factors which control dentine permeability have been identified by Pashley (1985). These include dentine diffusional area, dentine thickness, temperature, proximity to the pulp and factors unique to the diffusing substance, such as molecular size, charge, concentration and solubility. Greater diffusion can, therefore, be anticipated when dentine is more permeable such as in deep dentine with increased diffusional surface area

due to increased tubule orifice diameter and reduced tubule length, or when tubules are patent. Another important factor in a consideration of diffusion is the presence of smear layer. This layer is capable of establishing a protective diffusion barrier by contributing a large "resistance to convective transport" (Pashley *et al.*, 1981a). This layer was formed after cavity preparation in the present work but was very probably removed during etching of the cavity floor with phosphoric acid. The extent to which smear layer may modify diffusion will be considered in Chapter 6.

A comparison of the amount of TEGDMA *released* to that which *diffused* can reveal the extent to which dentine is a "protective barrier". TEGDMA was detected reliably in samples from 43.2 min in the tooth model whereas TEGDMA was detected in all samples for the entire time course in the release experiment. The difference seen in comparing peak release and diffusion is probably due to delay in passage through dentine and may well be the principal determinant of the protective effect of dentine. In this way, dentine behaves, as suggested by Pashley (1979) as a "reservoir....coupled in series with the pulp". The delay in passage retarded arrival of TEGDMA molecules at the odontoblast layer and muted the TEGDMA concentration slightly. The delay might be simply due to diffusion delay through dentine or to a combination of diffusion delay and reversible binding interactions with dentine, as suggested by Hanks and co-workers (1993).

The release experiment identified variables associated with increased TEGDMA release, such as increased surface area, air inhibition during cure, thickness beyond 2 mm and decreased exposure time to photoinitiation. In the tooth model, it was necessary to control or standardize these variables since the effect of dentine was the principal variable of interest. Several aspects of the tooth model, however, did not allow duplication of conditions used in the release experiment and each of these may have contributed to the difference observed between release and diffusion data. These aspects arose as a consequence of the simulation of clinical constraints and, for this reason, retain relevance to the potential for diffusion to occur *in vivo*. In the release study, photoinitiation of the composite resin was air-excluded, preventing formation of the air-inhibited layer and leaving the contour smooth. In the tooth model, the surface of composite resin from which release occurred was that which was closely adapted to the dentine floor of the cavity in contact with dentinal tubules. This very probably caused the formation of resin tags by permeation into dilated tubules thereby

increasing the inherent porosity of composite resin (Gjerdet and Hegdahl, 1979; Ogden, 1985) and, perhaps, causing some areas of the composite resin to be air- or moisture-inhibited during photoinitiation. In addition, the release and diffusion protocols necessitated different sample volumes to be used. The use of 1 ml as the collection volume with the diffusion model presented physical difficulties but gave results which appeared identical to 4 ml; that is, the collection volume did not appear to limit the rate of diffusion or release. Calculations for amount of TEGDMA released took collection volumes into account in each case. It should be noted that sterilization of the teeth by gamma radiation, as was done in the present study, has been found to not affect permeability (White *et al.*, 1994a) although cryopreservation may also have been used (Camps *et al.*, 1994).

Diffusion of the component TEGDMA from a composite resin through dentine is an important possible consequence of composite resin placement in a prepared cavity. Dentine structure offers some barrier to the early diffusion, which may indicate an early protective effect, but this is largely overcome as diffused TEGDMA leaves the dentine "reservoir" to enter the pulp space. The clinical implications of TEGDMA arrival in the pulpal space directly relate to the ability of the pulpal circulation to clear the diffused molecules to the general circulation and thereby greatly reduce their toxic potential by dilution. Pashley (1979) has postulated that the degree of inflammatory reaction in the pulp is a function of the toxin concentration in pulp fluid and that this concentration is dependent on a balance between two factors: the rate of permeation across dentine and the rate at which pulp circulation can remove the toxin. Reduced flow occurs in inflamed pulpal states (Olgart *et al.*, 1977; Kim *et al.*, 1982; Kim, 1990) or when the tooth is under occlusal trauma (Pashley, 1979) and unpredictable consequences could be anticipated if the balance between permeation across dentine and this flow is disturbed unfavourably to promote toxin accumulation in the pulp space.

In the experimental model used the dentine surface was pre-treated with 37% phosphoric acid for 60 s prior to resin placement. This was done in an attempt to reduce variability in diffusion characteristics which might occur due to variations in smear layer and because, as will be discussed in detail in the next Chapter, such treatment is advocated as part of many "dentine bonding" procedures. It was relevant to the experimental objectives to consider the effect on diffusion of other agents used in dentine pre-

treatment and which are known to interact with the inorganic phase only or smear layer only, such as EDTA and maleic acid. A consideration of these variables is presented in Chapter 6.

CHAPTER 6

Diffusion of Components of Composite Resin through Dentine, *in vitro*: Influence of some Dentine Pre-treatments

6.1 Introduction

6.2 Experimental methods

6.2.1 Tooth model

6.2.2 Materials

6.2.3 Procedure

6.2.3.1 No pre-treatment

6.2.3.2 Pre-treatment of dentine with EDTA

6.2.3.3 Pre-treatment of dentine with maleic acid

6.2.4 Sample analysis

6.2.5 Statistical analysis

6.3 Results

6.3.1 Component identification

6.3.2 TEGDMA quantification

6.3.2.1 The effect of different dentine pre-treatments on the cumulative diffusion of TEGDMA through dentine, *in vitro*

6.3.2.2 The effect of different dentine pre-treatments on the rate of diffusion of TEGDMA through dentine, *in vitro*

6.4 Discussion

6.1 Introduction

A common procedure in clinical restorative dentistry is pre-treatment of the dentine surface prior to cavity restoration with composite resin. These pre-treatments aim to improve adaptation and retention of composite resin to dentine by modification of the dentine or management of the smear layer and to exclude microleakage. One type of dentine pre-treatment, 37% phosphoric acid applied to dentine for 60 s (Fusayama *et al.*, 1979) was used in the experiment described in Chapter 5. The effects of two other suggested pre-treatment solutions were individually examined here; ethylenediamino tetraacetic acid (EDTA) (Asmussen and Munksgaard, 1985) and maleic acid (Pashley, 1990).

These treatments may modify dentine permeability sufficiently to alter TEGDMA diffusion. The following sections describe the influence these dentine pre-treatments had on component diffusion through dentine *in vitro* by comparing the observed diffusion with that presented in Chapter 5 (section 5.2.3). In each case, the only aspects which differed amongst the experimental procedures, that is, the experiment described in Chapter 5 and those in the current Chapter, were the use of the agents and procedures in dentine pre-treatment. Composite resin placement, photoinitiation and sample retrieval and analysis were as described in section 5.2.

6.2 Experimental methods

6.2.1 Tooth model

The same tooth model as that described in Chapter 5 was used. Prepared teeth, with chambers, were divided into three groups (EDTA, maleic acid and no pre-treatment) with $n=7$ for each group.

6.2.2 Materials

Materials used in the experiment were identical to those described in section 5.2.2 but in two groups, one of two dentine treatment solutions, either maleic acid or EDTA, was also used. The three experimental groups are defined in this Chapter as **pre-treatment of dentine with maleic acid**, **pre-treatment of dentine with EDTA** and **no pre-treatment**. The composite resin used in each of the groups was Foundation (Nulite Systems International, Hornsby, NSW, Australia).

6.2.3 Procedure

In general, cavity preparation as described in section 5.2.3 was carried out for all teeth. In brief, an occlusal cavity 6 mm in diameter, achieving a remaining dentinal thickness to the pulpal chamber within the range of 1.6 - 2.0 mm was prepared in each tooth. Pre-treatment of the dentinal cavity floor prior to composite resin placement was carried out as described in detail in each of the following sections for the experimental groups. These are summarized in Fig. 6.1. The composite resin was then applied in increments to the cavity floor to a thickness of 2 mm and light activated for 30 s. Sample removal from the pulpal chamber and its replacement was conducted under the same conditions and timing as that described previously (Chapter 5).

6.2.3.1 No pre-treatment of dentine

In this experiment no agents were applied to, or subsequent procedures carried out on, the dentine before composite resin was placed. Following cutting of the cavity, the dentine floor was irrigated with water and lightly dried with air from an air syringe. Composite resin was then adapted to the cavity floor, to a thickness of 2 mm, and light activated for 30 s. The remaining procedure was carried out as described in section 5.2.3.

6.2.3.2 Pre-treatment of dentine with EDTA

Following cavity preparation, dentine of the cavity floor was lightly coated with a freshly prepared solution of 0.5 M EDTA applied with a small cotton pellet. This solution was kept agitated on the dentine floor for a total of 60 s and then washed off with water for 15 s. Dentine was then air-dried using the triple syringe and composite resin was directly applied to the cavity floor, to a thickness of 2 mm, and activated for 30 s with a white activating light.

6.2.3.3 Pre-treatment of dentine with maleic acid

A 10% solution of maleic acid liquid (Scotchbond Multi-Purpose etchant, 3M, St Paul, MN, USA) from a dentine bonding system kit (Scotchbond Multi-Purpose Adhesive System, 3M, St Paul, MN, USA) was used as recommended by the manufacturers. This solution was applied, using a disposable dental bristle brush, to the dentine for 15 s during which the solution was agitated over the surface. The solution was then washed from the dentine surface with distilled water from the triple syringe for 15 s and dried lightly using

air from the triple syringe. Composite resin was then directly adapted to the cavity floor.

As the effect of the pre-treatment alone was of interest in these groups, the other steps in this adhesive system (that is, primer application and bonding agent application) were omitted.

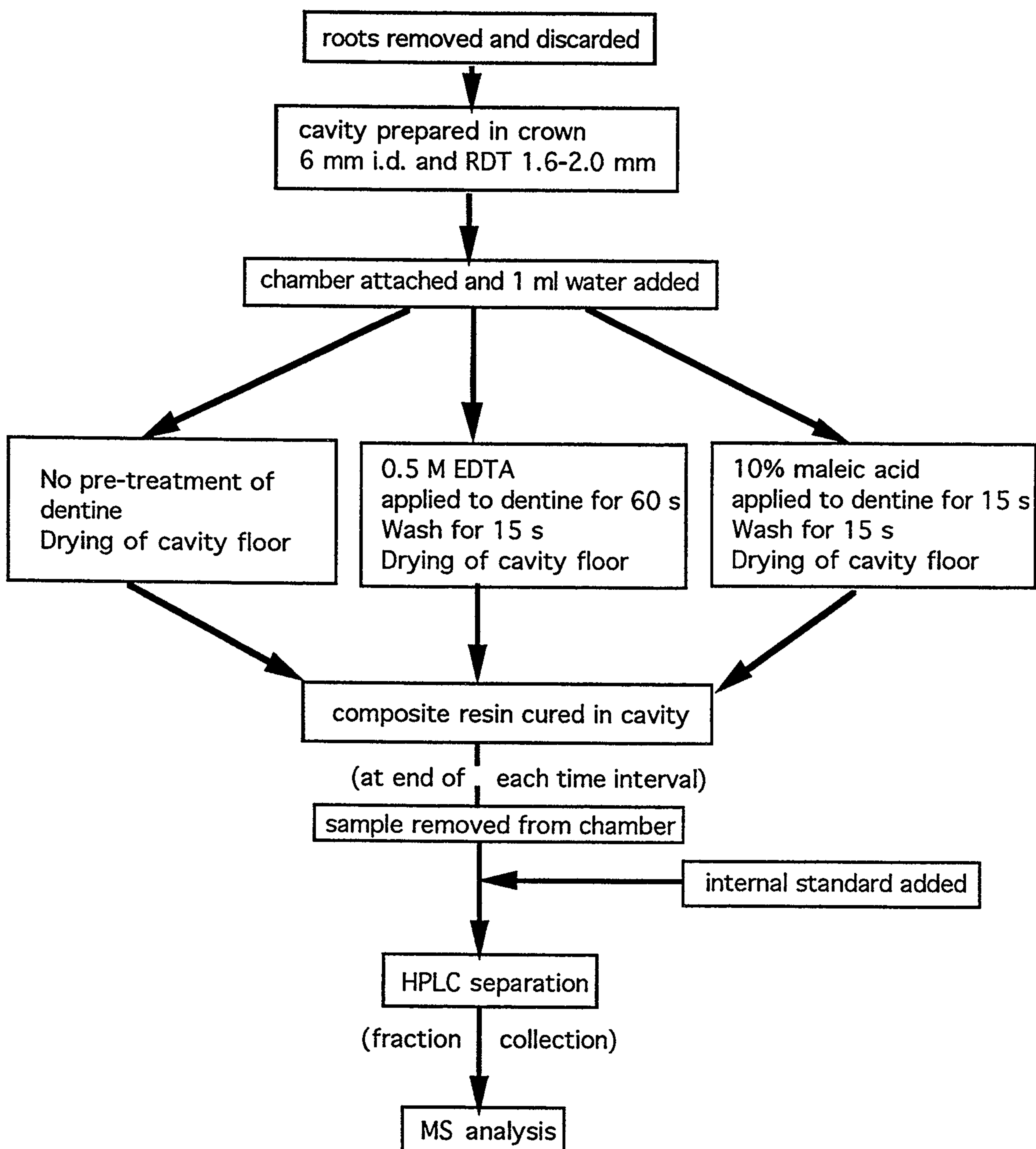


Figure 6.1 Diagram of the experimental procedure used in the tooth model experiments: the dentine pre-treatment groups

6.2.4 Sample analysis

Samples retrieved from the pulpal space were then prepared for separation by HPLC by addition of a volume of the internal standard solution and immediate separation or storage at 4°C for no more than 3 days prior to separation. Chromatographic conditions were as presented in Table 2.5. Collected fractions, taken at retention times of peaks of interest, for some duplicate HPLC separations were submitted for MS analysis. A standard solution was prepared for all known components of composite resin using concentrations shown in Table 2.6. Injectionless controls, distilled water samples and system controls were similarly analysed. Quantification of the identified TEGDMA was completed by integration and standard calibration curve fit from chromatograms carried out by the HPLC software (Millenium 2010, version 4.1, Millipore Corporation, Waters Chromatography Division, Milford MA, USA) as described in Chapter 2. This enabled the calculation of amount of TEGDMA found in each HPLC injection volume and conversion of this amount to a concentration in the sample volume was then completed.

6.2.5 Statistical analysis

Means and standard deviations about the means were calculated and significant differences discerned using statistical tests as described in section 5.2.5. That is, one-factor ANOVA-repeated measures and the Scheffé test were used to determine comparisons among data at different time points and among different groups (at $p < 0.05$).

6.3 Results

6.3.1 Component identification

Chromatograms of samples taken before 43.2 min differed little from those of the controls (no sample) and of the distilled water controls. A comparison of chromatograms generated for all samples with those of the controls after 43.2 min, confirmed that a compound was present in all samples removed after 43.2 min. Comparison of the retention time for this peak with that of the standard solution chromatogram (Fig. 2.2) suggested this compound was TEGDMA. MS analysis of the collected fraction taken at this retention time confirmed this identification. Other components, if present, were below reliable HPLC detection.

6.3.2 TEGDMA quantification

Data are presented graphically in the following sections for cumulative diffusion and for diffusion rate. Data presented in Chapter 5, for the pre-treatment with phosphoric acid, are included for comparison ("phosphoric acid" group). In some cases, data for TEGDMA release from composite resin presented in Chapter 3 are also included.

6.3.2.1 The effect of different dentine pre-treatments on the cumulative diffusion of TEGDMA through dentine *in vitro*

A comparison between cumulative diffusion of TEGDMA for the dentine pre-treatment groups is shown in Fig. 6.1. Data for the phosphoric acid group from Fig. 5.5 have been included for comparison.

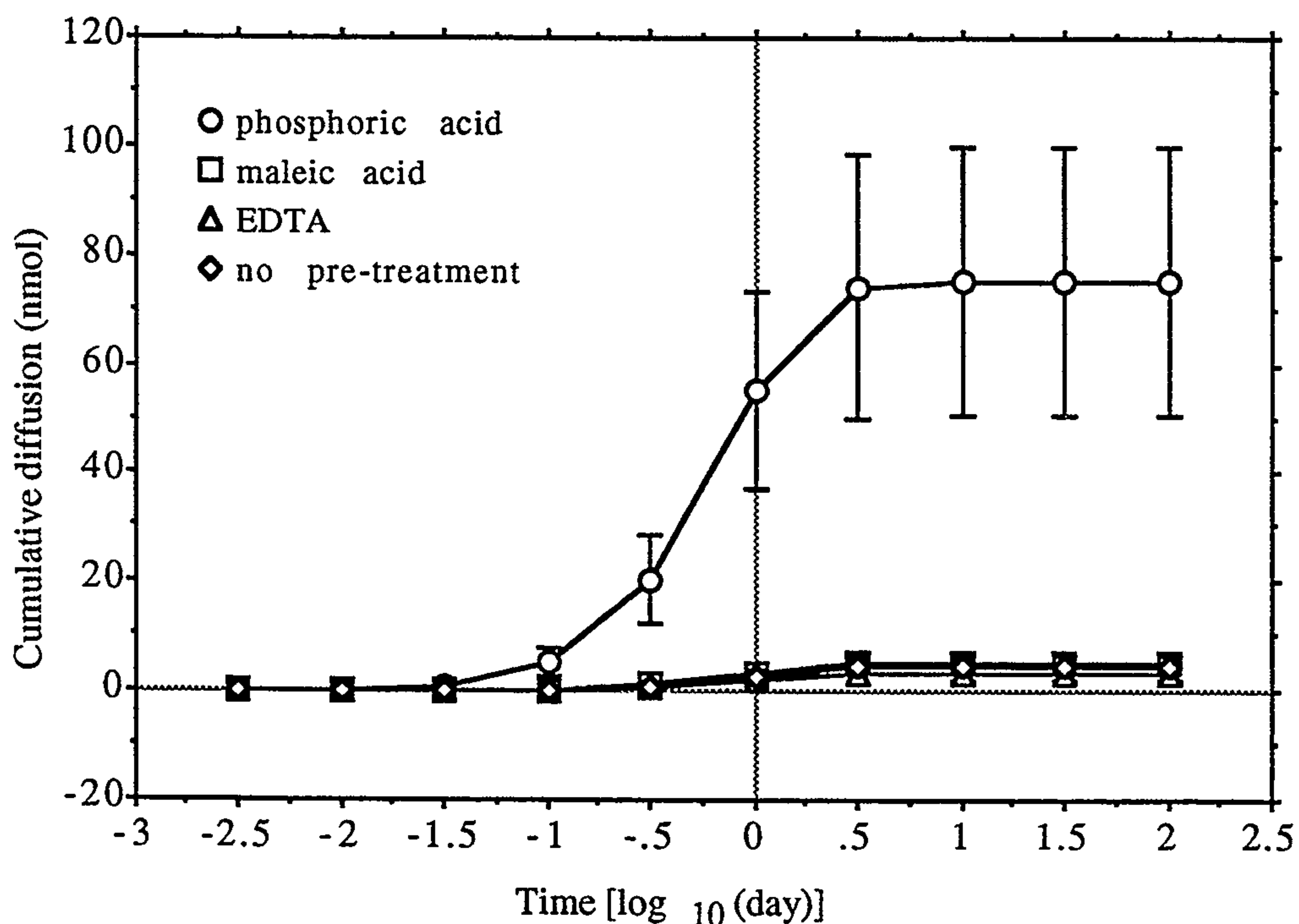


Figure 6.1 Cumulative diffusion of TEGDMA through dentine *in vitro* -comparison between pre-treatment groups

Table 6.1 shows a comparison between amounts accumulated by 3 days. Means and standard deviations about the means are shown; vertical lines unite means which are not significantly different ($p < 0.05$).

Table 6.1 Comparison between cumulative amounts of TEGDMA released and cumulative amounts of TEGDMA diffused through dentine *in vitro* after 3 days for the pre-treatment groups

Dentine Pre-treatment	Cumulative amount released (nmol)	Cumulative amount diffused (nmol)
Phosphoric acid	n/a	74.21 ± 24.45
No pre-treatment	n/a	4.79 ± 1.78
EDTA	n/a	3.42 ± 1.74
Maleic acid	n/a	5.32 ± 0.87
(mold confined)	120.71 ± 23.13	n/a

After 3 days, there was no significant difference between the cumulative amounts diffused in the EDTA, maleic acid and no pre-treatment groups. The cumulative amount found in the phosphoric acid group was significantly higher than that for any other pre-treatment group (at $p < 0.05$). The cumulative amounts *diffused* through dentine by 3 days in the pre-treatment groups can be compared with the cumulative amounts of TEGDMA *released* (from data in Chapter 3). If it is assumed that all released TEGDMA was available to diffuse through dentine in each of the groups, the proportion which did diffuse can be calculated. For the phosphoric acid group 61.5% of released TEGDMA had diffused through dentine; for the no pre-treatment group this was 4.0%. For the maleic acid group, 4.4% of the released TEGDMA had diffused through dentine and for the EDTA group this value was 2.8%.

6.3.2.2 The effect of different dentine pre-treatments on the rate of diffusion of TEGDMA through dentine *in vitro*

Fig 6.2 presents a comparison of the rates of diffusion for all the dentine pre-treatment groups. Data for the phosphoric acid group from Fig. 5.6 has been included for comparison. Since the amounts of TEGDMA detected in samples from the first two time intervals were well below reliable HPLC quantification, the rates at this time are depicted lower than graph scale. Rates of diffusion for the phosphoric acid group were significantly different from those of all other groups for all time intervals from 144 min (at $p < 0.05$). Rates of diffusion were not significantly different for the no

pre-treatment, EDTA and maleic acid groups from 144 min. In the interval 14.4 to 43.2 min, rates for the maleic acid group and the phosphoric acid group were not significantly different from each other, but were significantly different from those of the EDTA and no pre-treatment groups.

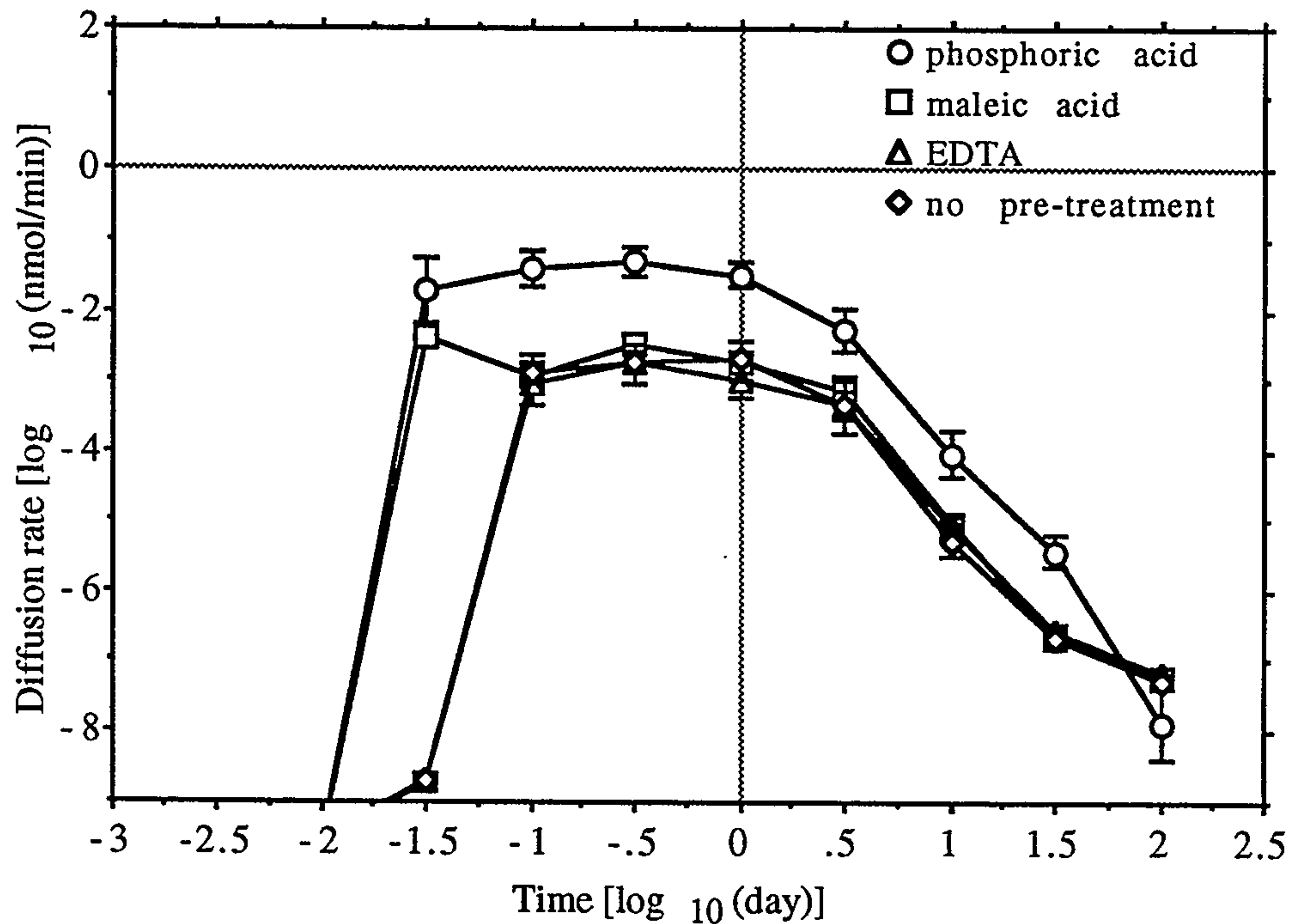


Figure 6.2 Rates of diffusion of TEGDMA through dentine *in vitro* -comparison between pre-treatment groups

The time interval of the highest diffusion rate of TEGDMA through dentine and the cumulative amount at that time are shown in Table 6.2. Data for the release of TEGDMA from the composite resin are also included. Means and standard deviations about the mean are shown. Vertical lines unite means, for the same time interval, which were not significantly different (at $p < 0.05$).

Table 6.2 Highest diffusion rate of TEGDMA and cumulative amount of TEGDMA at that time for the pre-treatment groups

Dentine pre-treatment	Rate (nmol/min)	Interval of highest rate	Cumulative amount (nmol)
Phosphoric acid	0.050 ± 0.020	144 - 432 min	20.44 ± 7.98
No pre-treatment	0.002 ± 0.001	144 - 432 min	0.68 ± 0.19
EDTA	0.002 ± 0.001	144 - 432 min	0.68 ± 0.34
Maleic acid	0.004 ± 0.001	14.4 - 43.2 min	0.12 ± 0.01
(mold-confined)	7.38 ± 2.12	0 - 432 min	31.87 ± 9.14

Highest *diffusion* rates for TEGDMA diffusion through dentine *in vitro* for all groups were significantly lower than highest *release* rate (at $p < 0.05$) and were attained later. Attainment of highest diffusion rate occurred during the same time interval (144-432 min) for phosphoric acid, EDTA and the no pre-treatment groups but was earlier in the maleic acid group (14.4-43.2 min). Highest diffusion rate and cumulative amount, for the EDTA group, by this time (144-432 min) was not different from those of the no pre-treatment group.

A comparison between data in Tables 6.1 and 6.2 shows that 26.4% of the total accumulated amount of TEGDMA *released* by 3 days, occurred during the time interval of highest release rate. This was similar for the phosphoric acid group where, after 3 days, 27.5% of the accumulated amount of TEGDMA had *diffused* during the interval of highest cumulative diffusion. However, in contrast, in the no pre-treatment group this was 14.3%; in the EDTA group, 19.9% and in the maleic acid group, 2.2%.

6.4 Discussion

Dentine possesses several structural and chemical properties which may complicate interaction with resin materials (Van Meerbeek *et al.*, 1992b). A high protein content, for example, results in low surface energy which prevents adequate resin wetting and the heterogenous distribution of organic and inorganic components places added chemical demands on the adhesive ability of resins. These features are modified when dentine is cut

and properties are further complicated by the formation of smear layer. For this reason various surface treatments may be used before resin contact with the dentine surface.

In the present work, 37% phosphoric acid applied for 60 s was used as the "basic" treatment of the dentine surface. This procedure has been suggested by several authors (Fusayama *et al.*, 1979; Kurosaki *et al.*, 1990; Fusayama, 1990; Bertolotti, 1990; Kanca, 1990) and the effect of such acid pre-treatment of dentine structure has been described (Gwinnett, 1977; Pashley *et al.*, 1981a; Pashley *et al.*, 1983; Kurasaki *et al.*, 1990). In the work described in this Chapter, dentine was either pre-treated with EDTA or maleic acid or was untreated prior to resin placement.

