Review Article

International Journal of Dental and Health Sciences
Volume 02,Issue 01

CASEIN PHOSPHOPEPTIDE - AMORPHOUS CALCIUM PHOSPHATE: A REVIEW

Deepa Bullappa[1],Manjunath P Puranik[2],Uma S.R[3]

1.Senior Lecturer,Department of Public Health Dentistry,Bangalore Institute of Dental Sciences,Bangalore
2.Prof and HOD,Department of Public Health Dentistry,Government Dental College and Research Institute,Bangalore
3.Assistant Professor,Department of Public Health Dentistry,Government Dental College and Research Institute,Bangalore

ABSTRACT:

Casein phosphopeptides-amorphous calcium phosphate (CPP-ACP) products have been widely used in the field of preventive dentistry. CPP exerts its main effect through binding and stabilizing calcium and phosphate ions (ACP) in an amorphous, non-crystalline state where they can enter enamel and enhance remineralization. This review summarises the research on Casein phosphopeptide-amorphous calcium phosphate complex and provides information related to its benefit and clinical applications in dentistry.

Keywords: Casein, CPP-ACP, remineralisation, dentistry

INTRODUCTION:

Tooth enamel consists predominantly of the mineral hydroxyapatite (HA). Although the tooth surface is continually bathed in saliva that is normally supersaturated in calcium phosphate with respect to HA, the production of acid by plaque bacteria during fermentation of dietary sugars results in saliva and plaque fluid becoming undersaturated with regard to HA and demineralization occurs, which is the initiation of dental caries.[1] Although dental caries is a highly prevalent disease and remains a major public health problem; in most developed countries, its prevalence has declined.[2]

A goal of modern dentistry is to manage non-cavitated caries lesions non - invasively through remineralization in an attempt to prevent disease progression and improve aesthetics, strength and function.[2] The precipitation of calcium phosphate phases in saliva normally does not occur due to the presence of salivary proteins particularly statherin and proline-rich phosphoproteins.[2]

The clinical use of calcium and phosphate ions for remineralization has not been successful in the past due to the low solubility of calcium phosphates, particularly in the presence of fluoride ions.[3]Fluoride is the cornerstone of the non-invasive management of non-cavitated caries lesions but its ability to promote net remineralization is limited by the availability of calcium and phosphate ions.[2] For every 2 fluoride ions, 10 calcium ions and 6 phosphate ions are required to

*Corresponding Author Address: Dr.Deepa Bullappa,Senior Lecturer,Department of Public Health Dentistry,Bangalore Institute of Dental Sciences,Bangalore-29. Email: deepabullappa@gmail.com

form one unit cell of fluorapatite [$Ca_{10}(PO_4)_6F_2$]. Hence, on topical application of fluoride ions, the availability of calcium and phosphate ions can be the limiting factor for net enamel remineralization to occur, and this is highly exacerbated under xerostomic conditions. A delivery system for bioavailable calcium and phosphate ions therefore may have a role as anadjunct to fluoride treatment in the management of early caries lesions.[4]

Dairy products have long been known to exhibit anticaries activity.[1] The components of milk responsible for this anticariogenic activity have been identified as casein (CN), calcium and phosphate. In milk, calcium and phosphate ions are stabilized in micelles in combination with the caseins. Casein is often used as a reference protein in nutritional studies but it is well known to have a lower nutritional value than several animals and a few vegetable proteins.[5]

Casein has a sequence containing a cluster of phosphoserine residues in the molecule. The phosphoserine-rich group of peptides thus formed and accumulated in the small intestine is called Casein Phopho-Peptide (CPP).[3]

Amorphous Calcium Phosphate (ACP) occurs in many biological systems, especially in primitive organisms, where they are believed to serve as a reservoir of calcium and phosphate ions. ACP is also used to refer to micelles of calcium phosphate (CaP) in milk and cheese.[6]

Casein phosphopeptide - amorphous calcium phosphate (CPP-ACP) is a milk derived peptide that has the ability to stabilize ionic calcium and phosphate and limit aggregation to a size that prevents crystal nucleation creating supersaturated solutions which drive remineralization and inhibit demineralization. Numerous invitro, in situ and clinical studies have reported that CPP-ACP is efficacious in remineralizing White Spot Lesions and preventing demineralization.[6]

