

Physical methods used to determine milk replacer structure

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Abstract

Physical methods of determination (infrared spectroscopy – IR, atomic absorption and electronic paramagnetic resonance – EPR) were used to determine the structure of milk replacers and of their constituents.

The experimental results have shown that IR spectra provide the most information supplying both the print for recognizing compound class and proportion. EPR spectra revealed the presence of some essential trace elements such as Mn^{2+} , Fe^{3+} , Cu^{2+} , Zn^{2+} , as also supported by the atomic absorption measurements.

Keywords: *milk replacers, IR spectroscopy, atomic absorption, electronic paramagnetic resonance*

Introduction

During the past decades, the progress achieved in chemistry in general and in organic chemistry in particular requires the widespread use of modern physical methods both in laboratory research and industrial production.

The low requirement for time and substances as well as the wealth of information on the milk replacers, some of which impossible to obtain by chemical methods, made the physical methods replace the old destructive chemical analyses, laborious and less specific.

In the absence of physical methods the classical research way applies exclusively the usual chemical methods. By reactions of degradation, transformation in derivatives, etc., one attempts to assess each structure S_1 - S_n . These chemical methods supply several positive or negative information, such as “P is a carbonylic product since it forms oxime” or “P is a carboxylic acid since it does not develop in $NaHCO_3$ solution and does not yield functional derivatives (acid chlorides, esters, amides etc.)”. Such information obtained by laborious methods which use large amounts of reagents may provide the (possible) structure of the product P (1).

The modern way, using physical methods, is the following: product P is assessed with several physical determinations such as IR (infrared), UV-VIS (ultraviolet-visible) spectra, EPR (electronic paramagnetic resonance), atomic absorption. Each of these spectra supply several positive or negative information (showing that some

structures are not compatible with spectral results) on certain structures.

Material and methods

A total of 16 samples were assayed for IR spectra and trace elements content: powder milk, glucose, starch, corn flour, yeast polyculture, choline, lard, lecithine, vitamin-mineral premix, antioxidant (AVOX), carrier, choline on soybean carrier, lactose, milk replacers (imported – Totallact, Protillac and a product developed by us). The following procedure was used to plot IR spectra: solutions of 1g of each sample and 10 ml CCl_4 (carbon tetrachloride) were stirred magnetically for 15 ± 2 minutes and thereafter filtered on blue band filter paper.

The spectra were determined with an M 80 IR spectrophotometer using 3 mm cesium iodure (CsI) cuvettes and 0.5 mm sodium chloride (NaCl) cuvettes (3,4). We analyzed the components with hydrocarbon structure – mono- and polysaccharide compounds, phospholipids, the vitamin complex; the powder milk and the indigenous and imported milk replacers were analyzed for solubility in a polar solvent such as carbon tetrachloride. The plotted spectra were compared with the spectra from literature (1).

Another method of investigation was the electronic paramagnetic resonance (EPR). Among the analyzed milk replacers constituents, structural information can be achieved from organic and inorganic paramagnetic species. Among the organic components, substances forming free radicals can be detected, such as natural and synthetic antioxidants, vitamins. These phenol compounds acting as antioxidants get stabilized as dimers or fix on a carrier as diamagnetic species. The inorganic paramagnetic species are much more stable. They may act as transition ions (Mn^{2+} , Fe^{3+} , Cu^{2+}) in nutrient formulations.

Different microelements can be detected by atomic absorption, such as Fe, Mn, Cu, Zn. The atoms have the ability to absorb light at different wave lengths and becoming excited due to absorption they release the supplementary energy by hits or by the emission of radiation. The simplest way of obtaining atoms from a substance is to heat it at high temperature and light it concomitantly; also needed are a monochromator, a detection system, an amplifier and a display system (computer) (3,4).

We used a VARIAN 250 to detect microelements by atomic absorption spectroscopy and a flame of air-acetylene.

Results and discussion

Among the hydrocarbon components that were analyzed (Table 1), the polysaccharide compounds displayed a good solubility and

yielded very intense spectra of good resolution. Among the very intense bands the main attributions can be done to the deforming vibrations δ_{C-H} observed in the range of 940-950 cm^{-1} , and to the skeleton valence vibrations ∂_{C-O-C} in the range of 1050-1090 cm^{-1} glucosidic rings, valence vibrations ∂_{C-OH} in the range of 1100-1200 cm^{-1} , correlated with vibrations of binding deforming δ_{C-H} between 1500-1550 cm^{-1} . Symmetric and antisymmetric vibrations were observed in the methylene group (2850-2900 cm^{-1}), while the terminal group methyl displayed antisymmetric vibrations at 3003 cm^{-1} (5).