Smear "plugs" can be dissolved by phosphoric acid; an effect which enhances tubule patency. When unfilled resin is applied to this dentine, resin enters patent tubules to form tags which are a source of diffusable TEGDMA, particularly in view of the morphology described by Gwinnett (1977) and Marshall and co-workers (1993). It is possible that small tags may also form when filled composite resin is similarly applied. Asmussen and Munksgaard (1985) suggested that the unfilled resin tags may be incompletely polymerized due to impaired conversion in these areas. Factors which have caused impaired conversion in this area include remoteness from the light source and air-inhibition (and moisture-inhibition) during polymerization, by virtue of the high density of dentinal tubules.

Differences seen between the three pre-treatments groups described in this Chapter and the "basic" protocol (described in Chapter 5) reflect the different effects which these systems have on the dentine surface and on the nature of the hybrid layer. Since, in the no pre-treatment group, dentine was not chemically or morphologically modified after cavity preparation it is pertinent to discuss this group first. In this group, dentine was ground away during cavity preparation with a water-cooled high speed handpiece. This cavity floor probably featured an array of obliquely cut and parallel cut tubules (Brännström, 1981). Much of the gross debris created in this procedure was irrigated away with water but what was presumably retained on the dentine surface and within dentinal tubules constituted smear layer (and smear plugs). This 1 μm -thick layer has been identified as "a uniform, amorphous structure which appears to completely

occlude orifices of all dentinal tubules" (Pashley *et al.*, 1981a; Pashley *et al.*, 1993). Since dentinal tubules are the most efficient avenue of transport of molecules across dentine, when orifices are occluded it is likely that dentine permeability will be reduced. Dentine with intact smear layer exhibits low rates of fluid filtration (Pashley *et al.*, 1981a). In the current work, the detection of small amounts of TEGDMA in the no pre-treatment group after 14.4 min indicated that dentine with smear layer present *in vitro* possesses an intrinsic but low level permeability. TEGDMA diffusion was seen in the "no pre-treatment group". This may be because ground dentine and smear layer prevent adequate "intradentinal permeation" (Pashley *et al.*, 1993) of filled composite resin which results in diminished resin tag size and restricted resin contact with intratubular fluid. Ground dentine may also be sufficiently hydrophilic to inhibit TEGDMA release from the composite resin which would reduce the amount of TEGDMA able to diffuse into the intratubular fluid.

Diffusional surface area may be defined as the product of the average surface area of tubules and the number of tubules per unit area (Pashley *et al.*, 1978). Highest diffusional area equates with higher trans-dentinal permeation particularly when tubules are of larger diameter and more numerous than in the unaltered state (such as when dentine is newly fractured). When ground by a rotating carbide bur, grinding debris blocks tubule orifices and so reduces diffusional area. Reduced tubular surface area therefore reduces the size and length of resin penetration. Imaging of the resin/treated dentine interface by several workers has confirmed the presence of resin tags which penetrate tubules (Spencer *et al.*, 1992; Nakabayashi *et al.*, 1992; Van Meerbeek *et al.*, 1992a; Van Meerbeek *et al.*, 1992b; Youngson and Grey, 1992; Van Meerbeek *et al.*, 1993; Van Meerbeek *et al.*, 1994) but the quality or stability of these tags has not been considered. Although these workers described the development of the zone with a bonding resin, it is possible that this zone is similarly characterized when composite resin, rather than primer/bonding agents, are directly applied to dentine since basic chemistry and polymerization kinetics are similar in the materials. Pashley and co-workers (1993) observed no apparent tubule orifices in ground, smear-covered dentine and so it is likely that any resin tags which form are only poorly developed and irregular. This would result in considerably less diffusional surface area with intratubular fluid and very much lowered diffusion when compared with that which would occur when the dentine was pre-treated, for example, by acid etching. The

majority of released TEGDMA may then be retained within the dentine layer immediately subjacent to the composite resin at least for some time acting as a slow-releasing "reservoir". If it is assumed that all released TEGDMA was available for diffusion, more than 95% of the available released TEGDMA was retained within untreated dentine. It may be possible to deny or confirm this suggestion by chemically analyzing the dentine retrieved after the different intervals in the current model and comparing this with the diffusion data already presented.

Several factors evident in the current work may have acted to inhibit TEGDMA release from the composite resin. The diminished length of resin tags may have contributed to better resin conversion by improved penetration of light to these short tags and by reducing the influence of air-inhibition, both of which have been found to decrease TEGDMA release from the composite resin (Chapter 3). In addition, following cavity preparation, dentine was lightly air-dried prior to immediate composite resin placement. Although dentine was most probably hydrated throughout the experiments, air-drying of the cavity floor may have caused evaporation at the dentine surface, as suggested by Pashley and co-workers (1984a), and therefore the inducement of capillary force drawing pulpal space water into the tubules. This effect is probably more prominent when dentine is acid etched. The dentine was then likely rendered hydrophilic which probably interacted poorly with a hydrophobic composite resin thereby reducing monomer kinetics with an aqueous medium. Since, in addition, hydrophobic composite resins are less cytotoxic than hydrophilic composite resins (Hume and Massey, 1988) it may be suggested that the reason for both the lower cytotoxicity and the low diffusion seen for hydrophobic resins may be the weak hydrolytic reaction with water.

In contrast with the nature of the dentine in the no pre-treatment group, dentine structure following pre-treatment with phosphoric acid is markedly changed, as described previously. Smear layer, smear plugs and peritubular dentine are variably dissolved, tubule orifices are enlarged, elliptical pores are formed in intertubular dentine and collagen fibres of the dentine matrix are selectively demineralized (Pashley *et al.*, 1993a). The significantly higher diffusion observed in this group compared with that observed in all other pre-treatment groups strongly suggests that the tubule patency below the demineralized area, which is improved by etching, and surface energy, which is lower in demineralized dentine, are

crucial to the permeability of dentine (Van Meerbeek *et al.*, 1992a) enhancing composite resin adaptation to the dentine surface.

Current clinical procedures favour etching dentine for shorter periods. Reducing etch time of phosphoric acid to 30 s (the treatment time in the present study was 60 s) could influence the diffusion seen since, as suggested by Pashley and co-workers (1993a), this shorter etching time "reduces the potential porosity of dentine...by creating a very thin surface film of condensed collagen fibres". This collagen layer was thicker when smear layer was present prior to etching and its presence severely restricted porosity of the dentinal surface. The nature of this layer has implications for hybrid layer formation when resin bonding agents are used. Recent work by Hamid and Hume (1995c) supports the use of shorter etching time. These workers used the same tooth model as that used here but reduced phosphoric acid pre-treatment to 10 s, and found a substantial reduction in TEGDMA diffusion from Z100, although a bonding agent used in combination may have contributed to the reduction. In the current work, a comparison of diffusion seen in the no pre-treatment group with that seen in the phosphoric acid group suggests that ground dentine structure and smear layer may have a significant influence on diffusion but this comparison does not allow a distinction to be made between these structures in their *relative* influence on dentine permeability.

The other pre-treatment groups, however, in their different effect on dentine and smear layer illustrated the importance of dentine structure exclusive of smear layer. Diffusion seen in both the EDTA and maleic acid groups was similar to that seen in the no pre-treatment group but differed greatly from that seen in the phosphoric acid group. A possible explanation for these observations is the chemical nature of the different acids and their interaction with dentine and smear layer. Unlike phosphoric acid, these weak acids are likely to be less effective in demineralization of dentine structure and smear layer. The similar diffusion seen in the maleic acid and EDTA pre-treatment groups suggests that these two agents similarly affected dentine permeability. Both agents remove smear layer but have only minor effects on peritubular dentine so the lower diffusion (compared with the phosphoric acid group) may be due to the small dentine surface area for diffusion afforded by these treatments, confirming the comments of Pashley and co-workers (1993a). The excellent buffering ability of dentine (Wang and Hume, 1988) probably contributed only a small degree to the neutralizing of maleic acid and EDTA since these acids dissociate only

weakly. In view of this, a low pH at the dentine surface may remain after these acid applications, changing the surface energy and perhaps influencing the adaptation and penetration of composite resin. The slightly lower cumulative amount of TEGDMA diffused in the EDTA group by 3 days compared with that observed in the no pre-treatment group, suggests that the effect of this pre-treatment (EDTA) is somewhat protective in interfering with TEGDMA diffusion perhaps by precipitation of removed material onto dentine.

In the Chapters which follow the release and diffusion through dentine of components of a range of composite resins and some bonding resins will be described.

CHAPTER 7

Diffusion of Components of Composite Resin through Dentine, *in vitro*: Various Composite Resins

7.1 Introduction

7.2 Experimental method

7.2.1 Description of tooth model

7.2.2 Materials

7.2.3 Procedure

7.2.4 Sample analysis

7.2.5 Statistical analysis

7.3 Results

7.3.1 Component identification

7.3.2 TEGDMA quantification

7.3.2.1 Cumulative diffusion of TEGDMA through dentine *in vitro* from various composite resins

7.3.2.2 Rate of diffusion of TEGDMA through dentine *in vitro* from various composite resins

7.3.3 Comparison between release of TEGDMA and diffusion of TEGDMA through dentine *in vitro*, from various composite resins

7.3.3.1 Comparison between cumulative amounts of TEGDMA diffused through dentine *in vitro* from various composite resins

7.3.3.2 Comparison between rate of release of TEGDMA and rate of diffusion of TEGDMA through dentine *in vitro* from various composite resins

7.3.4 Summary of results

7.4 Discussion

7.1 Introduction

In Chapter 5 the diffusion of TEGDMA through dentine *in vitro* was examined for one Bis-GMA-based composite resin. A comparison of release and diffusion data showed that dentine had a delaying effect on TEGDMA diffusion but did not substantially reduce the diffusing amount. Since this observation had been made for only one type of composite resin, it is possible that the particular formulation of composite resin influenced the diffusion seen and that perhaps diffusion for other materials would differ from that seen for Foundation.

In the following sections, component *diffusion* from five other common composite resins are reported and then compared to component *release* for these materials.

7.2 Experimental method

7.2.1 Description of tooth model

To enable comparison of findings for the various composite resins examined to that for Foundation, the same tooth model as first described in section 5.2.1 was used. In brief, intact human third molar teeth were obtained and separated into 5 groups, a group for each composite resin (each group $n=7$). An occlusal cavity was prepared in each tooth crown, approximately 6 mm in diameter and leaving a remaining dentinal thickness to the pulp within the range 1.6 to 2.0 mm. This was identical to that prepared for Foundation (Chapter 5). The positioning of a polypropylene chamber simulating the pulpal chamber was similarly completed.

7.2.2 Materials

The five composite resins selected were those for which component release had been reported in Chapter 3, namely Occlusin (GC, Tokyo, Japan), Z100 (3M Dental Products, St. Paul, MN, USA), Conquest (Jeneric/Pentron, Wallingford, CT, USA), Perfection (Den-Mat Corporation, Santa Maria, CA, USA) and Herculite XR (Kerr Manufacturing, Romulus, MI, USA). Data for Foundation (Nulite Systems International, Hornsby, NSW, Australia), the composite resin used in all experiments reported in previous chapters, except in section 3.8, are included for comparison.

Some characteristics of these materials are shown in Table 7.1 including the reported oligomer and diluent monomer contents, the filler content and the

mean particle size (MPS). Data have been adapted from Willems and co-workers (1992), Ruyter and Øysaet (1987) and from information provided by the manufacturer.

Table 7.1 Characteristics of the various composite resins used to examine component diffusion through dentine *in vitro*

Resin	Class ¹	MPS (μm)	Bis-GMA (%wt)	TEGDMA (%wt)	UDMA (%wt)	Filler ² (%vol)

Occlusin ³	F C-F	3.6-9.6	-	50	50	69.0
Foundation ⁴	f/U	14-16 (2-5)	73	25	-	71.6
Z100	U C-F	1.0-3.6	46	52	-	71.0
Conquest	U M-F	0.7-3.6		34	65	68.5
Perfection	MFspf	0.04	52	45	?	67.5
Herculite XR	U M-F	0.7-3.6	72	25	57	57.0

1 Classes as listed in Table 1.1

2 Refers to total filler content

3 Contains a mixture of two forms of UDMA

4 Manufacturer data for fibre rods, microfine silica MPS shown in brackets

7.2.3 Procedure

All experimental procedures for the five composite resins followed that described in section 5.2.3. Briefly, dentine of the cavity floor was irrigated with water from an air-water syringe and lightly dried with air. Dentine was then pre-treated in the same manner for all teeth, namely 37% phosphoric acid gel (3M Dental Products, St Paul, MN, USA) was applied to the dentine for 60 s using a disposable brush. The cavity was irrigated with distilled water for 30 s and dried with air. Following this, increments of composite resin were applied to the dentine to attain a composite resin thickness of 2 mm. The composite resin was then activated using a dental activating visible light for 30 s (Optilux 400, Demetron Research Corporation, Danbury CN, USA). The end of activation was taken as time zero.

Pulpal chamber contents (1 ml) were removed at the same intervals used previously and either immediately prepared for analysis or stored at 4°C until analysed.

7.2.4 Sample analysis

Samples from both the release and diffusion experiments were filtered and a volume of an internal standard solution was added. Analytical procedures were carried out as described in section 3.2.2. Briefly, a standard solution for known components was prepared for HPLC separation using concentrations shown in Table 2.6. A set of control samples and a series of dilutions of the standard was separated by HPLC using conditions shown in Table 2.5. Following confirmation that the standard solution peaks were valid (by comparison with the system and solvent controls) calibration curves were generated.

A HPLC series of samples was then arranged; controls, standards and unknowns and MS spectra were generated for collected fractions of selected samples to confirm the identity of the component in the samples. Following this, the amount of component in each HPLC injection volume was determined by fitting signal response of peaks in the samples to that component's calibration curve. The amount of component in the entire sample was computed and cumulative diffusion and diffusion rates were calculated from this amount.

7.2.5 Statistical analysis

Statistical analysis of data was completed as previously described. Means plus or minus standard deviations about the means for cumulative amounts and rates of release and diffusion were derived. Comparisons between data at different points for each group were made using one factor ANOVA-repeated measures and Scheffé statistical tests at $p < 0.05$.

7.3 Results

7.4.1 Component identification

TEGDMA was detected in all samples from all composite resins in the component release experiment and in samples from 43.2 min in the diffusion experiment. Other components diffusing were below the detection limit of the HPLC.

7.3.2 TEGDMA quantification

7.3.2.1 Cumulative diffusion of TEGDMA through dentine *in vitro* from various composite resins

In all data presented, those of Foundation (from Chapter 5) are also included for comparison. The cumulative diffusion of TEGDMA through dentine, *in vitro*, is presented in Fig. 7.1. Cumulative diffusion was highest for all composite resins by 3 days, with only small further increases after this time. Highest cumulative amount diffused by 3 days was seen for Occlusin and lowest for Herculite XR.

Statistically, the composite resins could be divided into two different groups where cumulative amounts by 3 days were not significantly different (at $p < 0.05$): Occlusin, Foundation and Z100 (as one group) and Conquest, Perfection and Herculite XR (as another group). Cumulative diffusion of TEGDMA through dentine *in vitro* for the first group was significantly higher than diffusion for the second group.

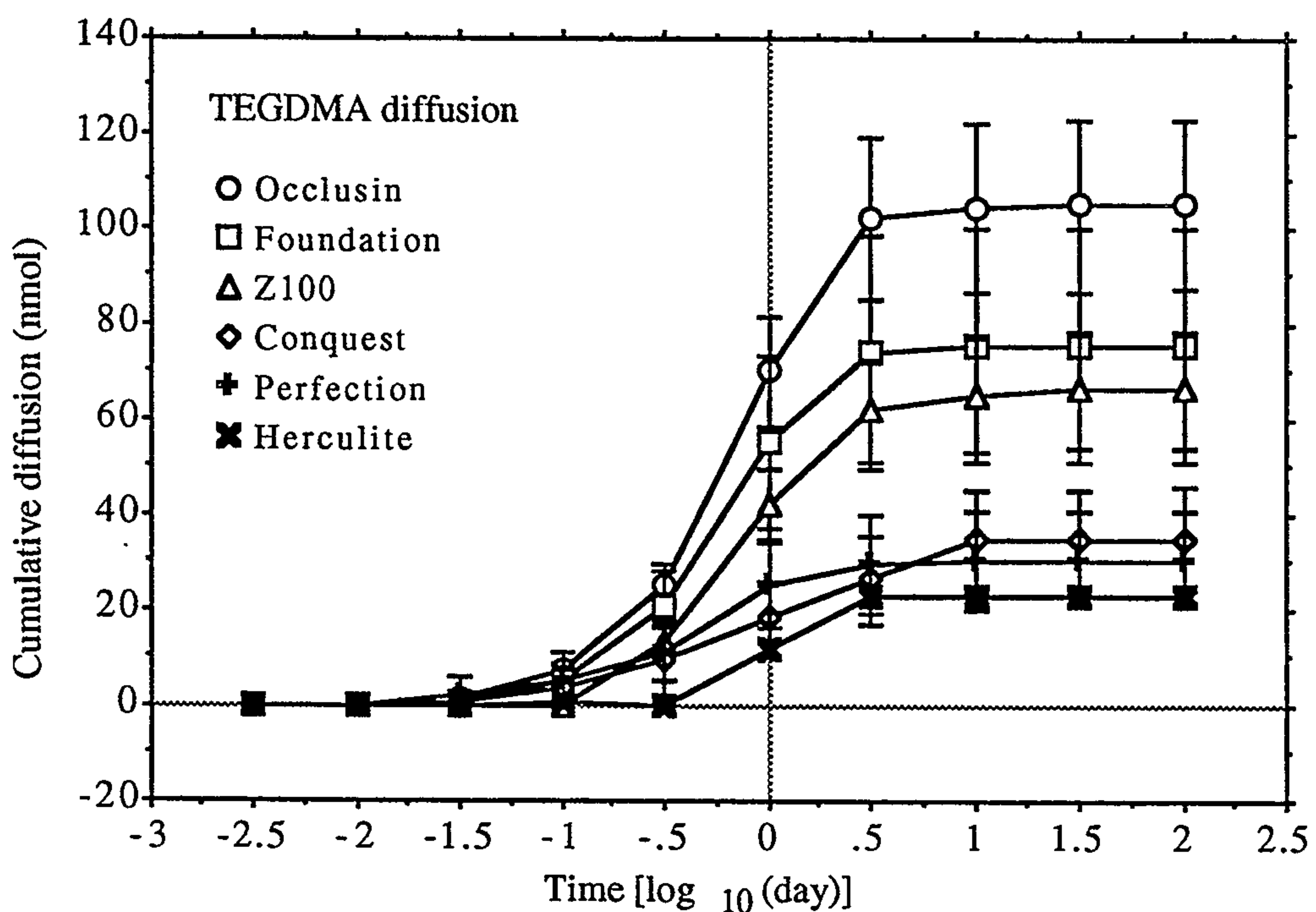


Figure 7.1 Cumulative diffusion of TEGDMA from various composite resins through dentine *in vitro*

7.3.2.2 Rate of diffusion of TEGDMA through dentine *in vitro* from various composite resins

The rates of diffusion of TEGDMA for the other composite resins examined are shown in Fig. 7.2. The diffusion rate patterns were similar to that seen for Foundation. With the exception of Z100, no significant difference was found between rates of diffusion for the composite resins examined through the time course. After 10 days, the diffusion rates for Z100 were significantly different from those rates for all other composite resins examined (at $p < 0.05$).

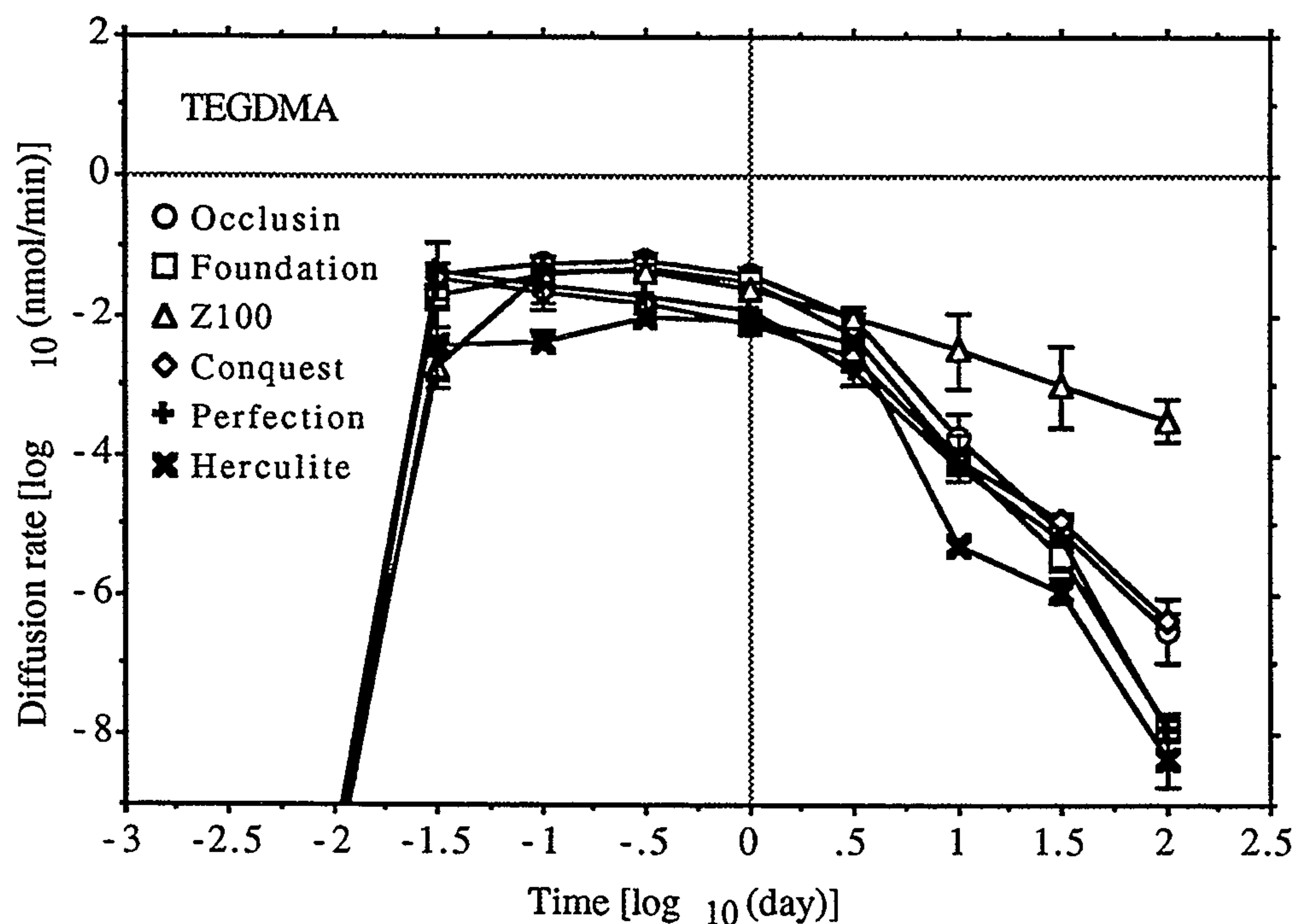


Figure 7.2 Rates of diffusion of TEGDMA from various composite resins through dentine *in vitro*

7.3.3 Comparison between release of TEGDMA and diffusion of TEGDMA through dentine *in vitro*, from several composite resins

For each composite resin, the amounts and rates of TEGDMA released from the mold-confined material were compared with the amounts and rates of TEGDMA diffusing through dentine *in vitro*.

7.3.3.1 Comparison between cumulative amounts of TEGDMA
diffused through dentine *in vitro* from various
composite resins

A comparison between cumulative diffusion and cumulative release for each of the other composite resins is shown: for Occlusin, this is shown in Fig. 7.3, in Fig. 7.4 for Conquest, in Fig. 7.5 for Perfection, in Fig. 7.6 for Herculite XR and in Fig. 7.7 for Z100 (that for Foundation was shown in Fig. 5.7.)

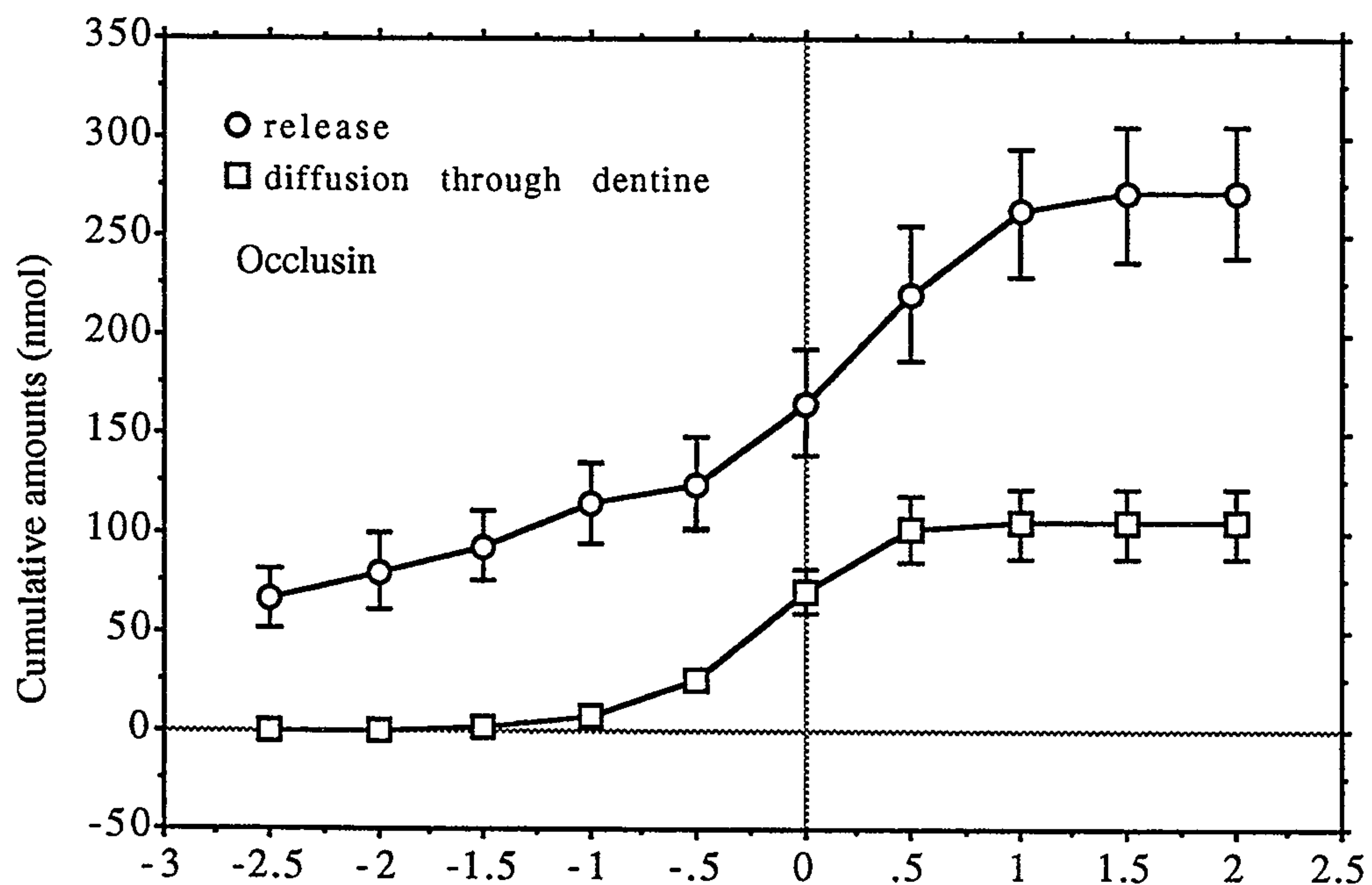


Figure 7.3 Comparison between cumulative amounts of TEGDMA released and diffused through dentine, *in vitro*, from Occlusin