The concept of CPP-ACP as a remineralising agent was first postulated in 1998.[7] CPP-ACP was developed by Prof. Eric Reynolds at the school of Dental Science at the University of Melbourne in Australia.[8] The CPP-ACP complex was patented by the University of Melbourne, Australia and the Victorian Dairy Industry Authority, Abbotsford, Australia. Bonlac Foods Limited (an Australian company owned by 2,300 dairy farmers in Victoria and Tasmania) has exclusive manufacturing and marketing rights for CPP-ACP and is the owner of the trademark (Recaldent). In early 1999, the U.S. Food and Drug Administration (FDA) accepted Recaldent as “generally recognized as safe” for its intended use as a texturizer in chewing gum (Trident White, Cadbury Adams USA, Parsippany, N.J.) at up to 5 percent weight per weight.[9]

The FDA has approved products marketed in the United States (MI Paste and MI Paste Plus containing 900 parts per million fluoride, GC America, Alsip, Ill.) for use primarily as abrasive prophylaxis pastes and secondarily for the treatment of tooth sensitivity (after in-office bleaching procedures, ultrasonic scaling, hand scaling or root planing). However, its use for

remineralizing dentin and enamel and preventing dental caries is an off-label application.[9]

According to the manufacturer (GC America), CPP-ACP is a useful cariostatic agent for the control of dental caries and it can be used as an adjunct preventive therapy to reduce caries in high-risk patients to reduce dental erosion in patients with gastric reflux or other disorders, to reduce decalcification in orthodontic patients, to repair enamel in cases involving white-spot lesions, orthodontic decalcification or fluorosis or before and after tooth whitening) and to desensitize teeth (for example, reducing hypersensitivity resulting from whitening procedures, treating sensitive dentin in patients with dental erosion and reducing sensitivity resulting from exposed root surfaces after professional tooth Cleaning.[8]

More and more applications are being suggested for CPP-ACP and so the intent of this review is to compile it's most common applications.

HISTORICAL BACKGROUND OF CPP:

Mellanby (1930) proposed milk as an important nutritional factor affecting pre-eruptive tooth mineralisation and post-eruptive caries resistance.

Nyvad and Fejerskov (1984) used transmission and scanning electron microscopy to show that milk of various fat contents could substantially modify the structure of the pellicle formed in vivo.

Reynolds (1987) using an insitu caries model showed that exposure of inset-enamel plaque to solutions containing tryptic peptides of casein significantly reduced enamel subsurface demineralisation. It was concluded that the tryptic peptides responsible for caseinate's anticariogenic activity were the calcium-phosphate - stabilising casein phosphopeptides.

Rolla and Rykke (1994), found that the surface of a recently formed pellicle was composed of micelle-like protein globules formed in the presence of saliva or milk.

ACP- DEVELOPMENT AND STRUCTURE:

ACP was firstly described by Aaron S. Posner in the mid 1960s. It was obtained as an amorphous precipitate by accident when mixing high concentrations (30 mM) of calcium chloride and sodium acid phosphate (20 mM) in buffer.

In X-ray diffraction, it was shown to have only two broad and diffuse peaks, with maximum at 25° 2è. No other features were obvious and it was clearly not apatite. This pattern is typical for substances that lack long range periodic regularity.

In 1965, Eanes et al. identified as a bone component. ACP in bone, along with the apatite might account for the broad diffraction pattern and variable.

In 1965, Eanes et al. identified ACP as a bone component. ACP in bone,along with the apatite might account for the broad diffraction pattern and variable composition of bone minerals.

In 1975, ACP was found in the mineralized cytoplasmic structure isolated from the blue crab hepatopancreas, with a very similar short-range atomic structure to synthetic amorphous calcium phosphate.

After the discovery of amorphous calcium phosphate, the early studies were focused on the structure of ACP. It was suggested that synthetic ACP particles, which appear as 300- 1000 Å spheres in the electron microscope, consist of a random assembly of ion clusters 9.5 Å in diameter, dimensions consistent with the chemical composition of $Ca_9(PO_4)_6$.

Wuthier et al reported that ACP, with Ca/PO4 molar ratio as low as 1.15, precipitated at more acidic preparative pH, i.e. 6.9. More importantly, it has been shown that ACP particles are nanometer particles. Primary particle sizes of ACP is about 40-100 nm.