Table 1 IR spectra characteristics for carbohydrates, ∂ , cm^{-1}

Glucose	Starch	Corn flour	Yeast polyculture	Attribution
950 m	980 fi 1025	990 fi 1030	940 fi 950	δ_{C-H} not flat ∂^s_{C-O-C}
	1080 fi 1120	1090 m 1125	1050 m 1090 m	∂_{C-OH} $\partial^s_{C-O-C}, \nu^s_{C-O}$
			1170 fi 1200	
1220	1220 1275 fi	1225 1250 fi		δ_{C-H} skeleton ∂^{as}_{C-O-C}
1450			1500 fi	$\delta_{CH}, \delta_{CH_2-CO}$
		1550 fi 1750 1770		δ_{O-H} $\delta_{C=O}$
	1860 m 2020 m			$\delta_{C=O}$
2850 m		2700 m 2900 fi 3000 fi 3020	2850 m 2910 i	$\partial^s_{CH_2}$ $\partial^{as}_{CH_2}$ $\partial^{as}_{CH_3}$
3300 m	3300 3400 l		3300 s	δ_{O-H} , associated polymer
3500 m				
	3600 s	3600 s		δ_{O-H} , nonassociated polymer

where: δ =deforming, s=faint, i=intense, l=wide, ∂ = of valence, m=medium, fi=very intense, s=symmetrical, α s =antisymmetrical.

Transmittance variation according to the wavelength displayed a similar general shape of starch and yeast polyculture spectra, while the corn flour spectrum was predominated by the band of methylene

chains (16-19 carbon atoms) specific to the oleic acid, as well as frequencies specific to the carboxyl group in which the carbonyl group appears around 1750 cm^{-1} correlated with the intense band at 3600 cm^{-1} of the nonassociated $\delta_{\text{C-H}}$.

Table 2 IR spectra characteristics for phospholipids, ν, cm^{-1}

Choline	Lard	Lecithin	Attribution
625 m			$\delta_{\text{C-Cl}}$
	925 fi		
950 i		955 fi	$\partial_{\text{P-O-C}}$
		1050 fi	$\partial_{\text{C-OH}}$
	1130 fi	1150 fi	$\partial_{\text{P-O}}$
1250 s	1200 fi	1200 fi	$\partial_{\text{P-O-C}}$ $\partial_{\text{C-N}}$
	1350 i	1350 i	$\delta_{\text{CH}_2\text{-CO}}$
	1425 fi		$\delta_{\text{CH}_2\text{-CO}}$
		1440 fi	
1430			
1625 s	1720 fi	1725 fi	$\delta_{\text{C=C}}$ $\delta_{\text{C=O}}$
2800 fi	2800 fi	2800 fi	$\partial_{\text{N}^+\text{-CH}_3}$
2850 s	2880	2900 fi	$\partial^{\text{s}}_{\text{CH}_3}$
2900 s	2950	2950 fi	$\partial^{\text{as}}_{\text{CH}_2}$
3000	3000	3000 fi	$\partial^{\text{as}}_{\text{CH}_3}$
3300		3300	$\partial_{\text{O-H, associated}}$
3500 m	3500 m	3400 fi	$\partial_{\text{N-H}}$ $\partial_{\text{O-H, free}}$

Tables 2, 3 and 4 show IR spectra for the phospholipid derivatives and for the vitamin complex, powder milk and milk replacers. Among the analyzed phospholipid compounds, choline has a lower solubility, which yielded an inconclusive IR spectrum, while the spectra for lecithin and lard were intense and with proper resolution.

Table 3 IR spectrum characteristics for the vitamin complex, ∂ , cm^{-1}

Premix	AVOX	Carrier	Choline on soybean carrier	Attribution
970 m			970 fi	
1000 m	1000 i	1000 fi 1030	1000 1050 m	∂_{C-H} ∂_{P-O-C}
1150	1100 fi 1180 fi			
1200 m	1200 1250 fi	1240 fi 1260 fi	1200 1250 fi	∂_{P-O} ∂_{C-N}
	1300 fi 1340 fi 1370 fi			
		1470 m 1575 fi 1740 l	1575 fi 1725 i 1755	δ_{CH_2-CO} δ_{CH_3-CO} $\partial_{C=C}$ ∂_{C-O} $\partial_{N=CH_3}$
	2740 fi 2790 fi 2820 fi		2890 i	$\partial^s_{CH_2}$
	2920 s	2900 l 2970 fi	2980 fi 3020 i	$\partial^{as}_{-CH_2-}$ $\partial^{as}_{-CH_3}$
3300 s				∂_{O-H} associated
3500 s	3540 fi			∂_{O-H} nonassociated

Table 4 IR spectra characteristics for powder milk, lactose and milk replacers, ∂ , cm^{-1}

Powder milk	Lactose	Indigenous milk replacer	Totallact	Protolact	Attribution
			930		
960 s		950 fi			∂_{C-H} , not plane
	980 fi	975			
	1070 fi	1090 l	1110 s	1050 m	∂^s_{C-O-C}
	1100 fi	1140	1180 s	1120 m	∂_{P-O-C}
		1200		1190 m	∂_{C-O-C}
		1220 fi			∂_{C-N}
		1350	1350 s		δ_{CH_2-CO}
		1425			δ_{CH_3-CO}