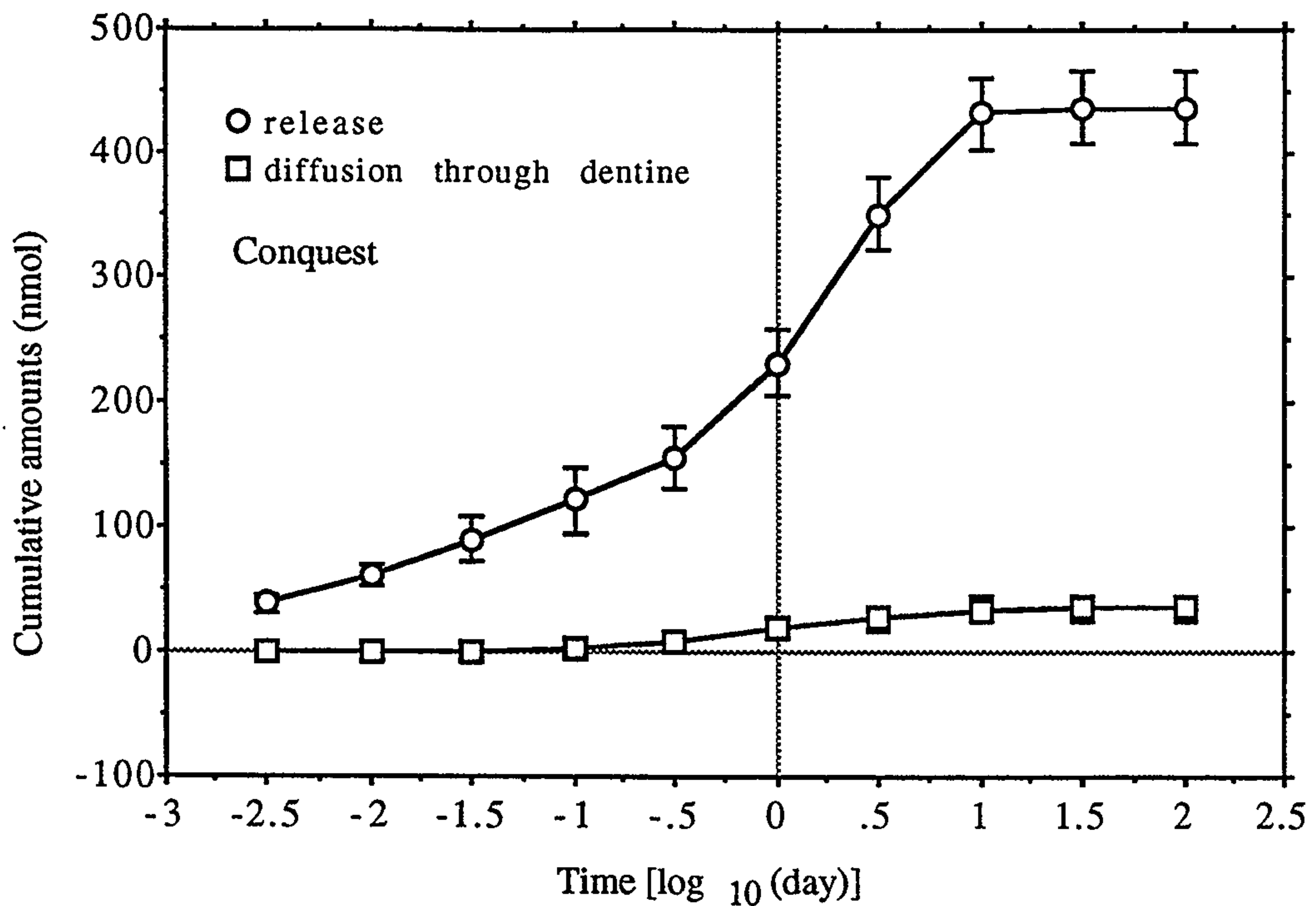


Figure 7.4 Comparison between cumulative amounts of TEGDMA released and diffused through dentine, *in vitro*, from Conquest

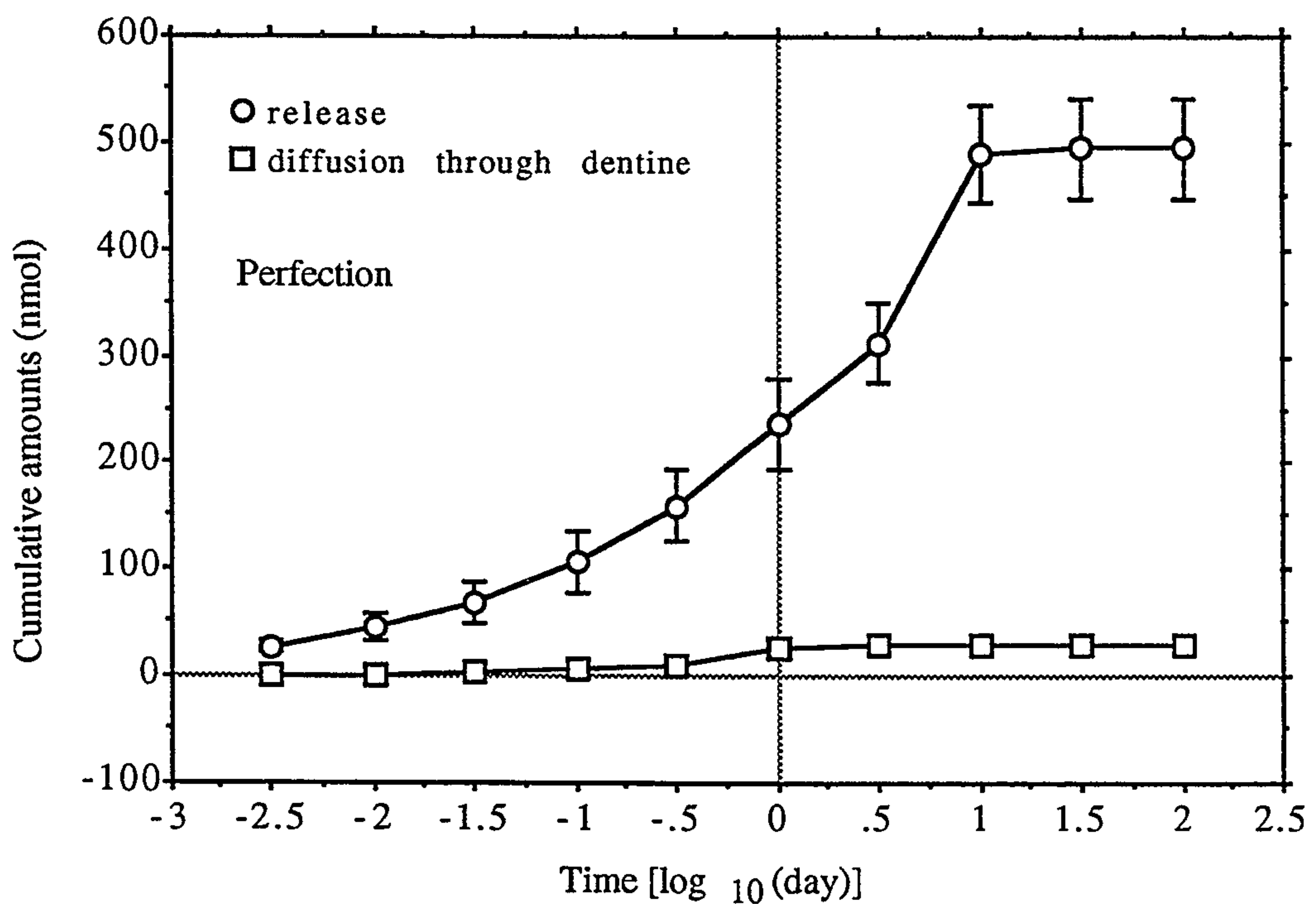


Figure 7.5 Comparison between cumulative amounts of TEGDMA released and diffused through dentine, *in vitro*, from Perfection

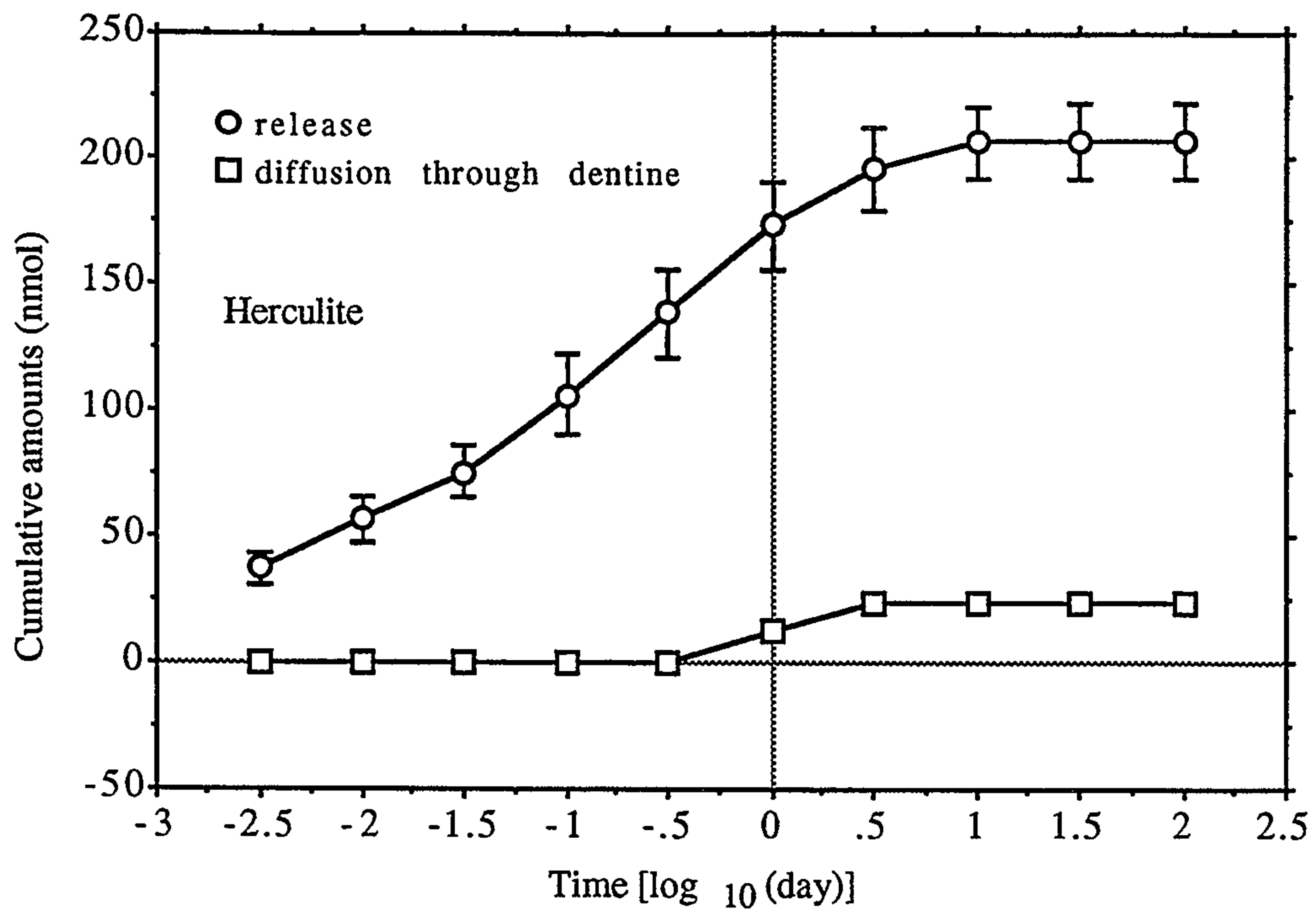


Figure 7.6 Comparison between cumulative amounts of TEGDMA released and diffused through dentine, *in vitro*, from Herculite XR

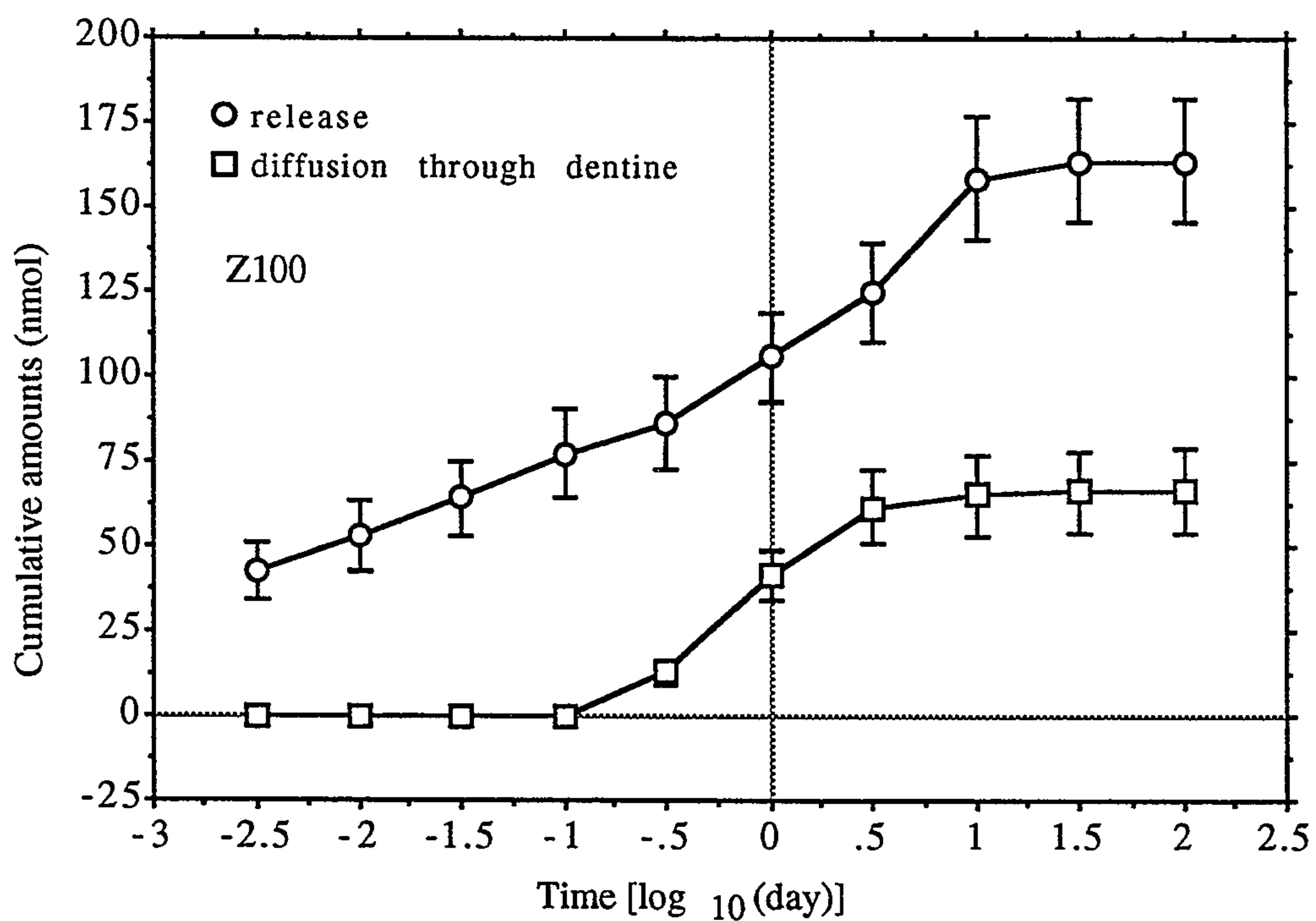


Figure 7.7 Comparison between cumulative amounts of TEGDMA released and diffused through dentine, *in vitro*, from Z100

A significant difference between cumulative release and diffusion was seen for each composite resin (at $p < 0.05$) but the magnitude of the difference for each composite resin was not consistent. Greatest difference between amounts of TEGDMA diffused through dentine and released was seen for the two ultrafine midway-filled composite resins, Herculite XR and Conquest and for Perfection. Smallest difference between released and diffused amounts was seen for Foundation (Fig. 5.7).

7.3.3.2 Comparison between rate of release of TEGDMA and rate of diffusion of TEGDMA through dentine *in vitro* from various composite resins

Comparisons between rates of release and rates of diffusion of TEGDMA through dentine *in vitro*, for each of the other composite resins examined are shown: in Fig. 7.8 for Occlusin, in Fig. 7.9 for Conquest, in Fig. 7.10 for Perfection, in Fig. 7.11 for Herculite XR and in Fig. 7.12 for Z100.

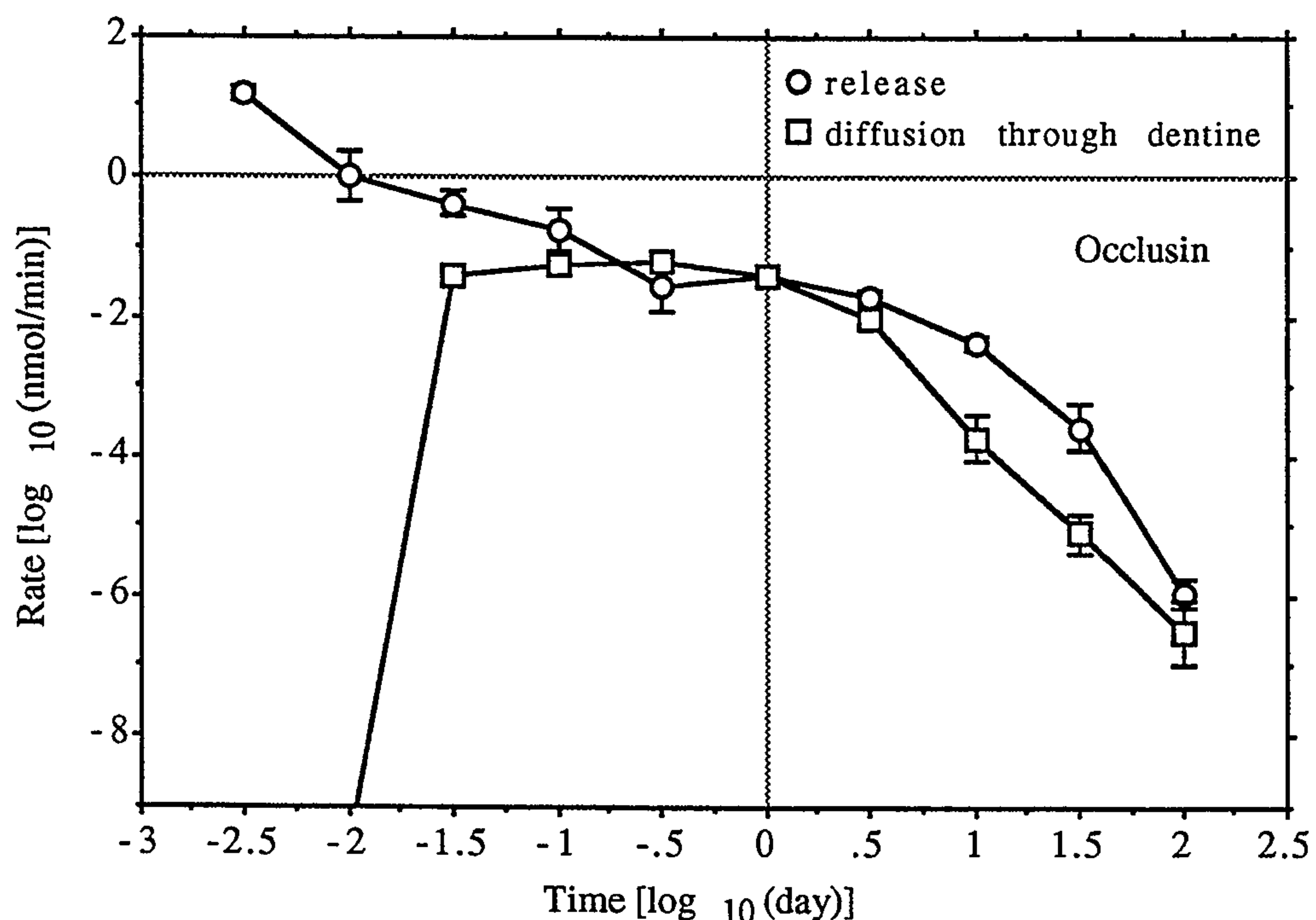


Figure 7.8 Comparison between rates of TEGDMA released and rates of TEGDMA diffused through dentine, *in vitro*, from Occlusin

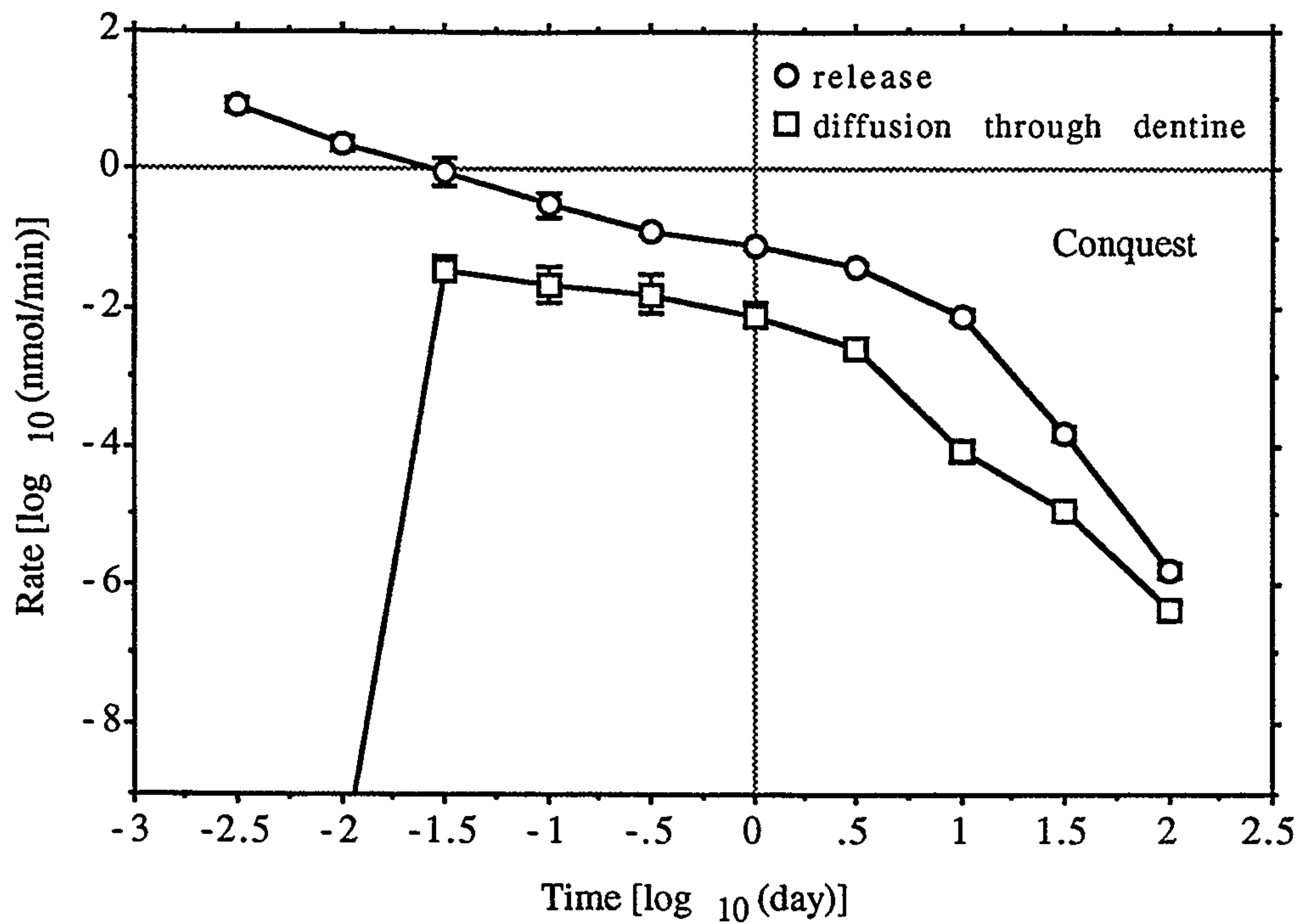


Figure 7.9 Comparison between rates of TEGDMA released and rates of TEGDMA diffused through dentine, *in vitro*, from Conquest

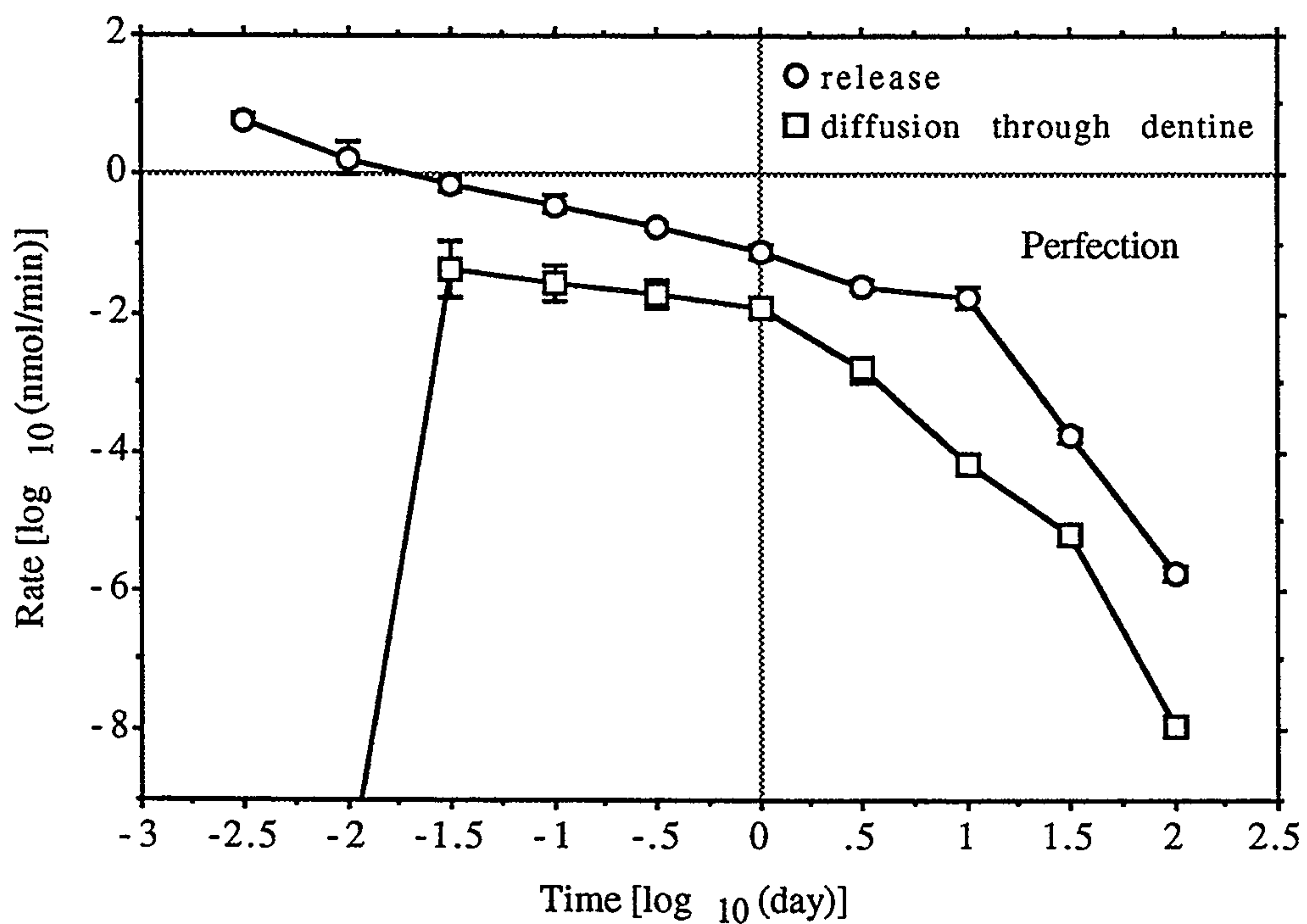


Figure 7.10 Comparison between rates of TEGDMA released and rates of TEGDMA diffused through dentine, *in vitro*, from Perfection