The ACP precipitate, with little long-range order, is a highly unstable phase and hydrolyzes almost instantaneously to more stable phases. In the presence of other ions or under in vivo conditions, ACP may persist for appreciable periods due to kinetic stabilization.

Although the exact mechanism of stabilization of ACP is not understood, the presence of Mg^{2+}, F^-, carbonate, pyrophosphate, diphosphonates, or polyphosphorylated metabolites or nucleotides, in sufficient quantity will prevent the transformation of synthetic ACP to hydroxyapatite.[10]

CPP-ACP

Casein - Phosphopeptides (CPP) are naturally occurring molecules which are able to bind calcium and phosphate ions and stabilize Amorphous Calcium Phosphate (ACP).[11]

Casein phosphopeptides (CPP) contain the cluster sequence of -Ser (P)-Ser (P)-Ser (P)-Glu-Glu from casein has a remarkable ability to stabilize calcium and phosphate as nanoclusters of ions in metastable solution (Cochrane et al., 2008). Through these multiple phosphoseryl residues, CPP has a remarkable ability to stabilize clusters of ACP into CPP-ACP complexes, preventing their growth to the critical size required for nucleation, phase transformation and precipitation. It adheres easily to soft tissue, pellicle, plaque and even hydroxyappatite.[10]

It reacts similar to the mineral/statherin relation in saliva supplying bio available calcium and phosphate required for remineralisation.[11] Through the active sequence, the CPP binds to forming nanoclusters of calcium and phosphate ions to form nanocomplexes of around 1.5 nm radius, preventing the growth of the nanoclusters to the critical size required for nucleation and phase transformation.[4]

MECHANISM OF ACTION:

Calcium-phosphate reservoir:

(CPP-CP) have been found to increase the levels of calcium and phosphate in plaque up to five folds in humans in *in situ* caries models and short-term mouthwash studies.[4] The proposed mechanism of their

anticariogenicity is that they act as a calcium-phosphate reservoir, buffering the activities of free calcium and phosphate ions in the plaque fluid helping to maintain a state of supersaturation with respect to enamel minerals, thereby depressing enamel demineralization and enhancing remineralization.[12] The binding of ACP to CPP is pH dependent; with binding decreasing as the pH falls. CPP-ACP have been demonstrated to have anticariogenic potential in laboratory, animal, and human in situ experiments.[8]

Inhibition of bacterial adhesion:

The immunolocalisation studies have revealed that CPP-ACP can be incorporated into supragingival dental plaque by binding to the surfaces of bacterial cells to components of the intercellular plaque matrix and to adsorbed macromolecules on the tooth surface. All these interactions may then lead to the formation of a less cariogenic plaque. Rose (2000) demonstrated that CPP-ACP competes with calcium for plaque calcium binding sites and this will reduce the degree of calcium binding between the pellicle and adhering cells and between the cells themselves as supposed by Schupbach et al (1996). It was suggested that casein glycomacropeptide (CGMP) and CPP adsorb to the surface of the pellicle and mask receptors on salivary molecules for these streptococci (Nyvad and Fejerskov, 1984).[7]

Dose-dependent response:

CPP-ACP reduces caries activity in a dose-dependent mechanism and the subsequently formed mineral is more resistant to acid attack (Shen et al, 2001; Reynolds et al, 1995; Cai et al, 2003; Iljima et al, 2004) CPP-ACP solutions, applied twice daily to the teeth of specific-pathogen-free rats orally infected with *Streptococcus sobrinus*, a bacterium that causes tooth decay in humans, significantly reduced caries activity with 0.1% w/v CPP-ACP producing a 14% reduction, and 1.0% w/v CPP-ACP producing a 55% reduction on smooth surfaces and 0.1% and 1.0% w/v CPP-ACP respectively, produced a 15% and 46% reduction in fissure caries activity relative to the distilled water control (Reynolds et al, 1995).[7]

Rate of remineralisation:

The CCP can stabilise over 100 times more calcium phosphate than is normally possible in aqueous solution at neutral and alkaline pH before spontaneous precipitation (Holt and van Kemenade, 1989). In the process of mineralisation, ACP and the crystalline phases dicalcium phosphate dihydrate (DCPD) and octacalcium phosphate (OCP) have been implicated as intermediates in the formation of hydroxyapatite (HA), depending on pH and degree of saturation.