		1525 fi			$\delta_{\text{O-H}}$ $\delta_{\text{C=C}}$
			1680 i	1680 i	
1725 s		1710 fi			$\delta_{\text{C=O}}$
	1850 m				
	1990 m				$\delta_{\text{N=CH}_3}$
		2780 fi	2780 i	2780 i	$\delta^{\text{s}}_{\text{CH}_2}$
2800 s		2875	2820 i	2820 i	$\delta^{\text{s}}_{\text{CH}_3}$
2890	2900 s	2950 fi			$\delta_{\text{O-H, associated}}$
3300 s	3300 s	3300 s	3300 s	3300 s	$\delta_{\text{O-H, notassoc.}}$
3500 s	3500 s		3500 s	3500 s	

Table 5 Trace element level in milk replacer components, ppm

Sample	Trace elements			
	Cu	Fe	Mn	Zn
Premix	0.0055	479.25	0	0.2002
Powder milk	15.98	0.130	11.040	10.803
Glucose	0	0.251	0.047	0.113
AVOX	0.072	5.340	0.010	0.081
Totlact	0.676	1.768	0.009	69.57
Protolact	0.813	0.086	0.002	0.808
Indig. milk replacer	0.70	0.013	0.049	1.241
Choline	0.076	0.182	0.004	2.983
Lard	0.122	0.406	0.025	0.118
Lecithin	0.047	0.028	0.012	0.133
Lactose	0.723	0.328	0.009	0.112
Starch	0.404	0.531	0.004	0.762
Corn flour	0.929	0.075	0.036	0.805
Carrier	0.791	0.019	0.460	3.595
Choline on soybean carrier	0.842	0.188	0.154	0.630

The vitamin-mineral premix displayed a low resolution, less conclusive spectrum. The structure of AVOX antioxidant revealed a concentration of very intense bands in the range 1100-1400 cm^{-1} that can be attributed to the aromatic ring and to specific C-N frequencies, methyl and methylene-bound carbonyl, as well as N-CH₃ frequency. Its structure may correspond to a steric-bound phenol-type antioxidant in position o-o' or it may be a compound with pyrimidinic structure.

The carrier and choline on soybean carrier spectra are very similar displaying double C=C bonds at 1575 cm^{-1} as well as C-OH and possible P-O and C-N bonds.

The powder milk and milk replacers Totallact and Protillac yielded spectra of lower resolution due to their lower solubility. The lactose and the milk replacer preparation displayed spectra with more intense bands. Compared to the spectrum of lactose, which displays all the bands characteristic to polysaccharides (Table 1), with very low absorption at 3000 cm^{-1} , the IR spectrum of the milk replacer preparation displayed very intense bands at 2800 cm^{-1} coming from the lecithin, from the corn flour and from AVOX antioxidant (2).

The results of the atomic absorption analysis are in agreements with the observations acquired by EPR, which showed the presence of Mn^{2+} ions in AVOX antioxidant and the preponderance of Fe^{2+} ion in the vitamin complex of the premix and the preponderance of Cu^{2+} and Zn^{2+} ions in the powder milk (Table 5).

Figure 1 shows in detail the 6 components of the hyperfine structure of Mn^{2+} ion, while Figure 2 shows the same spectrum for a narrower field scale, compared to the blank spectrum of Mn^{2+} (below), which supports the attribution. Unlike the spectrum of the blank, however, which displayed isotrop and equidistant main lines, the spectrum of AVOX antioxidant displayed a fine structure of rhombic anisotropy, showing that Mn^{2+} ion is not as salt, but complexed by a covalent bond to the organic carrier. Figure 3 shows the EPR spectrum for the premix vitamin complex. The signal corresponds to the Fe^{2+} ion chelated on an organic carrier.

Conclusions

Imported and Romanian milk replacers were analyzed by IR, EPR and atomic absorption.

IR spectra provided the most information displaying the group vibrations of the characteristic covalent bond, supplying both the print required to recognize a class of compounds and the proportion of some components.

EPR spectra showed the presence of essential trace elements such Mn^{2+} and Fe^{2+} , which are essential to nutrition, as supported by the atomic absorption measurements.

The imported milk replacers had a lower solubility in CCl_4 and vibrations of lower intensity than the indigenous milk replacer preparation. This suggests that the imported products contain mainly glucose and lactose in the polysaccharide component, powder milk in the phospholipid component and chiefly premix compounds in the vitamin component.

The milk replacer prepared by us contains corn flour, yeast polyculture, lard, AVOX antioxidant, most of these components being highly soluble in CCl_4 displaying very intense IR bands.

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