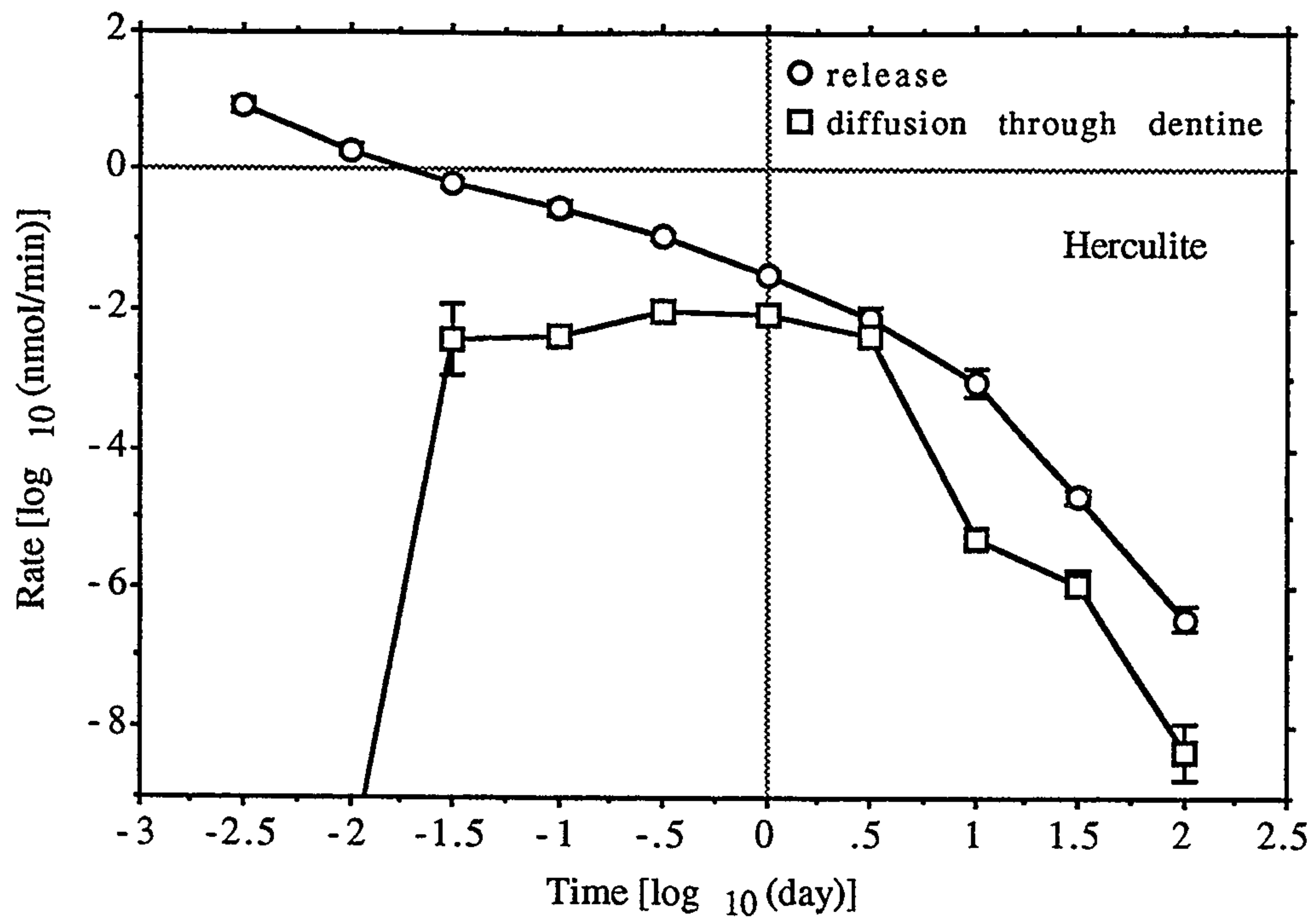


Figure 7.11 Comparison between rates of TEGDMA released and rates of TEGDMA diffused through dentine, *in vitro*, from Herculite XR

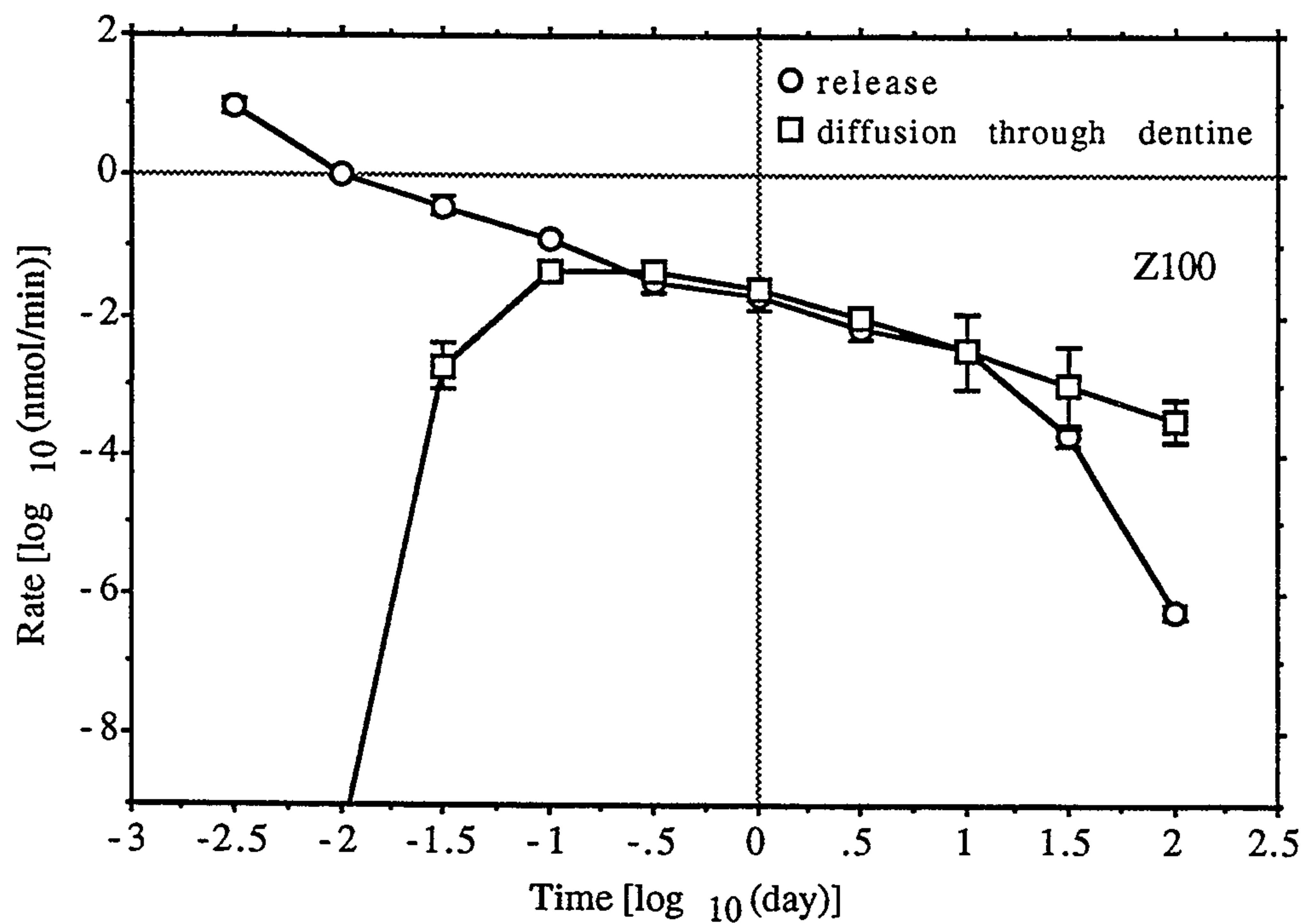


Figure 7.12 Comparison between rates of TEGDMA released and rates of TEGDMA diffused through dentine, *in vitro*, from Z100

7.3.3 Summary of results

Summarized data are presented in Tables 7.2 and 7.3. In each table, means and standard deviations about the means are shown and vertical lines unite means which are not significantly different (at $p < 0.05$). Table 7.2 shows a comparison between release and diffusion data of cumulative amounts by 3 days for the composite resin examined. A calculated ratio of the accumulated amount of TEGDMA *diffused* compared with the amount *released* for each composite resin by 3 days is also shown.

Small increases only were seen in TEGDMA detected after 3 days in all experiments. With the exception of Foundation, the amount of TEGDMA *diffused* was significantly less than the amount which had *released* for all composite resin examined (at $p < 0.05$). The ratios comparing the amount of diffused TEGDMA with that released were similar for Occlusin and Z100, and were similar for Conquest, Perfection and Herculite XR.

Table 7.3 shows the time of highest diffusion rate for each composite resin, when these rates occurred and the accumulated amount of TEGDMA by that time.

Table 7.2 Comparison between cumulative amounts of TEGDMA released and cumulative amounts of TEGDMA diffused through dentine *in vitro* by 3 days for several composite resins

Composite Resin	Release (nmol)	Diffusion (nmol)	Ratio: diffused/released (%)
Conquest	351.11 ± 28.19	26.42 ± 9.02	7.5
Perfection	313.08 ± 35.91	29.78 ± 10.24	9.5
Occlusin	220.78 ± 34.08	102.25 ± 16.92	46.3
Herculite XR	196.33 ± 16.80	23.13 ± 2.28	11.8
Z100	125.00 ± 14.90	61.90 ± 10.99	49.5
Foundation	120.71 ± 23.13	74.21 ± 24.45	61.5

Table 7.3 Maximum diffusion rate of TEGDMA through dentine and cumulative amount of TEGDMA at that time, for several composite resins

Composite Resin	Rate (nmol/min)	Interval of highest rate	Cumulative amount (nmol)
Foundation	0.05 ± 0.02	144 - 432 min	20.44 ± 7.98
Occlusin	0.06 ± 0.01	144 - 432 min	25.30 ± 4.04
Z100	0.04 ± 0.03	144 - 432 min	18.23 ± 4.02
Herculite XR	0.009 ± 0.002	144 - 432 min	3.31 ± 0.63
Conquest	0.04 ± 0.02	14.4 - 43.2 min	1.09 ± 0.61
Perfection	0.08 ± 0.13	14.4 - 43.2 min	2.33 ± 0.81

For all the composite resins, highest diffusion rate occurred before 432 min and the accumulated amount of TEGDMA by this time varied between the composite resins. Although highest rate for Herculite XR, an ultrafine midway-filled composite resin, occurred at the same time as the three composite resins, the rate was significantly lower than that of other composite resins.

7.4 Discussion

Two important observations were made in the work described. First, TEGDMA release and diffusion through dentine was seen for all composite resins tested. Secondly, the relationship between amounts of TEGDMA *released* (see Chapter 3) and *diffused* through dentine was remarkably different for each of the various composite resins.

The first observation indicates that composite resins, in general, release diluent monomer and that dentine offers some protection for pulpal tissue in delaying and reducing the amount which diffuses through to the pulp space. It would be of interest to determine to which physical or chemical characteristic of composite resin component release is most closely related, and further study could clarify this issue. The current data and the limited number of composite resins examined, do not clearly support any one characteristic, but rather strongly suggest that component release is itself a

complex mechanism in composite resins. The data suggests that *release* was not proportional to TEGDMA content in the uncured composite resins themselves, which would have been an expected direct determinant. This was particularly illustrated in the lack of similarity in the data for Foundation and Herculite XR, which contain similar amounts of TEGDMA. Occlusin, however, with the highest content of TEGDMA, showed the highest amount of TEGDMA diffused through dentine *in vitro* by 3 days for all the composite resins examined. However, from Table 7.2, when release and diffusion are expressed as a ratio, this ratio is directly proportional to TEGDMA content of the uncured composite resins.

Apart from inherent characteristics of composite resin which directly determine TEGDMA release, it is also likely that any factors which affect the degree of conversion of the resin are likely to affect residual unsaturation and the availability of unconverted methacrylate groups. Such factors may include optimal light-activation wavelength and time, light transmittance qualities of the composite resin, and basic resin chemistry. For example, although all the composite resins tested contained TEGDMA, Conquest and Occlusin contained UDMA, whereas the other resins are Bis-GMA-based. The relative rigidity of UDMA-containing composite resins may contribute to the ease with which residual diluent monomer diffuses out of the cured material. The identification of the characteristics which most closely determine TEGDMA release could assist in the development of composite resins which would show reduced release. Also of interest is the importance that TEGDMA release may have on the physical properties of the composite resin in the long term, for example, susceptibility to hydrolytic degradation, decrease in physical strength, increased susceptibility to wear or breakdown of intrinsic structure.

The second observation stems from the marked difference between the composite resins when the ratios of the amount of TEGDMA which had cumulatively *diffused* through dentine by 3 days to the amount of TEGDMA which had been *released* by 3 days, were compared. These ratios were presented in Table 7.2. If the difference between the composite resins was only a chemical "phenomenon" and composite resin interaction with dentine was similar amongst the composite resins, it would have been expected that the ratios would be similar although absolute values of diffused and released TEGDMA would be expected to be proportional to TEGDMA content of the uncured materials. The finding that the ratios were

significantly different suggested some other factor (or factors) was operating. The nature of this factor (or factors) is unknown. The ratios appeared to more closely relate to the mean particle size in the composite resins than to any other characteristic in the table. That is, for the composite resins examined, those materials containing particles of a larger mean size tend to be associated with greater diffusion of TEGDMA through dentine and composite resins with smaller particle size tend to have very much smaller amounts of TEGDMA diffusing relative to the amounts released. This may relate to the surface area of composite resin exposed to the fluid at the cavity floor which may, in turn, relate to the ability of composite resin materials to adapt to dentine. Both filler particle size and filler loading can influence viscosity, a characteristic which has been found to influence the adaptation of composite resin to acid etched enamel (Low *et al.* 1978). A similar phenomenon may be responsible for the substantial differences observed for diffusion:release ratios. It has also been suggested (Söderholm, 1990) that manufacturer's silane treatment of filler may influence the propensity of the resin to suffer aqueous reactions which in turn may influence monomer release.

The second observation may also indicate that the interaction of specific composite resins with etched dentine may be quite variable and, perhaps, may be unique to the composite resin. Resin adheres poorly to etched dentine (Rider *et al.*, 1977; Bowen *et al.*, 1982; Bowen and Cobb, 1983), perhaps because dentine is strongly hydrophilic (Qvist and Qvist, 1985; Braem *et al.*, 1986; Pashley and Pashley, 1991; Prati *et al.*, 1991), and this may reduce the penetration of dentine by the composite resin. This would cause the development of smaller or shorter resin tags. Differences in physical characteristics of the composite resins, such as viscosity and wetting, or differences in adhesive potential, such as adsorption, may also contribute to reduced tag formation. Adsorption is dependent on an appropriate interaction with water and may differ for different composite resins because the intrinsic hydrophobic nature of composite resins may differ. This reduced resin tag length for the different composite resins would reduce the diffusional surface area. Thus, the interaction at the dentine surface may also be an important factor in TEGDMA diffusion for the different composite resins.

The release and diffusion through dentine of TEGDMA was seen for a variety of composite resins, confirming the patterns seen for Foundation. A very

common clinical procedure, in the use of composite resin, is the application of components of a bonding agent system to the etched dentine prior to composite resin placement. It remains to question whether these systems are able to prevent TEGDMA diffusion by perhaps reducing dentine permeability or influencing TEDGMA release from the composite resins themselves. This is considered in the next Chapter.

CHAPTER 8

Diffusion of Monomers from two Dentine Bonding / Composite Resin Systems through Dentine *in vitro*

8.1 Introduction

8.2 Materials and methods

8.2.1 Experimental method

8.2.1.1 Tooth model

8.2.1.2 Materials

8.2.1.2.1 Dentine bonding systems

8.2.1.2.2 Composite resins

8.2.1.3 Procedure

8.2.1.3.1 Composite resin alone

8.2.1.3.2 Composite resin and bonding agent

8.2.1.3.3 Composite resin and bonding system

8.2.1.3.4 Comparison between component release and component diffusion through dentine *in vitro* for two bonding agent/composite resin combinations

8.2.2 Sample analysis

8.3 Results

8.3.1 Component identification

8.3.2 TEGDMA and HEMA quantification

8.3.2.1 Cumulative diffusion of TEGDMA through dentine *in vitro* for two bonding agent/composite resin combinations

8.3.2.2 Cumulative diffusion of HEMA through dentine *in vitro* for two bonding agent/composite resin combinations

8.3.2.3 Diffusion rate of TEGDMA through dentine *in vitro* from two bonding agent/composite resin combinations

8.3.2.4 Diffusion rate of HEMA through dentine *in vitro* from two bonding agent/composite resin combinations

8.3.3 Comparison between component release from a bonding agent and component diffusion through dentine *in vitro*, from a bonding agent

8.3.3.1 Comparison between cumulative amount of HEMA released from a bonding agent with cumulative amount of HEMA diffused through dentine *in vitro* from a bonding agent

8.3.3.2 Comparison between rate of HEMA released from a bonding agent with rate of HEMA diffused through dentine *in vitro* from a bonding agent

8.4 Discussion

8.1 Introduction

Common clinical procedures used to enhance adaptation of composite resin to dentine and to exclude microleakage include the use of low viscosity bonding agent systems which are applied to treated dentine before restoration of the cavity with composite resin. These agents are designed to penetrate into tubules of etched dentine (Qvist *et al.*, 1977). Components of bonding agents may also penetrate intertubular dentine forming an interdiffusion or hybrid zone (Asmussen *et al.*, 1991). This zone is relatively ill-defined but its presence is associated with improved bond strengths (Erickson, 1989b; Pashley *et al.*, 1993), the dissipation of interface stress (Van Meerbeek *et al.*, 1992b; Krejci *et al.*, 1993; Tam and Pilliar, 1993) and the reduction of interface contraction gaps (Harashima and Hirasawa, 1989). Little has been determined about the stability of this zone over time and therefore it is worthwhile to examine the potential for components of bonding agents to diffuse through dentine. If this occurs, the data may indicate the contributions of bonding agents to the pulpal toxicity of bond-composite combinations (Qvist, 1980; Qvist and Stoltze, 1982; Qvist *et al.*, 1989) and may also give insight into the nature of resin-dentine interactions.

In the work to be described below, bonding agents used in combination with composite resins were examined in the tooth model described in Chapter 5, in order to examine the potential for this combination of resin materials to influence component diffusion through dentine *in vitro*.

8.2 Materials and methods

8.2.1 Experimental method

8.2.1.1 Tooth model

Freshly extracted unerupted human third molar teeth were collected from consenting donors aged between 18 and 24 years. Each tooth was stored in distilled water at 4°C for up to one week then prepared as previously described in Chapter 5. In brief, the root system was removed from each tooth and discarded. A circular occlusal cavity 6 mm in diameter was prepared with a tungsten carbide bur at high speed with water spray, leaving a remaining dentinal thickness to the roof of the pulpal chamber within the range of 1.6-2.0 mm. A 1 ml-capacity polyethylene chamber filled with distilled water, simulating the pulp chamber, was attached to each tooth at the cemento-enamel junction.

8.2.1.2 Materials

8.2.1.2.1 Dentine bonding systems

Two dentine bonding systems were used, Scotchbond Multi-purpose (3M Dental Products, St. Paul MN, USA) and Visar Seal (Den-Mat Corporation, Santa Maria CA, USA). Visar Seal is the bonding agent in the Tenure/Perfection system (Den-Mat Corporation, Santa Maria CA, USA).

8.2.1.2.2 Composite resins

Two composite resins were used, Occlusin (GC, Tokyo, Japan) and Z100 (3M Dental Products, St. Paul MN, USA). The components of these materials are shown in Table 8.1. The acronyms used to identify the materials are shown in brackets.

Table 8.1 Components of resin materials and bonding systems used to examine the diffusion of components through dentine *in vitro*

Bonding system	Element	Components
Scotchbond Multi-purpose ¹ (SMP)	etchant	10% maleic acid, polyvinyl alcohol, dye, water
	primer	HEMA, methacrylate functional polycarboxylic acid, water
	bonding agent	Bis-GMA, HEMA, photoactivators
Z100 ¹ (Z100)		44% Bis-GMA, 52% TEGDMA (resin)
Visar Seal ² (VS)	bonding agent	Bis-GMA, TEGDMA (resin)
Occlusin ³ (O)		UDMA mixture, 55% TEGDMA (resin)

1 Manufacturer data

2 Information from Erickson (1992)

3 Information from Ruyter and Øysaød (1987)

8.2.1.3 Procedure

Sixty-seven human third molar teeth, featuring prepared occlusal cavities as described in Chapter 5 and with polyethylene "pulpal chambers" attached, were divided into four groups to receive either one of the two composite resins alone or a dentine bonding system-composite resin combination. These combinations were Scotchbond Multi-purpose with Z100, which will here be listed as Z100 + SMP in graphs and Visar Seal with Occlusin, which will be termed Occlusin + Visar Seal in graphs.

Following cavity preparation and placement of bonding agent and/or composite resin, teeth in all four groups were stored at 37°C until the 1 ml water sample was removed from the pulp chamber and replaced with fresh distilled water. Samples were removed at 4.32, 14.4, 43.2, 144 and 432 min and at 1, 3, 10, 30 and 100 days.

8.2.1.3.1 Composite resin alone

For teeth restored with Z100 (n=21) or Occlusin alone (n=17) and without bonding systems, the cut dentine was treated as described in Chapter 5, that is, etched with 37% phosphoric acid for 60 s and dried with air. The composite resin was then applied to the dried dentine floor to a thickness of 2 mm and cured for 30 s with a dental activating visible light (Optilux 400, model 401, Demetron Research Corporation, CN, USA). Pulpal chamber samples were then retrieved and replaced as described in section 5.2.1.

8.2.1.3.2 Composite resin and bonding agent

For those teeth to be restored with Visar Seal and Occlusin (n=8), the dentine was treated with 37% phosphoric acid for 60 s then rinsed and dried. Visar Seal was then applied to the dried dentine with a dental bristle brush, air thinned, cured for 30 s and immediately covered with an increment of Occlusin to a thickness of 2 mm. A diagram of the experimental procedure when Occlusin was used with Visar Seal is shown in Fig. 8.1.

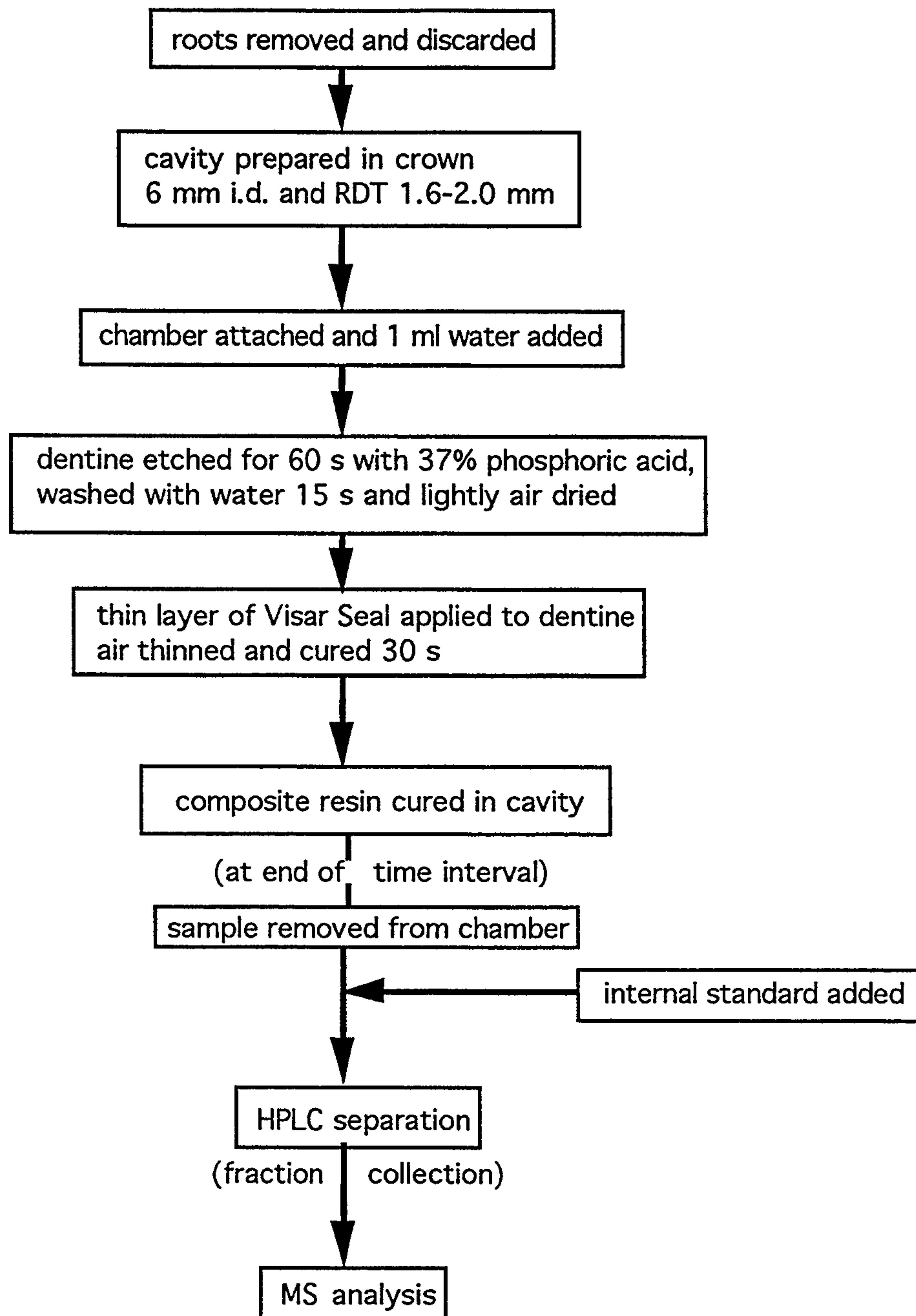


Figure 8.1 Diagram of the experimental procedure used in the tooth model experiments: the use of Visar Seal and Occlusin

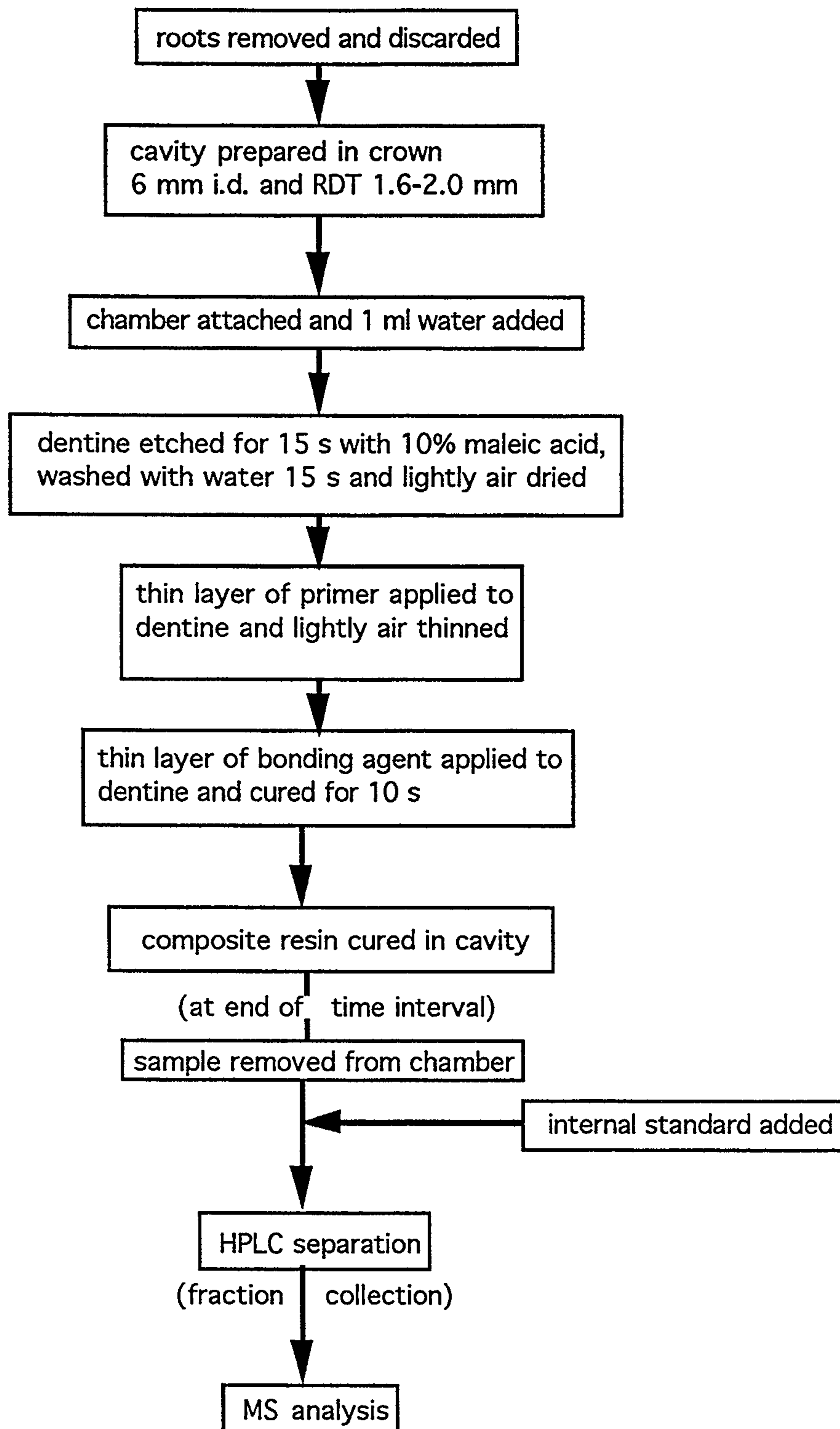


Figure 8.2 Diagram of the experimental procedure used in the tooth model experiments: the use of Scotchbond Multi-purpose and Z100

8.2.1.3.3 Composite resin and bonding system

For those teeth restored with Scotchbond Multi-purpose and Z100 (n=21), dentine pre-treatment, priming and bonding agent application were carried out as recommended by the manufacturer. Dentine pre-treatment included application of maleic acid to the dentine and enamel of the cavity for 15 s with a dental bristle brush. The surfaces were then washed with distilled water from a triple syringe for 15 s and air dried for 15 s. Primer was then applied to the etched surfaces in a thin layer with a dental bristle brush. The primer was then air dried lightly. Bonding agent was then immediately applied to the "primed" surface and gently air thinned. This layer was then light activated for 10 s. An increment of Z100 was then applied directly to the adhesive surface to a thickness of 2 mm, and light activated for 30 s. A diagram of the experimental method when Scotchbond Multi-Purpose was used with Z100 was shown in Fig. 8.2.