Prevention of tooth erosion:

CPP-ACP might prevent tooth erosion by suppressing demineralisation, enhancing remineralisation or a combination of these two processes. The presence of CPP-ACP might permit a rapid return to resting calcium concentrations and allow earlier remineralisation of the enamel substrate.[7]

Interaction of CPP-ACP with fluoride:

Plaque enzymes such as phosphatases and peptidases partially degrade CPP-based products, consequently increasing pH due to the production of ammonia. Adding fluoride to CPP limits phosphatase action by extending the action of molecular complexes (Vitorino et al, 2005). The adjunct anti-cariogenic effect obtained with CPP-ACP plus fluoride could relate to fluoride also being incorporated into the CPP-ACP complex. The casein phosphopeptide-amorphous calcium phosphate interacts with fluoride ions to produce an amorphous calcium fluoride phosphate stabilised by the CPP at the tooth surface. Casein phosphopeptide with amorphous calcium fluoride phosphate (CPP-ACFP) provides all the elements necessary for dental remineralisation on the tooth surface and in the dental biofilm. This provides soluble calcium, fluoride and phosphate ions to promote remineralisation with fluorapatite that is more resistant to future acid challenge. CPP can adhere to 25 calcium ions, 15 phosphate ions and five fluoride ions per molecule and can stabilise calcium phosphate in solution (Cross et al, 2005). In this way the CPP could act as an efficient delivery system, not only for amorphous calcium phosphate but also for fluoride.

Prevent de-mineralisation and improve re-mineralisation:

This mechanism has been observed in laboratory and animal studies and in situ studies covering human subjects. Explanation of this potential has been based on the ability of casein phosphopeptide (CPP) to stabilize calcium phosphate by binding amorphous calcium phosphate (ACP) and thus forming CPP-ACP clusters. These CPP-ACP clusters act as a calcium and phosphate reservoir that attaches itself to dental plaque and tooth surfaces. On acid challenge, the attached CPP-ACP releases calcium and phosphate ions, thus maintaining a supersaturated mineral environment, thereby reducing demineralization and enhancing remineralization. It has been shown that enamel remineralized by CPP-ACP is relatively more acid-resistant than normal tooth enamel.[12]

It has been proposed that the remineralization mechanism of CPP–ACP involves localization of ACP at the tooth surface which buffers free calcium and phosphate ions. By maintaining a state of supersaturation with respect to the hydroxyapatite, these ions depress demineralization and promote remineralization.[13]

CPP-ACP complex and GIC:

The release of CPP-ACP and fluoride from the CCP-ACP containing GIC as the acid erodes the cement was associated with enhanced protection of the adjacent dentin during acid challenge *in vitro*. It was concluded that the 1.56%-CPP-ACP-containing GIC might be a superior restorative/base with an improved anticariogenic potential. Furthermore, CPP has been shown to keep calcium, phosphate and fluoride as ions in solution, thereby enhancing the efficacy of the fluoride as a remineralizing agent.[8]

CPP-ACP and enamel bleaching:

Casein phosphopeptide-amorphous calcium phosphate (RecaldentTM, Cadbury Schweppes), has been successfully incorporated into oral health products such as a mouthrinse, sugar-free chewing gums and a sports drink to reduce enamel erosion. A CPP-ACP paste (Tooth Mousse (MI Paste); GC Corp. Japan), is recommended during or after enamel bleaching to reduce tooth sensitivity and anectodal reports have indicated the success of the paste in this regard.[14]

Anti-cariogenicity of CPP-ACP:

Casein phosphopeptide calcium-phosphate complexes (CPP-CP) have been found to increase the levels of calcium and phosphate in plaque up to five folds in humans in insitu caries models and short-term mouthwash studies. The proposed mechanism of their anticariogenicity is that they act as a calcium-phosphate reservoir, buffering the activities of free calcium and phosphate ions in the plaque fluid helping to maintain a state of supersaturation with respect to enamel minerals, thereby depressing enamel demineralization and enhancing remineralization. The binding of ACP to CPP is pH dependent; with binding decreasing as the pH falls. Casein phosphopeptide-amorphous calcium phosphate compounds (CPP-ACP) have been demonstrated to have anticariogenic potential in laboratory, animal and human in situ experiments.[8]

COMMERCIALLY AVAILABLE FORMS

CPP-ACP has been incorporated into various products in order to exert a topical effect.