8.2.1.3.4 Comparison between component release from a bonding agent and component diffusion through dentine *in vitro*, from a bonding agent

Release of components from the bonding agent in the Scotchbond Multi-purpose kit was examined using the mold, conditions and procedures as described in section 3.2. In brief, the basic stainless steel mold (cylindrical, 2.0 mm depth and 6 mm internal diameter) was filled with the bonding agent, cured for 30 seconds and immersed in 4 ml of water. Sample removal and analysis was carried out as described in section 3.2.

8.2.2 Sample analysis

Analysis of water samples was carried out by HPLC and MS using a modular unit (Millipore Corporation, Waters Chromatography Division, Milford MA, USA) under conditions described in Chapter 2.

Comparisons between data at different time points for each group were made using one factor ANOVA-repeated measures and Scheffé statistical tests at $p < 0.05$ (StatViewII 512+ software, Abacus Concepts Inc, Berkeley, CA, USA).

8.3 Results

8.3.1 Component identification

TEGDMA was detected in all samples from all groups of teeth. In the group where Scotchbond Multi-Purpose was used, another component, HEMA, was also detected. Other resin components were well below the level of valid detection by HPLC. The detection limit for valid HPLC detection of HEMA was determined as less than 0.02 nmol and less than 0.014 nmol for TEGDMA. A chromatogram of the mixed standard of resin components was presented in Fig. 2.2. A typical chromatogram of a sample from the Visar Seal with Occlusin group taken at 1 day is presented in Fig. 8.3 and shows TEGDMA only; an internal standard peak is shown at approximately 7.0 min. A typical chromatogram of a sample from the Scotchbond Multi-Purpose with Z100 group, again at 1 day shows both TEGDMA and HEMA (Fig. 8.4).

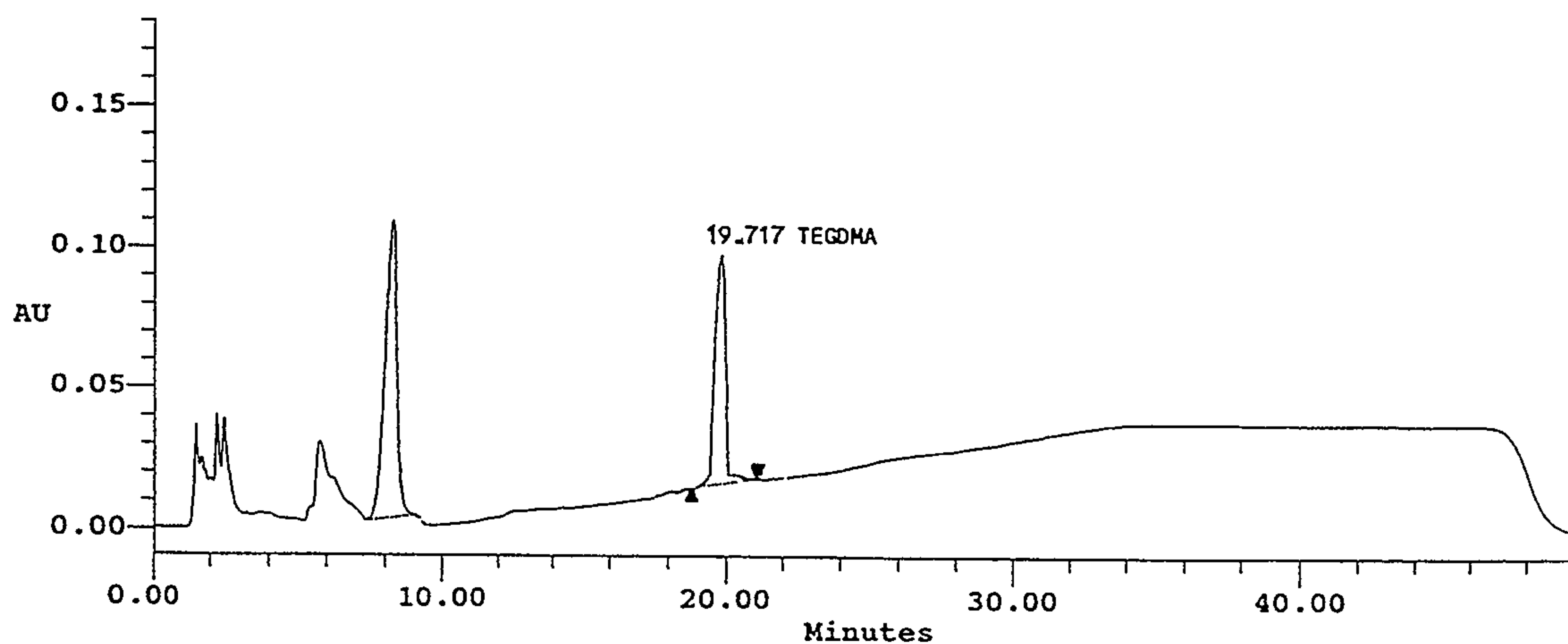


Figure 8.3 Chromatogram of a sample from the Visar Seal with Occlusin group (taken at 1 day)

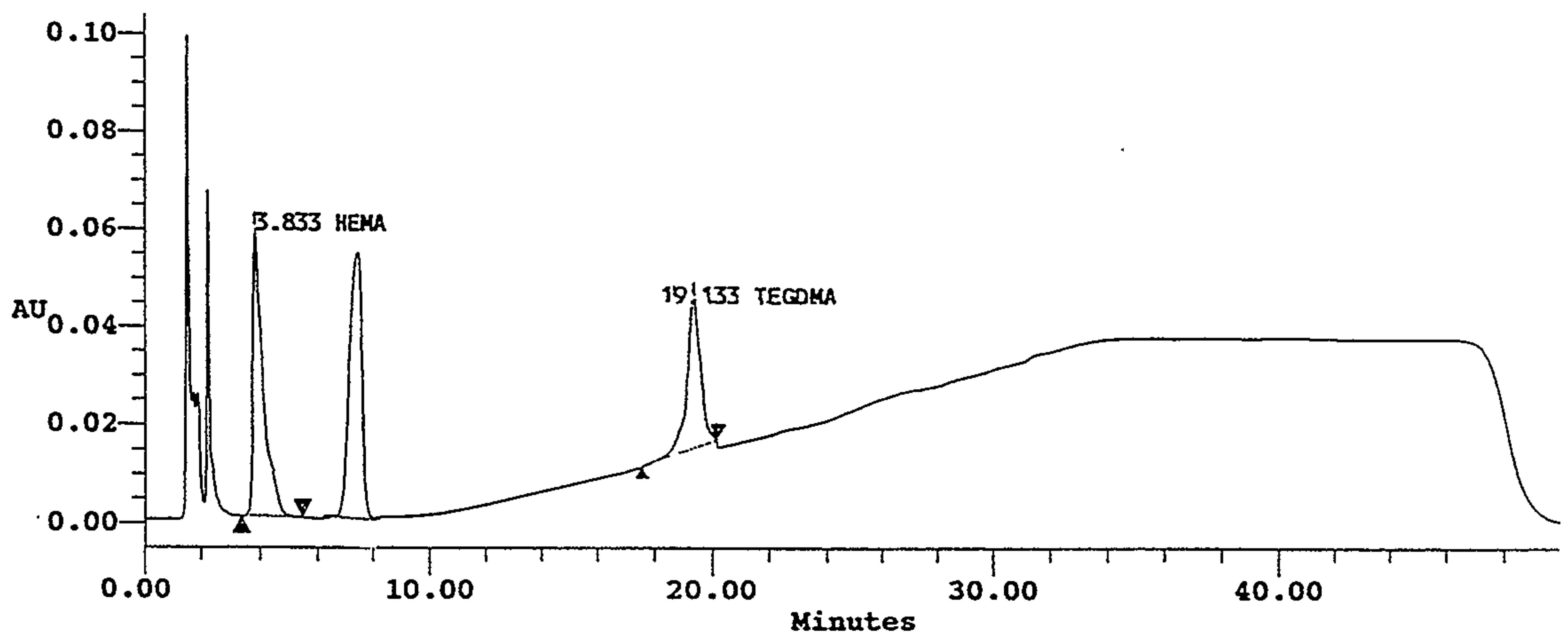


Figure 8.4 Chromatogram of a sample from the Scotchbond Multi-purpose with Z100 group (taken at 1 day)

8.3.2 TEGDMA and HEMA quantification

8.3.2.1 Cumulative diffusion of TEGDMA through dentine, *in vitro* for two bonding agent/composite resin combinations

A graph showing the cumulative amounts of TEGDMA released for the Occlusin alone and Visar Seal with Occlusin groups is presented in Fig. 8.5. Cumulative amounts for TEGDMA are shown for the Z100 alone group and Scotchbond Multi-purpose with Z100 groups in Fig. 8.6. Significantly higher amounts of TEGDMA had accumulated in the Visar Seal with Occlusin group than when Occlusin was used alone (at $p < 0.05$). There was no significant difference between the amounts of TEGDMA accumulated during the time course in the Z100 groups.

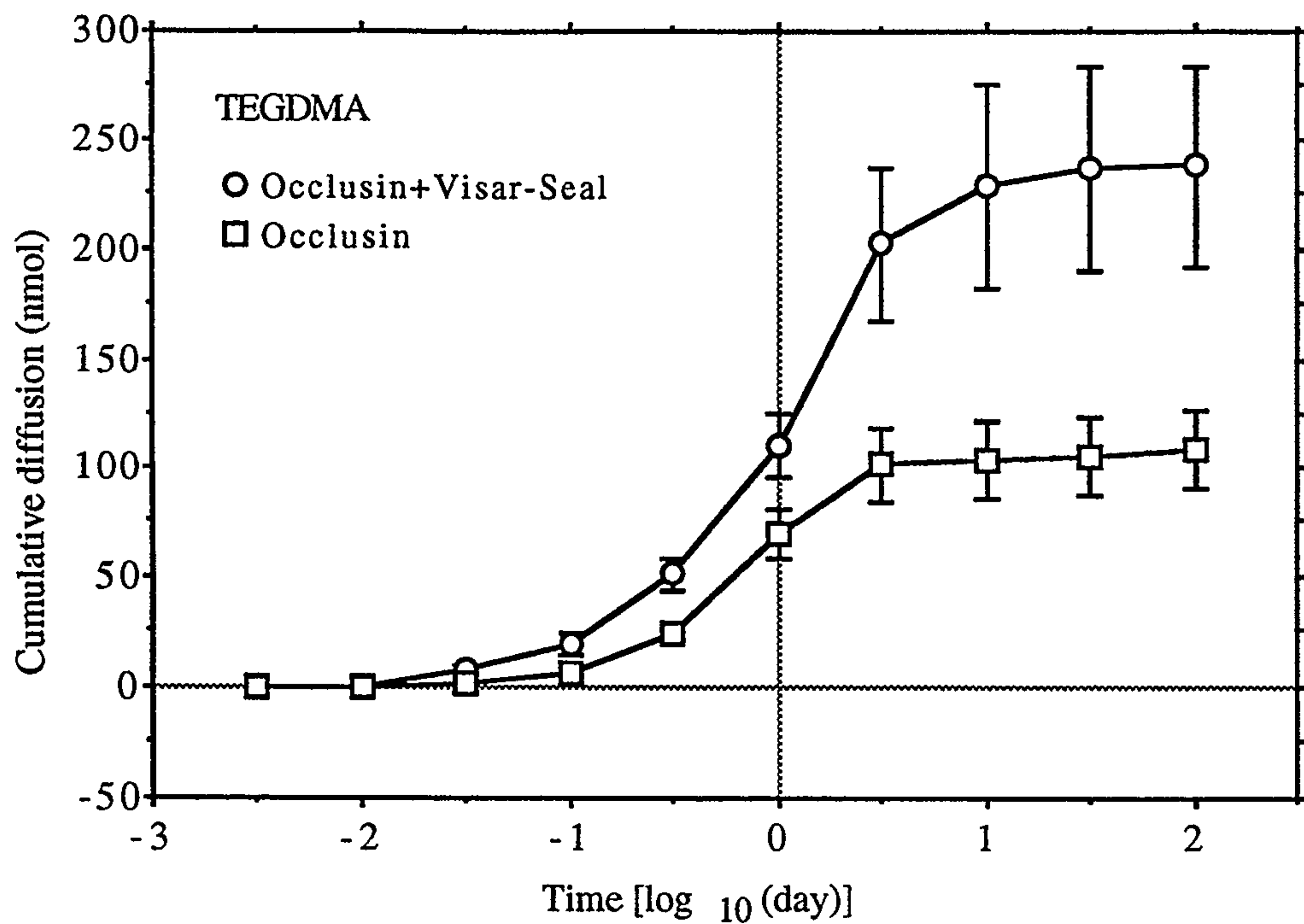


Figure 8.5 Comparison between cumulative diffusion, through dentine *in vitro*, of TEGDMA from Visar Seal and Occlusin with that from Occlusin alone

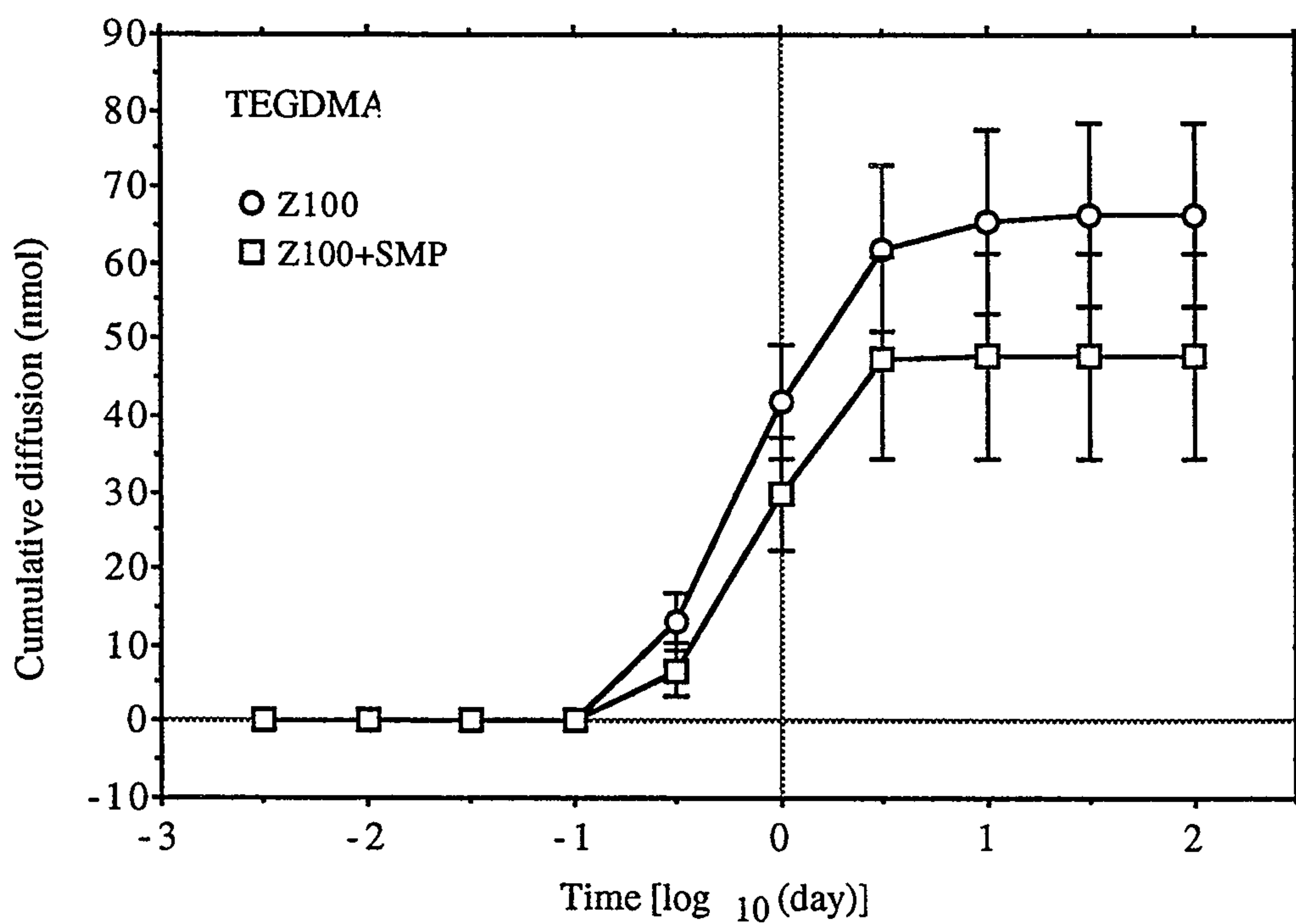


Figure 8.6 Comparison between cumulative diffusion, through dentine *in vitro*, of TEGDMA from Scotchbond Multi-purpose and Z100 with that from Z100 alone

8.3.2.2 Cumulative diffusion of TEGDMA and HEMA through dentine *in vitro* for a bonding agent/composite resin combinations

Fig. 8.7 shows the cumulative amounts for TEGDMA and HEMA in the Z100 with Scotchbond Multi-purpose group, and TEGDMA in the group where Z100 was used alone. HEMA diffusion occurred earlier than TEGDMA, from 4.32 min but, like TEGDMA, little more HEMA was detected after 3 days. No HEMA was detected in the Z100 alone group.

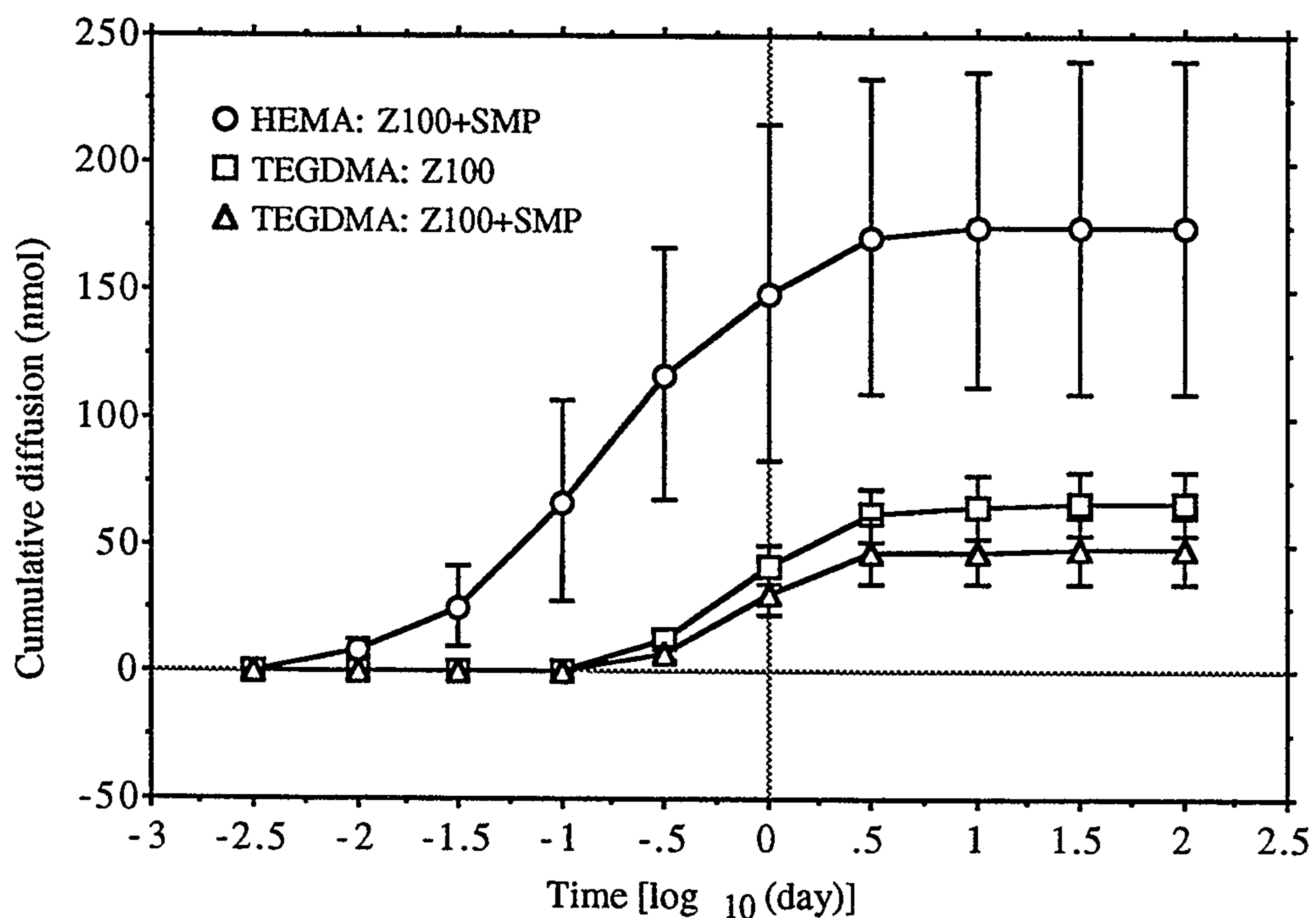


Figure 8.7 Comparison between cumulative diffusion of TEGDMA and HEMA, through dentine *in vitro*, for Scotchbond Multi-purpose and Z100

8.3.2.3 Diffusion rate of TEGDMA through dentine *in vitro* from two bonding agent/composite resin combinations

Fig. 8.8 and 8.9 show the diffusion rates for TEGDMA in the Occlusin and Z100 groups respectively. For all groups, greatest increase in rate occurred up to 43.2 min and rates diminished after 3 days. In the Occlusin groups, TEGDMA diffusion rates between 43.2 min and 1 day were similar, with rates diminishing thereafter. In comparing the Z100 groups, less variation was seen between the rates over the time course than was seen in the Occlusin groups.

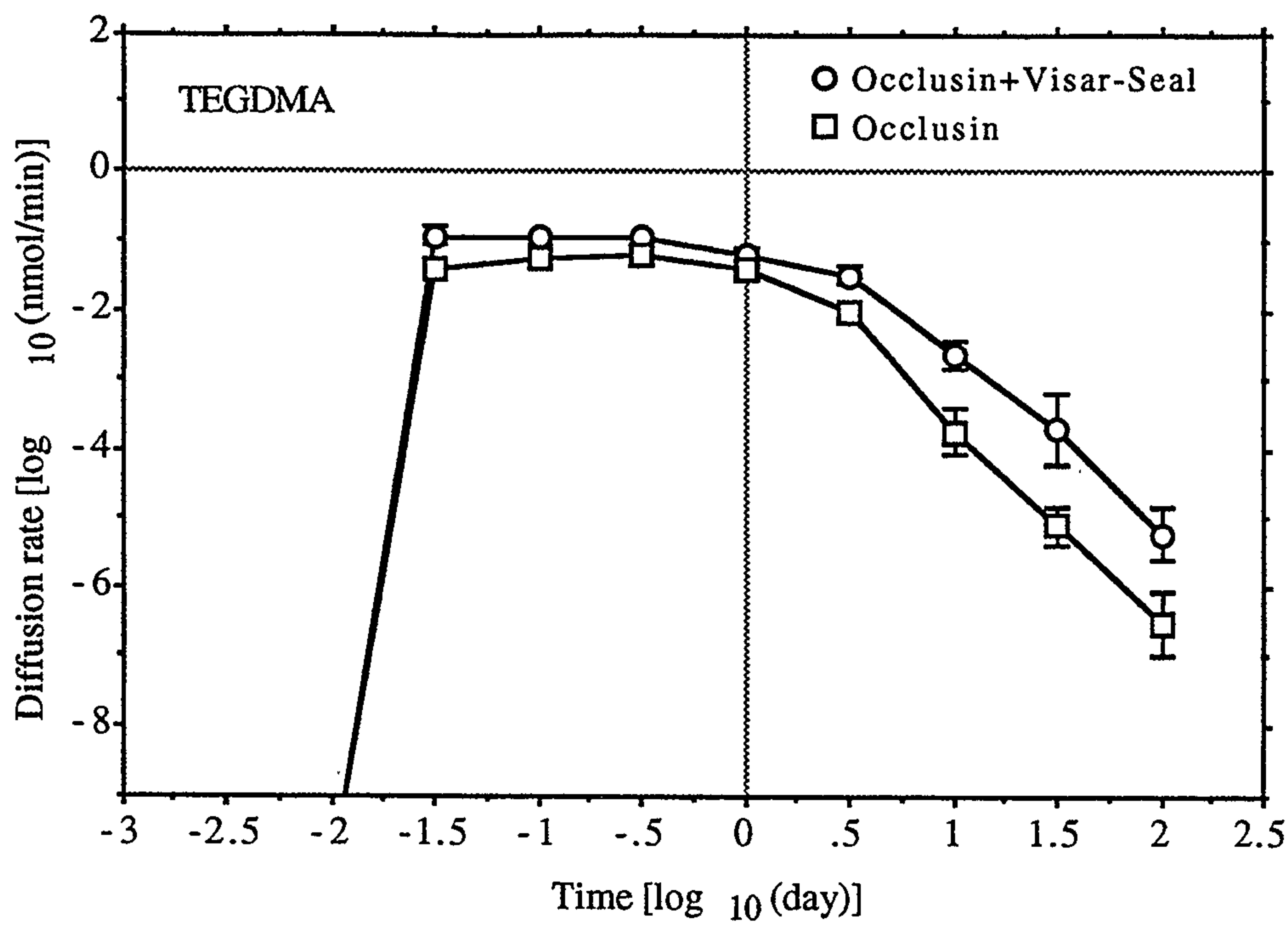


Figure 8.8 Diffusion rates of TEGDMA, through dentine *in vitro*, for Visar Seal and Occlusin

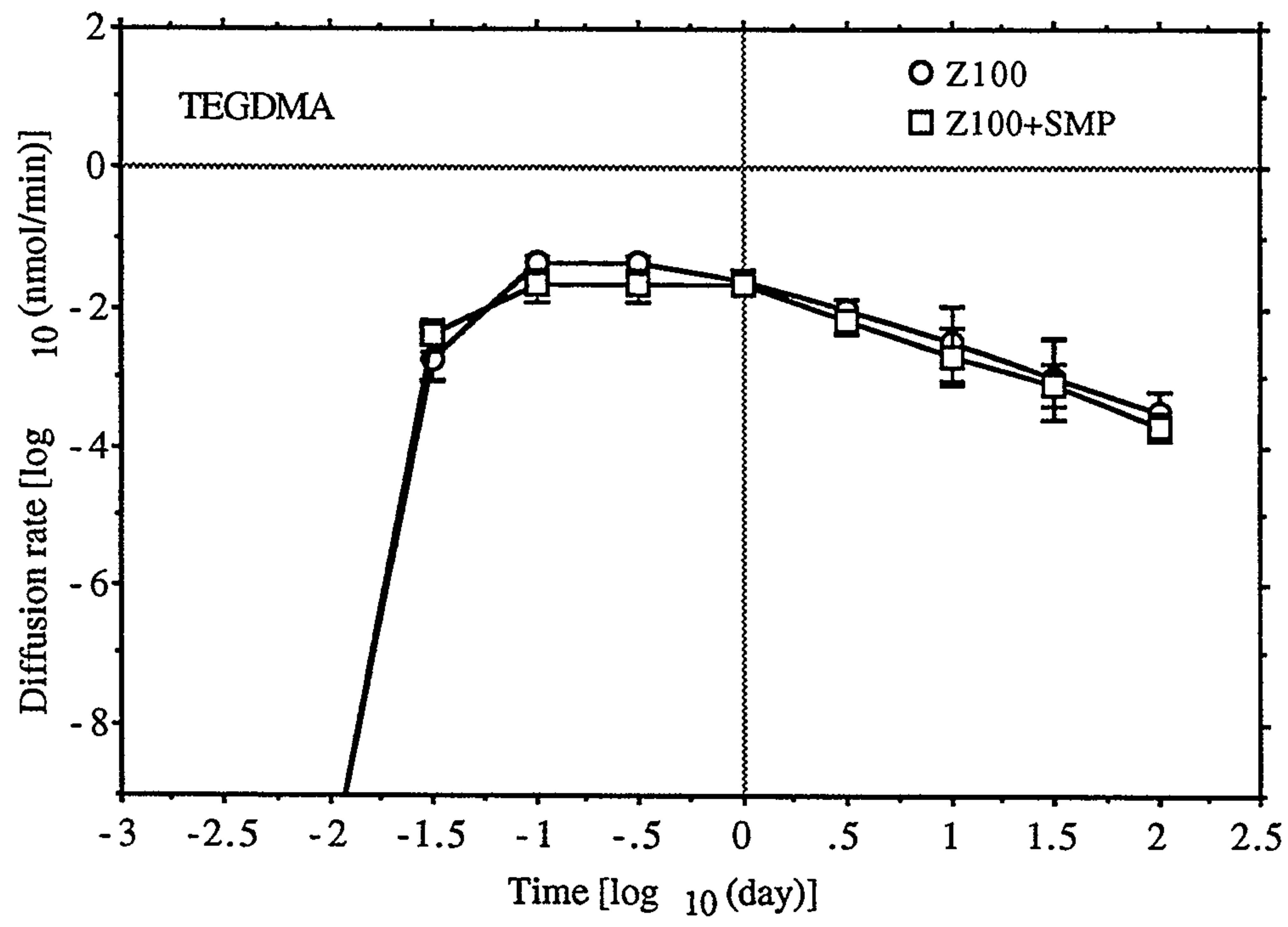


Figure 8.9 Diffusion rates of TEGDMA, through dentine *in vitro*, for Scotchbond Multi-purpose and Z100

8.3.2.4 Diffusion rates of HEMA and TEGDMA through dentine *in vitro* from a bonding agent/composite resin combinations

Fig. 8.10 shows the diffusion rates for TEGDMA and HEMA in the Z100 and Scotchbond Multi-purpose group. Diffusion rates for HEMA up to 1 day were significantly different from those of TEGDMA (at $p < 0.05$). HEMA rates were higher and occurred earlier than TEGDMA.

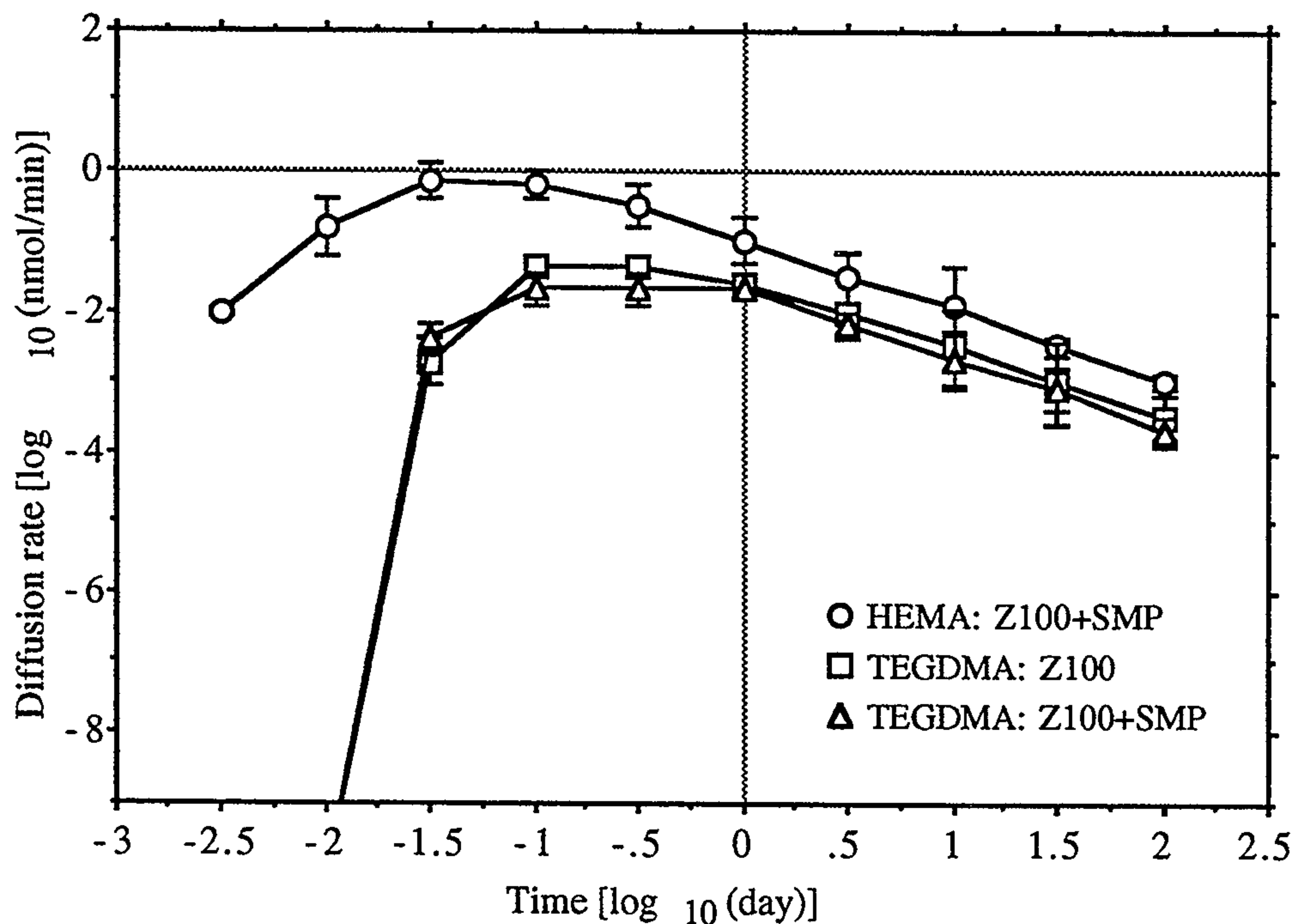


Figure 8.10 Comparison between the diffusion rates of TEGDMA and HEMA, through dentine *in vitro*, for Scotchbond Multi-purpose and Z100

Data for highest rates of diffusion and for cumulative release at 3 days for the four groups of teeth are shown in Tables 8.2 and 8.3 respectively. Means and standard deviations about the means are shown. The vertical bars unite means which are not significantly different, for the same time interval (at $p < 0.05$). Highest diffusion rates were attained earlier for the monomers when bonding agents were used.

Table 8.2 Highest diffusion rates of TEGDMA and HEMA and time intervals attained, for the combinations of bonding agent systems and composite resins examined

Bonding agent/ composite resin	HEMA (nmol/min)	Time interval	TEGDMA	Time interval (nmol/min)
Occlusin with Visar Seal	-	n/a	0.27 ± 0.06	14.4 - 43.2 min
Occlusin	-	n/a	0.06 ± 0.01	144 - 432 min
Z100	-	n/a	0.04 ± 0.01	144 - 432 min
Z100 with Scotchbond Multi-purpose	0.89 ± 0.55	14.4 to 43.2min	0.02 ± 0.01	144 - 432 min

Table 8.3 Cumulative amounts of TEGDMA and HEMA diffused through dentine, by 3 days, from the combinations of bonding agent systems and composite resins examined

Bonding agent/ composite resin	HEMA (nmol)	TEGDMA (nmol)
Occlusin with Visar Seal	-	202.31 ± 35.62
Occlusin	-	102.52 ± 17.43
Z100	-	63.14 ± 12.40
Z100 with Scotchbond Multi-purpose	165.2 ± 66.6	47.13 ± 11.82

A significantly higher maximum rate of release and a significantly higher cumulative release of TEGDMA at 3 days was seen in the Visar Seal with Occlusin group than occurred with Occlusin alone (at $p < 0.05$). SMP with Z100 resulted in a relatively high release of HEMA and a delayed and slightly reduced release of TEGDMA than occurred with Z100 alone.

Fig. 8.11 presents a comparison of the cumulative diffusion of TEGDMA for all the groups examined. Fig. 8.12 presents a comparison of all the TEGDMA diffusion rates for the groups.

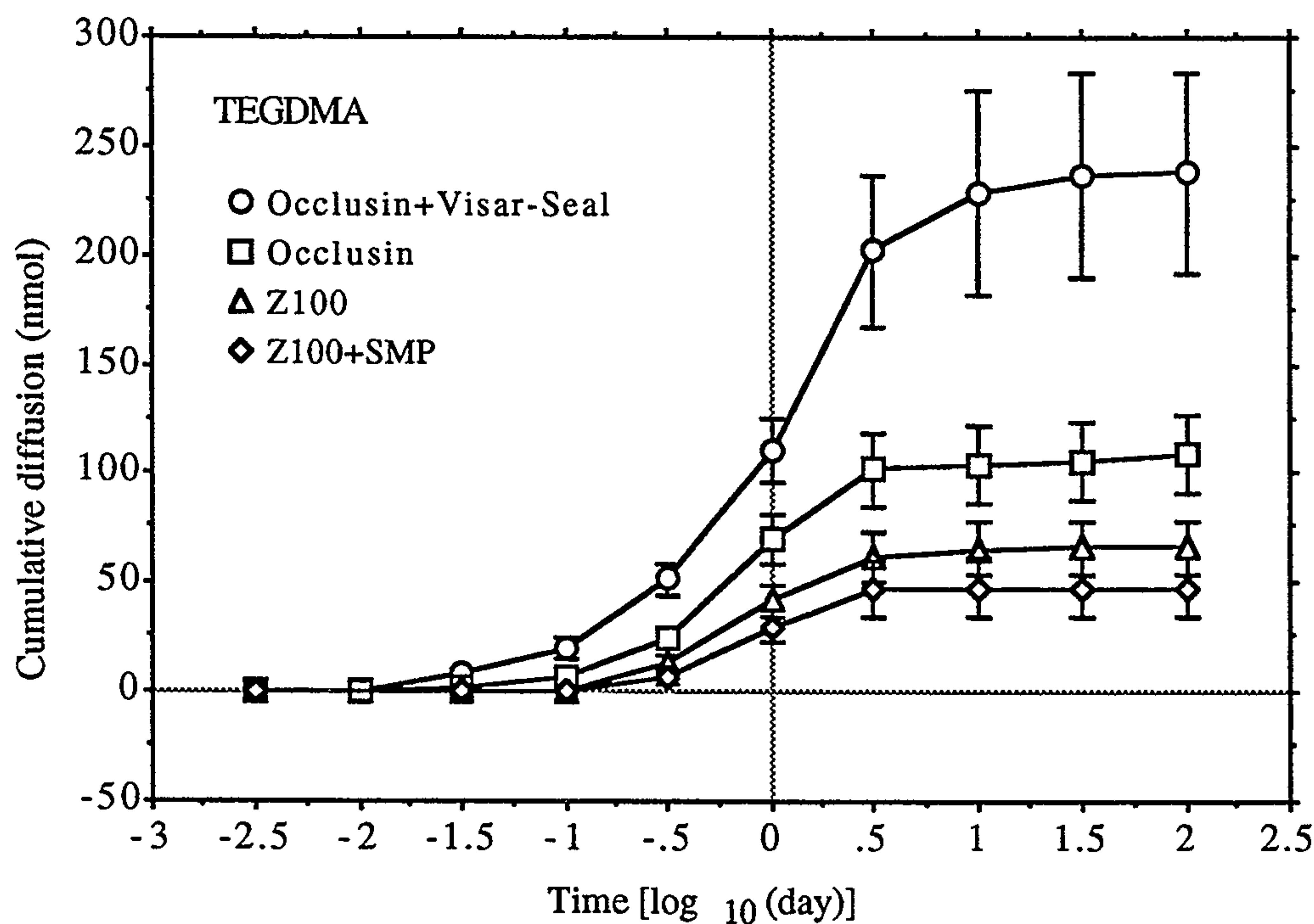


Figure 8.11 Comparison between cumulative diffusion of TEGDMA through dentine *in vitro*, for the combinations of bonding agent systems and composite resin examined

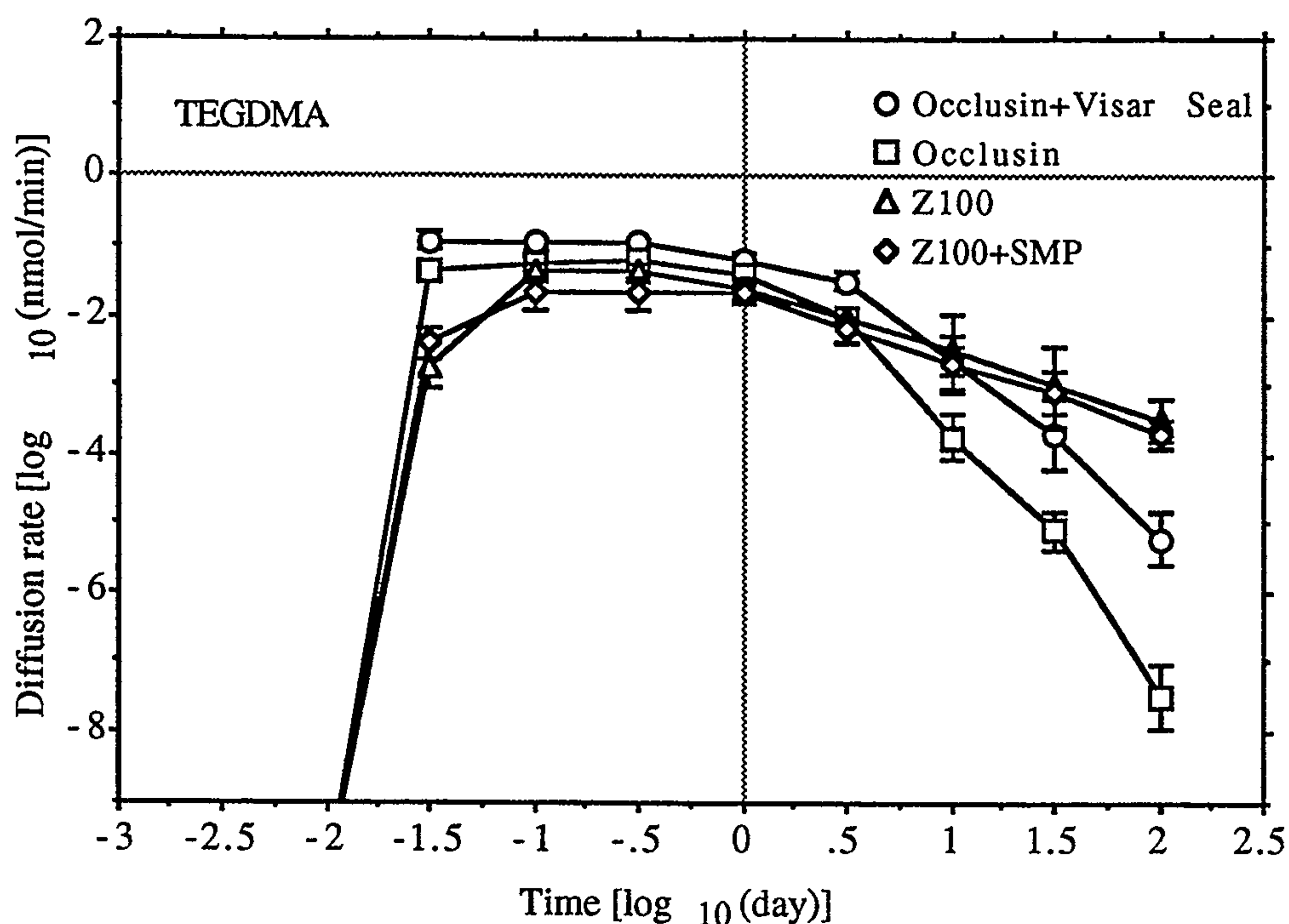


Figure 8.12 Comparison between TEGDMA diffusion rates, through dentine *in vitro*, for the bonding agent systems and composite resin examined

8.3.3 Comparison between release of HEMA and diffusion of HEMA through dentine *in vitro* from a bonding agent

8.3.3.1 Comparison between cumulative amount of HEMA released from a bonding agent with cumulative amount of HEMA diffused through dentine *in vitro* from a bonding agent

In order to examine the pattern of HEMA *release* from the bonding agent used in the Scotchbond Multi-purpose system over the time course, an experiment was conducted as described in section 8.2.1.3.4. Cumulative HEMA release from the bonding agent in the SMP system is presented in Fig. 8.13. For the purposes of comparison (see Discussion, below) HEMA diffusion from teeth in the Scotchbond Multi-purpose with Z100 group is shown on the same graph although the amount of Scotchbond Multi-purpose bonding agent used in the release and diffusion experiments were very different.

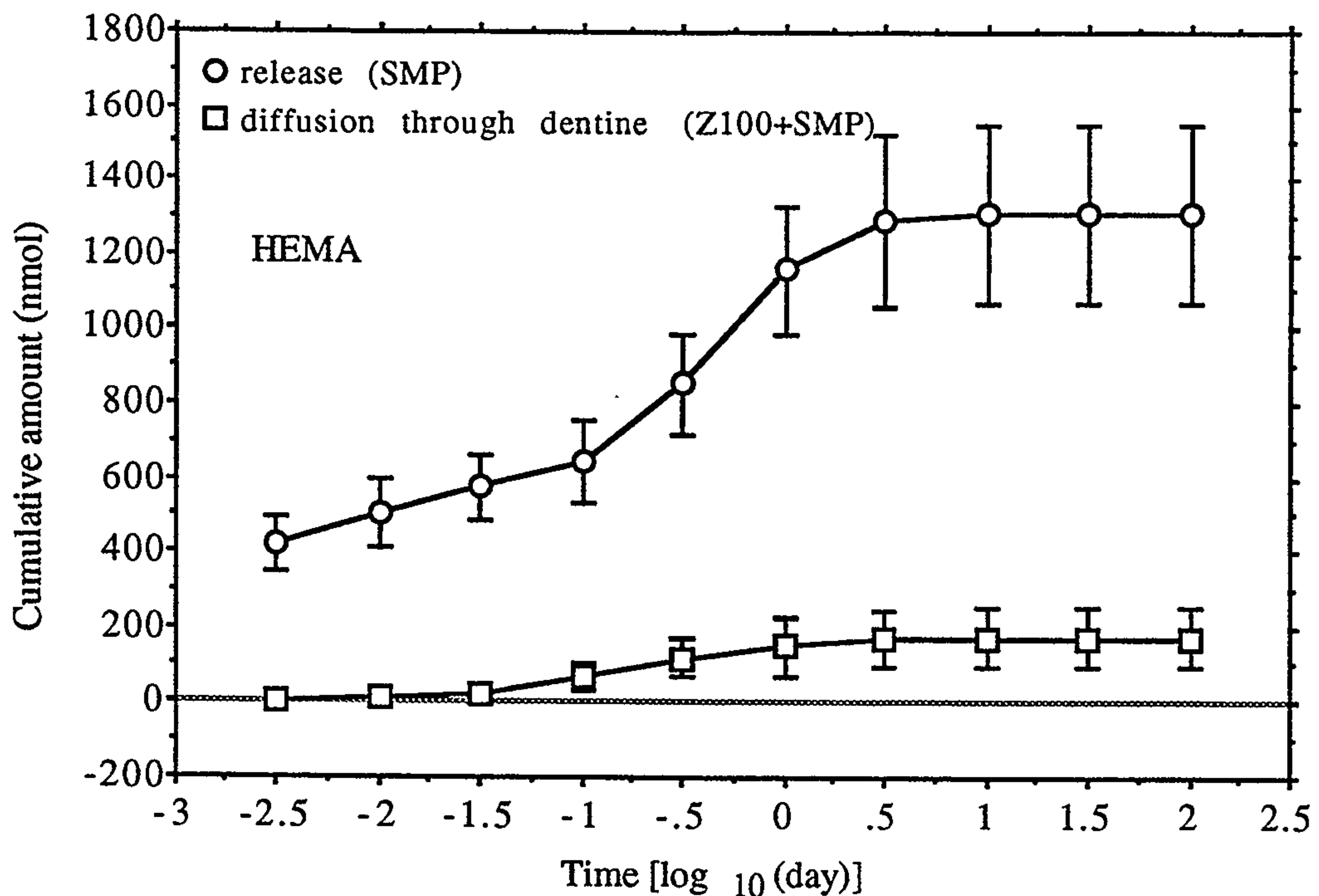


Figure 8.13 Comparison of cumulative *release* of HEMA from the bonding agent in the Scotchbond Multi-purpose system with cumulative HEMA *diffusion* through dentine *in vitro* for Scotchbond Multi-purpose with Z100

HEMA release was immediate and was essentially completed by 10 days. HEMA diffusion through dentine *in vitro*, was also immediate but significantly retarded by dentine.

8.3.3.2 Comparison between rate of release of HEMA from a bonding agent with diffusion rate of HEMA through dentine *in vitro* from a bonding agent

Fig. 8.14 presents a comparison of HEMA release rate with HEMA diffusion rate from the bonding agent in the Scotchbond Multi-purpose system. Again, data for HEMA diffusion from Scotchbond Multi-purpose with Z100 through teeth are shown for purposes of discussion to follow.

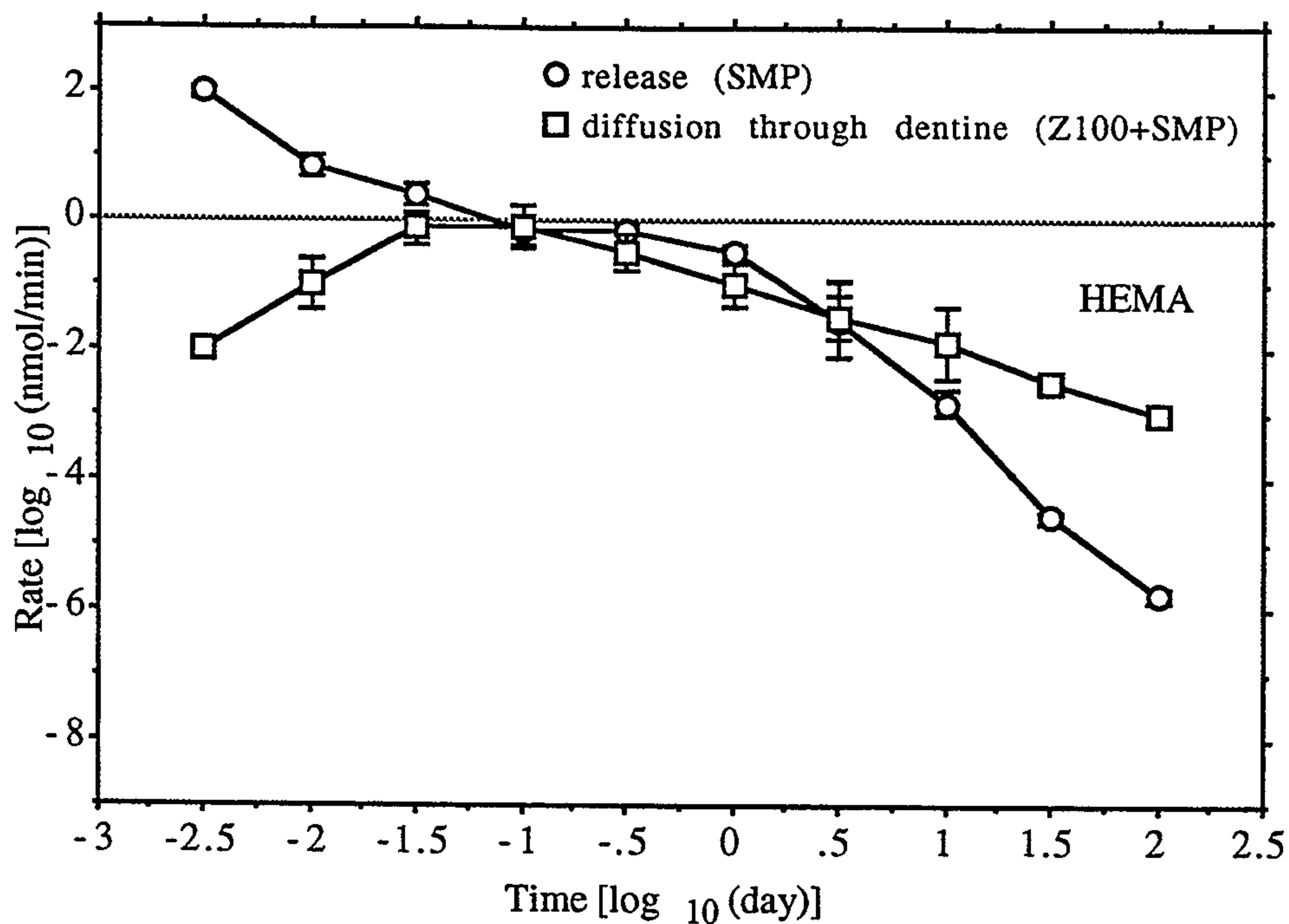


Figure 8.14 Comparison of HEMA release rate from Scotchbond Multi-purpose with HEMA diffusion rate through dentine from Scotchbond Multi-purpose with Z100

8.4 Discussion

The experiment demonstrated monomer diffusion from two bonding agent/composite resin combinations through dentine *in vitro*. The data show that both bonding agents examined make substantial contributions to the amount of monomer detected in the pulp space. The two combinations examined, Visar Seal with Occlusin and Scotchbond Multi-purpose with Z100, differed in several ways. First, the basic composition and physical presentation of the agents differed. Secondly, the dentine pre-treatments modified dentine and smear layer in different ways (see Chapter 6). Diffusion seen in both groups illustrated the influence of these differences on the resin-reinforced layer, that is, facilitation of monomer release. Varying amounts of diluent monomers TEGDMA and HEMA were able to diffuse through dentine *in vitro* and be detected in the pulp space. Bonding resins did not prevent monomer diffusion from the composite resin. These aspects will be discussed.

The two bonding systems used in the study differ both in composition and intended effects and represent two dentine bonding strategies. Visar Seal contains TEGDMA and Bis-GMA and is a low viscosity unfilled resin "liner".

It is an element of a typical second generation dentine bonding system designed to incorporate smear and to wet dentinal substrate (Erickson, 1989). Its potential as a bonding agent lies in its ability to act as an "intermediary" resin, simultaneously bonding to the composite resin and facilitating dentinal tubule penetration. The potential for facilitated dentinal bonding is thus an important use of this agent but clinical sensitivity has been reported after the use of Visar Seal directly onto etched dentine (Bertolotti, 1991). This bonding agent was included in the current study to examine its influence on component diffusion, exclusive of primers commonly used in more recent dentine bonding systems. Dentine pre-treatment with 37% phosphoric acid was used to allow comparison of these data with those presented in Chapter 5. When both bonding agent and composite resin contained TEGDMA, as in the cases of Occlusin and Visar Seal, diffusion of this monomer originating from *both* materials probably occurred since the amounts of TEGDMA observed to diffuse were higher than when the composite resin was used alone. The use of the Visar Seal in the Occlusin and Visar Seal group resulted in an earlier and higher maximum diffusion rate of TEGDMA which did not return to levels seen in the Occlusin-only groups until after 3 days. This shows that adverse effects to the pulp as a result of TEGDMA release would be more likely to occur when bonding resin and composite resin are used together in cavity restoration than when composite resin is used alone. These data are consistent with the clinical effects reported by Qvist and co-workers (1989) who demonstrated pulpal micro-abscesses, in the absence of bacterial microleakage, when the dentine of deep cavities in human teeth was acid-treated, a bonding agent applied and then cavities were restored with composite resin.

In contrast with Visar Seal which contains TEGDMA, the components of Scotchbond Multi-purpose, according to manufacturer's data (Table 8.1), include the monomer HEMA in both the primer solution and bonding agent. Dentine pre-treatment in the Scotchbond Multi-purpose system uses a 10% maleic acid solution (with polyvinyl alcohol, dye and water), which removes smear layer and increases flow across dentine *in vivo* (Pashley *et al.*, 1983) but does not remove peritubular dentine to the same extent as does phosphoric acid etch. This is followed by surface activation with primer and then the application of bonding resin. The adhesion promoter component (HEMA) of the primer solubilizes the smear layer, wets dentine and enables penetration of the bonding agent into dentinal tubules. When a HEMA-containing bonding resin system was used in combination with a

TEGDMA-containing composite resin (Scotchbond Multi-purpose with Z100) both monomers diffused through dentine. This observation has been recently demonstrated to occur for another bonding resin system/composite resin combination, All-Bond (Bisco, Downers Grove, IL, USA) with Z100 by Hamid and Hume (1995c), who found HEMA and TEGDMA similarly diffused through dentine, in the same tooth model.

In the current work, the highest diffusion rate of HEMA occurred earlier than that of TEGDMA, which may reflect the order of application of the materials to the dentine. It cannot be determined from the present data, however, whether the HEMA detected in the pulp space was derived from the primer or from the bonding agent. Significantly, the data for these groups show that TEGDMA diffusion from Z100 was not prevented by the presence of the Scotchbond Multi-purpose primer and bonding resin. Further, cumulative amounts of diffused TEGDMA detected in the two Z100 groups (with and without Scotchbond Multi-purpose) was not significantly different. This suggests that the resin reinforced layer of dentine (created with the use of the dentine bonding agent system) is permeable to the diffusion of TEGDMA from composite resin placed following the application of the bonding system on the cavity floor. The bonding resin did, however, delay the attainment of highest diffusion rate of TEGDMA and reduced the level of this highest rate compared to the data when Z100 was used alone. It can be suggested, then, that the resin reinforced layer may act as a longer term and subsequent source of TEGDMA and HEMA.