These products include:

- commercially available sugar-free chewing gum (Recaldent™; GC Corp, Japan and Trident White®; Cadbury Adams USA, Parsippany, New Jersey, USA),
- mints (Recaldent Mints™; Cadbury Japan Ltd, Japan),
- topical gels (Tooth Mousse™, Tooth Mousse Plus; GC Corp, Japan and MI Paste and MI Paste Plus; GC America, Alsip III) and
- experimentally tested sports drinks and glass ionomer cements

Chewing gum: Clinical trials of sugar-free chewing gum and mint have shown that they are non-cariogenic and can have an anticariogenic effect through the stimulation of saliva (Reynolds et al, 2003). G D Walker and co-workers[15] evaluated the effect of incorporating CPP-ACP into sugar-free gum on enamel remineralisation.

Mouth rinse: The fact that the remineralising effect is not confined to chewing gum as a vehicle for RecaldentTM was demonstrated by Cai et Al (2003) with rinsing solutions which also produced as much as 176% higher remineralisation than the controls.

Lozenges: Microradiographs and densitometry have shown the use of lozenges with different CPP-ACP

concentrations increases remineralisation in subsurface caries lesions which is dose-dependent. Two percent CPP-ACP solutions have also shown their effectiveness in reducing subsurface caries lesions, obtaining higher remineralisation with longer application times.

Topical gels: Lennon and colleagues applied a tooth cream containing 5% casein/calcium phosphate to bovine enamel specimens for 120 seconds twice daily. They found no significant difference with respect to erosive loss after seven and 14 days of erosive cycling. Higher remineralisation was observed in the specimens treated with Tooth Mousse GC after demineralization than the untreated specimens.

Glass ionomer cement: Studies have also been done to investigate the effect of adding the active complex to glass ionomer cements. Incorporation of 1.56% w/w CPP-ACP into the GIC was shown to increase compressive strength and microtensile bond strength, enhance the release of calcium, phosphate, and fluoride ions and enhance protection of the adjacent dentin to acid demineralisation.

Sprays: Hay and co-workers observed good moistening and lubrication with the CD-CP mouth rinse, when used as an atomised spray in the mouth. CCP-ACP preparations hold promise as caries preventive agents for individuals with dry mouth.

Energy drinks: Introducing CPP-ACP nanocomplexes to soft drinks and other frequently consumed acid products, especially for the adolescent and young adult population could help to reduce the erosive action of these products by Ramalingam et al. According to Walker et al, the addition of 2·0-5·0 g CPP-ACP/l to milk substantially increases its ability to remineralise enamel subsurface lesions. Casein phosphopeptide amorphous calcium phosphate (2%) is an important constituent of T.F.S.D.(Tooth Friendly Soft Drink).

Safety of CPP-ACP: The CPP-ACP formulation is non-toxic and In view of its broad spectrum of action and virtually unlimited usability, CCP complex can be of use to all patients at any time – from infants through senior citizens but patients with milk protein allergies should not consume products containing CPP-ACP. It can be used for both primary and permanent teeth, in patients with special needs/high-caries risk patients such as those with intellectual impairment, developmental and physical disabilities, Cerebral palsy, Down syndrome and those with any medical problems such as those undergoing radiation therapy and in cases of molar incisor hypomineralization (MIH).[16;17;18;19.]

SOME RECOMMENDED PROFESSIONAL APPLICATIONS FOR CPP-ACP COMPLEX

1) Bleaching
2) After application of topical fluoride, to provide a topical coating for patients suffering from erosion, caries and conditions arising from xerostomia (GC Europe, Recaldent).
3) In gerostomatology, the necks of the teeth are often a problem because of recession. If root caries is present,

the cream together with toothpaste can promote remineralisation as part of preventive treatment.

4) Professional tooth cleaning and root smoothing in periodontology can often result in hypersensitivity of the neck of the teeth which can be controlled very quickly with this new paste.

5) In restorative dentistry, the sensitivity of prepared abutment teeth can be reduced. White spot prevention/removal and enamel remineralisation after interdental stripping in orthodontics.

6) Remineralisation of the early natural enamel caries of fluorosed teeth.

7) Enhances remineralisation and decreases postoperative sensitivity following tooth whitening and microabrasion procedures in hypomineralised teeth.

8) As a transport medium for avulsed teeth. When highly diluted, the CPP-ACP preparation may help preserve L929 cell viability in the short term without inducing apoptosis.