The two composite resins used in the study also differ in composition. Occlusin contains a mixture of oligomeric urethane dimethacrylates and TEGDMA, as diluent (Ruyter, 1988). Z100 is a Bis-GMA-based composite resin and also contains TEGDMA. It is relevant that from these four resin materials, only the diluents TEGDMA and HEMA could be detected in the pulp-space fluid. This indicates that other components of each of the systems were either not released into the aqueous phase or were released in such small amounts that they were below the level of sensitivity of the detection system.

The difference between the monomers, TEGDMA and HEMA, is also of interest. TEGDMA is a common component of both bonding agents and composite resin. It is found in amounts varying from 30-50% in bonding agents and 15-65% in composite resins (Ruyter and Sjøvik, 1981). HEMA is

used in some bonding resin systems, in both primer and bonding agent, in amounts from 30-55% in the primer and up to 55% in the bonding agent. The efficacy of monomer conversion in different formulations of composite resins is an important factor determining the proportion of unsaturation of the material (Ruyter and Svendsen, 1977). The different amounts of diffusing TEGDMA, seen for Occlusin and Z100, illustrates this difference. The observed ability of TEGDMA and HEMA to diffuse through dentine, originating from either the bonding agent or composite resin, points to the possibility of these monomers to be available to participate in copolymerization with bonding agents which probably occurs in the resin-reinforced layer. The interaction of fluid monomers with patent dentinal tubules, however, and the clinical consequences remain ill-defined.

HEMA is a unique component and it appears to have several functions in bonding systems. Its inclusion in bonding agents reduces viscosity and enhances bond strengths to dentine (Nakabayashi *et al.*, 1992) and several characteristics probably contribute to this facility. For example, HEMA is hydrophilic and readily penetrates smear layer where this monomer can impregnate dentinal collagen bundles; if smear layer is removed, HEMA can encapsulate hydroxyapatite crystals (Nakabayashi *et al.*, 1992). Van Meerbeek and co-workers (1992) also suggested that this monomer may enhance micromechanical and chemical bonding to the dentine as well as participating in copolymerization with the adhesive resin. Agents containing HEMA have been demonstrated to penetrate deeply into dentine tubules of etched dentine (Qvist *et al.*, 1977) and enhance dentine softening (Asmussen *et al.*, 1991). Further, Drenzo and co-workers (1993) suggested chemical reaction of the primer hydroxyl group with dentinal collagen functional groups may also occur. This interaction probably results in the formation of the resin-reinforced layer (Asmussen and Munksgaard, 1985; Eick *et al.*, 1993) although in the current work it was not conclusively established that this layer, as described *in vitro*, was actually formed. The detection of HEMA in the Z100 with Scotchbond Multi-purpose group suggests that this resin-reinforced layer exhibits potential to allow HEMA diffusion out of the layer in vital dentine. This points to the possible chemical instability of this layer of dentine (Drenzo *et al.*, 1993) or may demonstrate the innate permeability of the layer (Sano *et al.*, 1994). Deep dentine, close to the pulpal space, is most likely to allow monomer diffusion in view of the findings of Pashley and co-workers (1993b). An SEM evaluation of this layer, in teeth extracted after formation of the layer in

prepared cavities *in vivo*, showed that the layer was sometimes non-uniform with variable gap formation and featured variably sized resin tags (Walshaw and McComb, 1994). Larger areas of gap-free attachment and the formation of a resin-reinforced layer 5 - 8 μm thick were seen when the Scotchbond Multi-purpose system was used, whereas the group most similar to the Visar Seal group (that is, 35% phosphoric acid etch of dentine for 40 s, then Scotchbond 2 application) showed large areas of poor attachment and short resin tags.

It is of interest to compare the relative rates, over time, of HEMA release from the Scotchbond Multi-purpose in molds with the HEMA diffusion through dentine in the Scotchbond Multi-purpose with Z100 teeth (Figs. 8.13 and 8.14). Since the amount of Scotchbond Multi-purpose in the molds was substantially greater than that applied to dentine in the teeth, the cumulative amounts cannot be compared. However, what is of note is the later diffusion pattern of HEMA through dentine. The pattern indicates both substantial delay and an unexpected persistence of low HEMA diffusion, with additional HEMA reaching the pulp space even in the 30 - 100 day period. It might be suggested that this continued detection of HEMA may be due to the possible chemical instability of the resin-reinforced layer.

The difference between the dentine pre-treatments used in the two Z100 groups is also noteworthy in view of TEGDMA diffusion seen. In the Z100-alone group, phosphoric acid was used; in the Scotchbond Multi-purpose with Z100 group, maleic acid was used. Maleic acid is a weak acid and data already presented showed that, for Foundation, TEGDMA diffusion was significantly less when this pre-treatment was used (section 6.3.2). This was not seen here for Z100 where similar amounts of TEGDMA were seen in the two groups although pre-treatment procedures differed. The only procedure which differed here to that in section 6.3.2 was primer and bonding agent application. This suggests that primer and bonding agent application not only contributed diffusable monomer, HEMA, but perhaps facilitated the diffusion of TEGDMA from the Z100 composite resin. In support of this suggestion, Erickson (1989) noted that HEMA modifies dentinal permeability by improving dentine diffusivity and by facilitating penetration of polymerizable monomer into the microporosities of the dentinal collagen network. HEMA has also been associated with the formation of a "hybrid" layer in dentine which, however, exhibits acid-resistance (Nakabayshi and Takarada, 1992). This impregnated dentine, as

noted by Tam and Pilliar (1993), also exhibits poor fracture toughness, suggesting changed physical properties, which would also possibly contribute to the continued HEMA diffusion. It would be of interest to examine this aspect further by substituting other different bonding agent systems, not containing HEMA, in this basic experiment.

Van Meerbeek and co-workers (1991a) have suggested that the resin reinforced layer may "have a protective potential" by blocking "the normal passage of microorganisms and toxins." In contrast with this, the current data showed that when Z100 was used alone, TEGDMA was detected in the pulp space but when Scotchbond Multi-purpose was used prior to the Z100, both TEGDMA and HEMA was detected in the pulp space. This showed that the resin reinforced layer did not prevent monomer diffusion originating from the composite resin, but itself also contributed a diffusing monomer. With the continued rapid development of dentine bonding systems, including self-etching primers (Watanabe and Nakabayashi, 1993; Chigira *et al.*, 1994) and in view of the monomer diffusion reported, it is apparent that implications of the resin-reinforced layer require further characterization relative to its chemical and structural stability over time.

Whilst extrapolation to the *in vivo* situation can only be tentative, in the current context the tooth model was designed to simulate clinical constraints and it is likely that some aspects of laboratory procedures, such as control of conversion by consistent and optimal light activation, may have in fact been optimal in the model. Optimization of technique is not always clinically possible, so the diffusion seen *in vitro* may, at times, understate the potential *in vivo*. An important factor to consider, in this vein, is the effect of hydrostatic pulpal pressure on component diffusion through dentine. This will be considered in the next Chapter.

CHAPTER 9

Effect of Pressure on Diffusion of Components through Dentine *In Vitro*.

9.1 Introduction

9.2 Materials and methods

9.2.1 Experimental method

9.2.1.1 Pressure model

9.2.1.2 Materials

9.2.1.3 Procedure

9.2.2 Sample analysis

9.3 Results

9.3.1 Component identification

9.3.2 TEGDMA and HEMA quantification

9.3.2.1 Comparison of cumulative diffusion of TEGDMA and HEMA through dentine *in vitro*, from a bonding agent system/composite resin combination (Z100 with Scotchbond Multi-purpose) when subjected to hydrostatic pressure

9.3.2.2 Comparison of rates of diffusion of TEGDMA and HEMA through dentine *in vitro*, from a bonding agent system/composite resin combination (Z100 with Scotchbond Multi-purpose) when subjected to hydrostatic pressure

9.4 Discussion

9.1 Introduction

Much has been learned about diffusion of chemicals through dentine using *in vitro* models and the predominant factors which determine such diffusion have been described. These are solute concentration and molecular size, tubule density, diameter and length, and temperature. Pashley has gone on to demonstrate that dentine permeability in dog's teeth was unchanged after extraction. However, the effects of intrapulpal pressure on diffusion have yet to be directly examined in human teeth. The monomer, TEGDMA, is present as a diluent in most Bis-GMA-based composite resins (Ruyter and Sjøvik, 1981) and is present in some bonding resins. HEMA also functions as a diluent in both the primer and bonding resin of several commonly-used bonding resins (Van Meerbeek *et al*, 1992a). Both of these monomers have been shown to diffuse through etched dentine to the pulp space in an *in vitro* tooth model in the current work. When used in combination with composite resins, bonding resins were found to contribute to monomer passage to the pulp space but do not prevent movement of monomer from the composite resin to the pulp. In the experiment described here, the effect of positive hydrostatic pressure on the passage of resin monomers through dentine *in vitro*, from a bonding resin/composite resin combination, was examined. Hydrostatic pressures, equivalent to those found *in vivo* in normal and in inflamed pulps, were applied to the contents of the pulpal chamber of each tooth and maintained throughout sample removal and replacement. Two levels of positive pulpal pressure were incorporated in the model described in Chapter 5.

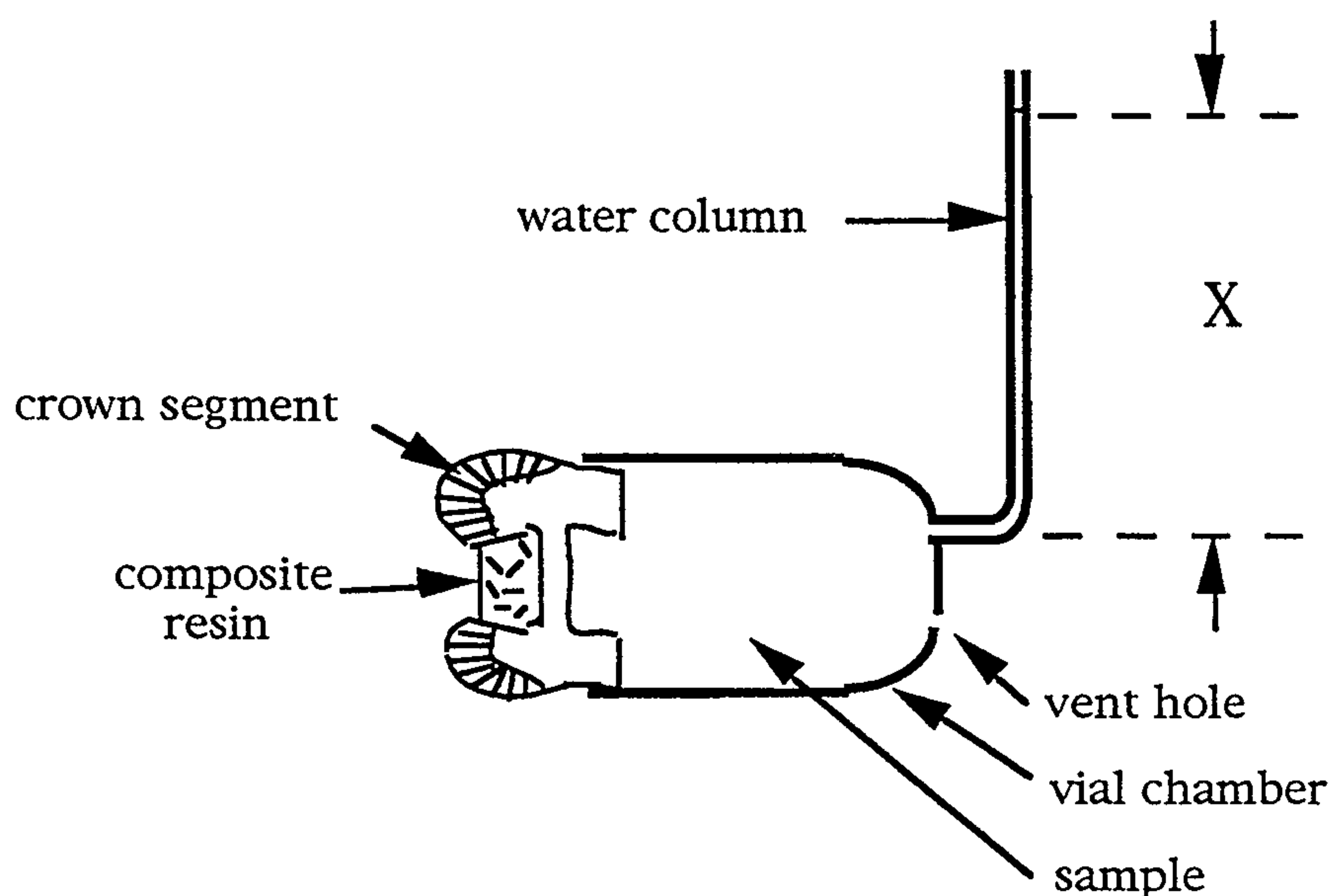
9.2 Materials and methods

9.2.1 Experimental method

9.2.1.1 Pressure model

Freshly extracted unerupted human third molar teeth were collected from consenting donors aged between 18 and 24 years and prepared as described in the basic protocol in Chapter 5. Briefly, the root system was removed from each tooth and discarded. A circular occlusal cavity 6mm in diameter was prepared with a tungsten carbide bur (Brasseler, Germany, 600 691) at high speed with water spray, leaving a remaining dentinal thickness to the roof of the pulpal chamber within the range of 1.6-2.0 mm. A 1 ml-capacity polypropylene chamber filled with distilled water, simulating the pulp chamber, was attached to each tooth at the cemento-enamel junction.

In one group of teeth, $n=15$, a length of flexible polyvinyl tubing of 1.5 mm internal diameter, which was fixed vertically to a support a column of water 15 cm in height (here known as **low pressure**, equivalent to approximately 1.5 kPa or 11 mm Hg), was attached to the pulpal chamber. This length of tubing itself had a volume capacity of 200 μl and communicated directly with the pulpal chamber so that the sample included both volumes to a total of 1 ml. In another group of teeth, $n=10$, the same tubing was used but at a height of 36 cm (here known as **high pressure** equivalent to approximately 3.5 kPa or 27 mm Hg), similarly filled with water and had an internal capacity of 400 μl . An illustration of the modified tooth model is shown in Fig. 9.1. Data from these groups were compared with that data presented in Chapter 7, where $n=15$, here known as **no pressure**.



Note: $X = 15\text{cm H}_2\text{O}$ in "low" pressure group, $X = 36\text{cm H}_2\text{O}$ in "high" pressure group

Figure 9.1 Diagram of the modified tooth model used to examine the diffusion of TEGDMA and HEMA through dentine *in vitro*, when subjected to hydrostatic pressure

9.2.1.2 Materials

All teeth received the same bonding resin/composite resin combination: Z100 with Scotchbond Multi-purpose (SMP) (both 3M Dental Products, St.Paul, MN, USA) as used previously. The components of Scotchbond Multi-purpose were presented in Table 8.1.

9.2.1.3 Procedure

Dentine structure was pre-treated in a method recommended by the manufacturer. Dentine was treated with 10% maleic acid for 10 s, rinsed and air dried then primer and bonding resin were applied, also as recommended by the manufacturer. A 2 mm thickness of Z100 composite resin was used to restore the cavity in each case. For each sample taken at each time interval, the 1 ml water sample, contained in the pulp chamber and in the tubing, was removed and replaced with fresh distilled water. Samples were removed at 4.32, 14.4, 43.2, 144 and 432 min and at 1, 3, 10 and 30 days.

9.2.2 Sample analysis

Analysis of water samples was carried out by reversed phase HPLC/MS as described in detail in Chapter 2. Resin components were used to prepare chromatographic standards and were all approximately 99% pure. Sample separation by reversed-phase HPLC used the conditions shown in Table 2.4. Sample fractions containing peaks of interest, which matched the retention times for the pure components, were collected from the HPLC and subjected to analysis by CIMS using conditions described in Chapter 2. Resin components found in the samples were then integrated and processed using Millenium 2010 Version 1.1 software (Millipore Corporation, Waters Chromatography Division, Milford, MA, USA) and the amounts of each component detected in the samples were calculated. Means plus or minus standard deviations about the means for cumulative amounts and rates of release were derived. Comparisons between data at different time points for each group were made using one factor ANOVA-repeated measures and Scheffé statistical tests at $p < 0.05$ (StatViewII 512⁺ software, Abacus Concepts Inc, Berkeley, CA, USA).

9.3 Results

9.3.1 Component identification

Both TEGDMA and HEMA were detected in samples from all groups. Other resin components were well below the level of detection by HPLC.

9.3.2 TEGDMA and HEMA quantification

9.3.2.1 Comparison of cumulative diffusion of TEGDMA and HEMA through dentine *in vitro*, from a bonding agent system/composite resin combination (Z100 with Scotchbond Multi-purpose) when subjected to hydrostatic pressure

For all the pressure groups, patterns of cumulative amounts of TEGDMA and HEMA diffusing through dentine *in vitro* were similar but significantly more HEMA was detected than TEGDMA (at $p < 0.05$). The amounts of TEGDMA arriving during the first time intervals were below HPLC detection limits for the component and so were plotted below these limits. Fig. 9.2 illustrates the cumulative amounts of TEGDMA found in the groups examined.

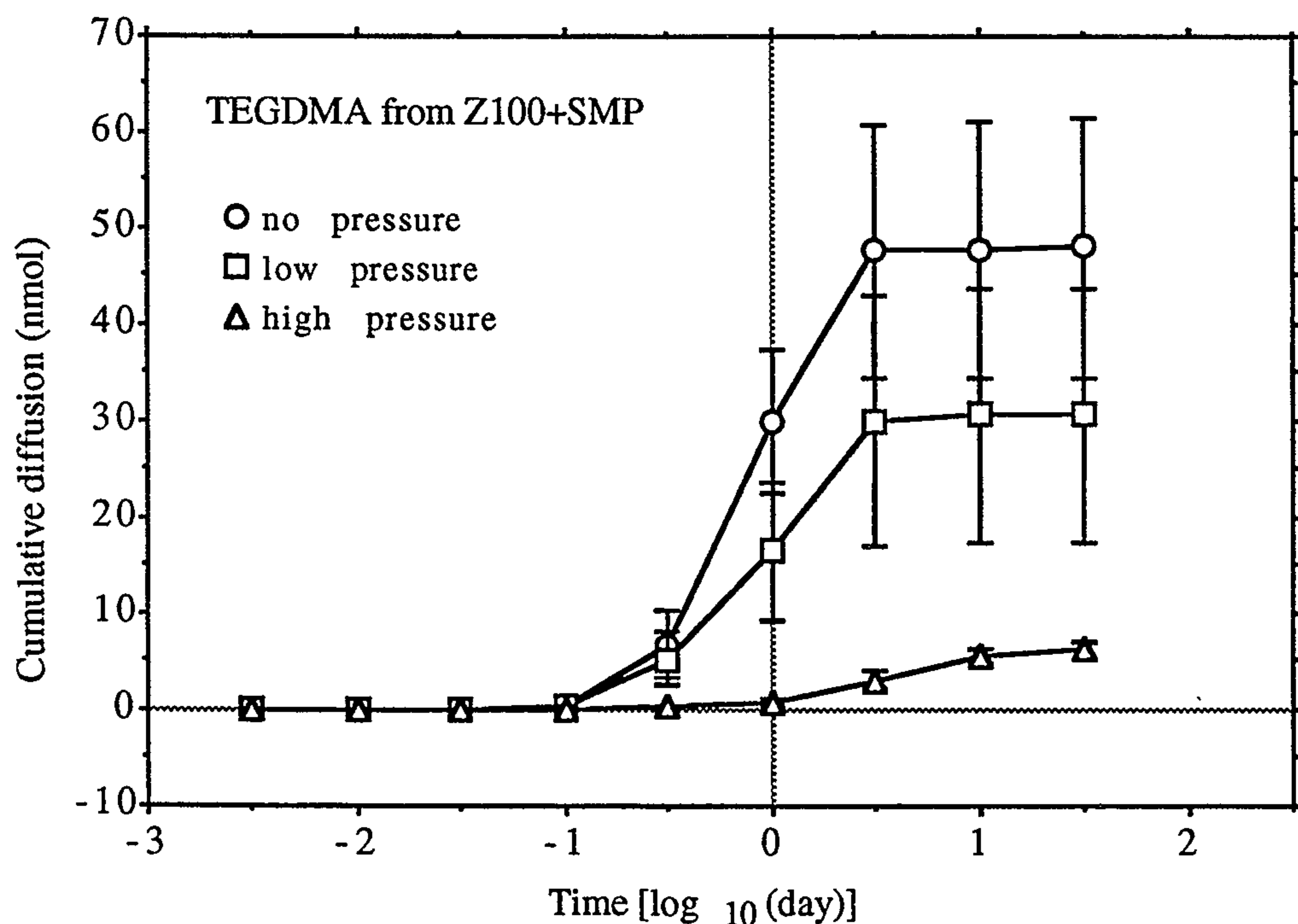


Figure 9.2 Cumulative diffusion of TEGDMA through dentine, *in vitro*, from Z100 and Scotchbond Multi-purpose when subjected to hydrostatic pressure

There were no differences noted between cumulative amounts of TEGDMA which had diffused in the groups for each of the time intervals up to 432 min (at $p < 0.05$). There were no differences noted between cumulative amounts of TEGDMA which had diffused in the no pressure and low pressure groups for the entire time course (at $p < 0.05$). However, after 432 min, significant differences were seen in the accumulated amounts of TEGDMA diffusing to the pulpal space, between the high pressure and both the no pressure and low pressure groups (at $p < 0.05$). After 3 days, little additional TEGDMA was detected in any group.

The cumulative diffusion of HEMA through dentine *in vitro* for the three groups is illustrated in Figure 9.3. HEMA was detected from the earliest time interval (0-4.32 minutes) and maximum diffusion rate occurred during this interval. Only very small amounts were detected after 3 days.

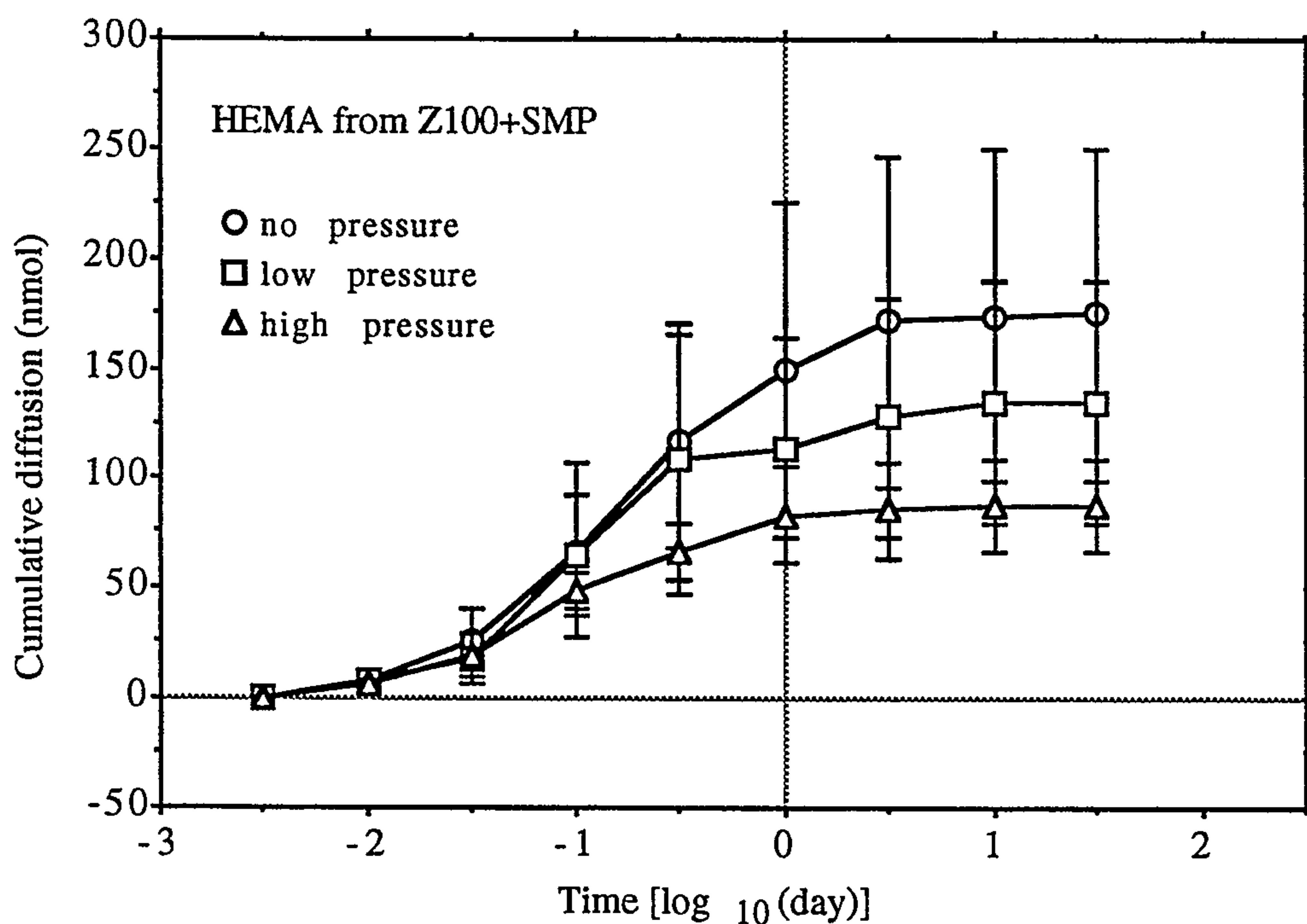


Figure 9.3 Cumulative diffusion of HEMA through dentine, *in vitro*, from Z100 and Scotchbond Multi-purpose when subjected to hydrostatic pressure

No significant differences were found for cumulative HEMA diffusion in the three groups over any of the time intervals (at $p < 0.05$) although there was a tendency suggesting HEMA diffusion was resisted by high pressure.

9.3.2.2 Comparison of rates of diffusion of TEGDMA and HEMA through dentine *in vitro*, from a bonding agent system/composite resin combination (Z100 with Scotchbond Multi-purpose) when subjected to hydrostatic pressure

Rates of diffusion of TEGDMA for these groups are shown in Fig. 9.4. There was no significant difference noted between the no pressure and low pressure groups (at $p < 0.05$) for intervals up to 3 days. After 10 days, rates differed for all groups. A significant difference was found between the rates for high pressure and both no pressure and low pressure groups after 144 minutes and for the duration of sample collection (at $p < 0.05$). A significant difference was also seen when low and high pressure groups were compared for all intervals after 43.2 minutes (at $p < 0.05$). The diffusion rates for HEMA are shown in Fig. 9.5.