CONCLUSION:

The calcium phosphate-based remineralisation technologies show promise as adjunctive treatments to fluoride therapy in the non-invasive management of early caries lesions. Remineralisation of early carious lesions by CCP-ACP complex may continue to emerge in importance as fluoride did in the past for caries prevention and reduction. Their use seems to be rewarding in the field of preventive dentistry. we should shift our ways of caries prevention to include products such as CPP-ACP in our prevention schemes for patients through remineralization of enamel and application of minimal invasive approaches in dentistry. Further research is required to provide a scientifically supported recommendation for other clinical applications.

REFERENCES:

1. Cross J, Huq NL, Stanton DP, Sum M, Reynolds EC. NMR studies of a novel calcium, phosphate and fluoride delivery vehicle-aS1-casein(59–79) by stabilized amorphous calcium fluoride phosphate nanocomplexes. Biomaterials. 2004;25:5061–5069.
2. Cochrane NJ, Cai F, Huq NL, Burrow MF, Reynolds EC. New Approaches to Enhanced Remineralization of Tooth Enamel. J Dent Res. 2010;89(11):1187-1197.
3. Takanori Kasai. CPP, casein phosphopeptides. Foods Food Ingredients J Jpn. 2003;208(3).
4. Reynolds EC. Casein Phosphopeptide-Amorphous Calcium Phosphate: The Scientific Evidence. Adv Dent Res. 2009 Aug;21:25-29.
5. C. G De. Kruif, C.Holt. Casein Micelle structure, functions and interactions. Advanced dairy

chemistry, vol 1: Proteins, 3rd Ed. Plenum Publishers 2003.
6. Bailey DL, Adams GG, Tsao CE, Hyslop A, Escobar K, Manton DJ, Reynolds EC, Morgan MV, Messer LB. An investigation into the remineralization of 'white spot' lesions of dental enamel with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and sodium fluoride following orthodontic band removal. ADRF Special Research Supplement. 2009 Dec;54(4).
7. Gupta R, Prakash V. CPP-ACP Complex as a New Adjunctive Agent for Remineralisation: A Review. Oral Health Prev Dent. 2011;9:151-165.
8. Ola B. Al-Batayneh. The Clinical Applications of Tooth Mousse TM and other CPP-ACP Products in Caries Prevention: Evidence-Based Recommendation. Smile Dental Journal 2009 Volume 4, Issue 1, p8-12.
9. Azarpazhooh A, Limeback H. Clinical efficacy of casein derivatives: A systematic review of the literature. JADA. 2008;139(7):915-924.
10. Zhao, Liu Y, Sun W, Zhang H, Jie Zhao et al. Amorphous calcium phosphate and its application in dentistry. Chemistry Central Journal. 2011;5:40:p1-7.
11. Karthikeyan M K, Ramaswamy K. How to combat white spot lesion in orthodontic cases-review study. Annals and essences of dentistry, 1 Jan - Mar 2012: Vol. IV Issue:p99-100.
12. Yengopal V, Mickenautsch S. Caries preventive effect of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP): a meta-analysis (Review Article). Acta Odontologica Scandinavica. 2009;67:321-332.
13. Srinivasan N, Kavitha M, Loganathan SC. Comparison of the remineralization potential of CPP-ACP and CPP-ACP with 900 ppm fluoride on eroded human enamel: An in situ study. Arch Oral Biol. 2010 Jul;55(7):541-4.
14. Adebayo OA, Burrow MF, Tyas MJ. An SEM evaluation of conditioned and bonded enamel following carbamide peroxide bleaching and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) treatment. J Dent. 2009 Apr;37(4):297-306.
15. Walker GD, Cai F, Shen P, Adams GG, Reynolds C, Reynolds EC. Casein Phosphopeptide-Amorphous Calcium Phosphate Incorporated into Sugar Confections Inhibits the Progression of Enamel Subsurface Lesions insitu. Caries Res. 2010;44:33–40.
16. http://www.recaldent.com/c_faq.asp accessed on 15/1/15 at 7:00.
17. http://www.healthwarehouse.com/solr/result/?q=MI+aste accessed on 15/1/15 at 13:20.
18. Azarpazhooh A, Limeback H. Clinical efficacy of casein derivatives : a systematic review of the literature. J Am Dent Assoc. 2008 Jul;139 (7):915-24; 994-5.
19. www. Recaldent.com accessed on 14/1/15 at 12:30.