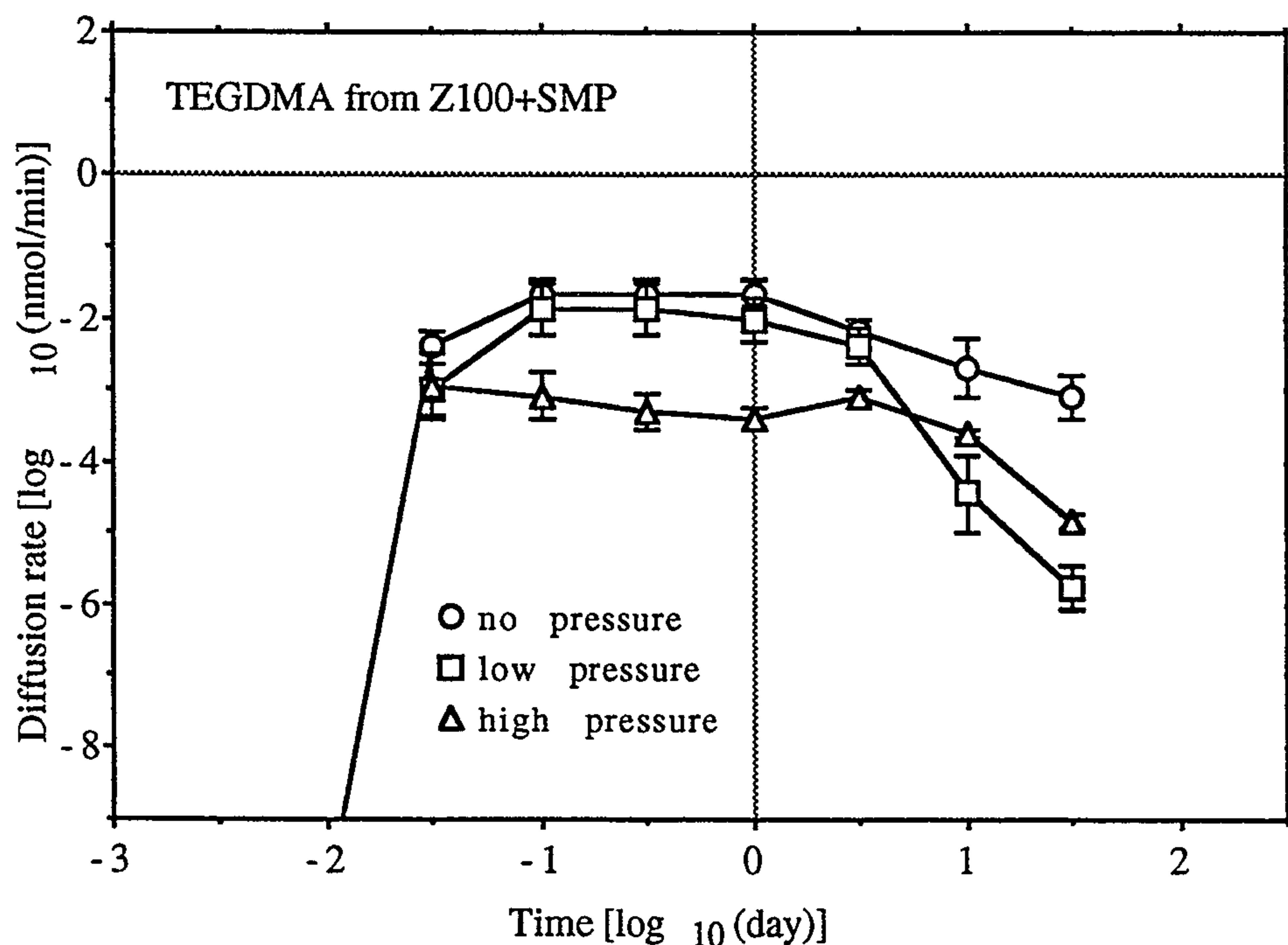


Figure 9.4 Rates of diffusion of TEGDMA through dentine, *in vitro*, from Z100 and Scotchbond Multi-purpose when subjected to hydrostatic pressure

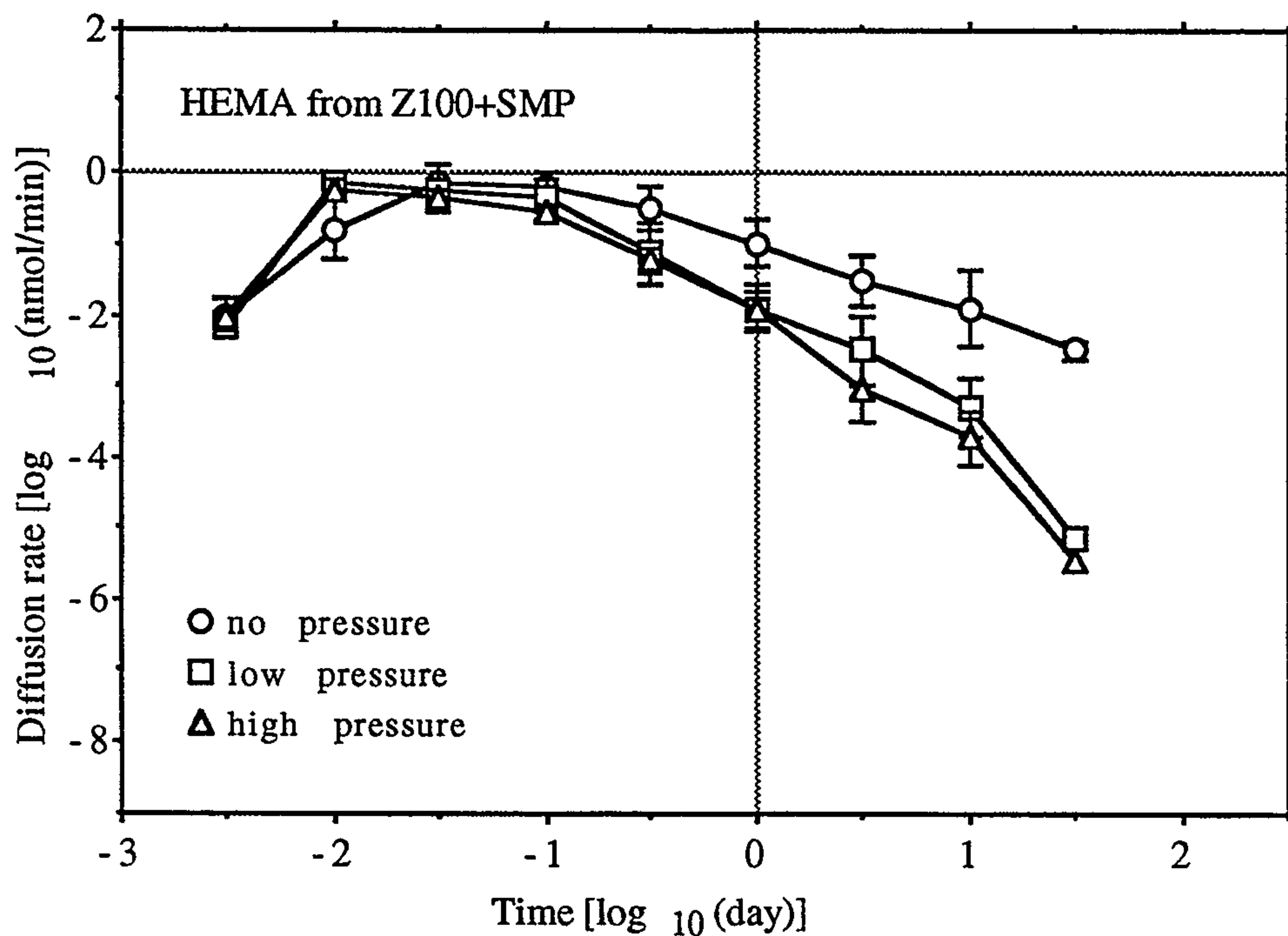


Figure 9.5 Rates of diffusion of HEMA through dentine, *in vitro*, from Z100 and Scotchbond Multi-purpose when subjected to hydrostatic pressure

Up to 432 minutes, there was no significant difference observed between diffusion rates of HEMA through dentine *in vitro*, observed for the three groups (at $p < 0.05$). From 1 day and for the remaining time course, rates in the no pressure group remained higher than both the low and high pressure groups, which were similar.

The data showed that positive intrapulpal pressure did not prevent the diffusion of TEGDMA or HEMA through dentine from the bonding resin-composite resin combination examined. Amounts and diffusion rates for HEMA were not significantly affected by intrapulpal pressure. In contrast, intrapulpal pressure did influence the diffusion rate of TEGDMA in that, at a high pressure, the diffusion rate was significantly less than at a lower

pressure. Significantly more HEMA than TEGDMA had accumulated by 3 days.

9.4 Discussion

The pulpal chamber may be considered an enclosed system of low interstitial compliance, containing a volume of fluid which exerts a definite fluid pressure (Tønder and Kvinnsland, 1983; Heyeraas, 1989). This pressure is known to vary in magnitude in response to differing stimuli. It is significantly higher in inflammatory states, observing physiological dynamics, and in the uninflamed condition it is positively directed toward the dentinal walls. The potential of this pressure, acting in an outward (positive) direction to oppose diffusion of substances across dentine, has been explored in relatively few *in vivo* experiments (Maita *et al.*, 1991; Vongsavan and Matthews, 1991; Vongsavan and Matthews, 1992). These studies have often involved superficial dentine examined under optimal conditions. Several investigators have measured intrapulpal pressure in the cat (Tønder and Kvinnsland, 1983; Heyeraas, 1989; Vongsavan and Matthews, 1991; Vongsavan and Matthews, 1992), in the dog (Maita *et al.*, 1991) and in humans (Andrews *et al.*, 1972). Generally, this has involved penetration of the pulp chamber and the unavoidable influence of this on pressure recorded. Hydrostatic intrapulpal pressure has also been considered in the examination of resin bonding to tooth structure.

Passive monomer diffusion from the resin combinations used in this work has been reported in Chapter 8. Both monomers TEGDMA and HEMA were identified in water samples during the time course. In the current work, these monomers were similarly traced through dentine *in vitro* under the influence of positive pulpal pressure.

Two levels of pressure were used in the current study. The lower pressure, 15 cm H₂O, is considered to be physiologically relevant (Pashley and Matthews, 1993). The higher pressure, 36 cm H₂O, is similar to that indicating irreversible inflammation in humans and in excess of the "hyperemic" state of about 18 cm H₂O (Andrews *et al.*, 1972) although other authors consider this level to be another simulation of pulpal pressure (Prati *et al.*, 1991). These higher pressure levels in inflamed conditions may

be localised to the inflamed area only and not extend to remaining pulpal tissue, dropping to near "uninflamed" levels as close as 2 mm from the inflamed area (Tønder and Kvinnsland, 1983). Therefore, the higher pressure used in this study will not be a close representation of the inflamed state. In order to examine the effect of this positive pulpal pressure on diffusion through dentine, it was necessary to simulate both pressures in parallel experiments. Pressure was maintained continuously throughout the experiment as far as possible but was relieved for a brief time during sample removal.

Data presented in Chapter 8 has shown that when SMP is used in combination with Z100, the bonding resin retards TEGDMA release from Z100 but does not prevent it. HEMA, a component of both the primer and bonding resin in SMP also diffuses through dentine. These tendencies were again noted here. Positive pulpal pressure did not prevent monomer diffusion from the resins examined. In the three groups examined and following an initial steep rise in rate over the first time interval (0-4.32 min), the diffusion rate of HEMA decreased linearly. This relative insensitivity to pressure may be partly explained in terms of some physical properties of HEMA. HEMA is a low viscosity, hydrophilic monomer, of relatively low molecular weight (130), with a density of 1.07 g/ml. and surface tension of 37.3 ± 0.03 dyne/cm (Dulik *et al.*, 1981). HEMA inclusion in composite resins has been found to increase water sorption values to as high as 1.24 ± 0.3 mg/cm² (Dulik *et al.*, 1981) which may increase solubility and water exchange in composite resins. These characteristics probably contributed to the diffusivity of HEMA through dentine. The relatively low density and surface tension of HEMA compared with TEGDMA probably also contribute to the diffusivity of this monomer. Density, in addition to other factors, may be used to derive a solubility parameter which measures the ability of the material to soften the receiving surface (Asmussen *et al.*, 1991). Softening of the surface is also achieved by the solvent (acetone) present in the primer solution. The solvent also enhances air and liquid removal by evaporation (Van Meerbeek *et al.*, 1992). Asmussen and co-workers (1991) suggested that HEMA, applied in both primer and bonding resin, may sufficiently wet and 'soften' the dentinal surface enhancing dentinal penetration. This probably allows passage of sufficient concentration of HEMA to overcome molecular interference by water molecules within tubules. HEMA is readily soluble in water and is known to improve diffusivity and penetrability of composite resin monomers (Erickson, 1989).

Deep dentine, such as that used in the experiment, is considerably wetter than superficial dentine. This additional water content probably facilitates the dissolution of low viscosity molecules in pulpal fluid. Deep dentine also contains more porous peritubular dentine (66%) and less intertubular dentine (12%) compared with superficial dentine (Pashley, 1991). In the Scotchbond Multi-purpose with Z100 group, dentine was prepared using 10% maleic acid, which removes smear layer and so increases the permeability of dentine to some degree which may have enhanced passage of monomers. The interactions of primer and bonding agent used in Scotchbond Multi-purpose enhances composite resin adaptation to substrate promoting penetration (Van Meerbeek, *et al.*, 1992).

The early arrival of HEMA and its relative insensitivity to positive pulpal pressure, can be discussed in terms of the theoretical factors contained in physical laws, although, as noted by Pashley, several factors unique to dentine act to diminish the applicability of these laws to diffusion through dentine.

Diffusion, or passive transport, is the net movement of particles from an area of high concentration to an area of low concentration due to the random molecular movement of particles. Fick's first law (equation 9.1) allows the calculation of a permeability coefficient (adapted from Pashley and Livingston, 1978).

$$P = \left(\frac{J}{(C_c - C_p)} \right) \quad J_v = 0 ; \Delta\pi = 0 \quad (9.1)$$

where:

J = net flux of permeating solute

C_c = concentration of solute on the cavity side

C_p = concentration of solute on the pulpal side

P = permeability coefficient

$\Delta\pi$ = change in osmotic pressure between cavity
and pulpal chamber = 0

J_v = volume flow between the cavity side and the pulpal side

This law basically relates "flux" of a permeating solute across a barrier to the concentration gradient, when pressure change across the barrier is zero. Therefore, according to this law, the diffusion observed for HEMA and TEGDMA in the no pressure group occurred simply by the observance of a concentration gradient between the cavity floor and the pulp space. The difficulty in applying this relationship to diffusion through dentine is associated with the nature of the state of the solute on the cavity side which is actually a complex interface of polymerized resin with dentine. The law also does not account for the different molecular weights of the diffusing molecules. Diffusion through dentine, as suggested in Chapter 5, is therefore not simply a case of fluid "flux".

The Fick equation (equation 9.2) relates factors to derive a solute "flux" across a barrier. Flux is directly related to concentration, the solute diffusion coefficient and the surface area for diffusion but is inversely related to dentine thickness.

$$F_s = D_s A \frac{dC}{dX} \quad (9.2)$$

where;

F_s = solute flux

D_s = diffusion coefficient of solute

A = diffusional surface area

C = applied concentration

X = dentine thickness

The equation makes no provision for pressure gradient across the barrier or for temperature and assumes uniform tubular diameter. Of interest in the current study is the importance of the diffusion coefficient which is inversely related to molecular weight. This suggests that HEMA (MW=130) would have a higher diffusion coefficient than TEGDMA (MW=286.3).

Hydraulic conductance, as defined by Reeder and co-workers (1978), is a "measure of the ease with which fluid, under hydrostatic or osmotic pressure, can pass through a permeable barrier under defined conditions". This relationship is shown in equation 9.3.

$$L_p = \frac{F_v}{A \times t \times P} \quad (9.3)$$

where:

L_p = hydraulic conductance

F_v = fluid flow

A = dentine area

t = time

P = hydrostatic pressure

Thus, the ease with which fluids flow across dentine is inversely related to dentine area and to hydrostatic pressure. Pressure was the only variable in equation 9.3 which varied amongst the groups and the inverse relationship between pressure and flow was illustrated by TEGDMA diffusion. In this regard, TEGDMA diffusion could be predicted by hydraulic conductance.

In the current study, HEMA diffusion was unaffected by the different pressure levels used whereas TEGDMA diffusion was reduced only by the higher pressure level. The difference between TEGDMA and HEMA noted in the study is of interest. It has been shown (in Chapter 8) that the bonding agent, despite its presence as a physical barrier within the resin-reinforced layer, does not prevent the diffusion of TEGDMA through to the pulp space. The findings in the current study indicate that there is some characteristic of the TEGDMA molecule that causes it to be more susceptible to high positive pressure than is the case for HEMA. TEGDMA, a diluent monomer component of the composite resin is a larger molecule than HEMA. HEMA is readily available both in solution (primer) and in being able to diffuse out of a polymerized, unfilled layer (bonding agent). It is reasonable to suggest that, on encountering positive hydrostatic pressure at the dentine/pulpal interface, the larger TEGDMA molecules are initially obstructed from

movement away from this interface, and into the pulp space, and that these molecules may remain within the dentine. As sufficient TEGDMA arrives later in the time course, a critical concentration is approached which then allows entry to the aqueous phase as shown by the increased diffusion rate observed at 3 days.

As was noted in chapter 7, dentine may act as a volume reservoir for diffusing molecules which are not significantly obstructed from entry into the pulpal space by positive hydrostatic pressure. For HEMA, it is significant that positive pressure on pulpal contents did not prevent monomer diffusion through dentine from the resins examined. This suggests that monomer diffusion is probably more influenced by other determinants of diffusion such as dentinal thickness and condition, and molecular size, and by clinical techniques. Whilst the current study examined only a few clinical variables in diffusion through dentine, it can be suggested that diffusion mechanisms in deep dentine *in vivo* may be facilitated by hydrostatic pressure which may change direction under differing circumstances.

CHAPTER 10

General Discussion

Release of components from materials used in tooth restoration is very probably unavoidable. At times, release is pharmacologically advantageous, such as when caries-arresting procedures are employed or topical antibacterial agents are administered. Unfortunately, sometimes responses are untoward.

Resin-based restorative materials are directly applied to dentine to create or enhance a retentive bond between tooth and restoration, to create or enhance an anti-microbial seal and to simplify restorative procedures. All these goals are not always achieved. However, despite the observation that individual components and composite resin materials have a definite toxic potential, the clinical use of such restorative materials generally results in few apparent ill-effects, provided that a good anti-microbial seal is created.

The current work provides new insights into some interactions between restorative resin materials and the dentine-pulp complex. In this Chapter, a summary of the principal findings will be given, followed by a brief consideration of the observed diffusion patterns through dentine, in light of established physical principles. Some possible biocompatibility issues, namely toxic and allergic responses, will then be discussed. Finally, future lines of investigation including the potential for increasing our understanding of the nature of the "interdiffusion zone" between resin materials and dentine using the present study method will be considered.

It was shown in the current work that the monomers TEGDMA and HEMA were released from various resin-based restorative materials into the adjacent aqueous phase. Chemical analysis of aqueous eluates by HPLC/MS confirmed the identities and quantities of the released components, using comparisons with solutions of the known components. The time course of release directly into water was determined. Release was initially very rapid and declined exponentially thereafter. Comparison between release data from samples of different thickness suggested a predominantly surface release phenomenon. TEGDMA release was increased markedly by air inhibition during activation, an occurrence which very probably reduced conversion. Release from non-activated ("uncured") composite resin was even greater. HEMA release from a bonding agent showed a similar pattern of release over time.

The potential of dentine to modify TEGDMA release when composite resin was directly applied to that tissue in a tooth model was then examined. TEGDMA was found to diffuse through dentine in detectable amounts from 43.2 min after composite resin placement in the cavity. Diffusion was delayed but not prevented by dentine, which substantiated dentine's "lack of barrier properties" (Pashley, 1989). Dentine retarded the movement of TEGDMA into water substantially but reduced total diffusion only slightly. The highest diffusion rate through dentine occurred much later, at about 432 min, than the highest *direct* release rate, which was in the first few minutes.

The use of dentine bonding systems contributed to overall monomer diffusion by supplying another diffusible monomer from either the primer or bonding agent, HEMA. When TEGDMA was present in both the bonding agent and the composite resin the amount of TEGDMA diffusing increased. A bonding resin containing HEMA but no TEGDMA, had little effect on the amount of TEGDMA released from the composite resin diffusing through the dentine. In addition, recent work by Hamid and Hume (1995b) using the same tooth model, have demonstrated that debrided inner carious dentine was highly permeable to both HEMA and TEGDMA diffusion from the same bonding resin/composite resin combination (Scotchbond Multi-purpose with Z100) as used in the current study; significantly larger amounts of the monomers were found.

It is worthwhile to consider the diffusion data presented in the light of established physical laws which describe diffusion and fluid flow.

First, the Fick equation (equation 9.2) relates factors which are pertinent to diffusion. It states that solute flux or movement is directly related to the diffusional surface area (in the case of dentine, tubule diameter), length of the diffusion path (in the case of dentine, tubule length), the concentration gradient and temperature. The equation describes flux across a homogeneous barrier. However, diffusional surface area in dentine varies in different depths of dentine; the diffusional surface area of the prepared dentine surface, comprising tubules, is in the order of 1% at the dentine-enamel junction and increases to 22% at the pulp (Pashley, 1991). As diffusional surface area increases, it can be expected that monomer diffusion through dentine may also be directly related to dentinal thickness. This has been shown by Hamid and Hume (1995) who examined monomer

diffusion through dentine in the Hume tooth model using four ranges of remaining dentinal thickness. These workers found substantial increases in TEGDMA and HEMA diffusion amounts and rates when remaining dentinal thickness was reduced.

Second, the Poiseuille-Hagen equation (below) relates factors determining fluid movement through small tubules (Pashley, 1989).

$$V = \frac{\pi \Delta P r^4}{8 \eta l} \quad (10.1)$$

where,

V = volume flow

ΔP = hydrostatic pressure difference across dentine

η = viscosity of fluid

r = radius of tubule

l = length of tubule

This states that fluid flow or filtration (Fogel *et al.*, 1988) varies directly with the fourth power of tubule radius and with hydrostatic pressure change across dentine, but varies inversely with tubule length. Therefore, greater flow across dentine would occur where tubules are of greater diameter, such as close to the pulp chamber, or when tubules have been dilated and a large increase in flow occurs with small changes in tubule diameter. Flow across dentine is likely to be less when tubule patency is reduced, for example, by the presence of debris.

In the current work, it was shown, using a bonding resin - composite resin combination that released both HEMA and TEGDMA into dentine, that HEMA diffusion was not influenced by positive intrapulpal pressure, but pressure similar to that in inflamed pulp (36 cm H₂O) reduced TEGDMA diffusion. If it is assumed that flow from the pulpal space to the cavity floor existed in the high-pressure groups, such flow would be likely to impair the diffusion of larger molecule (TEGDMA) more than smaller molecules (HEMA), which is consistent with the observations. In addition, the relative hydrophilicity of HEMA aiding its penetration into dentine has been demonstrated (Qvist *et al.*, 1977) and this characteristic may be sufficient to overcome positive pulpal pressure.

As can be seen from the above discussion, diffusion through dentine is a complex phenomenon which cannot be predicted fully by known laws. The present data show that physical characteristics of agents applied to dentine, such as viscosity, hydrophilicity and degree of conversion, may also be significant determinants of the dynamics of release and, therefore, of biological effects. It remains, then, to consider the biological significance of the diffusion of molecules through dentine, which initially must consider the toxic potential of the diffusing molecules.

There are many data showing that resin materials, including composite resins, bonding agents and various components of these products are cytotoxic in cell-culture test systems (for example, Spångberg *et al.*, 1973; Tyas, 1977; Hensten-Pettersen and Helgeland, 1977; Fujisawa *et al.*, 1978; Browne and Tyas, 1979; Hanks *et al.*, 1981; Meryon and Browne, 1983; Terakado *et al.*, 1984; Miller *et al.*, 1986; Fujisawa *et al.*, 1988; Hanks *et al.*, 1988; Hume and Massey, 1988; Imai and Nakamura, 1989; Kaga *et al.*, 1989; Meryon and Brook, 1989; Hume and Hood, 1990; Hood and Hume, 1990; Li *et al.*, 1990; Rathbun *et al.*, 1991; Hanks *et al.*, 1991; Caughman *et al.*, 1991; Rouse *et al.*, 1993). However, usage tests indicate that the same classes of material are in general either slightly toxic or are not toxic when applied to dentine clinically (for example, Baume and Fiore-Donno, 1968; Suarez *et al.*, 1970; Stanley *et al.*, 1972; Spangberg *et al.*, 1973; Baharloo and Moore, 1974; Ericksen, 1974; Stanley *et al.*, 1975; Heys *et al.*, 1976; Heys *et al.*, 1977; Brännström and Nordenvall, 1978; Stanley *et al.*, 1979; Leidal and Eriksen, 1985; Hörsted *et al.*, 1986; Plant *et al.*, 1986; Cox *et al.*, 1987; Stanley *et al.*, 1988; Lind, 1988; Anderson *et al.*, 1989; Bowen *et al.*, 1989; Qvist *et al.*, 1989; Fuks *et al.*, 1990; Dejou *et al.*, 1993).

These differences can be explained by the present data. Substantial and immediate release of TEGDMA and HEMA directly into water was observed when there was no intervening dentine. This, coupled with data on the cytotoxicity of those compounds (Hanks *et al.*, 1991), was consistent with data on the cytotoxicity of composite resins and bonding agents in tissue culture systems reported by others. However, intervening dentine delayed substantially the arrival in the pulp space of both chemicals, 'muting' the probable peak concentration sufficiently to explain the relatively low pulpal cytotoxicity in clinical use. Inherent in this proposal is the suggestion that the vector responsible for those adverse responses noted (in, for example, the studies of Qvist *et al.*, 1989 and of Fuks *et al.*, 1990) was

unconverted monomer from the composite resin or from the dentine bonding system.

The possibility exists of adverse responses other than those due to direct cytotoxicity. The term *allergy* encompasses disorders of immune function in response to repeated exposures to antigens which are severe enough to cause discomfort, illness or death (Kirkpatrick, 1987). *Immediate hypersensitivity* occurs within a few minutes after exposure, due to the release of histamine, leucotrienes and other inflammatory mediators. Severe, immediate hypersensitivity responses (also termed *anaphylaxis*) may include bronchoconstriction, vasodilatation, decreased blood pressure and cardiac failure. *Delayed hypersensitivity* is mediated by T-lymphocytes which release lymphokines to produce inflammation and, in severe cases, substantial local tissue injury (Roitt, 1988). The tissue reaction typically occurs several days after exposure. Once acquired, the state of hypersensitivity may persist for many years (Dahl, 1982).

In dentistry, hypersensitivity reactions to many dental materials have been identified, for example (with highest incidence first) cobalt-chrome-nickel alloys, local anaesthetics and methacrylate-based materials (Fisher, 1956; Franz, 1982; Yontchev *et al.*, 1986; Kaaber, 1990). Reports on the latter category include denture base resins (Turrell, 1966; Stungis and Fink, 1969; Nealey and del Rio, 1969; Danilewicz-Stysiak, 1971; Rickles, 1972; Olsen, 1975; Giunta and Zablotzky, 1976; Weaver and Goebel, 1980; Fernström and Öquist, 1980a; Fernström and Öquist, 1980b; Kallus, 1984). Hypersensitivity responses to older dentures also occur (Crissey, 1965; Stungis and Fink, 1969; McCabe and Basker, 1976). "Burning mouth syndrome" has been ascribed to allergic reaction to a range of compounds including those found in dentures such as acrylates and epoxy curing agents (Bergdahl *et al.*, 1994; Lamey *et al.*, 1994). Hypersensitivity to resins similar in formulation to composite resins, namely orthodontic adhesive resins, has been reported in animals (Davidson *et al.*, 1982; Söhoel *et al.*, 1994) and continues to be a concern. These reactions have generally been attributed to the slow leaching over time of components from the materials when used clinically (Ruyter and Györösi, 1976; Lygre *et al.*, 1993; Lygre *et al.*, 1994) and some of these reports have, in fact, prompted withdrawal of materials from the market (Davidson *et al.*, 1982). Composite resins have caused untoward subcutaneous tissue responses in dogs (Harsanyi *et al.*, 1974) and oral lichenoid reactions have

also been observed in soft tissues adjacent to composite resin restorations in humans (Lind, 1988).

Nathanson and Lockhart (1979) reported a case of allergy to composite resin restorations in a 30-year old woman. The clinical signs included erythema, vesiculation which appeared several hours after restoration placement reaching a maximum by 24 to 48 hours. The patient's first resin restorations (Adaptic) had been placed 4 years earlier. Two or three days after that first appointment, the patient developed an acute measles-like rash. These authors made several conclusions, that previous exposure to the allergen was essential, desensitisation was difficult and antihistamines were not effective in reducing the reaction. A negative patch test result to acrylic resin in this case suggested to these authors that some component unique to composite resin was the cause.

The present data show that both TEGDMA and HEMA are very likely to enter the body in one or more of three ways. Absorption through skin in dental personnel handling composite resins or bonding resins is highly likely. In patients, the chemicals are very probably available in microgram quantities via saliva in the minutes and hours after placement and via dentine and pulp in the hours and days after placement. Whether or not the increasing use of materials containing these components will lead to an increase in allergic responses to them has yet to be determined.

Several areas can be suggested for further study. Determination of component diffusion through dentine *in vivo* is an important next step. There is also much to be learned about the potential of HEMA and TEGDMA to induce allergic responses. Animal usage studies would be extremely worthwhile to meet both of these goals. Much also remains to be clarified about the resin reinforced layer in dentine. Interactions at the resin/dentine interface are at present poorly understood, with the exception of some morphologic data (Nakabayashi *et al.*, 1992; Van Meerbeek *et al.*, 1992b; Spencer *et al.*, 1992). The degree of polymerization (conversion) within the interfacial zone and the propensity of the layer to suffer hydrolysis over time, for example, are currently unknown. Monitoring the release of resin components and resin breakdown products over time using approaches derived from the present studies will give data both on the degree of immediate conversion (which can be expected to influence strength) and on the long term integrity of the layer.

The current work has provided data which may streamline such studies by defining the chemicals most likely to diffuse from present restorative resins, namely HEMA and TEGDMA, by establishing the probable amounts and time courses of their bioavailability and by creating a valid assay method to determine the identity and quantity of diffusing components. As new resin-based materials and bonding systems evolve, their potential for component release and diffusion can be examined using what is now an established model.

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